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Rosane Schwan, Brasil (ICY)
Disney Dias, Brasil
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<tr>
<th>30TH. - SUN.</th>
<th>1ST. - MONDAY</th>
<th>2ND. - TUESDAY</th>
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<tbody>
<tr>
<td>CRUB - UNCOMAHUE</td>
<td>NH-EDELWEISS</td>
<td>NH-EDELWEISS</td>
<td>NH-EDELWEISS</td>
</tr>
<tr>
<td>09.00 - 9.30</td>
<td>OPENING.</td>
<td>09.00 - 10.00</td>
<td>09.00 - 10.30</td>
</tr>
<tr>
<td>09.30 - 10.00</td>
<td>Invited speaker Pietro Buzzini.</td>
<td>Keynote Lecture - KEVIN VERSTREPEN</td>
<td>SESSION 5: Evolutionary genomics and domestication of yeast.</td>
</tr>
<tr>
<td>10.00 - 10.30</td>
<td>ORAL SESSION 1</td>
<td>10.30 - 11.00</td>
<td>Coffee break.</td>
</tr>
<tr>
<td>10.30 - 11.00</td>
<td>Coffee break.</td>
<td>11.00 - 12.30</td>
<td>SESSION 6: Genetic and metabolic improvement of yeasts.</td>
</tr>
<tr>
<td>11.00 - 12.00</td>
<td>ORAL SESSION 2</td>
<td>12.30 - 13.30</td>
<td>Lunch.</td>
</tr>
<tr>
<td>12.00 - 12.30</td>
<td>Invited speaker Marcois Morais.</td>
<td>12.30 - 14.30</td>
<td>Lunch in MANUSH.</td>
</tr>
<tr>
<td>15.30 - 16.00</td>
<td>Coffee break.</td>
<td>15.00 - 16.30</td>
<td>SESSION 7: Yeast in Biorefineries.</td>
</tr>
<tr>
<td>16.00 - 16.30</td>
<td>ISSY34 registration at NH Edelweiss.</td>
<td>16.00 - 17.00</td>
<td>Coffee break.</td>
</tr>
<tr>
<td>17.00 - 17.45</td>
<td>ORAL SESSION 4</td>
<td>16.30 - 17.00</td>
<td>Keynote Lecture - ROSANE SCHWAN</td>
</tr>
<tr>
<td>17.45 - 18.15</td>
<td>Invited Speaker Amparo Querol.</td>
<td>17.00 - 17.45</td>
<td>+ beers by ANTARES.</td>
</tr>
<tr>
<td>18.30 - 20.00</td>
<td>OPENING CEREMONY</td>
<td>17.45 - 18.45</td>
<td>Conference Dinner.</td>
</tr>
<tr>
<td>18.30 - 28.00</td>
<td>Closure Drinks.</td>
<td>18.00 - 19.00</td>
<td>+ beers by Heineken.</td>
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**NH Edelweiss Hotel**

14.00 - 17.00
Free transfers with sightseeing stops to LLAO LLANO Hotel
(Opening Ceremony)

18.00 - 20.00
OPENING CEREMONY

LLAO LLANO Hotel

**SESSION 5:**
Cletus Kurtzman’s Yeast Taxonomy and systematics workshop: The impact of genomics.

**SESSION 6:**
Evolutionary genomics and domestication of yeast.

**SESSION 7:**
Yeast in Biorefineries.

**SESSION 1:**
Ecology and Biodiversity of yeasts.

**SESSION 2:**
Industrial applications of non-conventional yeasts.

**SESSION 3:**
Natural variation and applied opportunities.

**SESSION 4:**
Bioprospection of extremophiles.
## 1ST. INTERNATIONAL WORKSHOP ON BREWING YEAST.

### 4TH. — THURSDAY

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</tr>
<tr>
<td>10.45 - 11.15</td>
<td>Coffee break.</td>
</tr>
<tr>
<td>11.15 - 13.00</td>
<td>SESSION 8B: Industrial fermentations.</td>
</tr>
<tr>
<td>13.00 - 14.00</td>
<td>Lunch.</td>
</tr>
<tr>
<td>14.00 - 15.00</td>
<td>e-posters 3.</td>
</tr>
<tr>
<td>15.00 - 15.30</td>
<td>Coffee break.</td>
</tr>
<tr>
<td>15.30 - 16.00</td>
<td>e-posters 2 + beers by Berlina.</td>
</tr>
<tr>
<td>16.30 - 17.30</td>
<td>Keynote Lecture — LUIS LARRONDO</td>
</tr>
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### 5TH. — FRIDAY

<table>
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<tr>
<th>Time</th>
<th>Event</th>
</tr>
</thead>
<tbody>
<tr>
<td>09.00 - 10.30</td>
<td>WELCOME / LECTURES Verstrepen, Hittinger and Sampaio.</td>
</tr>
<tr>
<td>10.30 - 11.00</td>
<td>break/posters.</td>
</tr>
<tr>
<td>11.00 - 12.30</td>
<td>LECTURES Walker, Powell and Dunham.</td>
</tr>
<tr>
<td>12.30 - 14.00</td>
<td>Lunch + Expo.</td>
</tr>
<tr>
<td>14.00 - 16.00</td>
<td>LECTURES Libkind, Fischborn, Janssens, and Thevelein.</td>
</tr>
<tr>
<td>16.00 - 16.30</td>
<td>Break and Beers.</td>
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### 6TH. — SATURDAY

<table>
<thead>
<tr>
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</thead>
<tbody>
<tr>
<td>10.30 - 11.00</td>
<td>break/posters.</td>
</tr>
<tr>
<td>11.00 - 12.30</td>
<td>LECTURES Daran, Gibson, Hutzler, and Prahl.</td>
</tr>
<tr>
<td>12.30 - 14.00</td>
<td>Lunch + Expo.</td>
</tr>
<tr>
<td>14.00 - 16.00</td>
<td>LECTURES Libkind, Fischborn, Janssens, and Thevelein.</td>
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<tr>
<td>16.00 - 16.30</td>
<td>Break and Beers.</td>
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### BEER TOUR

- 19:00 S. eubayanus CRAFT BEER TOUR.
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6TA. JORNADAS SUDAMERICANAS DE BIOLOGÍA Y BIOTECNOLOGÍA DE LEVADURAS
ANTARCTIC PERMAFROST AND BRINES: THE DISCOVERY OF NEW HABITATS COMPATIBLE WITH MICROBIAL FUNGAL LIFE

Pietro Buzzini¹, Luigimaria Borruso², Ciro Sannino¹, Sara Filippucci¹, Giorgia Tasselli¹, Benedetta Turchetti¹, Mauro Guglielmin³.

¹Department of Agricultural, Food and Environmental Sciences, University of Perugia, Italy. ²Faculty of Science and Technology, Free University of Bozen-Bolzano, Bozen-Bolzano, Italy. ³Department of Theoretical and Applied Sciences, Insubria University, Varese, Italy.

Presenting author: pietro.buzzini@unipg.it

Abstract ID: 215
Type: Keynote speaker

Antarctica may be considered a sentinel area for monitoring the changes of microbial biodiversity due to the global climate changes. Their effects on Antarctic ecosystems are apparently amplified respect to the other continents. Although the presence of extremely harsh conditions apparently makes difficult its habitability, Antarctic habitats are compatible with the life of psychrophilic and psychrotolerant microbial communities, including yeast and filamentous fungi, which have developed and evolved specific physiologic and metabolic adaptations to overcome the adverse effects on cell physiology of extreme low temperatures. In the present study, the mycobiota of different Antarctic ecosystems has been studied using a culture-independent approach (metagenomic techniques), which have shown the presence of diverse Antarctic microbial communities exhibiting a diversity much higher than that previously observed with culturable methods. Samples, including brines, ice and permafrost, were collected from two perennially frozen lakes (Tarn Flat and Boulder Clay) in Victoria Land (East Antarctica). Fungal diversity and community structure were investigated via NGS targeting the ITS region. Globally, an unexpected high alpha-diversity has been found. Beta-diversity analysis revealed a high phylogenetic differentiation among samples even at the small scale level. At the phylotype level, yeasts dominated the fungal communities: the most frequently found genera were Candida (among Ascomycota), Leucosporidium, Malassezia, Naganishia and Sporobolomyces (among Basidiomycota).
DEKKERA  BRUXELLensis  EMERGING  AS  BIOTECHNOLOGICAL YEAST

Marcos Antonio de Morais Junior, Will de Barros Pita, Fernanda Cristina Bezerra Leite, Rafael Barros de Souza.

1Departamento de Genética, Universidade Federal de Pernambuco, Recife, Brasil; 2Departamento de Antibióticos, Universidade Federal de Pernambuco, Recife, Brasil; 3Departamento de Biologia, Universidade Federal Rural de Pernambuco, Recife, Brasil; 4Instituto de Ciências Biológicas, Universidade de Pernambuco, Recife, Brasil.

marcos.moraisjr@ufpe.br

Abstract ID: 363
Type: Keynote Speaker

The yeast Dekkera bruxellensis is very known and feared by wine producers worldwide by the synthesis of off-flavours, mainly during aging in barrels. On the other hand, it well desired by producers of lambic beers in Belgium due to the characteristic acidic teste added to the beverage. Despite its evolutionary diversion to S. cerevisiae both yeasts converged to a set of similar characteristics such as Crabtree-positive and petite-positive phenotypes and tolerance to high concentrations of ethanol. Hence, this non-conventional yeast has downed in the biotechnological scenario. In the case of fuel-ethanol fermentation, we have shown that this yeast is capable to attaining high cells counts in the yeast population of sugarcane juice fermentation within a period of days, sometimes resulting in the complete replacement of the original S. cerevisiae population. Experiments in laboratory revealed that, despite that astonishing adaptability to anaerobic processes, this yeast is preferably oxidative, meaning that it tends to respire and produce biomass instead of fermentation products (except acetate). Apparently, its capacity to convert sugars to ethanol is very diminished in relation to S. cerevisiae, either in laboratory media or in industrial substrates. However, a breakthrough has been achieved when we showed that its already-known capacity to assimilate nitrate conferred advantages over S. cerevisiae. It makes possible for this yeast not only grow better but also ferment sugars more efficiently in anaerobiosis. These results lead us to finally understand the contradictions of industrial results. In the meantime, results were being accumulating on assimilation of sugars of industrial importance, like cellobiose produced during hydrolysis of cellulose. It expands its application for second generation ethanol. The ongoing works on the identification of regulatory mechanisms, with converging data from quantitative physiology, gene expression, proteomics and stress-tolerance profile, are helping to uncover the molecular mechanisms that turn possible to use of this yeast as platform for a variety of biotechnological purposes.
MECANISMOS MOLECULARES IMPLICADOS EN LA ADAPTACIÓN DE LAS LEVADURAS A LOS PROCESOS FERMENTATIVOS Y SUS POSIBLES APLICACIONES

Amparo Querol\textsuperscript{1}; Javier Alonso-del-Real\textsuperscript{1}; M Lairón-Peris\textsuperscript{1}; Romain Minebois\textsuperscript{1}; R. Pérez-Torrado\textsuperscript{1}; E. Eladio Barrio\textsuperscript{1,2}

\textsuperscript{1} IATA (CSIC)/Departamento de Biotecnología los Alimentos. C/ Catedrático Agustín Escardino Benlloch, 7 46980 Paterna, Valencia (España), \textsuperscript{2} Universitat de València/Departament de Genètica. C/ Doctor Moliner, 50 46100 Burjassot - Valencia (España).

Presenting author: aquarel@iata.csic.es

Abstract ID: 150

En las últimas décadas el sector productor de vino está demandando nuevas necesidades, algunas relacionadas con las demandas del mercado (menor contenido alcohólico y aromas más afrutados) o con los retos relacionados con el cambio climático que tienen como consecuencia una menor acidez, incremento de azúcares y de la astringencia producida por los taninos y aumento del alcohol, todos estos parámetros están que afectando a la calidad del producto final. A pesar de que \textit{S. cerevisiae} es la especie más utilizada en enología, las especies \textit{S. uvarum}, \textit{S. kudriavzevii} o híbridos entre las especies del género \textit{Saccharomyces}, pueden resolver estas demandadas del sector. En el presente trabajo analizaremos mediante la aplicación de metodologías ‘ómicas’ y de flujos metabólicos, las diferencias enológicas y metabólicas de estas especies. A pesar del interés reciente por la utilización de estas especies en enología, presentan problemas de implantación, siendo la poca tolerancia al etanol uno de los problemas de estas especies. Algunas de las aplicaciones que propinemos para su uso industrial son la coinoculación, la inoculación secuencial, la formación de híbridos o la evolución adaptativa para mejorar la tolerancia de estas especies. Agradecimientos: Proyectos AGL2015-67504-C3-1-R y AGL2015-67504-C3-3-R to A.Q. and E.B. respectively
ESTUDIO DEL EFECTO DE CAMBIOS BRUSCOS DE TEMPERATURA DURANTE LAS FERMENTACIONES ALCOHÓLICAS

Andrea Vargas-Trinidad¹, María Cecilia Lerena¹, Braulio Esteve-Zarzoso², Laura Analía Mercado¹, Albert Mas³, Amparo Querol⁴, Mariana Combina¹.


Presenting author vargas.andrea@inta.gob.ar

Abstract ID: 27
Type: Oral

Las levaduras del género Saccharomyces son las responsables de la fermentación alcohólica (FA). Las paradas o enlentecimientos de las fermentaciones son un problema recurrente en la industria del vino. Los cambios bruscos de temperatura (shocks térmicos) como causas de este problema no han sido estudiados en profundidad. Las fermentaciones son reacciones exotérmicas, donde ocurren elevaciones de temperatura a causa del metabolismo de las levaduras en la primera etapa de fermentación. Este aumento se ve favorecido por la nutrición de los mostos, la cual es una práctica enológica habitual. Por otro lado, los descensos bruscos de la temperatura ambiental, frecuentes en los primeros días del otoño, pueden alterar la cinética fermentativa. El objetivo de este trabajo fue identificar las condiciones térmicas predisponentes a una fermentación problemática. Se utilizaron cepas de Saccharomyces: PDM y T73 (levaduras comerciales), y SBB11 (aisladas de fermentaciones en Mendoza). Las fermentaciones fueron realizadas en mosto sintético. Se evaluó el efecto del shock térmico a 36°C y 40°C el día 3 de la FA, asociado a la nutrición de los mostos. Paralelamente, se evaluó el efecto del descenso abrupto de la temperatura (1.5°C, 8°C y 10°C) aplicado los días 2, 6, 10 y 14 de la FA. La cinética de fermentación fue monitoreada mediante medición de la densidad y la viabilidad/vitalidad por citometría de flujo. Los aumentos bruscos de temperaturas mostraron una visible alteración de la cinética fermentativa con diferente intensidad dependiendo de la temperatura y la cepa evaluada. En ningún caso se observó detención completa de la fermentación alcohólica, pero si marcados enlentecimientos. Este efecto fue mayor a 40°C, donde se evidenció una mayor reducción de la viabilidad/vitalidad de las levaduras. Por otro lado, se observó que un descenso brusco de la temperatura no produjo detención o enlentecimiento de la FA para ninguno de los casos evaluados.

O GENE AMDS COMO UMA MARCA DE SELEÇÃO DOMINANTE E RECICLÁVEL EM PICHIA PASTORIS

Luiza Cesca Piva¹, Viviane Castelo Branco Reis¹, Fernando Araripe Gonçalves Torres¹.

¹Laboratório de Biotecnologia de Leveduras, Departamento de Biologia Celular, Universidade de Brasília, Brasil.

Presenting author: piva.luiza@gmail.com

Abstract ID: 121
Type: Oral

A manipulação genética de Pichia pastoris depende de um número limitado de marcadores de seleção. Nesse contexto, o gene amdS de Aspergillus nidulans representa uma ferramenta como potencial marca de seleção dominante. Sua seleção é baseada no uso de acetamida como única fonte de nitrogênio, enquanto sua contrasseleção depende da produção do composto tóxico fluoroacetato a partir de fluoroacetamida pelas linhagens que carregam a marca. Para testar amdS em P. pastoris, foram construídos cassetes de expressão contendo a marca juntamente com
um gene reporter ou sequências homólogas para substituição de genes nativos. Primeiramente, uma amidaise putativa foi deletada do genoma da levedura com uma marca de resistência a antibiótico, que foi posteriormente reciclada. A linhagem resultante, LA1, foi usada juntamente com a linhagem X-33 em transformações com a marca \textit{amdS}. O cassette da marca de seleção e o gene EGFP foram ligados a um plasmídeo derivado do vetor pPIC9 (Invitrogen), gerando o vetor pAMDS. A integração do DNA exógeno foi direcionada para o \textit{locus HIS4} ou 3’\textit{AOX1}. Clones foram analisados por PCR e análise de fluorescência. Foram encontradas intensidades variáveis de fluorescência, indicando uma possível variação no número de cópias do gene EGFP. O \textit{locus HIS4} de integração forneceu mais clones fluorescentes que o \textit{locus 3’AOX1}. LA1 não apresentou nenhum resultado falso positivo, ao contrário da linhagem X-33. Em seguida, a marca \textit{amdS} foi usada na deleção dos genes \textit{ADE2} e \textit{URA5}. O cassette foi flanqueado por sequências homólogas para substituição das sequências codantes. A primeira transformação forneceu mutantes \textit{ade2} e a marca foi reciclada com pYRCre2, vetor contendo a recombinase CreA em. A seleção dos clones com marca reciclada foi realizada com sucesso em fluoroacetamida. A transformação seguinte substituiu o gene \textit{URA5}, gerando clones resistentes a 5-FOA e provando que a marca \textit{amdS} pode ser amplamente usada em \textit{P. pastoris}.

**PRODUÇÃO DE BEBIDA FERMENTADA NÃO LÁCTEA A BASE DE MANDIOCA E ARROZ UTILIZANDO A LEVEDURA \textit{TORULASPORE DELBRUECKII} COM COMBINAÇÃO COM BACTÉRIAS LÁTICA POTENCIALMENTE PROBIÓTICAS**

Cíntia Lacerda Ramos\(^1\), Ana Luiza Freire\(^2\), Patrícia Nirlane da Costa Souza\(^3\), Mauro Guilherme Barros Cardoso\(^2\), Rosane Freitas Schwan\(^2\).

\(^1\)Departamento de Ciências Básicas, Universidade Federal dos Vales do Jequitinhonha e Mucuri, Brasil.
\(^2\)Departamento de Biologia, Universidade Federal de Lavras, Brasil. \(^3\)Instituto de Engenharia, Ciência e Tecnologia, Universidade Federal dos Vales do Jequitinhonha e Mucuri, Brasil.

Presenting author: cintialacerdaramos@gmail.com

Abstract ID: 133
Type: Oral

Os consumidores em todo o mundo estão cada vez mais conscientes sobre a relação existente entre alimentação e saúde. Assim, o mercado dos chamados alimentos funcionais vem crescendo substancialmente nos últimos anos. O objetivo deste trabalho foi desenvolver uma bebida fermentada não láctea a partir da mistura de mandioca e arroz utilizando a levedura \textit{Torulaspora delbrueckii} em co-cultivo com bactérias lácticas probióticas. A cepa de levedura \textit{T. delbrueckii} CCMA 0235 (isolada do tarubá) e as bactérias lácticas (BAL) \textit{Lactobacillus plantarum} CCMA 0743 (cauim) e \textit{L. acidophilus} LAC-04 (comercial) foram utilizadas como culturas iniciadoras em culturas únicas e co-cultivo. As populações bacterianas atingiram valores aproximados de 8.0 log (UFC/mL) no final de todas as fermentações, de acordo com o recomendado para produtos probióticos. Maiores valores residuais de amido foram observados nos cultivos únicos de BAL (10.6% m/m) que aqueles em co-cultivo (6% m/m), mostrando que a fermentação em co-cultivo pode melhorar a digestibilidade da bebida. Para todos os ensaios, o ácido lático foi o principal ácido orgânico detectado (1.6 g/L) e a concentração de etanol foi menor que 0.5% (m/v), caracterizando como bebidas não alcoólicas. As fermentações realizadas com a levedura \textit{T. delbrueckii} apresentaram maior atividade antioxidante, aproximadamente 10% pelos métodos DPPH e ABTS). Contudo, bebidas fermentadas não lácteas foram obtidas com sucesso, e o co-cultivo da levedura \textit{T. delbrueckii} com as BAL avaliadas foram capazes de aumentar a segurança do produto, uma vez que apresentaram rápida e adequada acidificação e produção de ácidos orgânicos, inibindo o crescimento de patógenos. Além disso, o ensaio contendo a levedura em co-cultivo
aumentou a digestibilidade do produto, aumentou atividade antioxidante e ainda estimulou o crescimento da BAL probiótica durante a fermentação.

DISEÑO BASADO EN MODELOS DE FERMENTACIONES CON CULTIVOS MIXTOS DE LEVADURAS S. CEREVISIAE Y NO CEREVISIAE

Eva Balsa-Canto¹, Javier Alonso-del-Real², David Henriques¹, Amparo Querol².

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El uso de fermentaciones mixtas con inoculaciones controladas de Saccharomyces cerevisiae y levaduras Saccharomyces no convencionales tiene el potencial de producir vinos a la medida de los nuevos mercados. Sin embargo, el diseño de fermentaciones mixtas requiere investigar las interacciones ecológicas y metabólicas entre las especies consideradas en diferentes condiciones ambientales. En la práctica, las cepas de S. cerevisiae tolerantes al alcohol dominan las etapas finales de las fermentaciones. En este trabajo, utilizamos un enfoque basado en modelos para explicar las interacciones ecológicas entre la cepa vínica S. cerevisiae T73 y S. kudriavzevii CR85 en diferentes condiciones fermentativas. Finalmente el modelo se ha utilizado para diseñar fermentaciones mixtas. Se diseñó un plan experimental para analizar la capacidad de supervivencia de S. kudriavzevii en fermentaciones mixtas a diferentes temperaturas y condiciones de inoculación; los datos revelaron efectos de exclusión significativos en la mayoría de las condiciones. Para describir tal competencia, se propuso un modelo Lotka-Volterra modificado, que incorpora la competencia por nutrientes, e incluye una fase de decaimiento dependiente de la densidad celular y que agregaria varios mecanismos que contribuyen a la exclusión celular (producción de etanol u otros metabolitos tóxicos, agregación, etc) observados a altas densidades celulares. El modelo incorpora, además, el papel de la temperatura tanto en el crecimiento individual como en la interacción entre levaduras. Se identificaron los parámetros del modelo mediante una calibración multi-experimento utilizando la herramienta software AMIGO2 y se utilizó validación cruzada para evaluar su capacidad predictiva. Los resultados demuestran que el modelo puede explicar de forma fiable los datos. Por último, el modelo se introdujo en un esquema de optimización dinámica para obtener la temperatura e inoculación que maximizan la biomasa final. Los resultados muestran que la inoculación secuencial, es concreto, la inoculación tardía de S. cerevisiae, es la clave para mejorar la coexistencia de ambas especies.

CAPACIDAD DE BIOSORCIÓN DE Cr VI UTILIZANDO DIFERENTES ESPECIES DE LEVADURAS COMO PARTÍCULAS MICROBIANAS

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En los últimos años, la creciente contaminación en el medio ambiente causada por metales pesados se ha convertido en una preocupación debido al riesgo para la salud
de humanos, animales y ecosistemas en general. Uno de los más frecuentes contaminantes que causan un gran impacto negativo es Cr VI, que se genera como un efluente líquido en una variedad de industrias diferentes. Una alternativa de remediaciión para estos efluentes es la biosorción, un fenómeno de adsorción de contaminantes por medio de biomateriales que poseen superficie adsorbente. La captura de iones se realiza a través de un proceso pasivo. En este trabajo se ha estudiado la eficiencia, cinética e isotermas de biosorción de diferentes especies de levaduras nativas de Ecuador (*Kazachstania yasuniensis*, *Kodamaea transpacifica*, *Saturnispora quitensis*). La especie *Saccharomyces cerevisiae* fue utilizada como control. Las células de levadura se enmascararon con un agente surfactante catiónico y se probó su capacidad de adsorción para Cr VI. Como resultado, se estableció que la combinación del tamaño de la célula y la carga superficial son factores determinantes en términos de rendimiento de biosorción. La especie de levadura *S. quitensis* mostró la mayor eficiencia, mientras que *K. yasuniensis* y *K. transpacifica* exhibieron una menor capacidad de adsorción, respectivamente. Este estudio allana el camino hacia el uso de especies de levadura no tradicionales en la remediación de aguas contaminadas con Cr VI.

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**ESTUDIO DE LA REGULACIÓN DEL GEN DBPAD EN LA LEVADURA CONTAMINANTE BRETTRANOMYCES BRUXELLENSIS LAMAP2480**

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*Brettanomyces* es el principal microorganismo contaminante en la industria vitivinícola debido a la producción de fenoles volátiles, formados a partir de los ácidos hidroxicinámicos (AHC) (ácidos p-cumárico, ferúlico y cafeico). La generación de estos compuestos por parte de este microorganismo implica la acción secuencial de dos enzimas, un ácido fenilacrílico descarboxilasa (PAD) y una vinilfenol reductasa. Los compuestos fenólicos derivados del ácido p-cumárico como 4-vinilfenol y 4-etilfenol, han sido descritos como los principales contribuyentes de aromas asociados a establo, ratón mojado, medicinal, entre otros, lo cual disminuye la calidad aromática del vino, generando importantes pérdidas económicas. El gen implicado en la producción de 4-vinilfenol a partir del ácido p-cumárico ha sido identificado en *B. bruxellensis* LAMAP2480 como *DbPAD*, el cual codifica para una enzima PAD. Estudios recientes han indicado la presencia de un marco de lectura abierto de menor tamaño denominado *DbPAD2*, el cual también codifica una enzima con la misma actividad. El objetivo de este trabajo fue identificar los sitios de inicio de la transcripción (SIT) pertenecientes al gen *DbPAD*, con la finalidad de describir un mecanismo de regulación transcripcional. La identificación de los SIT se realizó mediante la generación de cDNA a partir de los transcritos del gen *DbPAD*, posterior circularización y amplificación inversa. El producto obtenido fue clonado y secuenciado. Posteriormente se realizó una predicción de los sitios de regulación transcripcional en la región promotora, para evaluar el efecto de la presencia de los sitios de unión de los factores transcripcionales seleccionados en la expresión del gen. Este estudio permite continuar con el desarrollo de metodologías que permitan determinar las variables biológicas involucradas en la expresión y
EVALUACIÓN DEL USO DE BACTERIAS INACTIVADAS EN CO-CULTIVO CON SACCHAROMYCES CEREVISIAE L1039 PARA EL AUMENTO DE LA ACTIVIDAD ANTIMICROBIANA

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El control microbiológico es uno de los factores importantes a ser resguardado en la industria de los alimentos, y las metodologías asociadas contemplan el uso de preservantes químicos. Sin embargo, actualmente existe una tendencia en la cual los consumidores han aumentado su exigencia hacia consumo de productos más saludables. Por ello se ha dirigido la búsqueda hacia agentes antimicrobianos naturales, como microorganismos, para sustituir a los preservantes químicos actualmente utilizados. En este estudio se evaluó la actividad antimicrobiana (AA) de la levadura Saccharomyces cerevisiae L1039 crecida en presencia de bacterias inactivadas para aumentar la AA de ésta sobre Escherichia coli ATCC25922, Salmonella typhimurium ATCC14028 y Listeria monocytogenes ATCC13932. Para esto, se evaluó la AA en el pellet y en el sobrenadante del co-cultivo, y se determinó cuantitativamente mediante recuento en placa como UFC/mL respectivamente. Los resultados mostraron que el mayor efecto antimicrobiano se observó en el sobrenadante del co-cultivo, presentando una reducción del crecimiento de 0,59, 0,54 y 0,62 unidades logarítmicas para E. coli ATCC25922, S. typhimurium ATCC14028 y L. monocytogenes ATCC13932 respectivamente. Estos resultados son prometedores y podrían ser usados como potencial agente antimicrobiano en matrices alimentarias.

O POTENCIAL DE CEPAS DE LEVEDURAS FARMHOUSE ALES COMO CHASSIS PARA O DESENHO DE HÍBRIDOS INTRA E INTERESPECÍFICOS

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O desenvolvimento de híbridos de levedura é crucial para a indústria cervejeira, permitindo a elaboração de linhagens com fenótipos fermentativos otimizados. A eficiência fermentativa e a tolerância aos diferentes estresses da fermentação são dois fenótipos alvos para programas de hibridização. Dos chassis de leveduras cervejeiras distintos que podem ser utilizados para a geração de híbridos, cepas de farmhouse ales são bastante promissoras. O objetivo deste trabalho foi avaliar a adaptabilidade de três cepas de farmhouse ale belgas isoladas da região da Valônia (WLN I, II e III) aos estresses fermentativos em comparação com as cepas de leveduras SafAle™ US-05 (levedura ale), SafLager™ W34/70 (levedura lager), farmhouse ale norueguesa Voss Kveik (The Yeast Bay™), CAT-1 (levedura produtora de bioetanol) e BY4741 (levedura
RIBOPROTEOMA DE SACCHAROMYCES CEREVISIAE DURANTE QUIESCENCIA Y REACTIVACIÓN TRADUCCIONAL POR ESTIMULO DE NUTRIENTES

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Cuando los nutrientes se consumen Saccharomyces cerevisiae entra en quiescencia, y reingresa al ciclo celular cuando los nutrientes vuelven a estar disponibles. La regulación traduccional es fundamental para modificar el proteoma celular y determinar, por ejemplo, cambios en el ciclo celular. Para caracterizar la maquinaria traduccional en las fracciones monosomal y polisomal de células wt en fase estacionaria (SP) o luego de un estimulo con medio fresco de 30 o 60 minutos usamos nano-LC MS/MS y estimaciones de abundancia proteica usando el índice de abundancia proteica exponencialmente modificado (emPAI). En SP la traducción global se encuentra inactiva, luego de 30 minutos de estimulo aún no se reactivaron totalmente los niveles, y a los 60 minutos los perfiles polisomales son similares a los presentados en fase exponencial. El análisis proteómico mostró diversos grupos, además de traducción y plegamiento de proteínas, asociados a ribosomas en las condiciones en estudio. Sólo los monosomas de todas las condiciones mostraron un subset de proteínas involucradas en degradación de proteínas. Análisis de enriquecimiento indicaron que la condición de 30 minutos sería una condición regulatoria, altamente enriquecida en proteínas involucradas en el procesamiento de aminoácidos. Las proteínas ribosomales Rpl31B y Rps0B están presentes únicamente en el monosoma de SP. Análisis de abundancia proteica mostraron que factores como Stm1 -factor clave para la correcta traducción durante estrés- y chaperonas como Ssb1 y Ssb2 se asocian a monosomas en SP. Los polisomas de SP tienen asociados factores de elongación de la traducción y esta asociación disminuye con el estimulo. Hsp26 se encuentra asociada a monosomas y no en polisomas, y su asociación no depende del estimulo. Estos resultados profundizan en la regulación del control traduccional por estimulo de nutrientes y sugieren que las levaduras quiescentes modificarían su maquinaria traduccional vía cambios en el core ribosomal o proteínas accesorias.
Diversos subproductos y residuos agroindustriales son actualmente transformados en las biorefinerías en compuestos de mayor valor agregado. En este trabajo se propone la utilización de levaduras capaces de fermentar la lactosa presente en suero de quesería para producir etanol y mantenerse viables luego del proceso con el fin de poder ser utilizadas en otras aplicaciones. Kluyveromyces marxianus es una levadura respirofermentativa, generalmente reconocida como segura, de la cual ha sido explorada su bioactividad y capacidad de producir etanol. Comenzamos nuestro estudio con un screening de 30 cepas de K. marxianus, observando su crecimiento en placas con suero agarizado (7.5 g lactosa/L) en condiciones de aerobiosis y anaerobiosis, con una concentración de etanol del 5% y a pH 2,5 (alcanzado mediante adición de H₂SO₄). Se detectó que K. marxianus 9 (un aislado de leche fermentada en Argentina) y K. marxianusNCYC1429 mostraron buen desarrollo en las diferentes condiciones ensayadas y por ello fueron seleccionadas para la evaluación con el sistema miniaturizado de producción de etanol de primera generación teniendo como referencia el comportamiento de Saccharomyces cerevisiae en melaza de caña de azúcar. El sistema incluyó 5 ciclos de fermentación con reciclado de células y tratamiento ácido entre 2 ciclos consecutivos. Ambas cepas de K. marxianus presentaron buen rendimiento en la producción de etanol (≈40 g de etanol producido a partir de 100g de lactosa) y mantuvieron una viabilidad del 90%, incluso luego de 5 ciclos de fermentación. Actualmente nos encontramos analizando las propiedades bioactivas de estas dos cepas, con el objetivo de evaluar la posibilidad de emplearlas como ingrediente en alimentos funcionales.

CONTROL DE PATÓGENOS FÚNGICOS DE MANZANAS MEDIANTE COMPUESTOS VOLÁTILES PRODUCIDOS POR CEPAS DE CANDIDA SAKE

El almacenamiento de manzanas en cámaras refrigeradas (0-1ºC) ha permitido mantener una oferta relativamente constante de este producto a lo largo todo el año. El almacenamiento a bajas temperaturas no evita el desarrollo de patógenos fúngicos que actualmente provocan grandes pérdidas económicas. El uso de fungicidas químicos sintéticos ha sido el método tradicionalmente empleado para el control de estas
enfermedades, sin embargo, las exigencias actuales de los consumidores han llevado a la búsqueda de alternativas que impliquen menores riesgos para la salud y el medio ambiente. En estudios anteriores llevados a cabo por nuestro grupo, se aislaron y evaluaron levaduras de la Antártida como microorganismos antagonistas de estos patógenos, lo que se conoce como control biológico. En esta colección de levaduras, se encontraron dos cepas identificadas molecularmente como *Candida sake*, que produjeron compuestos volátiles capaces de inhibir el desarrollo in vitro de los patógenos de manzana *P. expansum, Botrytis cinerea, Alternaria tenuissima, Alternaria alternata* y *Alternaria arborescens*. En los ensayos llevados a cabo en fruta se observó que la presencia de los compuestos volátiles producidos por estas levaduras llevó a una disminución de la severidad de la enfermedad. La identificación de los compuestos volátiles se realizó empleando la técnica de microextracción en fase sólida (SPME) y posterior análisis por GC-MS. Los compuestos identificados fueron mayormente ésteres de ácidos de cadena corta. Es necesario continuar estudiando estos compuestos con el fin de conocer cuáles son los responsables de la actividad antifúngica, así como las concentraciones que provocan la inhibición de los patógenos. El uso de estos compuestos se plantea como una alternativa para la prevención del desarrollo de patógenos fúngicos en manzana.

**EVALUACIÓN DEL RESIDUO DE SIDRERAS PARA LA PRODUCCIÓN DE BIOMASA DE *PICHIA MEMBRANIFACIENS NPCC 1250*, POTENCIAL AGENTE DE CONTROL BIOLÓGICO PARA POSTCOSECHA DE PERA**

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*Pichia membranifaciens* NPCC 1250 fue seleccionada en trabajos previos por su capacidad antagónica frente a fitopatógenos en peras durante la poscosecha. El objetivo de este trabajo fue evaluar la producción de biomasa de *P. membranifaciens* con un sustrato regional, el residuo de sidreras como fuente de nitrógeno y carbono, con la intención de reemplazar insumos costosos en la elaboración a escala industrial. Se evaluó el efecto de cinco factores sobre el crecimiento *P. membranifaciens: pool* de aniones, cationes, vitaminas, urea y todos estos aditivos a 13°C y 20°C (10 condiciones). A fines comparativos se empleó también un medio a base de melaza a las dos temperaturas. La biomasa obtenida en las 12 condiciones fue evaluada en ensayos *in vitro*: producción de toxinas *killer* y enzimas quitinasas y glucanasas, contenido trehalosa intracelular y conservación en frio utilizando tres criopreservantes (sorbitol, glutamato de sodio y trehalosa). En ensayos *in situ* se evaluó la capacidad de colonizar heridas de pera y la actividad antagonista frente a *Penicillium expansum* y *Botrytis cinerea* en condiciones de cámara fría (-1/0°C, 95% RH). En cuanto a la actividad antagónica, el mayor control para los dos patógenos se obtuvo con las levaduras crecidas en residuo de sidrera al 15%, urea 0,6 g/L y pool de cationes (C3) y aniones (C4) a 13°C. Estas condiciones tuvieron altos niveles de colonización de superficie de peras y altos niveles de trehalosa intracelular (150 mg/g de peso seco). Las levaduras crecidas en C3 presentaron la mayor viabilidad con los tres criopreservantes a 4°C. Las levaduras obtenidas en las 12 condiciones no presentaron diferencias en la capacidad *killer* y de producción de enzimas hidrolíticas. Los resultados obtenidos permiten
comprobar que el desecho de sidrera con suplementos nutricionales podría ser empleado en la producción a gran escala de *Pichia membranifaciens*.

**SACCHAROMYCES EUBAYANUS: POTENCIANDO EL AMARGOR**

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*Saccharomyces eubayanus* es una levadura criotolerante aislada en la Patagonia Andina. Estudios genéticos confirmaron que la hibridación entre esta especie y *S. cerevisiae* (levadura Ale) dio origen a *S. pastorianus* (levadura Lager). La condición parental de esta nueva especie en relación a la levadura más utilizada para la producción de cerveza a nivel mundial despertó interés en el estudio de sus propiedades para su potencial aplicación en la industria cervecera. Los cerveceros eligen la cepa de levadura basándose en características como perfil organoléptico, floculación, atenuación y producción de etanol, mientras que el amargor generado (IBU) suele no tenerse en cuenta. El objetivo de este trabajo fue evaluar el desempeño fermentativo y el impacto en el amargo de cepas de *S. eubayanus* y de levaduras industriales. Se caracterizaron fermentativa y fisicoquímicamente 5 cepas de *S. eubayanus* y 7 cepas cerveceras industriales, evaluándose: velocidad de fermentación, atenuación, pH final, perfil de azúcares consumidos, etanol producido e IBU. Las cepas de *S. eubayanus* tuvieron un comportamiento fermentativo similar (62% atenuación), excepto CRUB1935 cuya performance resultó disminuida (48% atenuación). La cepa control Lager superó a las salvajes en velocidad de fermentación (35% mayor) y, al igual que las Ale, tuvieron una atenuación superior de 80%. La mayor atenuación de las cepas cerveceras domesticadas se condice con su consumo eficiente de maltosa y maltotriosa. La levadura salvaje *S. eubayanus* carece de la capacidad de metabolizar este último azúcar. Los IBU obtenidos para cervezas fermentadas con *S. eubayanus* resultaron superiores que para las cepas industriales (18% mayor), siendo esta diferencia notoria sensorialmente e incluso percibida como un amargo áspero (*harsh*). El conocimiento del comportamiento fermentativo de las levaduras a diversos niveles resulta necesario para adoptar criterios acertados a la hora de su aplicación y lograr predecir adecuadamente el perfil final de las cervezas a obtener.
INTERACCIONES ENTRE INFRAESTRUCTURAS, MICROORGANISMOS Y PAPERS. REFLEXIONES SOCIOLÓGICAS EN TORNO A DATOS Y MATERIALES EN LA MICROBIOLOGÍA

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Muchas áreas científicas se relacionan con infraestructuras de datos y materiales para realizar trabajos. El uso de bases de datos informáticas en biomedicina y biotecnología tienen pocas décadas, estas últimas comenzaron a desarrollarse hacia finales de los 60’s. Hacia los 80’s en la genómica con bases de datos genéticos para búsquedas de estructura y función con una difusión generalizada hacia fines de los 90’s. Estos eventos se han analizados desde la sociología, la historia y la filosofía. Las colecciones de microorganismo son otro tipo de infraestructuras que conservan y preservan materiales biológicos y microorganismos, y su inicio puede ser trazado a partir del desarrollo de los cultivos puros a fines del siglo XIX. Las colecciones de microorganismos y específicamente de levaduras en el mundo intentan difundir reglas y acciones para sus usuarios. Intentan, por otra parte, interesar a las agencias de financiamiento de su relevancia en biodiversidad y biotecnología. Las colecciones más importantes se encuentran en Europa, Estados Unidos y Asia, algunas están especializadas en diferentes tipos de microorganismos para distintas áreas: industriales, salud y biodiversidad. Los encargados de estas colecciones o expertos en la materia hacen publicaciones con recomendaciones, estados del arte metodológicos y legales. Tomaremos como fuentes discusiones en publicaciones de referentes de colecciones, referentes del campo científico y entrevistas a científicos especializados en levaduras. Exploraremos estas fuentes a la luz de conceptualizaciones de la sociología de la ciencia y la tecnología que tematizan bases de datos, la circulación de conocimiento y la biodiversidad.

EFECTO DE LA TEMPERATURA Y DE LA ACTIVIDAD DE AGUA EN LA INTERACCIÓN DE UNA CEPA DE METSCHNIKOWIA PULCHERRIMA CON ALTERNARIA ALTERNATA

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La presencia de Alternaria spp, principalmente A. alternata, ha sido observada durante poscosecha en uvas de mesa cv. Red Globe de Mendoza, Argentina. La tendencia actual es substituir el SO2 por agentes biológicos para reducir las pérdidas en poscosecha. Se aislaron levaduras epífitas de uvas cosechadas en viñedos de Mendoza. Las cepas fueron identificadas por métodos moleculares. El efecto de una cepa de Metschnikowia pulcherrima en fase de latencia y tasa de crecimiento de Alternaria alternata se evaluó en un medio a base de agar mosto al 3% bajo diferentes condiciones de actividad de agua (0.88, 0.991 y 0.995 aw) y temperatura (0, 4, 15 y 28 ºC). Dichas condiciones fueron seleccionadas considerando la actividad de agua de las uvas durante las etapas de maduración y el rango de temperatura existente durante el ciclo fenológico y poscosecha de la uva de mesa. La tasa de crecimiento y la fase de latencia fueron
influenciadas por todas las interacciones evaluadas (p < 0.05). Los estudios están en progreso para evaluar estas cepas in vivo bajo las condiciones de poscosecha.

ESTUDIO DEL PAPEL DE LAS ACUAPORINAS EN LA RESISTENCIA A LA CONGELACIÓN EN LAS ESPECIES SACCHAROMYCES CEREVISIAE, S. KUDRIAVZEVII, S. UVARUM, S. PARADOXUS Y HÍBRIDOS DE S. CEREVISIAE–S. KUDRIAVZEVII

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Hoy, los efectos del calentamiento global sobre la calidad de los mostos de uva y la demanda creciente de los consumidores para vinos menos alcohólicos y más aromáticos, hacen que el sector vitivinícola tenga que implementar nuevas estrategias en la vinificación. Una de ellas es la realización de fermentaciones a bajas temperaturas con cepas cuyo rendimiento en etanol es menor. Aunque S. cerevisiae es la principal levadura empleada en los procesos industriales, las otras especies S. uvarum, S. kudriavzvii, S. paradoxus y los híbridos S. cerevisiae x S. kudriavzvii han demostrado tener propiedades interesantes en vinificación, debido entre otro a la calidad aromática de sus vinos y a sus buenas características fermentativas a bajas temperaturas. Diferentes mecanismos están implicados en la adaptación a las bajas temperaturas y han sido estudiados en S. cerevisiae, S. uvarum y S. kudriavzvii. Entre ellos, las acuaporinas, codificadas por los genes parálogos AQY1 y AQY2, son pequeñas proteínas implicadas en el transporte del agua y en la resistencia a la congelación. A temperaturas bajo cero, facilitan la salida de agua intracelular y evitan la formación de cristales que pueden dañar a la célula. En el presente trabajo, se estudió la presencia o ausencia de genes funcionales de las acuaporinas en las otras especies del género Saccharomyces y sus híbridos para determinar su posible papel en los procesos de resistencia a la congelación. Para ello se analizó la resistencia a estrés por congelación en cepas aisladas tanto de ambientes fermentativos y naturales de las especies S. cerevisiae, S. uvarum, S. kudriavzvii y S. paradoxus, así como de híbridos S. cerevisiae x S. kudriavzvii. Se complementó el estudio con un análisis filogenético de las secuencias de los genes de las acuaporinas para determinar si las mutaciones de pérdida de función han ocurrido de forma independiente o no.

PRODUCCIÓN DE FACTOR DE CRECIMIENTO EPIDÉRMICO (HEGF) EN KOMAGATAELLA PHAFFII

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El sistema de expresión basado en la utilización de la levadura Komagataella phaffii ha sido exitosamente empleado en la producción de una gran variedad de proteínas heterólogas. Esta levadura combina diversas características como fácil manipulación
genética, rápido crecimiento celular, habilidad de realizar modificaciones post-traduccionales y eficiente secreción, además de crecer hasta altas densidades celulares. Una de las proteínas de gran interés en la industria biofarmacéutica y cosmética es el factor de crecimiento epidérmico (hEGF). El objetivo de este estudio fue desarrollar un sistema para la producción de hEGF en *K. phaffii*. Para la secreción, serán utilizados tres péptidos señales (αF, SUC2 and PHO1). Los tres vectores de expresión fueron construidos bajo el control del promotor PGK1 y usados para transformar *K. phaffii* M12-K, una cepa mutante para KEX1, gen que codifica para una carboxipeptidasa. Los clones recombinantes fueron confirmados por PCR de colonias y el crecimiento fue en medio mínimo para evaluar la cinética de crecimiento. Los clones positivos fueron seleccionados y usados para crecimiento y expresión en frascos usando medio complejo. Los resultados obtenidos mostraron la presencia de hEGF y fue confirmada usando western blot (WB) con un anticuerpo específico. Una muestra de cada sistema fue seleccionada para la producción y purificación de hEGF por cromatografía de exclusión molecular y RP-HPLC. A partir de esos experimentos la presencia de hEGF fue confirmada por WB y el polipeptídeo fue parcialmente purificado. Finalmente, la muestra αF::hEGF fue seleccionada para la optimización del proceso de purificación con un paso adicional en HILÍC. hEGF fue exitosamente purificado. El secuenciamento del N-terminal y espectrometría de masas confirmaron la integridad y la secuencia del hEGF. Los resultados obtenidos mostraron que *K. phaffii* es una plataforma eficiente para la producción de hEGF.

**RESERVORIOS DE LA BIODIVERSIDAD DE LEVADURAS VÍNICAS EN VIÑEDO: ¿DÓNDE HABITA SACCHAROMYCES?**

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Es un hecho aceptado que, el viñedo es el hábitat natural de las levaduras vínicas, no obstante, resulta un aspecto no dilucidado dónde y cómo persisten éstas, año a año, en este ambiente hostil y altamente cambiante en el tiempo. El presente trabajo tuvo como objetivo estudiar las poblaciones de *Saccharomyces cerevisiae* en diferentes nichos del viñedo durante un ciclo biológico completo de la vid, evaluando el aporte de estos nichos en las poblaciones finalmente presentes en uvas. Se seleccionaron 10 sectores de un viñedo cv. Malbec ubicado en la región vitícola Zona Alta del Río Mendoza (Argentina) y se realizó un muestreo en fases continuas de un ciclo fenológico completo, tomando muestras de bayas, corteza, yemas, suelo y de agua de riego. Las uvas se molieron asépticamente y las otras muestras se colocaron en mosto estéril (24ºBrix, pH 3,5). Todos los mostos se incubaron a 25ºC hasta que el 75% de los azúcares fueron consumidos y los aislados *S. cerevisiae* se diferenciaron intra-específicamente por PCR interdelta, se estimaron similitudes y se construyeron dendrogramas para estudiar el agrupamiento de las cepas y sus relaciones moleculares utilizando UPGMA. Cada etapa evaluada mostró una situación distinta en cuanto a presencia y número de cepas *S. cerevisiae*. En ambas cosechas, las uvas tuvieron una alta incidencia de *S. cerevisiae* en todo el viñedo, verificándose que este comportamiento sólo se manifiesta en esta etapa. En todo el ciclo estudiado, los suelos mostraron tener baja diversidad y contribución como reservorios de estas levaduras, mientras que las yemas en el invierno y las cortezas, desde la brotación a la madurez, sí funcionarían como buenos reservorios. Se
observó una dinámica de cambio de poblaciones de *S. cerevisiae* a lo largo del ciclo evaluado, mostrando conjuntamente, baja similitud entre los perfiles moleculares presentes en cada nicho y etapa.

**VINIFICACIONES CON LEVADURAS SELECCIONADAS DE VIÑEDOS DE LA PROVINCIA DE MENDOZA, ARGENTINA**

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En bodegas de nuestra región, el mosto se inocula con levaduras secas activas. Estos microorganismos son seleccionados e importados desde otras regiones vitivinícolas. La tendencia es realizar vinificaciones con levaduras provenientes de la misma zona de producción por estar mejor adaptadas al medio y la materia prima. La FCA-UNCUYO cuenta con una colección de levaduras de más de 450 individuos de regiones vitivinícolas de la provincia de Mendoza. Han sido evaluados a nivel de laboratorio y en microvinificaciones siguiendo protocolos de selección propuestos por la OIV. Se seleccionaron 3 representantes 115.4-San Martín, 512.4-Junín y 811.5-Rivadavia, los cuales se identificaron como *Saccharomyces cerevisiae* utilizando la técnica de diferenciación intraespecífica para PCR Interdelta. Las levaduras seleccionadas se vinificaron a mayor escala y degustaciones para determinar su comportamiento organoléptico. Cada tratamiento se realizó en 3,000mL de mosto cv. Cabernet Sauvignon, sulfitado con 0,5 ppm de SO2 molecular, inoculado con 2.106 cel/mL, a temperatura de 20±2ºC. Se monitorearon las fermentaciones mediante pérdida de peso y disminución de 6Be. Al finalizar se realizaron los análisis exigidos por el INV y se evaluaron las cinéticas de fermentación. Se degustaron los vinos siguiendo la Norma OIV-CONCOURS 332A-2009. Las técnicas de selección de levaduras en las condiciones de nuestro laboratorio presentaron correlación con los resultados de las vinificaciones a mayor escala, lográndose vinos con óptimos valores físico-químicos y dentro de los requerimientos legales para su comercialización. La degustación arrojó promedios de 70 puntos. Teniendo en cuenta las prácticas enológicas aplicadas y que no se utilizaron aditivos que favorezcan la actividad de las levaduras, podemos decir que las cepas seleccionadas fueron adecuadas y son factibles de ser utilizadas a nivel industrial, que generan vinos de calidad aceptable y con adecuado potencial enológico. La cepa 115.4 además, presentó fenotipo killer, lo que beneficiaría su desarrollo sobre cepas indígenas presentes en mostos.

**COMPORTAMIENTO DE LEVADURAS DEL GENERO SPATHASPORA Y SCHEFFERSOMYCES EN CONDICIONES DE ESTRÉS**

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La dinámica de los microorganismos para adaptarse a las perturbaciones ambientales es variada y específica para cada uno de ellos. El interés en entender estos mecanismos
para construir cepas robustas en diferentes procesos industriales ha permitido abordar los diferentes enfoques a nivel celular y molecular. Actualmente, la búsqueda de mecanismos moleculares resistentes a las condiciones de estrés en levaduras ha revelado un potencial todavía poco caracterizado en linajes salvajes, que han demostrado tener un alto nivel de tolerancia a los inhibidores. La aplicación de regímenes de cultivo bajo condiciones de estrés permite explotar la plasticidad del genoma microbiano. Por esta razón, se estudió el comportamiento de diferentes especies de dos géneros de levadura fermentadores de xilosa. Los cambios fenotípicos en la producción de biomasa y el rendimiento de etanol fueron los parámetros evaluados bajo varias condiciones de crecimiento. Los resultados mostraron que los antecedentes ambientales y la pre-exposición al estrés ácido y térmico en las cepas de los géneros \textit{Spathaspora} y \textit{Scheffersomyces} generan un cambio fenotípico en términos de parámetros de cinética de crecimiento y producción de etanol. Esto permite explorar un potencial natural para la adaptación de estas levaduras en procesos fermentativos.

**DESARROLLO DE APLICACIONES MÓVILES PARA PREVENIR LEVADURAS ALTERADORAS EN JUGOS CONCENTRADOS DE UVAS Y VINOS**

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La industria de vitivinícola es una importante actividad económica para la región de Cuyo, donde los principales productos son los vinos y jugos de uva concentrado. Ambos productos pueden sufrir alteraciones y defectos por la acción de levaduras contaminantes. \textit{Dekkera bruxellensis} desarrolla en los vinos tintos produciendo un defecto organoléptico asociado a aromas negativos como “fenolico”, “establo” y “sudor de caballo” entre otros. \textit{Zygosaccharomyces rouxii} es capaz de desarrollar y alterar los jugos de uva concentrados, produciendo gas y alcohol, haciendo que las partidas exportadas lleguen a destino alteradas, siendo inmediatamente rechazadas. Un producto defectuoso o alterado produce grandes pérdidas económicas para las empresas y daña la imagen del país obstaculizando futuras exportaciones. La prevención es el mejor camino para evitar estas contaminaciones. En nuestro laboratorio se han desarrollado dos modelos matemáticos de predicción para conocer el riesgo potencial para producir alteraciones o defectos de estas levaduras en vinos y jugos de uva concentrados. Los modelos predictivos se construyeron considerando variables de los alimentos que puedan medirse y modificarse en la industria, como el pH, la concentración de etanol y SO$_2$ en los vinos; y pH y concentración de azúcares en los jugos de uva concentrados. Los modelos matemáticos se han validado con éxito en sustratos naturalmente contaminados. En un esfuerzo por proporcionar herramientas útiles y prácticas a enólogos y productores, se desarrollaron dos aplicaciones móviles que facilitan el acceso y uso de estos modelos. Mediante la aplicación, los productores podrán conocer las combinaciones variables que inhiben el crecimiento de las levaduras y estimar la vida útil del producto durante el almacenamiento y exportación por barco. Además, esta herramienta permite el diseño de estrategias de monitoreo y control prevenir el deterioro, evitando las pérdidas económicas asociadas con el rechazo de los productos.
DETECCIÓN DE COMPUESTOS TIPO AUXINAS EN LEVADURAS DE SUELO DEL BOSQUE PATAGÓNICO

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La producción de fitohormonas del grupo de las auxinas ha sido propuesta como uno de los principales mecanismos de interacción microorganismo-planta. El ácido indolacético (AIA) es la auxina más estudiada y con mayor actividad en las plantas. Resultados previos indican que algunas levaduras de suelo de bosque de Patagonia serían capaces de producir compuestos tipo auxinas utilizando triptófano como precursor. El objetivo del presente trabajo fue evaluar la producción de AIA por levaduras de suelo seleccionadas por su potencial como promotores de crecimiento vegetal. Se utilizaron 7 aislamientos de diferentes especies de levaduras. Los cultivos se incubaron en medio DEV; con y sin adición de triptófano 0,1%, luego se centrifugaron y la fracción sobrenadante se empleó en determinaciones colorimétricas (reactivo de Salkowsky) y de Cromatografía Líquida de Alta Performance (HPLC). La detección colorimétrica indicó la presencia de compuestos tipo auxinas en 4 cultivos que incluyen triptófano, mientras que el análisis por HPLC reveló la presencia de AIA en 3 de los cultivos con triptófano, con valores inferiores a 0,05Mm. Estos resultados verifican la capacidad de algunas levaduras de producir AIA en presencia de triptófano. La presencia de otros compuestos de naturaleza indólica podrían corresponder a intermediarios de la vía metabólica. Estudiar la producción de auxinas en levaduras puede ser una forma de conocer su interacción con las plantas.

FERMENTANDO ESTRESSES NA CERVEJARIA: A RESPOSTA À PROTEÍNAS MAL ENOVELADAS E O ESTRESSE DE RETICULO ENDOPLASMÁTICO NA TOLERÂNCIA AO ETANOL

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As leveduras lagers (Saccharomyces pastorianus) constituem um dos principais microrganismos empregados na industria cervejeira. Contudo, pouco é conhecido sobre como essas leveduras toleram os estresses resultantes do processo de fermentação cervejeira. Dos vários mecanismos de tolerância à estresses descritos, os relacionados ao estresse de retículo endoplasmático (ERE) e de resposta a proteínas mal enoveladas ainda não foram estudados no contexto da fermentação cervejeira. A fim de compreender o papel do ERE e da resposta a proteínas mal enoveladas em S. pastorianus durante a fermentação cervejeira, foi realizada uma análise metatranscritômica e de biologia de sistemas. Os dados indicaram a ativação resposta à proteínas mal enoveladas ERE e proteção antioxidante nas fases finais da fermentação cervejeira.
EFECTO INHIBITORIO DE LEVADURAS EPÍFITAS DE UVA PARA VINIFICAR SOBRE EL CRECIMIENTO DE ALTERNARIA ALTERNATA

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La prevención del crecimiento de hongos toxicogénicos resulta la estrategia más efectiva para controlar la presencia de micotoxinas en alimentos. El género Alternaria es componente mayoritario de la micobiota de uva para vinificar en distintas regiones vitivinícolas de Argentina y del mundo. En estudios previos encontramos que cepas de Alternaria alternata aisladas de uva Malbec eran capaces de producir alternariol (AOH), alternariol monometil ether (AME) y ácido tenuazonico (ATe) in vitro y que además exhibían patogenicidad y producción de ATe en uva para vinificar. En el presente estudio se evaluó el efecto inhibitorio de 14 levaduras epífitas de uva para vinificar sobre el crecimiento radial de 3 cepas toxicogénicas y patogénicas de A. alternata (5.5, 7.5 y 25.1) en un medio sintético de composición similar a la uva (MSU) a 3 temperaturas distintas (15, 25 y 30 °C). Como resultado, todas las cepas de levadura Metschnikowia spp. evaluadas (6) inhibieron completamente el crecimiento de las cepas de A. alternata (5.5, 7.5 y 25.1) a las 3 temperaturas testeadas. Mientras que la inhibición producida por las cepas de levadura Starmerella bacillaris (3) varió según las condiciones ensayadas. Todas inhibieron el crecimiento de la cepa A. alternata 5.5 a 25 °C y a 30 °C, una de ellas inhibió el crecimiento de las cepas A. alternata 7.5 y 25.1 a todas las temperaturas y las otras 2 inhibieron el crecimiento de las cepas A. alternata 7.5 y 25.1 sólo a 30 °C. Finalmente, una de las cepas de levadura Hanseniaspora uvarum de las evaluadas (5) mostró un efecto inhibitorio sobre el crecimiento de las 3 cepas de A. alternata pero sólo a 30 °C. En conclusión, entre las levaduras epífitas de uva para vinificar evaluadas, las cepas de Metschnikowia spp. resultan prometedoras para el control biológico de A. alternata.

COMPORTAMIENTO FERMENTATIVO DE LEVADURAS RECUPERADAS EN DIFERENTES MOMENTOS DEL PROCESO DE ELABORACIÓN DE CERVEZA ARTESANAL

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La recuperación y reutilización de levaduras es comúnmente llevada a cabo en la industria cervecería. Durante la fermentación y hacia el final de la misma, las levaduras floculan y sedimentan en los tanques cilindroconicos empleados para el bioproceso. Este sedimento es posible recuperarlo y emplearlo en fermentaciones sucesivas. La dinámica fermentativa, viabilidad y vitalidad de las levaduras puede variar en función del momento y de la zona de recuperación en el tanque. En este estudio se propuso llevar a cabo 3 fermentaciones sucesivas con levaduras recuperadas (Saccharomyces cerevisiae Safe Ale US05) luego de 7 días de fermentación (T2) y con levaduras...
almacenadas durante 4 días a 1°C (T3), pertenecientes a la empresa Ancestral SRL. El sedimento se obtuvo de la zona media del cono. Una dosis de $1 \times 10^7$ cel/mL fue inoculada en frascos cilindroconicos con 170 mL de mosto de cerveza ($P=13.81$, pH=5.2). Un tratamiento control con la levadura comercial hidratada se realizó en simultaneo (T1). Todos los tratamientos se hicieron por triplicado. Al finalizar cada fermentación se determinó viabilidad (tinción azul de metileno) y vitalidad (test de acidificación) de las levaduras recuperadas. La dinámica fermentativa fue analizada mediante pedida de peso debido por la liberación de CO$_2$. En todos los casos las levaduras lograron atenuar el mosto correctamente. Durante los 3 ciclos sucesivos, las levaduras recuperadas (T2 y T3) presentaron mayor dinámica y vigor fermentativo respecto al control. Mientras que T2 presentó mayor dinámica que T3 durante los dos primeros ciclos de reutilización. En el tercer ciclo T3 mejoró el comportamiento imponiéndose ante T2. La viabilidad y vitalidad celular de las cepas levaduras, fue mayor a partir del segundo ciclo. Esto indica que las levaduras reutilizadas presentaron mejores condiciones respecto a la levadura comercial y además mejoraron su comportamiento en las fermentaciones consecutivas llevadas a cabo.

**FERMENTACIÓN ETANÓLICA DE MELAZA CON S. CEREVISIAE INMOVILIZADA EN UN SOPORTE MAGNÉTICO NANOESTRUCTURADO**

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El objetivo fue estudiar la producción de etanol mediante *S. cerevisiae* a partir de melaza en presencia de nanopartículas de ferrita de manganeso recubiertas con quitosán (NPM) en dos ciclos mediados por la separación magnética de biomasa. La fermentación se realizó utilizando dos cepas de *S. cerevisiae* presencia de 300mL de melaza a 20°Bx con 1.6 g/L de sulfato de amonio y 1.0g/L de fosfato de potasio a 32°C bajo condiciones anóxicas, en presencia de $10^7$ células en forma libre e inmovilizada en 480mg de NPM. El soporte nanoestructurado fue obtenido por coprecipitación en presencia de quitosán con posterior tratamiento hidrotérmico. Se caracterizó la isoterma de adsorción realizando la separación magnética de las células adsorbidas y conteo de células libres en microscopio. La cinética de producción de etanol y consumo de azucares se evaluó por HPLC. Las NPM presentaron saturación de 12.5 emu/g, remanencia de 5.1emu/g y coercitividad de 850Oe. La baja remanencia magnética demuestra que el soporte no posee magnetización en ausencia de un campo magnético. Se demostró que el isoterma de adsorción de levadura en NPM se ajusta al modelo de Freundlich. En el proceso de la fermentación de melaza se observó el consumo de glucosa y fructosa. La producción de etanol no fue afectada por la presencia de NPM, llegando a un valor de 46.3±2.3 g/L. En el caso del ensayo realizado con nanopartículas, la biomasa fue precipitada en un campo magnético externo y sometida a fermentación adicionando nueva porción de medio. Se observó que la producción de etanol llegó a un mayor nivel de 61.6±1.5 g/L, es decir 33% mayor que en el primer proceso. Los resultados obtenidos indican que la separación magnética de biomasa puede ser aplicada para facilitar el proceso de su reúso en ciclos de fermentación permitiendo obtener mayores niveles de etanol.
INFLUENCIA DE ADITIVOS INORGÁNICOS SOBRE LA EFICACIA BIOCONTROLADORA DE CYSTOFILOBASIDIUM CAPITATUM Y MEYEROZYMA GUILLIERMONDII FREnte A PATÓGENOS DE POSTCOSECHA

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Las enfermedades de postcosecha limitan la vida comercial de las cerezas. En Patagonia, los patógenos asociados a dichas enfermedades son Botrytis cinerea, Penicillium crustosum y Mucor piriformis. Se ha demostrado que aislamientos de levaduras indígenas de Cystofilobasidium capitatum y Meyerozyma guilliermondii son potenciales agentes de biocontrol (ACB) para este agroecosistema. En este marco, el uso de cloruro de calcio y BTH pueden mejorar la efectividad de los ACB seleccionados. Para evaluar esto se planteó: i) analizar el efecto de los aditivos sobre el crecimiento de los ACB, mediante el recuento de UFC de los ACB; ii) evaluar la eficacia in vitro de los ACB en combinación con los aditivos, analizando la germinación de conidios y crecimiento miceliar de los patógenos; iii) evaluar la eficacia de los ACB en combinación con los aditivos en ensayos sobre fruta almacenada, determinando la incidencia y severidad de la enfermedad a 0° y 22°C en los distintos tratamientos. Los resultados indican que i) no hay efecto de los aditivos sobre el crecimiento de los ACB. ii) La combinación BTH+C. capitatum resultó el tratamiento más eficaz para inhibir la germinación de conidios y el crecimiento miceliar de B. cinerea y de M. piriformis (≥ 98%). Mientras que C. capitatum+CaCl₂ resultó la combinación más exitosa para inhibir la germinación de conidios (≥ 99%) y el desarrollo miceliar de P. crustosum. iii) la intensidad de la enfermedad causada por los 3 patógenos se redujo por el efecto sinérgico de ambos ACB y aditivos a 0°C, mientras que a 22°C la combinación de C. capitatum+BTH resultó más eficaz (40 – 80% reducción de intensidad). Estos resultados permiten concluir que en las condiciones evaluadas la combinación BTH+C. capitatum podría ser la base para el desarrollo de un producto eficaz para el tratamiento de enfermedades de postcosecha.

EVALUACIÓN DE LA RESPUESTA DE GENES ASOCIADOS A ESTRÉS OXIDATIVO Y ESTABLECER SU RELACIÓN CON EL MECANISMO DE RESISTENCIA A ÁCIDO P-CUMÁRICO EN BRETTANOMYCES BRUXELLENsis

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Brettanomyces bruxellensis ha sido descrita como la principal levadura contaminante del vino, por su capacidad de metabolizar el ácido p-cumárico (ApC) a compuestos menos tóxicos. Estudios transcriptómicos han reportado que la entrada de ApC en la célula provoca una condición de estrés generalizado, induciendo la expresión de bombas de protones y mecanismos involucrado en la salida de compuestos tóxicos. Asimismo,
genes relacionados con respuesta a estrés oxidativo fueron fuertemente sobre-expresados. En este estudio, se comparó la expresión relativa de genes relacionados a estrés oxidativo mediante RT-qPCR en dos cepas de *B. bruxellensis* (LAMAP1359 y LAMAP2480) que presentan diferencias en la duración de la fase *lag* cuando son expuestas a *ApC*. Los resultados indican que existe una mayor expresión de los genes *SOD1*, *GCN4*, *HSP12* y *PAD1* en la cepa LAMAP2480, mientras que el gen *ESBP6* tiene una mayor expresión en la cepa LAMAP1359, no presentando diferencias significativas entre ambas cepas. Posteriormente estos genes fueron validados mediante transformación de *Pichia pastoris*. Evaluando la cinética de crecimiento de las transformantes, se observó que la presencia de una copia del gen analizado mejora la velocidad de crecimiento en comparación con la cepa no transformada crecida en la misma condición.

**OPTIMIZACIÓN DE LA PRODUCCIÓN DE BIODIESEL A PARTIR DE GLICERINA CRUDA Y VINAZA MEDIANTE LA LEVADURA OLEAGINOSA *R. GRAMINIS* S1/2R**

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La producción de biodiesel nacional ha involucrado una diversificación de la matriz energética, una disminución de la dependencia en los combustibles fósiles. Sin embargo, los altos costos de obtención de las materias primas vegetales utilizadas y la competencia con el sector alimentario, ha marcado la necesidad de explorar nuevas materias primas para la obtención de biodiesel, dentro de las que se incluyen los triglicéridos acumulados intracelularmente por levaduras oleaginosas. Sin embargo, para lograr un proceso competitivo es necesario utilizar para su producción sustratos fermentables de bajo costo tales como subproductos o desechos industriales. Nuestro grupo de trabajo cuenta con un aislamiento de una cepa de levadura identificada como *Rhodotorula graminis*, que es capaz de acumular triglicéridos en cantidades mayores al 40% de su peso seco, cuando es cultivada en un medio con una elevada relación C/N. En trabajo anterior s logró optimizar las condiciones de cultivo en un medio a base de glicerina cruda en batch y batch alimentado. En este trabajo se realizó la optimización de la producción de la levadura en un medio de cultivo preparado sustituyendo la base del medio optimizado por vinaza. Los ensayos fueron realizados en batch, en matraces con agitación y en fermentador, lográndose un porcentaje de acumulación de 39,6, siendo los rendimientos en la cantidad de biomasa y metiésteres similares a los obtenida en el medio anterior. La sustitución del medio base por este subproducto industrial abarataría costos de producción y daría un uso alternativo para la vinaza, logrando de esta manera abaratar aún más los costos de producción.
EFEITO DE FENÓIS VOLÁTEIS SOBRE O CRESCIMENTO DE SACCHAROMYCES CEREVISIAE

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A levedura Dekkera bruxellensis é um importante contaminante de fermentações etanólicas, com a habilidade de produzir fenóis voláteis (4-etilfenol e 4-vinilfenol) a partir de ácido p-cumárico. Esse ácido hidroxicinâmico está presente nas uvas e também em mostos de cana-de-açúcar, como caldo de cana e melão. Estudos realizados por nosso grupo de pesquisa verificaram que a levedura D. bruxellensis é capaz de produzir 4-etilfenol em ambos os mostos de cana em um processo fermentativo em batelada com reciclo celular, em concentrações superiores às encontradas em vinho. Não se sabe o impacto que a produção desses compostos pode ter sobre a viabilidade da levedura do processo, a espécie Saccharomyces cerevisiae, ou mesmo sobre os parâmetros fermentativos. No presente trabalho, inicialmente foi verificado o efeito do ácido p-cumárico, 4-vinilfenol e 4-etilfenol sobre o crescimento de uma linhagem industrial de S. cerevisiae (PE-2), em concentrações próximas às encontradas no mosto ou durante o processo fermentativo. Os experimentos foram realizados em microplacas de 96 poços, em triplicata, em meio YPD, no equipamento Infinite 200 PRO-Tecan durante 24 horas, a 30°C, com agitação, avaliando-se a densidade óptica a 600 nm a cada 15 minutos. A avaliação dos resultados foi realizada com base nos valores de velocidade específica de crescimento e duração da fase lag. Não houve efeito inibitório do ácido p-cumárico e dos fenóis voláteis sobre a velocidade máxima de crescimento e não ocorreu aumento da fase lag da levedura com as concentrações testadas (4 a 20 mg/L, 2 a 10 mg/L e 3 a 15 mg/L, para p-cumárico, 4-vinilfenol e 4-etilfenol, respectivamente). Outros experimentos estão sendo realizados para avaliar possíveis interações entre os efeitos do 4-etilfenol e do pH, concentração de etanol e concentração de açúcar sobre o crescimento e potencial fermentativo de S. cerevisiae. Apoio: Fapesp (2016/20680-4).

IDENTIFICAÇÃO E ANÁLISE FUNCIONAL DE NOVOS PEPTÍDEOS SINAIS ENDÓGENOS PARA KOMAGATAELLA PHAFFI

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Pichia pastoris, reclassificada como Komagataella sp, é considerada uma excelente plataforma para a produção de proteínas recombinantes. Mais de 600 proteínas já foram produzidas usando Komagataella como sistema de expressão. A maioria dos vetores de expressão utiliza a sequência pré-pro do fator α de Saccharomyces cerevisiae como um sinal de secreção, que fornece excelentes níveis de secreção, mas pode ser processado de forma inadequada em condições de hiperoxpressão. Usando um sinal de secreção nativo de Komagataella sp. este problema pode ser superado, mantendo a estrutura correta da proteína heteróloga. Neste trabalho foram identificados treze sinais de
secreção do secretoma de *Komagataella phaffii* por meio de análise *in silico*. Estas sequências e o factor α putativo de *K. phaffii* foram clonados em pPIC9 contendo α-amilase de *Bacillus subtilis* (*amyE*) como gene repórter. Todos eles, exceto o PS9, mostraram um halo de hidrolise em placa contendo amido. Todos os clones analisados tinham apenas uma cópia da α-amilase. Ensaio de atividade de α-amilase mostraram que exceto o PS9, todos os outros tiveram nível de expressão semelhantes ou superiores aos do fator α de *S. cerevisiae*. As amilases de quatro clones foram purificadas para subsequente sequenciamento do N-terminal. Estes novos peptídeos sinais podem ser usados como uma alternativa ao fator α de *S. cerevisiae*, principalmente quando o processamento N-terminal é mais específico ou para melhores níveis de proteína secretada.

**EFEITO DA QUITOSANA OBTIDA POR FERMENTAÇÃO LÂTICA EM ESTIRPES DE LEVEDURAS DO BIOETANOL**

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A quitosana é um biopolímero amplamente utilizado na agricultura e nas indústrias alimentícia, médica e farmacêutica. É obtida através da desacetilação da quitina, extraída de crustáceos ou fungos. Apesar de seu potencial como agente antimicrobiano, a quitosana ainda não é usada na indústria do bioetanol. *Dekkera bruxellensis* é uma levedura oportunista de grande interesse da indústria do vinho e dos processos fermentativos para produção de etanol no Brasil. Neste trabalho, a quitosana foi primeiramente obtida de resíduo de camarão através de um processo de bioconversão utilizando a bactéria *Lactobacillus plantarum* em meio MRS, em concentrações otimizadas de resíduo, inóculo e glicose. Após a fermentação, o resíduo foi lavado com água destilada e seco em estufa. O processo de desacetilação foi feito pelo método da autoclave utilizando hidróxido de sódio 45%. O efeito antimicrobiano da quitosana natural obtida comparado ao efeito de uma quitosana comercial (ambas diluídas em solução de ácido acético 2%) foi avaliado contra uma estirpe de *D. bruxellensis* (*Db*) e uma estirpe industrial (PE-2) de *Saccharomyces cerevisiae* (*Sc*). Este experimento foi realizado em placa de cultura celular de 96 poços no equipamento leitor de placas Infinite 200 PRO com meio YPD e concentrações de quitosana variando entre 50 mg/L e 500 mg/L, a 30oC, 24 horas. A quitosana natural reduziu a taxa específica de crescimento (µ) e aumentou a fase lag de *Db* na concentração 500 mg/L. Quanto a quitosana comercial, 100 mg/L foi eficiente mas não reduziu a fase lag. Um efeito somente na taxa de *Sc* foi observado com ambas as quitosanas. O aumento da fase lag em 4 horas causado pela quitosana natural é relevante para controlar o crescimento de *Db* na fermentação etanólica. Futuros experimentos são necessários para aumentar a solubilidade da quitosana natural, a fim de alcançar melhores resultados com concentrações mais baixas.
EXPRESSÃO DE AMILASES EM KOMAGATAELLA PHAFFII: COMPARAÇÃO ENTRE OS PROMOTORES PGK E AOX1

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A levedura metilotrófica Komagataella phaffii é amplamente usada como modelo para a expressão de proteínas heterólogas. A utilização é normalmente associada ao promotor induzido por metanol do gene que codifica a enzima álcool oxidase 1 (AOX1), embora o transporte, armazenamento e elevado custo do metanol criem uma demanda por promotores constitutivos de alta eficiência. A região promotora do gene que codifica a enzima 3-fosfoglicerato quinase (PGK) tem sido utilizada como uma alternativa efetiva para o promotor AOX1, mas a região completa é geralmente necessária (aproximadamente 2000 bp). Considerando que promotores menores facilitam a manipulação e aumentam a estabilidade de DNA heterólogos, neste trabalho foi comparada uma região menor e otimizada do promotor PGK (aproximadamente 400 pb) com o promotor AOX1 para a expressão heteróloga em K. phaffi. Três diferentes construções de amilases: uma α-amilase de Bacillus subtillis, uma glicoamilase de Aspergillus awamori e uma fusão entre as duas. Uma colônia de cada linhagem transformante com apenas uma cópia do vetor integrada no genoma foi selecionada para a análise de crescimento e atividade de produção das enzimas. O promotor AOX1 foi mais efetivo induzindo a produção de α-amilase, que é a menores das três proteínas (60,6 kDa). Para a glicoamilase (74,4 kDa), os dois promotores obtiveram resultados similares. E para a fusão (125,0 kDa), o promotor PGK foi o mais eficiente. Esses resultados sugerem que o promotor AOX1 pode ser muito forte para proteínas mais complexas, levando a erros de dobramento e sobrecarga do sistema de secreção da levedura. Essa versão menor do promotor PGK pode ser uma escolha mais balanceada quando a proteína heteróloga for muito grande ou complexa.

POTENCIAL DE RESÍDUOS AGROINDUSTRIAIS COMO FONTES DE COMPOSTOS ANTIMICROBIANOS COM APLICAÇÃO NA FERMENTAÇÃO ETANÓLICA

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A grande quantidade de resíduos produzidos pelas agroindústrias levanta sérios problemas de gestão, tanto do ponto de vista econômico, quanto ambiental. Muitos desses resíduos têm potencial para serem usados em outros sistemas de produção, como por exemplo, aditivos naturais ou antimicrobianos. O objetivo deste estudo foi avaliar o potencial de resíduos de frutas como fontes de compostos antimicrobianos e sua ação contra leveduras da fermentação etanolica. Foram obtidos extratos de resíduos de abacate (semente), laranja (bagaço) e uva (casca e sementes) utilizando etanol 80% (20 g de resíduo seco/200 mL) e o solvente evaporado em evaporador rotativo. Foi realizada a análise dos compostos fenólicos (CF) e em seguida os extratos foram diluídos para avaliação da inibição do crescimento das leveduras Saccharomyces cerevisiae.
(linhagem industrial PE-2) e Dekkera bruxellensis (contaminante da fermentação etanólica) em ensaios realizados em tubos e em microplacas de 96 poços no equipamento Infinite 200 PRO-Tecan, com meio YPD. Considerando-se os extratos brutos (sem diluição), o maior valor de CF foi observado em extrato de uva (1,355 mg/100g). O efeito inibitório contra D. bruxellensis foi consideravelmente alto utilizando-se extrato de semente de abacate a partir da concentração de 100 mg/L. Com extrato de resíduos de laranja, o efeito foi observado a partir de 300 mg/L. O extrato de resíduo de uva Cabernet mostrou uma tendência a reduzir o crescimento da levedura D. bruxellensis a partir de 200 mg/L, no entanto houve inconsistência nos efeitos deste extrato provavelmente devido à oxidação dos compostos, sendo necessária a repetição do experimento. O efeito dos extratos sobre a levedura S. cerevisiae foi menos extenso do que para D. bruxellensis. Estes resultados encorajam a realização de novos ensaios para confirmar a seletividade na inibição das leveduras e avaliar a composição dos extratos para determinar a composição química.

**SACCHAROMYCES UVARUM COMO HERRAMIENTA BIOTECNOLOGICA PARA LA DIFERENCIACION DE SIDRAS 100% PATAGONICAS**

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Algunas de las características fisiológicas de S. uvarum como su mayor criotolerancia y capacidad fructofílica que S. cerevisiae resultan interesantes para el desarrollo de cultivos iniciadores en fermentaciones realizadas a baja temperatura utilizando mostos con elevado contenido de fructosa como el de manzana y pera. La Patagonia resulta ideal para evaluar este recurso ya que concentra el 75% de la producción de sidra del país y una gran diversidad de cepas de S. uvarum, difíciles de encontrar en otros lugares del mundo. En este trabajo se evaluó en primera instancia la cepa S. uvarum NPCC1314 (aislada de chicha) en fermentaciones a escala semipiloto (10L) en mosto de manzana Granny Smith no estéril a 13°C y 25°C, en comparación con una levadura comercial de la especie S. cerevisiae. Se logró mejor implantación de Su a 13°C que a 25°C (100% vs 66%). Sin embargo, el análisis sensorial de las sidras fermentadas a 25°C demostró una preferencia por la inoculada con S. uvarum mientras que el análisis de las sidras fermentadas a 13°C evidenció preferencia por la fermentada con S. cerevisiae. A fin de mejorar la calidad sensorial se compararon dos cepas S. uvarum: NPCC1314 y NPCC1420 (aisladas de sidras regionales) en mostos de manzana Granny Smith y Pink Lady y en mosto de pera Packams. Esta vez se realizaron todas las fermentaciones a 13°C para garantizar la implantación máxima. La cepa NPCC1420 presentó una mayor velocidad de fermentación que NPCC1314 en los tres mostos utilizados. La implantación fue de un 100% para ambas cepas en pera, mientras que en ambos mostos de manzana la cepa NPCC1314 tuvo una implantación menor que la NPCC1420. Las propiedades aromáticas fueron significativamente distintas entre las dos levaduras evaluadas y en los análisis sensoriales se destacó NPCC1314 por sus aromas frutales y NPCC1420 por su mayor cuerpo y aceptabilidad general.
BIODIVERSIDADE DE LEVEDURAS EM LATICÍNEOS DO ESTADO DE PERNAMBUCO

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Desde a perspectiva de produção de biocombustíveis, existe um grande potencial ainda inexplorado na biodiversidade de leveduras selvagem presente em processos industriais. Certamente, no âmbito da produção de lácteos, a possibilidade de identificar leveduras inéditas com capacidade de produção de etanol, biosurfactantes e/ou acumulação de lipídeos a partir da bioconversão de lactose é elevada. Em vista do exposto, este trabalho teve como objetivo isolar, identificar e caracterizar fisiologicamente leveduras capazes de assimilar lactose em 26 queijarias do Estado de Pernambuco, Brasil. Foram isoladas 795 colônias em quatro pontos do processo de fabricação de queijo coelho (tanque de recebimento do leite, mesa de prensagem, mesa de corte, e no soro de queijo) em um período de seis meses. O isolamento foi realizado em placas de Petri (extrato de levedura 1%, peptona 2%, lactose 2%, ágar 2%, cloranfenicolo 6 mg/ml), e incubado a 30°C por 24h. As colônias foram re-isoladas para confirmar a pureza e estocados (glicerol stock 30% a -80°C). Os isolados foram submetidos à identificação pela técnica de espectrometria de massa (MALDI-TOF MS), através da análise do perfil proteico. Como resultado preliminar, foi possível identificar 532 isolados, representados por 15 géneros (sete fungos e oito bactérias), que correspondem a 25 espécies, com predominância do gênero Candida sp. (395 isolados), Escherichia sp. (33) Trichosporon sp. (32), Saccharomyces sp. (19), e Geotrichum. sp. (18). Destas, algumas espécies de leveduras com importância biotecnológica, como Candida kefyr (Kluyveromyces marxianus) (29), e Candida lipolytica (55) foram possíveis de associar à produção de etanol e acumulo de lipídios, respectivamente. Os resultados obtidos confirmam a ampla biodiversidade das leveduras presentes nos laticínios com aplicações biotecnológicas. Esses resultados também destacam a necessidade de realizar treinamento, implementação e monitoramento contínuo de boas práticas para prevenir contaminação microbiana nesta indústria.

EVALUACIÓN DEL BAGAZO DE MANZANA COMO SUSTRATO PARA LA PROPAGACIÓN DE LEVADURAS DE USO ENOLÓGICO

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En la región del Comahue, la producción de frutas de pepitas (manzanas y peras) constituye uno de los pilares de su economía. El 40% de la manzana se destina a la industria, principalmente para la producción de jugos concentrados o exprimidos. En este trabajo se evaluó la potencial capacidad del bagazo de manzanas, un desecho de la industria juguera regional, para ser usado como sustrato alternativo a las melazas de caña y remolacha azucareras en la propagación de la biomasa de especies de levadura nativas, de probada aptitud para uso enológico. Los bagazos fueron caracterizados por
lores en sus contenidos de azúcares reductores (AR), nitrógeno fácilmente asimilable (FAN), humedad, pH y polifenoles totales, según técnicas AOAC. A su vez, se estudiaron los distintos medios de cultivo mediante la metodología de Diseño Estadístico Experimental. Se analizaron los parámetros de crecimiento de dos levaduras vínicas patagónicas Saccharomyces cerevisiae F8 y Pichia kudriavzevii P15, con diseños Placket-Burman y comparando los sustratos bagazo de manzana y melaza de caña (control). Los resultados obtenidos en el análisis físicoquímico indican que el bagazo de manzana presenta una alta calidad como potencial sustrato para la propagación de biomasa, con valores similares a los reportados para manzanas regionales de la misma variedad. En el análisis de aplicación del bagazo como medio de cultivo, los resultados evidenciaron que este sustrato constituye una alternativa mejorada para la producción de biomasa de levaduras que la melaza de caña, dado que no requiere de suplementación nutricional para obtener el máximo crecimiento de los microorganismos. El bagazo de manzana generado durante la producción jugos regionales se presenta como una alternativa de calidad para su revalorización en la producción de biomasa de levaduras, resolviendo además el problema de su disposición sin generar pasivos ambientales, mejorando la rentabilidad de las empresas productoras y contribuyendo a la diversificación productiva regional.

CULTIVOS ADJUNTOS DE PICHIA KUDRIAVZEVII: ¿UNA ALTERNATIVA A LA FERMENTACIÓN MALOLÁCTICA PARA LA DEACIDIFICACIÓN BIOLÓGICA DE VINOS?

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En la región del Comahue, ubicada en la norpatagonia argentina a 40º LS, los mostos de uvas tintas presentan contenidos elevados de ácido málico (3 a 6 g/L). Mientras que la fermentación maloláctica (FML), una práctica de rutina utilizada para la deacidificación de estos vinos, es un proceso difícil de controlar a nivel industrial, el objetivo de este trabajo fue evaluar la capacidad de un cultivo adjunto de P. kudriavzevii de consumir ácido L (-) Málico durante la fermentación alcohólica (FA). Esta levadura presenta capacidad de degradar ese sustrato como única fuente carbonada, razón por la que proponemos su uso para la deacidificación de vinos. Las vinificaciones se realizaron con mostos Malbec a escala piloto (200 L) durante las Vendimias 2015 a 2017. Los vinos se elaboraron mediante FA conducidas por el cultivo iniciador autóctono Saccharomyces cerevisiae F8 (ScF8) y por el cultivo mixto ScF8 y P. kudriavzevii P15 inoculadas simultáneamente (relación 1/100) (CoC). Los vinos jóvenes se sulfitaron con el fin de inhibir la FML y su calidad físicoquímica y aromática se determinó una vez embotellados, utilizando métodos convencionales (INV) y cromatográficos (HPLC y GC-FID). La calidad sensorial se evaluó por cata y ensayos cualitativos realizados por un panel entrenado, y por ensayos de preferencia por los consumidores. Todos los vinos resultaron normales, secos y puntuados como muy buenos a la cata, pero los elaborados con el CoC presentaron concentraciones de ácido málico significativamente menores y contenido de ésteres significativamente mayores que los ScF8. El análisis sensorial evidenció diferencias significativas entre los vinos CoC y ScF8, con mayor aroma frutal en los primeros y preferencia significativa de los consumidores. La validación del CoC
en entornos reales durante tres años lo presenta como una interesante alternativa a la FML para la deacidificación de vinos patagónicos.

**LEVADURAS POLIEXTREMOTOLERANTES DE AMBIENTES GLACIARIOS**

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Los ambientes fríos conforman uno de los ambientes extremos más extendidos y poseen múltiples factores que afectan el desarrollo de la vida. Los microorganismos deben emplear estrategias que les permitan vivir y desarrollarse en estos ambientes, en donde la disponibilidad de agua es fluctuante y la temperatura afecta la permeabilidad y rigidez de la membrana plasmática y las actividades enzimáticas. Entre los microorganismos capaces de crecer en estas condiciones se encuentran las levaduras, que son capaces de habitar estos ambientes y presentan una amplia gama de estrategias para sobrevivir. La producción de metabolitos, como glicerol, micosporinas, ergosterol y trehalosa, así como la producción de enzimas extracelulares activas a bajas temperaturas, forman parte de estas estrategias. Nuestros estudios muestran que existe una gran diversidad de levaduras y hongos en los ambientes asociados a sistemas glaciarios. Los estudios de biodiversidad con metodologías de cultivo tradicional, se complementaron con metagenómica en glaciares no-polares de la Patagonia Argentina. Nuestros resultados, muestran la presencia de Chytridiomycetes, como integrantes importantes de la biota de estos ambientes, además se observó la prevalencia de Basidiomycetes en sustratos de hielo, nieve y agua, con las clases Microbotryomycetes y Tremellomycetes, entre las más abundantes. Se realizó además el estudio de la producción de metabolitos específicos para contrarrestar los efectos directos e indirectos de las bajas temperaturas. Todo esto muestra la especialización de ciertos grupos taxonómicos, presentes en estos ambientes extremos. Nuestro trabajo contribuye a un mejor conocimiento de la biodiversidad de las levaduras extremotolerantes y a la preservación de recursos genéticos que permitan el desarrollo de estrategias de conservación.

**GENERACIÓN DE NUEVOS HÍBRIDOS LAGER PARA FERMENTACIONES A BAJAS TEMPERATURAS**

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El descubrimiento de *Saccharomyces eubayanus* en la Patagonia Andina, ayudó a resolver el misterio de la especie parental del híbrido Lager, *S. pastorianus*. Desde entonces existieron varios intentos por generar híbridos interespecíficos entre *S. eubayanus* y *S. cerevisiae*, aunque todos ellos necesitaron de la formación previa de
mutantes auxótrofos mediante técnicas que producen organismos genéticamente modificados (OGM). En el presente trabajo nos basamos en características intrínsecas de cada especie, como es el consumo de diferentes fuentes de carbono, para la selección del híbrido resultante. Primero se realizó un screening de cepas de tipo Ale pertenecientes al Banco de Levaduras de Cervezas del IPATEC, buscando aquellas con alta atenuación y buena floculación. De las 17 cepas pre-seleccionadas, se eligieron finalmente 3 debido a su capacidad de producir esporas viables y con ellas se realizaron las cruzas con la cepa de S. eubayanus CRUB1568T mediante un micromanipulador (espora-espora). Las cruzas fueron sombreadas en medios selectivos y las colonias crecidas en los medios selectivos se chequearon por RLFP a partir de la región CBT1. Se obtuvieron un total de 9 híbridos a los cuales se les analizó la cinética de fermentación, el consumo de azúcares, la capacidad de floculación y el perfil sensorial. Los porcentajes de atenuación variaron entre un 65 – 70 % debido al consumo parcial o total de la maltosa del mosto, en cuanto a la floculación se vieron mejores respecto a S. eubayanus aunque muy por debajo de su parental Ale. Finalmente, se seleccionaron 3 cepas híbridas para fermentaciones a mayor escala. Si bien ninguno de los híbridos generados por espora-espora mostraron el vigor híbrido, todas las cepas mostraron una buena performance a bajas temperaturas donde se destaca el perfil aromático particular de las mismas, cualidad necesaria para la creación de cervezas diferenciales.

RESPUESTA DE SACCHAROMYCES UVARUM Y SACCHAROMYCES EUBAYANUS PATAGONICAS A CONDICIONES DE FERMENTACIÓN EN VINO BLANCO

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La demanda de vinos únicos, sumada a la mayor retención de compuestos volátiles (flavour) lograda a baja temperatura, generan la necesidad de contar con nuevas levaduras criotolerantes en vinificación. En este trabajo se evaluó el comportamiento de dos cepas patagónicas criotolerantes -S. eubayanus (Se) y S. uvarum (Su) en vinificación y en condiciones de estrés típicas del proceso. Inicialmente se inocularon las levaduras en mosto Sauvignon Blanc natural (21ºBrix; 10 L), y se fermentó a 13ºC a fin de garantizar la implantación. S. eubayanus logró porcentajes de implantación de 90,5% y produjo vinos con el máximo contenido de glicerol y mínimo de ácido acético (6,77 ± 0,01 y 0,57 ± 0,02 g/L, respectivamente). S. uvarum produjo vinos con elevado contenido de octanoato de etilo, y aunque logró una implantación de sólo 37.5% sus vinos fueron elegidos en un análisis sensorial de preferencia. En una segunda oportunidad se evaluaron las mismas cepas en mosto Sauvignon Blanc con 24ºBrix y la fermentación se realizó a 16ºC a fin de garantizar la implantación. S. eubayanus logró porcentajes de implantación de 90,5% y produjo vinos con el máximo contenido de glicerol y mínimo de ácido acético (6,77 ± 0,01 y 0,57 ± 0,02 g/L, respectivamente). S. uvarum produjo vinos con elevado contenido de octanoato de etilo, y aunque logró una implantación de sólo 37.5% sus vinos fueron elegidos en un análisis sensorial de preferencia. En una segunda oportunidad se evaluaron las mismas cepas en mosto Sauvignon Blanc con 24ºBrix y la fermentación se realizó a 16ºC. En este caso, para ambas levaduras, no se logró una buena implantación y las fermentaciones no finalizaron, dejando niveles promedio elevados de azúcares residuales y ácido acético (43,21 ± 5,40 y 0,89 ± 0,03 g/L, respectivamente). Del análisis de factores de estrés típicos de vinificación, realizado en microplacas, se evidenció que las levaduras criotolerantes crecieron mejor que S. cerevisiae (control) en medios con bajo contenido de nitrógeno fácilmente asimilable (evaluado entre 20 y 300 mg/L) y no fueron afectadas por las elevadas concentraciones de glucosa (evaluadas entre 0-300 g/L). Sin embargo, no pudieron crecer a concentraciones de etanol mayores a 8% v/v y en presencia de SO2. Los resultados
sugieren que debería mejorarse la tolerancia de las cepas al etanol y al SO$_2$ a fin de lograr una mejor implantación a temperaturas mayores que 13ºC.

**ESPECIFICIDAD DE LA RESPUESTA ANTIOXIDANTE DE DOS LEVADURAS ETANOLOGENICAS SOMETIDAS A ESTRES**

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Dos cepas de levaduras etanologenicas: una de laboratorio; Scheffersomyces stipitis (NRRL7124 (Crabtree-) y una industrial; Saccharomyces cerevisiae (Crabtree+). Genéticamente modificada, osmotolerante, se sometieron a compuestos tóxicos para el metabolismo de las mismas, a concentraciones crecientes hasta 10g/L de CH$_3$-COOH para S. cerevisiae y hasta 90%(v/v) de un hidrolizado ácido de cascaras de semillas de Simmondsia chinensis, (jojoba) para S. stipitis. Utilizando la metodología de evolución adaptativa (1). Se obtuvieron 2 clones isogénicos de las cepas parentales. Llamadas HA para S. stipitis y Y8A para S. cerevisiae; los clones fueron sometidos a distintos estreses físico-químicos, oxidativo, etanol, térmico, salino, osmótico, comp. Fenólicos, etc. Por sonicación se obtuvieron extractos libres de células de ambos clones y se valoraron las actividades de las enzimas catalasa (CAT), superoxido dismutasa (SOD) y glutatión transferasa (GST). En los clones adaptados Y8A y HA fueron más altas las actividades enzimáticas pero en forma diferencial. Mientras que en Y8A se observó solo aumento de CAT y GST pero no de SOD. En HA se observó aumento de CAT y de SOD, estos resultados se correspondieron con los valores de Especies Reactivas de Oxigeno (ROS) medidos. Conclusión: la tolerancia a múltiples estreses en levaduras estaría asociada a su capacidad de detoxificar ROS en forma diferencial.

**HIBRIDACION COMO MECANISMO PARA GENERAR NUEVAS CEPAS DE LEVADURAS INDUSTRIALES**

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Las levaduras del género *Saccharomyces* tienen una gran importancia industrial. *Saccharomyces cerevisiae* ha sido durante siglos, la principal productora de vinos y cervezas tipo ale. Además, su alta resistencia a los estreses industriales le ha permitido ser redomesticada para la producción de procesos como la obtención de bioetanol. No obstante, existe una gran diversidad de levaduras dentro del género *Saccharomyces* que se han aislado de procesos de producción de sidra, como es el caso de *Saccharomyces uvarum*, o de vinos Croatas, en el caso de *Saccharomyces paradoxus*. En los últimos años, y con la aplicación de los nuevos métodos moleculares hemos sido
capaces de detectar que otras especies de Saccharomyces también tienen un rol importante en la producción de cervezas lager, en el caso de Saccharomyces eubayanus y vinos y cervezas ale producidos en regiones Europeas caracterizadas por sus bajas temperaturas, como Saccharomyces kudriavzevii. Estas dos últimas especies se encuentran en forma de híbridos con S. cerevisiae y/o S. Uvarum o incluso triples híbridos. Actualmente, hemos desarrollado una metodología para la generación de dobles, triples o incluso híbridos más completos para aprovechar las mejores características de cada especie. Además, la herencia mitocondrial tiene un gran impacto en el fenotipo y cuya herencia estamos explorando en condiciones industriales.

MODELADO DE RUTAS METABÓLICAS EN SACCHAROMYCES CEREBVISIAE PARA LA PRODUCCIÓN DE ÁCIDO LÁCTICO A PARTIR DE GLICERINA

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La contaminación ambiental generada por los residuos plásticos resulta uno de los problemas críticos a tratar a nivel mundial. La utilización de nuevas tecnologías de materiales, tales como el uso de bioplásticos, resulta una alternativa interesante. En este trabajo de doctorado se propone la modificación genética de la levadura Saccharomyces cerevisiae para la obtención de ácido láctico, como monómero para la síntesis del bioplástico PLA (ácido poliláctico), y el diseño de un medio de cultivo que posibilite obtener altas concentraciones del producto y del microorganismo. El PLA es uno de los bioplásticos más económicos en términos de producción, que también resulta atractivo por tratarse de un material degradable bajo condiciones de compostaje. Por otro lado, la utilización de microorganismos robustos, que pueden tolerar altas concentraciones de ácido como las levaduras, posibilitarían la obtención de altas concentraciones de ácido láctico mediante procesos de fermentación, evitando la generación de efluentes tóxicos como en el caso de la síntesis química. La utilización de glicerol, residuo de la industria del biodiesel, como sustrato, brinda la posibilidad de minimizar la acumulación de este compuesto. Dentro de las estrategias de modelado propuestas en este trabajo, se ha llevado a cabo la expresión heteróloga de una enzima L-lactato deshidrogenasa de Bos Taurus en las cepas de S. cerevisiae BY4741 y BY4742. Actualmente se están llevando a cabo ensayos para realizar la deleción de algunos genes de la ruta de síntesis de etanol (PDC1 y ADH1) y la sobreexpresión de genes del catabolismo de glicerol. Dada la importancia de la composición del medio y de las condiciones de cultivo para favorecer determinada ruta metabólica, se llevará a cabo un diseño experimental que permita hacer un análisis estadístico de los resultados (Diseño factorial con punto central), variando los componentes del medio y sus diferentes niveles de concentración.
ESTUDIO DE LA IMPLICANCIA DEL CONSUMO DE NITRÓGENO EN EL FENOTIPO DE ADAPTACIÓN DE *BRETTANOMYCES BRUXELLENSIS* A ÁCIDO P-CUMÁRICO

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*Brettanomyces bruxellensis* es la principal responsable del deterioro aromático de vinos debido a la producción de derivados fenólicos, generados a partir de ácidos hidroxicinámicos (ácido p-cumárico, ácido ferúlico y ácido cafeico), presentes en el mosto de uva y descritos como agentes antimicrobianos. De esta forma, aquellos microorganismos que crecen en este medio de cultivo deben poseer mecanismos eficientes de adaptación. Estudios previos han demostrado que cepas de *B. bruxellensis* presentan diferencias en el crecimiento en presencia de ácido p-cumárico, sin embargo, se desconocen los mecanismos moleculares que confieren la resistencia a estos compuestos. Los datos aportados por la secuenciación de la cepa de mayor resistencia a ácido p-cumárico (LAMAP2480) mostraron que ésta posee un gran número de genes vinculados con la utilización de fuentes de nitrógeno. Mediante análisis transcriptómicos, se determinó que el ácido p-cumárico induce la expresión de genes relacionados con el flujo de salida de compuestos tóxicos, lo que podría estar relacionado con la disminución de la concentración intracelular de nitrógeno. En base a lo expuesto, este trabajo plantea la siguiente hipótesis: “La resistencia de *B. bruxellensis* a ácido p-cumárico se correlaciona con una mejor absorción de aminoácidos, en particular aquellos de naturaleza aromática”. Para responder a esta hipótesis se evaluará la eficiencia de la tasa de crecimiento entre dos cepas de *B. bruxellensis*, con diferente resistencia a ácido p-cumarico, en presencia de medios de cultivos con alta y baja disponibilidad de aminoácidos, en presencia y ausencia de ácido p-cumárico. Posteriormente, se evaluará la asimilación de aminoácidos, esperando encontrar diferencias en aminoácidos de naturaleza aromática.

LEVADURAS MULTIFUNCIONALES DE ORIGEN ENOLÓGICO: EVALUACIÓN DE SU POTENCIAL PROBIÓTICO Y CARACTERÍSTICAS TECNOLÓGICAS PARA SER EMPLEADAS COMO STARTERS EN PROCESOS FERMENTATIVOS

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Una tendencia actual en la microbiología de los alimentos es el empleo de microorganismos multifuncionales. Un cultivo starter es una preparación de un gran número de células de un microorganismo que puede agregarse a materia prima y producir un alimento fermentado, conduciendo un bioproceso y asegurando la inocuidad y vida útil de los alimentos. Los probióticos son microorganismos vivos y activos que se administran en cantidades adecuadas y confieren beneficios a la salud del huésped. Las levaduras asociadas al proceso de vinificación (*Saccharomyces*, *no-Saccharomyces*) están adaptadas a las condiciones hostiles presentes durante la
fermentación. Por lo tanto, la tolerancia a estas condiciones (con semejanzas a las del sistema gastrointestinal, SGI), sumado a sus similitudes estructurales y funcionales convierte a las levaduras enológicas en potenciales candidatas probióticas. Hipótesis: las levaduras aisladas de ambientes vitivinícolas se consideran multifuncionales dado que poseen aptitudes para ser empleadas como probióticos para humanos, starters de aceitunas de mesa y cerveza tipo Ale. Bajo este contexto el objetivo general es estudiar las propiedades probióticas y aptitudes fermentativas de levaduras enológicas para emplearlas como cultivos multifuncionales en alimentos fermentados. Se utilizarán levaduras autóctonas aisladas de ambientes vitivinícolas (cerapio IBT-UNSJ). Con el fin de emplearlas como probióticos se analizará la supervivencia de las levaduras a las condiciones presentes en el SGI. Posteriormente, se evaluará la adhesión a las células epiteliales del SGI in vitro de aquellas levaduras tolerantes. Las levaduras que presenten aptitudes probióticas se caracterizarán tecnológicamente con el fin de emplearlas como cultivos starters en los procesos fermentativos de aceituna de mesa y de cerveza artesanal tipo Ale. Por último, se evaluará la viabilidad y vitalidad de las levaduras multifuncionales empleadas en procesos fermentativos. Este proyecto prevé una aplicación tecnológica de productos regionales en el sector de la industria de alimentos, encuadrándose en temas prioritarios del sector Bioeconomía.

**USO DE LEVADURAS COMO AGENTES DE BIOCONTROL DE HONGOS AFLATOXICOGÉNICOS EN PISTACHO (PISTACIA VERA)**

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En Argentina existen alrededor de 800ha cultivadas con pistacho, las cuales se distribuyen entre las provincias de San Juan (75%) y La Rioja (25%). El principal destino de los pistachos argentinos es el mercado italiano, seguido por el japonés y el brasileño. Los pistachos pueden contaminarse con aflatoxinas (toxinas cancerígenas) producidas principalmente por los hongos *Aspergillus flavus* y *A. parasiticus*. Existen diferentes tipos de aflatoxinas, la aflatoxina B1 es considerada la más tóxica y es producida por ambos microorganismos. En Argentina, hasta el momento no existen investigaciones sobre la aplicación de levaduras autóctonas inhibidoras de hongos aflatoxicogénicos y/o reductoras de aflatoxinas en pistacho. Conociendo las consecuencias que ocasiona el uso de fungicidas químicos sobre el medio ambiente y la salud humana, como así también las pérdidas económicas que conlleva la presencia de aflatoxinas en alimentos, surge la necesidad de buscar e implementar estrategias de control de hongos aflatoxicogénicos más amigables con el medio ambiente. Las hipótesis del presente trabajo son: Levaduras aisladas de pistacho y de su ambiente de producción, como estrategia de prevención, disminuyen la contaminación con aflatoxinas y/o las poblaciones de *A. flavus* y *A. parasiticus*, en pistachos en condiciones de almacenamiento. Levaduras biosupresoras de *Aspergillus* sp. en uva y aceituna controlan a hongos aflatoxicogénicos en pistacho, en condiciones de almacenamiento. El objetivo general de este trabajo es evaluar el efecto de levaduras autóctonas sobre el crecimiento fúngico de *A. flavus* y *A. parasiticus* y sobre la producción de aflatoxinas. Los objetivos específicos de esta investigación son: aislar, identificar y caracterizar levaduras y hongos aflatoxicogénicos nativos de pistacho. Evaluar el efecto de las levaduras sobre la velocidad de crecimiento, evaluar los mecanismos de acción de las
levaduras como posibles agentes de biocontrol sobre los hongos y determinar el impacto de interacción microbiana sobre la acumulación de aflatoxinas.

PRODUCCIÓN DE BIOETANOL A PARTIR DE EXCEDENTES Y RESIDUOS DE LA ACTIVIDAD VITIVINÍCOLA COMO ESTRATEGIA PARA LA DIVERSIFICACIÓN PRODUCTIVA Y EL AGREGADO DE VALOR

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El bioetanol y el biodiesel son los principales biocombustibles utilizados en todo el mundo. El bioetanol que se produce a partir de azúcares y almidón, se conoce como etanol de “primera generación” (1G). Debido a que la materia prima utilizada compite con la alimentación humana su uso es controvertido. Para superar estas limitaciones aparece el concepto de biocombustible de “segunda generación” (2G) basado en la biomasa lignocelulósica no comestible como son los subproductos agrícolas. Mendoza y San Juan son las principales provincias productoras de uva y vino, representando un importante componente en sus economías productivas. El sector vitivinícola tiene un excedente de jugos de uva de baja calidad enológica y además produce una gran cantidad de residuos que pueden ser utilizados para la obtención de biocombustibles 1G y 2G, respectivamente. El orujo de uva es el principal residuo generado en la vinificación. El presente proyecto propone iniciar las investigaciones desde un enfoque microbiológico para la producción de bioetanol a partir de material de origen vitivinícola, generando una diversificación en el destino de los jugos de uva para la obtención de bioetanol 1G y aprovechando un residuo de la industria vitivinícola (orujo de uva) para la obtención de bioetanol 2G. En una primera etapa se aislaran, caracterizarán y seleccionarán levaduras con elevados rendimientos de alcohol para la obtención de bioetanol 1G y levaduras capaces de metabolizar azúcares C5/C6, resistentes a los furfurales y con capacidad de producir elevadas concentraciones de etanol para la producción de bioetanol 2G. Además, se optimizarán las condiciones de fermentación para obtener el máximo rendimiento en etanol con las levaduras seleccionadas. Los resultados obtenidos permitirán disponer de un paquete biotecnológico para la producción de bioetanol como estrategia para la diversificación productiva y el agregado de valor con elevado potencial para su transferencia.

ESTRATEGIAS SUSTENTABLES DE CONTROL DE BOTRYTIS CINEREA Y PENICILLIUM EXPANSUM EN UVA DE MESA BAJO CONDICIONES DE POSTCOSECHA

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Por sus características morfo-fisiológicas, la uva es sensible al ataque de diversos hongos patógenos, principalmente Botrytis cinerea y Penicillium expansum, aun
durante su conservación en cámara frigorífica. Estos patógenos pueden generar pérdidas significativas en términos de cantidad y calidad de la uva en fresco, afectando económicamente a San Juan, principal productor y exportador del país. El control tradicional de estas enfermedades a través de fungicidas químicos genera un impacto negativo sobre los alimentos, la salud y el ambiente por lo que se pretende -mediante su estudio- el incremento del uso de agentes de biocontrol microbiano, entre ellos ciertas levaduras. Aun así, los beneficios y costos del biocontrol generan incertidumbre en relación a: densidad de patógenos, nivel de reducción de fungicidas e impacto sobre organismos no objetivo. El manejo integrado de enfermedades fúngicas de plantas probablemente sea una manera efectiva de reducir el desarrollo de poblaciones de patógenos. Por lo expuesto se plantea como hipótesis que la producción de compuestos orgánicos volátiles antifúngicos (COVs) y la competencia por espacio y nutrientes son mecanismos de acción de levaduras biocontroladoras, compatibles con el uso de agentes antimicrobianos naturales, para el manejo integrado de enfermedades de hongos fitopatógenos. Los objetivos específicos son: comprobar la resistencia de diferentes cepas de B. cinerea y P. expansum, y levaduras biocontroladoras a agentes antimicrobianos naturales y radiación UV, en función de esclarecer la compatibilidad de hongos y levaduras. Verificar la compatibilidad de los agentes antimicrobianos naturales ensayados y la radiación UV, con los mecanismos de acción putativos de las levaduras biocontroladoras seleccionadas, a 2±1°C. Determinar el perfil de los COVs producidos por las levaduras que resultaron compatibles con los agentes antimicrobianos naturales y la radiación UV ensayados y realizar ensayos en racimos de uva de mesa con un formulado integrado por un agente antimicrobiano natural y una levadura biocontroladora.

ASPECTOS MOLECULARES E BIOQUÍMICOS DA FERMENTAÇÃO COM BRETTANOMYCES APLICADA NA INDÚSTRIA CERVEJEIRA

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O processo fermentativo na indústria cervejeira mudou com o emprego de cepas isoladas de levaduras. A utilização de cepas isoladas permitiu um melhor controle de processo fermentativo, aumentando a qualidade das cervejas. Nesse sentido, as levaduras Saccharomyces cerevisiae e Saccharomyces pastorianus tem sido usadas industrialmente em fermentações cervejeiras de ales e lagers, respectivamente. Entretanto, cervejas de fermentação espontânea, como Lambics e Gueuzes apresentam levaduras e bactérias selvagens distintas. Nesse sentido, um gênero de levadura semidomesticada que é componente crucial na fermentação de Lambics, Brettanomyces (Dekkera) vem ganhando crescente atenção na indústria cervejeira. Brettanomyces apresenta características semelhantes a Saccharomyces como efeito Crabtree positivo, biossíntese de etanol e tolerância a ambientes estressantes. Por outro lado, suas altas atividades de esterase e β-glicosidase, capacidade de formar compostos fenólicos, tetrahidropiridinas, assim como fermentação de dextrinas e metabolização de cellobiose a partir de barris de madeira tem tornado esse gênero atrativo para a indústria cervejeira. Embora Brettanomyces spp. seja reconhecida pela sua importância em alguns estilos cervejeiros, seus atributos moleculares e bioquímicos para a fabricação de cervejas são pouco compreendidos. Dessa forma, esse trabalho tem como objetivo...
abordar o atual conhecimento molecular e bioquímico que perfaz a performance de Brettanomyces na indústria cervejeira.

**EFECTO DEL QUITOSANO SOBRE LA EFICACIA BIOCONTROLADORA DE LA LEVADURA INDIGENA ccCIEFAP1204 FRENTE A PATÓGENOS DE CEREZA**

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En Patagonia, la producción de cerezas es una actividad en crecimiento. Las cerezas se comercializan en fresco y se almacenan en frío para ampliar su vida útil. Las enfermedades causadas por hongos provocan grandes pérdidas y en el país no existen fungicidas registrados para su uso en poscosecha. El uso de levaduras como agentes de control biológico (ACB) y la adición de aditivos, como el quitosano, son alternativas al uso de fungicidas químicos. Los objetivos del trabajo en curso son: i) evaluar el efecto del quitosano sobre el crecimiento del ACB nativo y de los patógenos de cereza; ii) determinar el efecto del quitosano sobre la eficacia antagónica del ACB contra los patógenos en ensayos *in vitro* donde se determinará el crecimiento de la levadura (en unidades formadoras de colonia), el crecimiento y la germinación de los conidios de los patógenos con y sin el agregado de quitosano a 0°C (T° almacenamiento de la fruta) y 22°C (T° comercialización). Los resultados de estos ensayos serán corroborados en ensayos de biocontrol con la levadura antagónica y el quitosano sobre fruta almacenada. Con este trabajo se espera aportar información acerca de un aditivo orgánico que ejerza un efecto sinérgico junto con el ACB y permita desarrollar nuevas estrategias de control biológico para patógenos poscosecha.

**SELECCIÓN E IDENTIFICACIÓN DE LEVADURAS STARTER CON PERFIL CERVECERO PARA EL VALLE DEL CAUCA. COLOMBIA**

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La cerveza es uno de los productos biotecnológicos más consumidos por el ser humano, el cual es obtenido a partir de la fermentación alcohólica de granos maltados mediante levaduras. Otros ingredientes incluyen lúpulo y agua. El estilo de cada cerveza producida va a depender del tipo de malta, lúpulo o levadura empleada. La producción industrial de cervezas, con el objetivo de estandarizar el producto para la venta, emplea cepas adaptadas industrialmente, un solo tipo de malta y un solo tipo de lúpulo, lo que genera un bajo valor agregado al sabor del mismo. En contraparte, la producción artesanal, que viene en crecimiento económico en Latinoamérica con el 1% del mercado, emplea gran diversidad de maltas y lúpulos especiales que abren campo a la oferta de estilos de cerveza diferenciados. No obstante, la levadura empleada continúa siendo la ofrecida a nivel industrial, lo que limita las posibilidades de producción de una cerveza con identidad local. En este sentido, el objetivo de este proyecto es buscar y
seleccionar cepas nativas de levaduras *Saccharomyces cerevisiae* y no *Saccharomyces* que consigan fermentar mosto cervecero y tengan potencial aplicación como starter en producción de cerveza artesanal con identidad local. Para ello, se aislarán levaduras usando un medio de cultivo nutritivo y/o selectivo para especies del género *Saccharomyces* a partir de pulpas de frutas y flores de árboles de la región. Posteriormente, se evaluará la capacidad de las mismas para asimilar y fermentar fuentes de carbono (glucosa, fructosa, sacarosa, maltosa, maltotriosa), se realizarán curvas de crecimiento en maltosa, análisis de producción de sulfuro de hidrógeno, tolerancia a estrés osmótico y etanólico, capacidad de floculación, crecimiento a diferentes temperaturas y producción de toxinas killer. Una o varias cepas con perfiles cerveceros serán seleccionados para posterior evaluación, lo que permitirá presentar alternativas, independencia y autonomía al mercado cervecero.

**OPTIMIZACIÓN Y ESCALADO DE LA PRODUCCIÓN DE BIOMASA DE UNA LEVADURA SELECCIONADA COMO AGENTE DE CONTROL BIOLÓGICO PARA POSTCOSECHA DE PERAS**

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Las peras, al ser un producto perecedero, requieren de tecnologías adecuadas para su conservación que permitan el mantenimiento en el tiempo de sus características organolépticas. No obstante, durante esta conservación la fruta es susceptible a diferentes enfermedades fúngicas. El Control Biológico resulta una de las alternativas más prometedoras para la substitución de fungicidas por las ventajas que presenta en cuanto a sostenibilidad ambiental y producción de fruta orgánica. En trabajos previos se seleccionó a *Vishniacozyma victoriae* NPCC 1263 por su capacidad antagonica para enfermedades postcosecha de pera. Esta levadura fue ensayada a escala comercial, obteniendo resultados muy alentadores. El plan de trabajo de esta Beca tendrá como objetivo optimizar un medio de cultivo a partir de fuentes de nitrógeno y carbono económicas con la intención de reemplazar insumos costosos, como la glucosa y el extracto de levadura. Se realizarán fermentaciones en lote y en lote alimentado (10 L) con la finalidad de evaluar la cantidad y calidad de la biomasa de levadura. Posteriormente, se realizará el escalado en planta piloto (100L) para la producción de biomasa de la levadura. Se evaluarán como metodologías de secado la liofilización y el secado spray para obtener un producto estable y que mantenga la efectividad antagonica. Finalmente, se comprobará la viabilidad y efectividad antagonica, tanto de la biomasa fresca como deshidratada en cada nivel de escalado, en bioensayos a nivel de laboratorio en heridas de pera y en línea de postcosecha en cámaras regionales de peras orgánicas. Estos ensayos permitirán establecer una condición ideal para el desarrollo de biomasa fresca y deshidratada. El objetivo de este proyecto lograr un producto biotecnológico regional para la conservación de fruta orgánica, con el empleo de materias primas y procesos económicos, factores fundamentales considerando los grandes volúmenes involucrados en la producción a escala industrial.
LEVADURAS ÁCIDO-TOLERANTES CAPTADORAS DE METALES: ESTUDIO GENÓMICO-FISIOLÓGICO Y POTENCIAL USO EN BIORREMEDIACIÓN

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Los cuerpos acuáticos ácidos constituyen ambientes extremos caracterizados por poseer bajo pH y, a menudo, altas concentraciones de metales. El incremento en la generación de este tipo de ambientes, debido a la actividad minera y a la erogación de efluentes industriales y cloacales, impone la necesidad de generar planes de contingencia para un saneamiento efectivo. La biorremediación resulta una estrategia económica, eficiente y eco-amigable. Muchas especies de levaduras han mostrado una alta capacidad de remover estos xenobióticos del ambiente. En este trabajo se aborda el estudio de especies aisladas tanto de ambientes extremos antropogénicos (drenajes de minas de Santos Domingos, Portugal), como natural (sistema Río Agrio-Lago Caviahue -RALC-, Argentina). La selección de estas cepas radica en su alta tolerancia a diversas especies metálicas y su supervivencia en ambientes extremadamente ácidos. Se seleccionaron 4 cepas de especies del género Goffeauzyma: G. iberica, G. aciditolerans y G. metallitolerans, aisladas de Portugal, y G. agrionensis, obtenida exclusivamente de agua del RALC; 4 cepas de una especie no descrita que podría constituir el primer registro de una levadura acidófila (dos de Portugal y dos del RALC) y dos especies con distribución ubicua pero alta frecuencia en el RALC (Coniochaeta fodinicola y Rhodotorula mucilaginosa). En este trabajo se obtendrán las secuencias genómicas de estas especies y se caracterizará su capacidad de captación de cobre y zinc, a fin de identificar las principales estrategias que les permiten tolerar estas condiciones. La identificación y comparación de estas adaptaciones entre distintas especies permitirá generar modelos genómico-fisiológicos que contribuyan a elucidar mecanismos de tolerancia al bajo pH y a metales pesados. Asimismo, se evaluará su capacidad de captar metales en pH ácidos y se establecerán los parámetros que permitan evaluar su potencial uso en biorremediación. Esto representa una estrategia clave de cara al diseño de procesos de biorremediación de ambientes antropogénicos, como los drenajes ácidos de minas.

RITMOS ENDÓGENOS EN LEVADURAS DE RELEVANCIA BIOTECNOLÓGICA

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Las levaduras poseen una enorme importancia en investigación básica y aplicada, siendo responsables de generar la mayor cantidad de productos biotecnológicos del mundo en campos relacionados con la alimentación, la salud y el medioambiente. Si bien estos microorganismos son ampliamente utilizados, poseemos un conocimiento limitado acerca de algunos procesos básicos y aparentemente universales, como el funcionamiento del reloj biológico. Los ritmos circadianos permiten a los organismos coordinar sus funciones fisiológicas y su patrón de comportamiento a los ciclos diarios...
de luz/oscuridad y cambios de temperatura asociados. Este fenómeno, que otorgaría ventajas adaptativas en la naturaleza, sería innecesario y hasta contraproducente desde un punto de vista aplicado, en condiciones de laboratorio e industriales. La falta de conocimiento respecto de las bases moleculares de los ritmos circadianos y sobre cómo afecta a productos biotecnológicos, limita en gran medida su manipulación genética para obtener mejoras en los rendimientos. Nuestra hipótesis es que el reloj biológico regula la producción circadiana de metabolitos de importancia biotecnológica. El conocimiento de los mecanismos reguladores podría ser aplicado a la construcción de mutantes independientes del reloj, potencialmente hiper-productores de compuestos de interés. El objetivo general de este proyecto es estudiar las bases moleculares de la regulación circadiana de metabolitos de interés biotecnológico (pululano y micosporinas) en *Aureobasidium pullulans*, una levadura nativa y de importancia industrial, con la perspectiva de generar estrategias que aumenten su producción y, por otro lado, de aplicar este conocimiento al biocontrol de fitopatógenos (*Botrytis cinerea*). Para alcanzarlo, realizaremos análisis bioinformáticos, estudios de expresión génica, generación de mutantes de deleción y evaluación de su efecto en la producción de los metabolitos en estudio. Se analizará el valor adaptativo del reloj molecular mediante experimentos de competencia. La mejora en los procesos biotecnológicos derivados de levaduras a través de la ingeniería genética de sus relojes biológicos es un terreno promisorio, teniendo un gran potencial en aplicaciones industriales.
WELCOME TO THE ISSY34 PROGRAM
CLETUS KURTZMAN´S WORKSHOP ON YEAST TAXONOMY AND SYSTEMATICS: THE IMPACT OF GENOMICS
A PHYLOGENOMIC ROADMAP FOR THE BUDDING YEAST SUBPHYLUM

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The budding yeasts in the subphylum Saccharomycotina (phylum Ascomycota) represent a diverse group that includes the premier eukaryotic model system, Saccharomyces cerevisiae; the common human commensal and opportunistic pathogen, Candida albicans; and over 1,000 other known species, with more continuing to be discovered. Yeasts are found in every biome and continent and are more genetically diverse than either plants or animals. Ease of culture, simple life cycles, and small genomes have made yeasts exceptional model organisms. Since only a tiny fraction of yeast biodiversity and metabolic capabilities has been exploited, expanding the taxonomic breadth of deep genomic investigations and reconstructing budding yeast evolutionary history holds great promise for understanding how genome function evolves to encode their diverse metabolisms and ecologies. As part of National Science Foundation’s Dimensions of Biodiversity program and in collaboration with Chris Todd Hittinger’s lab at the University of Wisconsin-Madison, the late trailblazer of yeast taxonomy Cletus Kurtzman at the USDA, and numerous key collaborators throughout the world, we undertook a large-scale comparative genomic study to uncover the genetic basis of metabolic diversity in the subphylum. In my talk, I will discuss our phylogenomic and molecular clock analyses of 332 genomes spanning the subphylum. Through these analyses, we have: a) established a very robust genus-level phylogeny that divides the subphylum into 12 major clades, at least two and up to eight more than previously recognized, b) identified the branches of the budding yeast phylogeny that remain unresolved and the possible underlying reasons for the observed lack of resolution, c) inferred a robust timetree of budding yeast evolution and the approximate times of divergence of key taxa and lineages. This phylogenomic roadmap provides a robust evolutionary framework for understanding the patterns and processes that sculpted budding yeast evolution from their last common ancestor to the present.

TAXONOMIC STUDIES IN SOME BASIDIOMYCETO YEST LINEAGES

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Next-generation sequencing technologies have unleashed a wealth of genetic and sequence data from yeasts and yeast-like fungi in every conceivable ecosystem. Interpretation of results—especially in relating operational taxonomic units (OTUs) to species—however, can be challenging given the paucity of described species with publically available baseline data. Compounding these taxonomic challenges are newly arisen nomenclatural issues that have risen as a result of recent changes in the International Code of Nomenclature for algae, fungi, and plants. With the change to one scientific name for pleomorphic fungi, separate names typified by sexual and asexual morphs of the same species are no longer permitted. In particular, the early diverging basidiomycete lineages (i.e., Pucciniomycotina and Ustilaginomycotina) comprise numerous species known only from yeast stages that must be both
taxonomically and nomenclaturally integrated within their pleomorphic sisters. This presentation will draw on several case studies from the Ceraceosorales, Microstromatales, and Sporidiobolales and on data from both culture-dependent and culture-independent methods to examine the following: (1) Can we use OTUs to estimate species richness? (2) Are there instances in which the most commonly employed methods may over- or underestimate species richness? (3) Are there any lineages that may not be detectable with current methods? And, (4) What are the best methods for taxonomically integrating members of asexual yeast genera within sexual and/or pleomorphic genera?

GENOME-BASED APPROACH FOR SPECIES DELINEATION IN THE GENUS HANSENIASPORA

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Species of the genus Hanseniaspora are mostly part of fermentative yeast communities on various fruits and with an ecological advantage of rapid growth they can predominate fruit surface microbiota. Since the introduction of DNA sequence analysis for species delineation the number of newly described Hanseniaspora species has increased from seven to eighteen, a similar trend as observed for other yeast genera. Among the newly described species are those which exhibit very small divergences in D1/D2 and ITS regions although protein-coding genes and DNA homology values indicated significant genetic discontinuities between species. With the introduction of whole genome sequencing, the genetically distinct species can be delineated using different algorithms for determining level of similarities between genomes, such as alignment-free distance measure (K_r) or Genome-to-Genome Distance Calculator. In general these analyses give results which are in agreement with DNA-DNA reassociation values. Additionally, from the genomic information the evolutionary adaptation to specific habitats of the novel yeast species was predicted. In the case of two novel Hanseniaspora species isolated from Cytalaria stromata as part of a fermenting microbiota, we found that the SSU1-like gene encoding a sulfite efflux pump, was present only in few closely related species suggesting that this gene might be one factor for diversification of the novel species. We also searched the genomes of the novel species for candidate genes underlying the physiological traits used for traditional identification. As species of Hanseniaspora can assimilate only a limited number of carbon sources, they also mostly lack homologs of the genes required for their utilization.

ONE FUNGUS = ONE YEAST IN BASIDIOMYCETES

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Yeast represents a live stage or live form that has evolved early in fungal evolution and has been retained in several phylogenetic lineages. Under certain conditions,
researchers have observed filamentous fungi or their spores giving rise to cultures proliferating as yeasts by cell budding, for example in molds, parasitic ascomycetes, jelly fungi, and smuts. Even though the ability to produce a yeast stage was often viewed from the perspective of systematics rather than from its functional side, mycologists accumulated substantial numbers of examples of parasitic fungi with predominantly unicellular (or yeast-like) asexual states. Sequencing technologies and molecular identification have made it possible to recognize the link between yeast and parasitic stages in several lineages, thereby changing our understanding of yeast biology in many ways. Subsequent studies showed that many known basidiomycete yeasts are phylogenetically related to parasitic fungi. The practice of using dual generic names for naming sexual and asexual species was common among fungi. In basidiomycetous yeasts, this resulted in rapid growth of polymorphic and polyphyletic asexual genera (e.g., Cryptococcus, Rhodotorula and Tilletiopsis) as counterparts of teleomorphic filamentous fungi. However, parasitic and saprobic lifestyles were not always associated with a reproduction mode, and several asexual parasites (e.g. Christiansenia, Quambalaria) and sexually reproducing yeasts (e.g., Bulleromyces, Papiliotrema, Rhodosporidium) were documented. Also, several parasitic sexual genera (e.g., Tremella, Trimorphomyces, Syzygospora, and Ustilago) were demonstrated to be polyphyletic. The taxonomic complexity in groups comprised by filamentous and yeast-like fungi was growing until the One Fungus = One Name principle was recently adopted by the ICBN. As a result, numerous changes and combinations were proposed to harmonize nomenclature of filamentous and yeast-like basidiomycetes. The current state of the yeast taxonomy, recent developments and future challenges for yeast systematics will be discussed.

EXTREMOPHILIC YEASTS FROM MICROBOTRYOMYCETES: A NEW PSYCHROPHILIC GENUS ISOLATED FROM ICE GLACIERS

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Class Microbotryomycetes (subphylum Pucciniomycotina), harbors more than 200 described yeast species with diversified morphological characters and ecological distribution. Camptobasidiaceae family, was recently considered as ‘incertae sedis’ in Microbotryomycetes, this family includes psychrophilic genera Camptobasidium and Glaciozyma. During different surveys in extreme cold environments from the world psychrophilic strains were isolated belonging to a new species within Camptobasidium (Camptobasidium gelus sp. nov.) and a new genus for which the name Giraudozyma gen. nov. is proposed. The species Giraudozyma psychrophilicus sp. nov. includes isolates from glacial ice from Alaska, Patagonia Argentina and Antarctica. The analysis of a draft genome for this species revealed the presence of genes encoding antifreeze proteins. The discovery of hidden diversity is essential for a better understanding of the phylogeny of Microbotryomycetes.
MOLECULAR TAXONOMY OF YEASTS CAN HELP TO REVEAL ANCIENT HUMAN CONTACTS

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Until very recent times, it has been generally accepted that yeasts species can be widely spread on Earth's ecosystems and its occurrence obeys mostly to geographical and environmental conditions that shape the local or regional yeast biodiversity composition. The occurrence of psychrophilic yeast species common to gelid habitats such as Antarctica and the Alps can be explained in such a manner. Nevertheless, we have found molecular evidence that other species would be incidentally spread by humans in ancient times. Here we report three yeast species found in Ecuador mainland, the Galápagos Islands, Costa Rica, Malaysia, Philippines, and Australia. We were able to link yeast occurrence in distant regions of the planet, through molecular evidence of the transpacific human-mediated dispersion of at least one yeast species (i.e. Kodamaea transpacifica); also, the potential historical link of two yeast species Candida theae and Saccharomyces fodiens. All the above mentioned yeast species have been described in recent years and the isolates are preserved at the Catholic University Yeasts Collection in Quito. The yeast biodiversity studies in mega diverse Countries such as Ecuador are contributing with new elements for a better understanding of yeasts by revealing fascinating links between yeast diversity, human history and molecular taxonomy as a tool for understanding obscure aspects of yeast biodiversity on Earth as well as a better comprehension of unwritten human history.

GENOME-BASED APPROACH FOR GENUS DELINEATION IN THE TRICHOSPORONACEAE

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DNA-based species identification has now been widely used, allowing an accurate assessment of species, and as a consequence, giving us insights into species richness and evenness in various habitats. For better understanding of ecosystems, a study of the richness at the genus or higher level is also important. A recent progress of genome analyses suggests the merit of use of genome data for this purpose. Genome data are useful to construct a highly reliable molecular phylogenetic tree and to identify phenotypic information that delineates one cluster from the others. In this study, we performed the genome-based approach for genus delineation in the Trichosporonaceae. We used 22 haploid and three “natural” hybrid genomes in Trichosporonales for the analyses with three Tremellales genomes as outgroups. Based on the cluster analyses of orthologous genes, we found 24 and 285 genus-specific genes for 8 Apiotrichum species (A. brassicae, A. domesticum, A. gamsii, A. gracile, A. laibachii, A. montevideense, A. porosum and A. veenhuisii) and 5 Trichosporon species (T. asahii, T.
coremiiforme, T. faecale, T. inkin and T. ovoides), respectively. Selected genes are not found in other species, indicating that they can be used for delineation between these genera. In the Cutaneotrichosporon, only one gene was found as common among 8 species, so further analyses should be required. Recent taxonomic revisions for accommodating 1F=1N have also caused taxonomic problems; for example, the genus that includes both teleomorphic and anamorphic taxa became morphologically heterogeneous. Genera divided and proposed solely based on phylogeny in one anamorphic genus are difficult to distinguish from each other. Nevertheless, genome data will be powerful to find and identify the trait that characterizes respective genera.

THE GENUS CANDIDA IN ITS PHYLOGENETIC CONTEXT

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The name *Candida* was applied to a multitude of unrelated asexual ascomycetous yeasts due to the morphological basis of the former fungal genus concept and the resulting dual naming system for sexual and asexual forms until the Melbourne Code, 2012. The numerous multigene phylogenies by Clete Kurtzman and authors inspired by his studies resulted in a yeast classification that increasingly reflects lines of decent, although non-symmetrical natural processes sometimes defy the application of monophyly as an absolute criterion of taxon delimitation. Reclassification of the majority of *Candida* species is pending to restrict the genus to species that are more closely related to the type species *Candida tropicalis* than to other type species. Such reclassification should not exclude the possibility of accepting potential paraphyly caused for example by *Nematodospora* and *Lodderomyces*, distinguished by their unique ascospore morphology. The clinically important species *Candida albicans* should remain part of this genus to avoid the break-down of the *Candida tropicalis*-clade into many small and barely distinguishable genera. Currently available data are not conclusive and the hypothetical character of phylogenetic reconstruction necessitates validation, e.g. from whole genome analyses. The assignment of *Candida* species to other genera is progressing, the accumulation of new species in this genus has slowed down, and ascomycetous yeast species without known sexual reproduction are now classified in a more informative system: 1) as *Candida* for species belonging to the *Candida tropicalis*-clade, 2) as *formae asexuales* in genera typified with sexually reproducing species, 3) in genera without known ascosporic state, or, 4) exceptionally, in *Candida pro tempore* if substantial phylogenetic study does not allow a better assignment.
BIRTH-AND-DEATH EVOLUTION AND RETICULATION MAKE rDNA UNSUITABLE FOR BARCODING IN PULCHERRIMIN-PRODUCING METSCHNIKOWIA SPECIES

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The nuclear ribosomal DNA units consisting of three rRNA genes and transcribed spacers form long tandem arrays of repeats and are highly homogenised in yeasts, owing to their concerted evolution. The sequence homogeneity makes their D1/D2 domains and ITS1-ITS2 spacers suitable for barcoding (taxonomic differentiation and identification) of species. The notion that the nuclear rDNA units/repeats evolve in a concerted manner has in effect become dogma. However, we found that the rDNA repeats of the pulcherrimin-producing Metschnikowia species evolve in a different way, according to the birth-and-death model, known in certain groups of protists, flatworms and plants but hitherto undescribed in yeasts. The birth-and-death mechanism has no homogenisation effect and hence the D1/D2 and ITS segments of these species are highly diverse in sequence and cannot be used for barcoding. We detected intragenomic sequence divergence higher than 10 % in these segments of the type strains of the species. This intragenomic diversity is nearly an order of magnitude higher than the taxonomic threshold proposed for species discrimination on the basis of the sequence analysis of the yeast strains of the CBS collection and even exceeds the threshold proposed for the discrimination of genera. In contrasts to the organisation of the rDNA repeats of other yeasts in continuous arrays, here the repeats are dispersed throughout the genomes, many of them have truncated non-functional structures (pseudogenes) and their transcripts show high structural diversity. These features are characteristic of multigene families evolving under a birth-and-death process. The phylogenetic and network analyses of the sequences indicate that intra- and interspecies reticulation are also involved in the evolution of the rDNA units. As the biological isolation of the species is incomplete, their rDNA units can interact (reticulate) during hybridisation and the segregation of the hybrid genomes.

SPENCERMARTINSIELLA NICOLII SP. NOV., A YEAST SPECIES ISOLATED FROM ROTTING WOOD IN BRAZIL

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Yeasts may be found in nature associated with rotting wood. This substrate consists of cellulose and hemicellulose, being a source of fermentable sugars for ascomycetous yeasts. The genus Spencermartinsiella belongs to the family Trichomonascaceae, and it accommodates three species isolated from rotting wood: Spencermartinsiella europaea, Spencermartinsiella ligniputridi, and Spencermartinsiella silvicola. Four strains representing a new yeast species, Spencermartinsiella nicolii sp. nov., were isolated from rotting wood samples collected at four sites in Atlantic Rain Forest, Amazonian Forest, and Cerrado ecosystems in Brazil. The sequence of D1/D2 domain of the large...
subunit rRNA gene of the new species is identical (100% identity) to the sequence of the strain *Spencermartinsiella* sp. isolated from infected tissues of crocodile, as reported by Carvalho et al. 2017. Morphological and molecular findings clearly demonstrated that the reptile developed systemic infection by this yeast. The crocodile was probably infected by the microorganism through contact with rotting plant materials. Sequence analysis of D1/D2 domain and ITS-5.8S region showed that this novel yeast is most closely to the type strain of *Candida cellulosicola*. This species differed by thirty-seven substitutions and thirty-one indels in the sequences of the ITS region from the novel species. The sequence analysis of the mitochondrial small-subunit rRNA (mtSSU rRNA) gene revealed that this yeast is related to the species of *Spencermartinsiella europaea*, that differed from the new species by seventy-six substitutions and twenty-five indels. mtSSU rRNA sequence of *Candida cellulosicola* is not available in the databases for comparison with the new species. The novel species is heterothallic and the sporulation was observed on 2% malt extract agar at 25ºC after three weeks. The strains produced asci with hemispherical ascospore. *Spencermartinsiella nicolii* sp. nov. is the first species of the genus related to causing infection, indicating that this species may be an opportunistic pathogen.

**ASK YOUR DATABASE WHERE A YEAST LIVES**

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Our understanding of yeast biodiversity and species concepts heavily relies on specimens preserved in herbaria and culture collections. Collection catalogues provide information about genetic, biochemical and physiological properties, as well as their origin, such as sampling locality and habitat description. Persons, who isolated a microorganism, usually deposit it with the associated metadata that include information about country and substrate of isolation. Description of the substrate of isolation does not follow any standardized format and varies in terms of complexity of the provided information, wording, spelling and heterogeneity of recorded environmental data. A substantial progress has been made to collect and unify the information in public databases such as GenBank, BacDive, MycoBank and StrainInfo. However, little effort has been made to present information of strain origin in a systematic way. Here we report a novel approach to classify and analyze data of the source of isolation from a collection of 700 yeast strains. We allowed a record to be described with several keywords, analogous to hashtags used in social media. Each keyword was characterized within an own hierarchical ontology-like structure. A total of 480 records were manually curated and 155 were modified using information from either publications or other collections. A total of 432 strains were associated with 1-6 (out of 85) keywords that were used to classify sources of isolation into 7 categories and 40 subcategories: a combination of at least 2, and 3, and 4 keywords were used to describe the origin of 75%, 50%, and 25% of strains, respectively. Using the advantage of the ontology-like structure, we applied a network analysis linking strains of similar origin on two hierarchical levels. With these results, we provide examples on how advanced classification of substrates can improve the use of data from culture collections for research in microbial ecology, medicine and biotechnology.
THE YEASTS, A COMMUNITY-BASED, ONLINE AND UPDATABLE PLATFORM

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Yeast diversity is rapidly expanding due to discovery of new species and new insight in their phylogenetic relationships. The Yeasts aims to present taxonomic and functional information on yeast species in an updatable and on-line, open access electronic format. Public information on authenticated yeast strains from public culture collections and other sources will be made available in a species-based electronic information platform. This species descriptions will contain information on morphology, nutritional physiology, biochemical and molecular features, reference barcodes and genomes, MALDI-TOF MS spectra, origin of strains, ecology, use in agriculture, food industry and biotechnology and clinical importance. We also aim to compile information on resistance-related genes. Protocols for the isolation and identification of yeast isolates will be provided. Moreover, introductory chapters will be expanded to cover aspects of e.g. food fermentation and spoilage, biotechnological applications, medical aspects, etc. The Yeasts will provide a highly useful resource for all those interested and working with yeasts, both from applied and fundamental points of view. During the workshop, we will discuss the current developments of The Yeasts aiming at getting the species descriptions available on-line as soon as is possible.

THE FATE OF THE ARTIFICIAL GENUS CANDIDA FROM A CLINICAL PERSPECTIVE

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In the last edition of The Yeasts the form genus Candida comprised 314 species that belong to almost all clades of Saccharomycotina (Lachance et al. 2011), and this increased > 400 species. Clinically important species also belong to various clades. For instance, C. albicans, C. dubliniensis, C. tropicalis and the C. parapsilosis complex belong to the Lodderomyces clade; the emerging C. auris and C. haemulonii belongs to the Metschnikowia clade; and C. glabrata belongs to the Nakaseomycoses clade. Importantly, susceptibility to antifungal drugs of these pathogens to a large extent correlate with this phylogeny supporting proposals to restructure the taxonomy of this artificial genus. This correlation also has consequences for diagnostics and treatment. Therefore, we have designed a qPCR strategy that focus on both taxonomic identity and susceptibility patterns of members of the C. haemulonii/auris clade to immediately reach a decision on treatment. One such group is C. auris and relatives and a qPCR has been developed to detect this species in serum. We feel it highly urgent to reach a
consensus on the taxonomy of Saccharomycotina yeasts in the near future and we expect that various kinds of data will contribute to this
KEYNOTE LECTURES
STUDYING THE YEASTS OF YESTERDAY TO GENERATE THE YEASTS OF TOMORROW

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Keynote Speaker Lecture

The common brewer's yeast *Saccharomyces cerevisiae* is used in a broad range of industrial applications, from the production of beer, wine and bread to biofuels and pharmaceuticals. Interestingly, there are hundreds of different industrial yeast strains, but their origins and specific characteristics are largely unknown. We combined large-scale phenotyping with genome sequencing to track the genealogy and evolution of today’s industrial yeasts. Using this knowledge allowed us to set up large-scale breeding programs to generate superior variants that increase production efficiency and expand the range of yeast-derived products and aroma’s, allowing more efficient beer fermentation, production of superior beers and the creation of novel products.
Cocoa research performed during the last century has elucidated the basic physiology and ecology of cocoa fermentation and the biochemical changes that occur during cocoa fermentation, drying and roasting that lead to the development of the chocolate flavour. Biotechnological manipulation of the steps of microbial fermentation (microorganisms, amount of pulp, selected strains) can result in understandable and reasonably predictable effects on chocolate quality. Many different species of microorganisms have been isolated from cocoa fermentation and have been characterised and the microbial succession has been defined. Yeast are essential to the fermentation process and development of chocolate flavour. The concept of using starter cultures to conduct cocoa bean fermentations is not new. Initially, around 1960-1980, the aim was to induce a faster, more consistent fermentation, without adverse impact on chocolate quality. More specific investigations on the use of starter cultures have now been conducted where the main goals have been to develop a faster, more consistent fermentation process that yields cocoa beans with predictable qualities. The dynamic of *Saccharomyces cerevisiae*, *Pichia kluyveri* and *Hanseniaspora uvarum* during spontaneous and inoculated cocoa fermentations and their effect on sensory characteristics of chocolate were investigated. Yeast populations were assessed by qPCR. *S. cerevisiae* was predominant during spontaneous (average 5.4 log cell/g) and inoculated (average 7.2 log cell/g) fermentations. The *H. uvarum* seemed to be suppressed by the other two yeasts, as it showed similar population (approximately 4.0 log cell/g) even in the inoculated assay. Carbohydrates were consumed quickly at inoculated fermentation (68% and 42% were consumed in the inoculated and control assays respectively, at 24 h). Ethanol content was higher in the inoculated (8.3 g/kg at 48 h) than in the control (4.6 g/kg at 96 h) fermentation. Chocolate produced from the spontaneous fermentative process presented dominance of the bitter flavour, while obtained through inoculated fermentation process presented bitter, astringent, coffee and acid as dominant flavours. The inoculation accelerated the fermentative process in 48 h. The inoculation of yeast influenced the microbial profile, which affected the volatile compounds that affect sensory characteristics, resulting in chocolate with dominant bitter, cocoa, and fruity attributes.
OPTOGENETIC CONTROL OF GENE EXPRESSION: PUTTING SOME LOV AND RED-LIGHT ACTION INTO YEAST BIOTECHNOLOGY

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Keynote Speaker Lecture

Light is a strong environmental cue. Learning how to harness it, as a means to control gene expression, opens the door to new strategies to reprogram cell function. Thus, we have adopted different optogenetics strategies to utilize light as an orthogonal signal to control transcription in yeast, as Saccharomyces cerevisiae is an organism naturally incapable of seeing light. Therefore, we have implemented in yeast an optogenetic system based on the molecular interaction of two blue-light photoreceptors from the filamentous fungus Neurospora crassa, WC-1 and VVD. This synthetic optogenetic switch, which we called FUN-LOV, can directly drive transcription of a gene of interest, providing low background levels in the dark, and over 1300-fold of induction upon blue-light, as measured with a luciferase reporter. We tested the efficiency of FUN-LOV for heterologous protein expression, obtaining higher levels of a tagged protein, when compared to a classical galactose induction system. Additionally, we have utilized FUN-LOV to control flocculation with the click of a light. Depending of the target gene controlled by FUN-LOV, Flocculation in Light (FIL) or Flocculation in Darkness (FID) were achieved. In addition, we have also implemented a red-light toggle switch, which activates transcription upon red-light, and cease such activity when far-red light is shined. This two-positions switch also shows low background in the dark, and fast ON/OFF dynamics. We are presently trying to combine both systems, in order build complex logic gates, combining red and blue light switches, in order to efficiently manipulate metabolic pathways. Current efforts, are also focused on the implementation of these optogenetic switches under bioreactor conditions, in a way that they can be easily utilized under industrial settings. Funding: MI-iBIO, FONDECYT 1171151, HHMI International Research Scholar Research Program.

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SESSION 1: ECOLOGY AND BIODIVERSITY OF YEASTS
Species of the genus *Metschnikowia* share the intriguing property of producing asci with only two ascospores, never more. These are genuine meiotic products. The spores are assembled in parallel with the first meiotic division, but the timing and localization of meiosis II has remained a mystery. An account of our progress towards determining the exact time course will be given. This involves labelling nuclei of complementary strains with different fluorescent proteins in species for which a genetic toolkit is not yet available. Many *Metschnikowia* species share a haplontic and heterothallic sexual cycle also found in the sister genus *Clavispora*, suggesting that this represents the ancestral condition, whereas later-emerging clades are mostly diplontic. The haplontic condition has made it easy to examine mating and ascus formation as indicators of species boundaries. Abundant mating takes place between closely related species, but fertile ascus only arise in conspecific matings. We have mined draft genomes to improve our understanding of genes involved in mating type determination and cell-cell recognition within and across many species, including those assigned to the floricolous beetle-associated large-spored group. The mating type locus and its flanking genes are conserved across the family Metschnikowiaceae and bear some similarities with those of the neighbouring Debaryomycetaceae. Genes responsible for the synthesis of mating pheromones vary across species in number of loci and number of coding regions per locus. Pheromone sequences follow other genes phylogenetically and their similarity or divergence among species made it possible to predict mating compatibility in some, but not all cases. It is clear that speciation in haplontic *Metschnikowia* species begins with genetic divergence between isolated populations and is only accompanied by prezygotic isolation much later in evolutionary time.
DIFFERENT INDIGENOUS YEAST POPULATIONS IN SPONTANEOUSLY FERMENTING MUSTS FROM V. VINIFERA L. AND V. LABRUSCA L. GRAPES HARVESTED IN A SHARED TERROIR

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Yeast communities associated with Vitis vinifera L. ecosystems (i.e., grapes and fermenting grape musts) have been widely characterized. Less is known, however, about yeast communities present in Vitis non-vinifera ecosystems. Moreover, there are no comparative studies concerning yeast communities in grapes from V. vinifera L. and non-vinifera Vitis species in vineyards from a shared terroir. In our work, we have used culture-dependent strategies, phenotypic analyses, and molecular genotyping, to study the most representative yeast species and strains present in spontaneously fermenting musts of grapes harvested from neighbouring V. vinifera L. (cv. Malbec) and V. labrusca L. (cv. Isabella) vineyards. Analyses of a small number of isolates from early stages of fermentation showed that Hanseniaspora uvarum was the predominant non-Saccharomyces species in both Malbec and Isabella ecosystems. Hanseniaspora vineae, Metschnikowia pulcherrima and Torulaspora delbrueckii, yeast species commonly found in V. vinifera L. grape musts, were isolated only from the Malbec ecosystem. Candida californica, on the other hand, was only isolated from the Isabella ecosystem. Phenotypic analyses of four randomly selected H. uvarum, Starmerella bacillaris and Sacharomyces cerevisiae isolates, as well as microsatellite genotyping of S. cerevisiae isolates from each Malbec and Isabella grape musts, suggest that V. vinifera L. and V. labrusca L. ecosystems could potentially harbour yeast strain populations that are specific to each Vitis species. It is tempting to speculate that specific as yet unknown characteristics of different Vitis species could underlie the assembly of communities of associated yeast species and strains from a given species. We propose that non-conventional Vitis ecosystems offer opportunities to look for unique yeast strains of potential relevance for the winemaking industry.

2,629 CACTOPHILIC YEAST STRAINS AND ASSOCIATED DATA ACQUIRED BY THE PHAFF YEAST CULTURE COLLECTION

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Two collections totalling 2,629 cactophilic yeast strains gathered by recently retired professors William T. Starmer and Phillip Ganter were transferred to the Phaff Yeast Culture Collection (UCDFST) at the University of California Davis, which already contained over 2,000 cactophilic yeasts. Mentored by Herman Phaff, these two researchers each amassed sizeable research collections of yeasts while studying evolutionary ecology, community ecology, biogeography, host-vector-yeast relationships and systematics. The yeasts had been isolated between 1976 and 2002, primarily from 17 genera of cacti in north, south and central America, and eastern
Australia. Yeasts were revived, cryopreserved, and identified by ribosomal sequencing. Yeasts belong to 81 known species plus at least 37 potentially novel species. Source information and phenotypic data include geographic origin, plant or insect host, assimilation of 40+ carbon compounds, assimilation of nitrogen compounds, and tolerance of stress conditions such as temperature extremes, high sugar, high salt, triterpene glycosides, and cycloheximide. The set includes large numbers of strains of the same species from different host organisms and locations, such as 300 strains each of Candida sonorensis and Pichia cactophila, and over 150 strains of Clavispora opuntiae. Data mining opportunities include microbial ecology, systematics, biogeography, and comparative, functional, and population genomics. It is also possible to compare naturally co-occurring strains of multiple species. As an exploratory taxonomic study, yeasts in the Sporopachydermia cereana complex were examined. Additional strains were identified for four of the ten genetically distinct taxa in the S. cereana complex proposed by M.-A. Lachance et al. in 2001. Furthermore, several additional taxa emerged, with between one and 17 strains per taxon. Additional clusters of new species with multiple strains are related to Starmera amethionina, Candida inconspicua, Pichia barkeri, Clavispora opuntiae, and Phaffomyces opuntiae. These strains are being added to the Phaff collection public catalog (http://phaffcollection.ucdavis.edu), and are available for research use.

YEASTS FROM THE EXTREME (DEEP) SEA ENVIRONMENT

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In 2011 a collection of more than 700 yeast isolates was send to the CBS yeast collection of the Westerdijk Institute by Jack Fell. These isolates were collected in the 1960s from different marine areas that range from regions in the Tasmanian Sea, Caribbean Ocean, Indian Ocean, sea areas around Florida (US) and the cold Southern ocean near the Antarctic. The water depth ranged from the water surface to 5 km. Most of these isolates were initially identified using physiological characteristics. After inclusion in the CBS collection, sequences of the ITS and D1/D2 domain of the LSU were generated for these isolates. These sequences were compared to sequences available from GenBank and to validated barcode sequences of more than 9 000 yeast isolates from the publically available CBS collection. As the CBS collection contains almost all ex-type isolates of currently recognized yeast species, it is a valuable reference dataset to use for species identification and it was successfully used to identify isolates of the deep sea collection. Approximately 80% of the isolates could be linked to an existing species of which 37% of the isolates were initially correctly identified based on the physiological profiles obtained. More than 100 different species were recognized with several potential novel species. Many of these strains/species can be tied to specific ocean currents and water masses, which may provide information on their origin and habitats. In addition to the isolates representing potential novel species, many of the isolates represented species of which only a few isolates or even only a single ex-type strain was available in the collection. Therefore, it is a clear indication that such valuable collections, such as this deep sea yeast collection, are worth saving for the future generations to study, using the up-to-date techniques available.
**MALASSEZIA FURFUR BIODIVERSITY**

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Malassezia spp. are basidiomycetous yeasts occurring on human and animal skin as commensals but under certain circumstances can also cause a number of skin disorders such as dandruff/seborrheic dermatitis (D/SD), pityriasis versicolor (PV), atopic dermatitis (AD), Malassezia folliculitis (MF), and psoriasis. They are the most abundant fungal component of the human skin microbiome but also have been encountered in other parts of the human body such as in the oral cavity and nasal vestibule. A limited number of species has been described to cause bloodstream infections in immunocompromised people and neonates receiving lipid parenteral nutrition. The most recently described species was isolated from bats and can grow at a much lower temperature than the other described Malassezia species. A number of recently published culture-independent studies have suggested that Malassezia may occupy a much more diverse ecological niche than previously believed, as Malassezia sequences have been found in terrestrial and marine ecosystems such as deep-sea sediments, (Antarctic) soils, corals, sponges, cone snails and nematodes. Here we zoom in on Malassezia furfur, a genetically heterogenic species complex with a diverse host background and report on its genetic diversity, host-genotype relationships and biology. A collection of 250 M. furfur strains from various hosts were characterized with Pulsed Field Gel Electrophoresis (PFGE), and Amplified Fragment Length Polymorphism (AFLP), Multi Locus Sequence Typing (MLST), Mating type PCR, Matrix Assisted Laser Desorption/Ionization Time-Of-Flight Mass Spectrometry (MALDI-TOF-MS), cell measurements and physiological data. Some strains were analyzed using Whole Genome Sequencing (WGS). We identify a number of genotypes in M. furfur, with various levels of host-specificity. Based on our data, we describe one of the genotypes as a new species. Various biologically relevant genetic and genomic mechanisms are identified, including a hybridization event.

**HYBRIDIZATION ENHANCES NATURAL YEAST DIVERSITY**

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Genome recombination by hybridization plays a major role in the domestication of yeasts, by promoting the emergence of new genotype combinations from pre-existing diversity in natural and anthropic reservoirs. Using population genomics, experimental crosses and fitness assays in Saccharomyces paradoxus inhabiting the North American temperate forests, we addressed the contribution of hybridization in yeast diversity in a completely natural context. We found (1) reproductive isolation between hybrid and parental lineages, (2) evidence for hybridization in the nuclear and mitochondrial genomes, and (3) a link between hybridization and reproductive isolation. In addition, we found that the hybrid lineage displays specific growth phenotypes that potentially reflect a new ecological niche. Our results show that hybridization contributes as well to diversity in natural yeast populations.
ENDOPHYTIC YEASTS ASSOCIATED WITH VITIS VINIFERA TANNAT GRAPE OF URUGUAY

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Endophytic microorganisms coexist and co-evolve with their host plant, without producing symptoms of disease and even sharing the same metabolic pathways. Endophytic bacteria and filamentous fungi are being intensively explored, while endophytic yeasts have received very little attention. Endophytic yeasts capable of synthesizing plant hormones, antimicrobial compounds, food aromas, and compounds of interest for the production of biofuels have been reported. In particular in Vitis the presence of endophytic filamentous fungi that could act as biological control agents and as producers of resveratrol has been described, but no endophytic yeasts have been reported in grapes so far. This work considers the isolation and identification of cultivable endophytic yeasts present in Tannat grapes, the emblematic variety of Uruguay. Cultivable epiphytic and endophytic yeasts were isolated and identified. Results were consistent in terms of the species that were found exclusively as endophytic. We conclude that from the total yeast flora associated with Tannat grapes, Aureobasidium, Rhodotorula, Debaryomyces, Cryptococcus and Rhodosporidium species were exclusively endophytes when mature berries were sample. Interestingly, during the early stages of berry set and growth Aureobasidium strains were detected within the epiphytic yeast flora. We further studied through molecular methods the presence of these species in skin and seed transcriptome data during Tannat berry development. Results are discussed to understand if there is a precise mechanism during grape berry growth in which some yeasts become part of the endophyte flora. The endophyte yeasts of grapevine constitute an unexplored niche of great biotechnological potential.

WINE YEAST BIODIVERSITY RESERVOIRS IN THE VINEYARD: WHERE DOES SACCHAROMYCES LIVE IN?

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It is generally accepted that the vineyard is the natural habitat of wine yeasts. However, questions such as where and how wine yeasts persist year by year in this ecosystem, sometimes hostile and highly changing, are not completely clarified. The objective of this survey was to study Saccharomyces cerevisiae populations in different vineyard niches during a biological cycle of the vine, evaluating the contribution of these niches as reservoirs of yeasts populations that are finally present in ripen grapes. Ten different plots of a cv. Malbec vineyard located in the Zona Alta del Río Mendoza (Argentina) were selected and samples were collected in 5 stages of a complete phenological cycle (from one harvest to the following one). Samples of berries, bark, buds, soil and
irrigation water were collected. The grapes were aseptically crushed whereas the other samples were introduced into sterile grape must (24° Brix, pH 3.5). All the musts were incubated at 25°C until 75% of the sugars were consumed and *S. cerevisiae* colonies were isolated and molecularly typed by interdelta-PCR. Similarity’s coefficients were estimated and dendrograms were performed to understand strain clustering and their molecular relationships using UPGMA. Results showed that each stage had a different number and diversity of *S. cerevisiae* strains. The grapes in both harvests showed a high number and wide distribution of strains across the vineyard’s regions, confirming that this behaviour is only present in this stage. Along the whole cycle, the soils showed low diversity suggesting their scarce contribution as reservoirs of these yeasts. Conversely, vine buds in winter as well as barks in sprouting to harvest showed to be good reservoirs, since a wide variety/quantity of yeast were isolated from those samples. A dynamic change of *S. cerevisiae* population was observed in the evaluated cycle, clustering according to their different isolation niches.

**CELLARS AND VINEYARDS SACCHAROMYCES CEREVISIAE POPULATIONS ARE CONNECTED BY ASSYMETRIC BIDIRECTIONAL GENE FLOW**

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Abstract ID: 137

Vineyards and wineries are ecological habitats that house a diverse community of yeast and bacteria. Grapes and cellars are two sources of *S. cerevisiae* strains involved in the winemaking process, but the relationship between both *S. cerevisiae* populations is still poorly understood. Indeed strains in vineyards samples are rarely the same as those isolated from vats. In order to better apprehend this issue, 1374 *S. cerevisiae* isolates were collected from 193 samples of Merlot grapes obtained across 5 Bordeaux regions and from 11 spontaneously fermenting must of 7 cellars. We obtained 402 different genotypes using 17 microsatellite markers. A first analysis of genotypes indicated that approximately ¼ of isolates presented more than 75% of similarity with commercial yeast starters, suggesting that they escaped from the cellar environment, but presented variations higher that could be detected from the analysis of their industrial batch production. The resulting *S. cerevisiae* populations of 302 grapes-associated and 225 cellar-associated unique profiles revealed a global low differentiation (Fst=0.036), but with differences from sites to sites. To limit potential geographic or sampling effects, a subset of 72 individuals were selected among the vineyard and cellar isolates, representing 5 cellars and the vines located in their immediate environment. Again a low differentiation was noticed (Fst= 0.03±0.001). The geneflow between vines and cellars was inferred, using a likelihood approach implemented in the software MIGRATE. Inferences showed that cellar and grape metapopulations present similar theoretical sizes and are connected by asymmetric geneflow: almost 4 times higher in the direction ‘grapes-to-cellar’ than for ‘cellar-to-grapes’ (number of migrants per generation 191 [166 -226] versus 55 [25 -83], respectively). This reveals that vines and cellars are two compartments of the same ecosystem, which has deep ecological significance.
THE ANCESTRAL GRAPEVINE VITIS VINIFERA SSP. SYLVESTRIS OF THE MEDITERRANEAN BASIN AS A SOURCE OF WINE YEASTS

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The wild vine Vitis vinifera L. ssp. sylvestris (Gmelin) Hegi is considered as the parental generation of today’s cultivars. Urbanization, industrialization of territories, climatic change and the arrival in Europe of pathogens and pests have led it to be included on the IUCN Red List of Threatened Species in 1997. Although numerous studies about the current health status of this species have been carried out, at best of our knowledge, the yeast population of the grape-berry surface has not been investigated yet. The present work focused on the study of yeast occurrence and diversity on grape samples collected from wild vines. The sampling plan was carried out in Azerbaijan, Georgia, Italy, Romania and Spain. Fifty species, including Saccharomyces cerevisiae were recognized by 26S rDNA D1/D2 domain and ITS region sequencing. In all, 163 different genotypes were obtained by SSR-PCR analysis. Final outputs allowed both to obtain a more precise information about yeast communities and to provide an objective framework for the classification of the broadest range of species according to their extinction risk. In conclusion, this study highlights that the biodiversity potential of pristine environments represents a fascinating source of attractive yeast strains that, for their biotechnological potential, could offer new opportunities to face common problems in winemaking.

INTEGRATED APPLICATION OF CHEMICAL AND PHYSICAL TECHNIQUES TO REDUCE THE NATIVE MICROBIOTA OF GRAPE MUST

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A wide qualitative and quantitative variability of yeast species are present at the beginning of winemaking. Metabolic activity of them can both impact on the sensorial quality of wine, and affect the implantation of yeast starters. The aims of this work were to analyze, select and optimize the application of chemical and physical techniques in order to reduce the initial native microbiota. Grape must (24°Bx, pH 3.6) was inoculated with a microbial consortium (Hanseniaspora uvarum (60%), Candida zemplinina (15%), Metschnikowia pulcherrima (5%), Pichia occidentalis (5%), Brettanomyces bruxellensis (5%), Zygosaccharomyces rouxii (5%), Saccharomyces cerevisiae (5%)) at 1x10⁶cell/mL initial total population in order to mimic the actual conditions of the winemaking beginning. Simultaneous application of chemical techniques (Chitosan, sulfur anhydride
(SO\textsubscript{2}) and sodium benzoate) and physical techniques (exposure to: UV light, ultrasonic and short periods of high temperatures (2 min, 60\degree C) alternating with low temperatures (2 min, 20\degree C) thrice, thermic shock (TS)) was analyzed by Placket-Burman experimental design (99.5\% confidence level, t value: 1.943). According to P-B design, techniques that significantly affect the reduction of initial microflora were chitosan and TS application. These techniques were selected to be optimized by response surface methodology using Box-Behnken experimental design. Also, we include SO\textsubscript{2} application to the experimental design (at low doses because it is the antiseptic commonly use in winemaking and it has other beneficial properties such as antioxidant power). The polynomial model gave a non-significant lack of fit (0.05) and the high-adjusted coefficient of determination ($R^2$: 94.26\%). In this context, the model is appropriate to describe the effect of the evaluated factors on reduction of microbiota in grape must. The maximum reduction of microbiota is achieved when applied 1.39mg/L of chitosan, 19.05mg/L of SO\textsubscript{2} and 2 min 60\degree C/ 2 min 20\degree C thrice at the beginning of the fermentation. Integrated management combining physical and chemical techniques is a possible strategy to mitigate the native microbiota of the grape must fermentation.

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**FUNGAL COMMUNITIES IN SOUTH AFRICAN CHENIN BLANC AND CABERNET SAUVIGNON GRAPE MUSTS**

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Microbial community structures associated with wine grapes and fermentation play a critical role in grape and wine quality. Furthermore, they are important determinants of wine aroma and wine typicity. Recent studies suggest that the grape and wine microbiota exhibits regionally defined patterns linked to the vineyard of origin as well as climatic conditions. In this study, Illumina Miseq amplicon sequencing was performed to investigate the fungal diversity in Chenin blanc and Cabernet Sauvignon grape musts derived from different areas within the Stellenbosch region of South Africa. The grapes were harvested at similar ripeness levels. The fungal community diversity was found to be similar between the samples. Similarity Percentages (SIMPER) showed an overall average dissimilarity of 33\% between the communities of the two varietals. Similar dissimilarity levels were observed across the three sampling areas. Principal component analyses based on correlations between communities showed that there is some structural separation according to grape varietals. Overall, the data revealed that grape varietal played a bigger role in determining the fungal community structure than origin. However, since only three vineyards were sampled it is not possible to draw conclusion on regional community patterns. Overall, yeast diversity was low, and showed not specific distribution along location or grape varietal. Commonly encountered genera including *Hanseniaspora*, *Lachancea*, *Torulaspora*, *Candida*, *Starmerella* and *Saccharomyces* were identified. Interestingly, *Saccharomyces* was detected only in Cabernet Sauvignon. Future work will compare the dynamics of these species throughout fermentation to determine the persistence of the individual species and their contribution to wine aroma.
ANALYSIS OF THE FUNGAL POPULATION OF CABERNET SAUVIGNON IN WINE-GROWING REGIONS OF DIFFERENT COUNTRIES COMPARED TO THE LOCAL CULTIVAR YEAST POPULATION BY NGS

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Type: e-Poster, Session 1

Wine is one of the most ancient fermented beverages, it has remained almost as originally conceived until recently, when new techniques were implemented mainly to solve fermentative problems. The selection and use of specific wine yeast strains with excellent fermentation capabilities is almost mandatory in new wine practices. The drawback of using these yeast strains is the loss of wine diversity, compared to spontaneous fermentations. Recently the industry is incorporating non-Saccharomyces strains in an effort to rescue some of the diversity that comes from spontaneous fermentations. In this context, consumers have a great acceptance for wines with autochthonous character, and the industry is looking to accomplish this with controlled multistarter fermentations. This autochthonous character has been linked to different parameters, from weather conditions to soil chemistry but also to grape yeast microorganisms. The grapevine associated microbiota is therefore a source of variability. This work aims to assess yeast biodiversity and how it is associated with the vine cultivar. Understand whether the population dynamics of yeast are based more on the vine cultivar with which it is associated or whether the local environment is the main driver. The mycobiome of grapes from different vineyards in four wine regions of Europe (Georgia, Italy and Spain) and South Africa has been analyzed in local autochthonous wine cultivars compared to Cabernet-Sauvignon cultivars in the same regions. The use of a metagenomic approach allows us to provide a new point of view on the mycobiome of grapes. Preliminary results showed that the country of origin was the main driver clustering the different samples, and that samples belonging to the Cabernet-Sauvignon cultivars were more similar to each other than samples belonging to different local cultivars, thus showing a common profile for the Cabernet-Sauvignon cultivar but with less weight than the country of origin.

EXPLORING SACCHAROMYCES POPULATIONS IN CANADIAN PINOT NOIR VINEYARDS: A TWO-YEAR SURVEY

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Yeasts, namely species of the genus Saccharomyces, are found in vineyards and wineries - and are increasingly recognized as an important contributor to a wine\'s regional character, or terroir. Studies suggest that there are unique sub-populations of
**Saccharomyces** strains in various winemaking regions, but Canadian vineyard-associated **Saccharomyces** populations have not been regionally profiled. This two-year study characterizes the **Saccharomyces** populations in Pinot Noir vineyards of British Columbia’s Okanagan Valley (OKV), one of the major winemaking regions in Canada. We sampled Pinot Noir grapes in the 2016 and 2017 vintages from thirteen vineyards in three OKV sub-regions spanning 100 km (Olever-Osoyoos, Penticton-Naramata and Kelowna) and fermented them in-lab to enrich for **Saccharomyces** yeasts. From the 2016 and 2017 vintages, 1632 and 1440 **Saccharomyces** yeasts were isolated, respectively. There was a high disparity in fermentation success rate among sub-regions and vintages. We found the presence of **Saccharomyces** species varied across vintages; **S. cerevisiae** was isolated in 2016 while both **S. cerevisiae** and **S. uvarum** were isolated in 2017. To genetically characterize **S. cerevisiae** and **S. uvarum** isolates, we performed microsatellite analysis of 11 genomic loci on all yeasts collected. Commercial **S. cerevisiae** strains were identified by comparing the microsatellite profiles of each isolate to a lab-compiled commercial **S. cerevisiae** database of over 150 strains. **Saccharomyces** strain richness and distribution was highly heterogeneous both within and among vineyards. Population structure of **S. cerevisiae** strains appears to be influenced by both sub-region and vintage. While commercial **S. cerevisiae** strains were found in all sub-regions, phylogenetic analysis suggests a subpopulation of **S. cerevisiae** strains are genetically distinct from commercial strains. This is first study to regionally profile Canadian vineyard-associated wine yeasts. Our long-term goal is to oenologically characterize these yeasts and develop fermentation starter cultures that can produce high-quality wines with regional character in a predictable manner.

**KLUYVEROMYCES MARXIANUS SESSILE LIFE STYLE: UPREGULATED GENES**

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Biofilms are formed by the aggregation of microorganisms into multicellular structures that adhere to surfaces. It is closely associated to mat formation defined as the ability to form complex multicellular structures. Both attachment to plastic and mat formation require Flo11p, a member of a large family of fungal cell surface glycoproteins involved in adherence. Adhesion and biofilm formation of yeasts to solid food matrices, like cheese surfaces, are an essential requirement for their establishment and growth on the surface and for the contribution to the final product quality. In this study we analyzed 8 **K. marxianus** strains (6M2, LM127, M135, 1SC4, CBS834T, FM09, M83, VG4) isolated from different dairy products, selected on the basis of their capacity to produce biofilm (in YPD and in whey) and to form mature mat structures. Moreover, we identified 4 **K. marxianus** orthologues genes known from studies in other yeast to be involved in biofilm formation. The number of sessile and planktonic cells was established by plate count. All tested strains were able to form biofilm in both substrates with higher values in whey, with a preference for sessile lifestyle that was about 1-2 fold higher than planktonic lifestyle. All the strains were able to produce a mature mat, creating different structures. The genes tested were: **FLO11** (**MUC1**) and **STE12** involved in invasive growth and pseudohyphal formation, and **TPK3** and **WSC4** involved in nutrient control of cell growth and division, and in stress response, respectively. The results showed that the genes **STE12** and **FLO11** were highly expressed, while **WSC4** and **TPK3** didn’t show upregulation in any of the tested strains.
THE DIGESTIVE TRACT OF PHYLLOICUS (TRICHOPTERA: CALAMOCERATIDAE) HARBOURS DIFFERENT YEAST TAXA IN CERRADO STREAMS, BRAZIL

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We investigated the occurrence of yeasts associated with the digestive tract (DT) of Phylloicus spp. larvae (Trichoptera: Calamoceratidae) from first-order streams of two Cerrado sites in Mato Grosso - MT and Pará - PA states in Brazil. Phylloicus spp. larvae were collected, disinfected superficially (70% ethanol; sterile distilled water) and dissected to obtain the DT content, from which the yeasts were cultivated. The isolates were identified based on sequence analysis of the D1/D2 domains of the large subunit of rRNA genes. The DT of Phylloicus harbored 23 yeast species belonging to six genera of Ascomycota and five Basidiomycota. The most frequent genera were Candida (19.3%), Issatchenkia (15.8%), Papiliotrema (12.3%) and Rhodotorula (19.3%). Species richness was higher in larval DT collected in streams from PA site in comparison to MT site possibly due to landscape fragmentation in the PA site that interferes with source of substrates to the shredders diet.

IDENTIFICATION OF EPIPHYTIC YEASTS ON APPLE AND LEMONS

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Yeasts are ubiquitous microorganisms in the environment and abundantly present on surfaces of fruits and vegetables. This study is aimed to clarify yeast biodiversity of different apple and lemon cultivars. Two international (‘Golden’and ‘Starking’) and one Turkish local (‘Amasya delicious’) apple cultivars were used as a raw material, similarly international cultivars ‘Meyer’, ‘Interdonato’ and local Turkish cultivar ‘Kutdiken’ were selected for lemon fruit. Yeasts were isolated and purified from yeast extract peptone dextrose agar and malt extract agar. Molecular identification of a total of 52 isolates was achieved by a combination of PCR-RFLP of the 5.8 S rRNA region and sequencing of the D1/D2 domain of the 26 S rRNA gene. Hae III, Hinf I and Cfo I restriction endonucleases selected as to differentiate of the 5.8S rRNA PCR product. Five different yeast species (Saccharomyces cerevisiae, Saccharomyces paradoxis, Rhodotorula mucilaginosa, Aureobasidium pullulans, Wickerhamomyces anomalus) belonging to four different genera were determined. S. cerevisiae (59 %) and A. pullulans (21 %) were found as most isolated species from cultivars of apple and lemon. S. cerevisiae was the main yeast species present in all the fruits. However, A. pullulans were determined in all apple cultivars, inversely could not isolated any of the lemon cultivars. On the other hand, R. mucilaginosa was isolated only from the surface of starking delicious apple and interdonato lemon, while S. paradoxis was determined in cultivars ‘Starking’ apple and ‘Kutdiken’ lemon. W. anomolus presents in both cultivars ‘Amasya’ and ‘Starking’ apples and ‘Meyer’ lemon. To sum up, yeast microflora of fruits is varying according to different cultivars in apples and lemons. In future studies, biocontrol potential of these yeasts against fungal pathogens on apple and lemon will be assessed.
YEAST ASSOCIATED TO PULP OF MANGO (MANGIFERA INDICA) AND POMARROSO (SYZYGIUM MALACCENSE) IN CALI, COLOMBIA

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The ecological role of yeast in different habitats in Colombia has been slightly studied. Even less is known about the diversity of yeast associated with different plant structures. For example, fruits are a potential ecological niche for growth and survival of yeast species in nature and yeasts can affect the productivity and quality of bioeconomic fruits, such as Mango. On the other hand, unexplored fruits, such as “Pomarroso”, can be niche from novelty yeast strains with biotechnological potential.

In this sense, the main objective of this study was to isolate and identify yeasts associated to Mango (Mangifera indica) and “Pomarroso” (Syzygium malaccense) pulp in Cali, Colombia. We obtained 90 isolates, 15 from Pomarrosos and 10 from Mangoes, which were grouped considering colony, cell morphology and MSP-PCR Fingerprinting profiles using the microsatellite (GTG)₅ as primer. The D1/D2 domain of the ribosomal large subunit (LSU) was sequenced for each representative isolate. We found the shared species Hanseniaspora thailandica and H. opuntiae from both fruits. Other yeast species were specific to Pomarroso (H. uvarum, Pichia terricola, Rhodosporidiobolus ruineniae, Candida albicans, Clavispora lusitaniae), or specific to Mango (Meyerozyma caribbica, M. guilliermondii, C. natalensis, Aureobasidium pullulans, Saturnispora diversa and C. jaroonii). One morphotype was not identified to taxonomic level of species and we reported as candidate for new species, belonging to genus Candida. Both fruits provide microenvironments that allow the growth of biotechnologically important species such as A. pullulans, M. guilliermondii and H. uvarum.

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YEASTS VECTORED BY STINGLESS BEES IN CERRADO REGIONS IN THE STATE OF TOCANTINS, BRAZIL

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In this study we identified the yeast species vectored by Frieseomellita varia, Scaptorigona polysticta, Sacaptorigona postica, Tetragonisca angustula angustula, Melipona compressipes manaoensis and Melipona scutellaris in areas of Cerrado. Sampling was done in natural Cerrado areas of Northern Brasil, and bees workers were captured in the nest entrance with sterile bags and put to walk on plates containing YMA. Yeasts were identified by molecular sequencing of D1/D2 sequence. Among all stingless bees, 61 yeast species belonging to 18 genera of Ascomycota and six Basidiomycota were identified, with a higher frequency of Torulaspora delbrueckii, Candida apicola, Pichia memranifaciens, Pichia klyveri and Starmerella meliponinorum that represented 41% of the strains. Significant differences in the yeast composition occur between Meliponini and Trigonini bee tribes, indicating different trends.
strategies of visitation in the substrates colonized by yeasts. The vectoring of yeasts differed among bee species of each tribe, indicating habitat partitioning for collection among the bees.

**YEASTS DIVERSITY ASSOCIATED WITH ANTS’ NESTS**

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Yeasts can interact with several species of insects in nature. These invertebrates act as vectors of yeast communities and use them as own food source and for their broods. Some yeast species associated with nests of *Atta sexdens rubropilosa* are potential producers of enzymes, which act in the breakdown of plant polysaccharides to release simple sugars to the ants. The purpose of this study was to describe the yeast species associated with ants’ nests. The collection was done in Serra do Cipo National Park, located in Brazilian Cerrado ecosystem. Yeasts were isolated from ants’ nest (located in tree hollows), corporal surface of ants and homopters present in the nest, samples of nests surface and samples of soil next to the nests. The material was processed on solid medium YM. The yeasts were grouped according to the colonies morphology and molecular profiles through PCR fingerprinting using the (GTG)₅ primer. One representative of each molecular group obtained was selected for molecular identification by the sequencing of the D1/D2 domain of the large subunit rRNA gene. The sequences were compared with the sequences in GenBank by BLASTn platform, available in the NCBI portal. A total of 108 yeasts were obtained, distributed in 20 yeasts and 8 yeast-like species. *Candida orthopsilosis* (*C. albicans/Lodderomyces* clade) was the most isolated species, with 29 strains. Eleven new possible species were identified belonging to the genera *Fellomyces, Kwoniella, Meyerozyma, Phaeoacremonium, Phaeomoniella, Pseudotremella, Symbiotaphrina, Vishniacozyma* and *Wickerhamomyces*. Although this study has isolated 28 yeasts species, the use of others isolation mediums or substrates associated with ants could favour the isolation of more yeasts species. Therefore, this work contributed to the knowledge of species associated with ants’ nests and provides insights to the study of the yeasts biodiversity in the Brazilian environments.

**DIVERSITY OF YEASTS ISOLATED FROM THE BRAZILIAN AMAZONIAN FOREST**

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The Amazon Forest contains the most species-rich terrestrial and freshwater ecosystems in the world. Few studies have characterized the yeast diversity of Brazilian Amazonian environments and much more research is needed to understand the enormous diversity and complexity of this region. In this work, we studied yeast
diversity in various plant materials, such as flowers, rotting wood, rotting fruits, tree barks and tree exudates. Samples were collected between January 2015 and May 2016 at Caxiuanã National Forest, in the state of Pará, northern Brazil. Several isolation media were used, such as YNB with different carbon sources (xylose, xylan, erythritol and raffinose), medium for osmotolerant yeasts (50% glucose, 0.5% yeast extract) and YM. Yeasts were identified by sequence analysis of the D1/D2 region and internal transcribed spacer (ITS) domains of the large subunit of the rRNA gene. A total of 682 yeasts strains was recovered, comprising 164 different species. The most frequent species belongs to genera *Cyberlindnera*, *Lodderomyces*, *Nakaseomyces*, *Kodamaea* and *Spencermartinsiella*. Few basidiomycetous yeasts were isolated (48 strains, 14 species) and among them the most common genera were *Vanrija* and *Moniliella*. Among the species isolated from rotting wood, the most common genera were *Cyberlindnera*, *Galactomyces*, *Scheffersomyces*, *Spencermartinsiella*, *Kazachstania* and *Sugiyamaella*. From rotting fruits samples, the most common genera were *Clavispora*, *Hanseniaspora*, *Kurtzmaniella*, *Nakaseomyces* and *Pichia*. In the flower samples, the most common genera were *Clavispora*, *Cyberlindnera*, *Hanseniaspora*, *Kodamaea*, *Metschnikowia*, *Moniliella* and *Starmerella*. Three *Saccharomyces cerevisiae* strains were obtained from tree bark samples, as well species of the genus *Clavispora*, *Cyberlindnera*, *Hyphopichia*, *Mejerzyma* and *Wickerhamomyces*. Fifty-nine new species have been identified in this work, some representing basal strains within *Saccharomycetaceae* and other belonging mainly to the genera *Clavispora*, *Cyberlindnera*, *Galactomyces*, *Kurtzmaniella*, *Metschnikowia*, *Mejerzyma*, *Moniliella*, *Pichia*, *Spathaspora*, *Spencermartinsiella*, *Sugiyamaella*, *Wickerhamiella*, *Wickerhamomyces*, *Yamadazyma* and *Zygotorulaspora*.

**STUDY OF VOLATILE COMPOUNDS AND GENE EXPRESSIONS IN KLUYVEROMYCES MARXIANUS**

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Type: e-Poster, Session 4

*Kluyveromyces marxianus* is a promising non-*Saccharomyces* yeast for biotechnology applications thanks to his traits such as rapid growth, thermostolerance and bioethanol production. As a lactose fermenting yeast, *K. marxianus* is associated with dairy products. In fermented dairy products, *K. marxianus* contributes to flavours and aroma, mainly through the production of acetate and ethyl esters. Commercially, the strong activity of the Ehrlich amino acid degradation pathway has been exploited to produce the higher alcohol 2-phenylethanol from phenylalanine. The yeast also produces high levels of acetate esters (2-phenylethyl acetate), relative to other yeasts and it is of interest to understand this better for applications as a dairy yeast and for industrial fragrance and flavour applications. This requires analysis of the genes and pathways involved in the use and breakdown of amino acids, and in the conversion of breakdown metabolites to acetate esters. Knowing what genes are involved, and how they are regulated, will allow us to manipulate or control expression as required to tailor the volatile profile of the yeast. We identified 8 *K. marxianus* orthologues of all the genes known from studies in other yeasts to be involved in these pathways and examined how their expression was regulated by different nitrogen sources. Volatile molecules were measured to assess whether production could be correlated with gene expression. We used a CRISPR-Cas9 system to individually knock out all eight genes and assessed mutant phenotypes under different conditions. The most striking result was severe growth impairment of a *bat1* mutant when growing on minimal medium with any
Innovation is one of the driving forces in craft beer industries. It is important from the choice of ingredients through to packing. Exploiting yeast diversity is one way to be truly innovative, extending known brewer’s yeasts beyond traditional lineages. In the 1990s, our group was working on yeast ecology in tropical habitats of Rio de Janeiro State and originally identified rain forest isolates as *S. cerevisiae* with positive fermentation of glucose and sacarose. Later genetic studies provided new data leading to the description of a new species *Saccharomyces cariocanus*. At that time maltose assimilation and fermentation were not observed in the lab. The culture was duly deposited in the CBS collection and further tested as a routine procedure. Surprisingly, results at CBS for maltose fermentation varied from delayed (IMUFJR51816.CBS7995) to positive (IMUFJR51791.CBS7994). Based on those findings, recently we re-activated lab strains and repeated those fermentation tests. The results confirmed a delayed but strong capacity to ferment maltose, the principal sugar component of beer wort. Thus the possibility of *Saccharomyces cariocanus* becoming a new brewer’s yeast arose. Fermentation trials were then repeatedly undertaken using the culture and a maximum wort concentration of 12°P was established with an ideal fermentation temperature ranging from 17°C to 25°C. An aromatic beer was produced using a wort of pale ale malt with 10°P, fermentation reached 74% attenuation with 3.9% ABV (calculated). Subsequently, several laboratory scale batches of beer were produced and drunk using this recipe and new recipes, changing malt grist and hop quantity. Consistent fermentation capacity was observed with all recipes. We report here on a tasty beer totally fermented by *Saccharomyces cariocanus*, a product with regional name and appeal for craft beer markets seeking novelty.

**DIVERSITY OF COLD-ADAPTED FUNGI IN NON-POLAR GLACIERS**

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Type: e-Poster, Session 4

Cold environments are one of the most widespread extreme environments, different microorganisms including yeasts are able to survive, live and colonize these environments. The objective of this work was to analyze the biodiversity of cold-tolerant cultivable yeasts associated with glaciers of Mount Tronador and compare them with
free culture analysis of the same environment. Soil, ice and water samples were collected from Castaño Overa and Ventisquero Negro glaciers (Mount Tronador). Ice and water samples were filtered in 45um filters, and placed in petri dishes with 10% MYP medium and YPD with antibiotics. Soil samples were cultivated by means of serial dilutions in YPD and MYP media, with Rose Bengal and antibiotics. All samples were incubated at 5 °C until the developments of colonies. For total DNA extractions, MOBIO® kits were used. Identified isolates yeasts corresponded to the genera: Solicozyma, Filobasidium, Naganishia, and Vishniacozyma among the most abundant Basidiomycetes, and Candida and Aureobasidium for Ascomycetous. Free culture method show a high abundance of Microbotryomycetes, and sequences of Tremellomycetes as Naganishia friedmannii and Filobasidium magnum were also abundant. Groups belonging to Chytridiomycetes were also registered. Identified species correspond to cosmopolitan, psychrophilic or psychrotolerant taxa. This study contributes to a better knowledge of the biodiversity of extremophile yeasts and to the preservation of genetic resources that allow the development of conservation strategies.

DIVERSITY OF SACCHAROMYCES YEASTS IN UTRECHT UNIVERSITY BOTANIC GARDEN

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Type: e-Poster, Session 4

Inspired by findings of Saccharomyces yeasts at various places on Earth we set up projects for undergraduate students at the Utrecht University of Applied Sciences and University of Amsterdam to investigate the presence of Saccharomyces yeasts on trees growing in the Botanic Garden of Utrecht University at De Uithof, Utrecht, The Netherlands. Five groups of students sampled trees, mainly oak, during two years in various seasons. Pieces of bark were collected and placed in ethanol containing media inside tightly closed plastic tubes that were fully filled with growth medium. Yeast isolates were identified by MALDI-TOF MS, sequencing and AFLP. Various species of Saccharomyces were found, such as S. arboricola, S. cariocanus, S. cerevisiae, S. kudriavsevii, S. paradoxus, and S. uvarum. In addition various hybrids were isolated belonging to different hybrid groups, i.e. S. cerevisiae × S. uvarum, S. cerevisiae × kudriavsevii and S. cerevisiae × S. eubayanus. Several trees were repetitively positive for Saccharomyces yeasts when sampled at all five occasions suggesting colonization of the trees by the yeasts. No correlation was observed between different exposure sides (N, W, E, S) nor with the seasons, but this may be due to the limited sampling effort. As we hypothesize that the tree-Saccharomyces interaction likely is more complex future project may focus on the involvement of microfauna in this ecosystem. Next to Saccharomyces several other fermentative yeasts were found that are also know from food fermentations. Is it possible that the use of oak-wood utensils in the past [prehistoric times] has contributed to the selection of such microbes for food and beverage fermentations?
SESSION 2:
INDUSTRIAL
APPLICATIONS OF
NON-CONVENTIONAL
YEASTS
CONTRIBUTIONS OF GENOMIC AND GENETIC DIVERSITY TO PHENOTYPIC VARIABILITY IN KLUYVEROMYCES MARXIANUS

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Abstract ID: 345
Type: Main Speaker

Kluyveromyces marxianus is traditionally associated with fermented dairy products, but can also be isolated from diverse non-dairy environments. Because of thermostolerance, rapid growth and other traits, many different strains are being developed for food and industrial applications but there is, as yet, limited understanding of the genetic diversity or population structure of this species. Our analysis of the genomes of over forty strains, mostly from culture collections, has revealed ploidy variation, multiple instances of aneuploidy, and extensive intra-species variation. Interestingly, all strains isolated from dairy environments are either diploid or triploid, whereas most strains from other environments are haploid. Diploid strains are intraspecies hybrids between lactose positive and lactose negative haplotypes. These data are consistent with a hypothesis that the natural reservoir of K. marxianus is non-dairy and its use to produce fermented milk products has selected for a set of strains with higher ploidy that are adapted for dairy fermentation. We found that the lactose positive trait is due to adaptive variation in the LAC12 gene, which encodes a permease also capable of transporting galactose and the disaccharide cellobiose. Further investigation revealed that there is unique expansion of genes encoding hexose transporters in K. marxianus with at least four other permeases capable of transporting galactose in this yeast. Analysis of cellobiose transport established that there are two cellobiose utilization systems that are ancestral in the Kluyveromyces genus but components of these show variable loss during evolution. In addition, we find evidence of gene transfer and acquisition that has played a major role in shaping the genus. Heterologous expression and targeted gene disruption has been used to dissect the function of the individual components of the flexible sugar transport systems in this biotechnologically-important yeast species.
BIOLOGICAL CONTROL ACTIVITY OF KILLER YEAST CLAVISPORA LUSITANIAE 146 AGAINST PENICILLIUM DIGITATUM IN LEMONS

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Abstract ID: 65
Type: Oral

Postharvest decays caused by *Penicillium digitatum* are responsible of significant economical losses on citrus industry worldwide. In spite of the disadvantages for human health and the environment, chemical fungicides are still widely used for the control of fungal diseases. A native killer strain of *Clavispora lusitaniae* 146, which previously proved to be effective against green mold in lemons, has been further tested in controlled conditions as a biological and safer alternative to the use of synthetic fungicides. The aim of this work was to determine *C. lusitaniae* 146 protection efficiency by varying yeast suspension concentrations and dipping times. On the other hand, wound colonization ability and compatibility with commercial waxes of the killer yeast were also evaluated. We showed that strain 146 protection efficiency remained high, even at the lowest yeast concentration used and for all different exposition times. Wounds analysis by scanning electron microscopy confirmed that *C. lusitaniae* 146 showed an evident wound colonization ability on lemons, being able to develop at both low and room temperatures. Besides that, *C. lusitaneae* 146 was able to survive in one of the major natural waxes used by local lemon packinghouses at both storage conditions, after 7 and 40 days at 25 and 8°C, respectively; while the tested synthetic wax was not compatible with strain 146. All these results support the potential practical application of *C. lusitaneae* 146 as a biological agent for postharvest protection of lemons fruits against *P. digitatum*.

SACCHAROMYCES EUBAYANUS POTENTIAL IN WINEMAKING FIELD

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Type: Oral

The literature data have pointed out the growing interest for the species *Saccharomyces eubayanus*, a recently described taxon, isolated from natural environments in Patagonia and discovered to be the unknown part, together with *S. cerevisiae*, of *S. pastorianus*, an allopolyploid hybrid used for lager beer production. Although up to now, *S. eubaynus* was associated with Nothofagus trees and some traditional fermented beverage in Patagonia, it is believed to have a great potential also in oenological sector. For this, principal aim of this research was to investigate the potential of *S. eubayanus* CBS 12357 for fermenting Chardonnay musts at 10, 16 and 26°C. Its technological potential was compared to that of *S. cerevisiae* VIN13, a commercial strain typically used for this purpose. For both the strains, the fermentation kinetics and the yeast cell loads, in relation to the adopted temperature, were monitored. The obtained wines were also characterized for oenological parameters and the volatile molecule profiles by
GC/MS/SPME. Moreover, also panel tests were performed. The data obtained pointed out the great cryotolerant aptitude of *S. eubayanus* which resulted, at 10 and 16°C, in faster fermentations with respect to *S. cerevisiae* VIN 13. Also, *S. eubayanus* gave rise to wines characterized by specific volatile molecule fingerprinting. According to the panel test performed, the wine obtained by *S. eubayanus* resulted not statistically different from those from *S. cerevisiae* but they were characterized by “body” and aroma persistence, highlighting as proper winemaking processes can be useful to exploit the *S. eubayanus* potential in winemaking.

**METABOLIC PATHWAYS INVOLVED IN AROMATIC AZO DYES DEGRADATION BY *T. AKIYOSHIDAINUM***

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Type: Oral

*Trichosporon akiyoshidainum* is basidiomycete yeast that proved to be a promising tool for biodegradation of textile dyes. The mechanism involved in this process likely involve enzymes like laccases and Mn-peroxidases, and other peroxidases, which activities have been detected in culture supernatants of cells grown in the presence of textile azo dyes. However, it is also possible that radical-generating reactions like Fenton-type reactions are involved in the process. In order to elucidate the metabolic pathways involved in aromatic azo dyes degradation, a genomics analysis was carried out allowing identifying several clusters of genes implicated in different steps of the dye degradation. Through a proteomic analysis, by nanoLC-MS/MS, a complete spectrum of peroxide-producing oxidases, radical-producing enzymes and other relevant compounds involved in iron homeostasis, iron reduction, and quinone cycle were identified in the presence of Reactive Black 5 (RB5) dye. These results are consistent with the biodegradation of dye by Fenton or Fenton-mediated reactions in which Fe (II) and H2O2 react to form hydroxyl radicals, highly reactive species capable of depolymerizing aromatic compounds. Those results were further verified by biomimetic assays, proving that Fenton-type reactions could drive dye removal. The degradation of the dye BR5 by *T. akiyoshidainum* demonstrated to be a complex process that comprises both enzymatic and non-enzymatic oxidative reactions. Both mechanisms are similar to those involved in the biodegradation of the lignocellulosic material by filamentous fungi. The elucidation of the metabolic pathways linked to the degradation of BR5 is an important contribution to the study of the aerobic degradation of aromatic dyes by yeasts and could be recognized as a starting point in the exploration of the lignocellulolytic potential of *T. akiyoshidainum* and related yeasts.
IMPROVEMENT OF THERMOTOLERANCE IN *LACHANCEA THERMOTOLERANS* USING A BACTERIAL SELECTION PRESSURE

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The use of thermotolerant yeast strains is an important attribute for a cost-effective high temperature bioethanol production. The availability of thermotolerant yeast strains remains a major challenge. Isolation of temperature resistant strains from extreme environments or the improvements of current strains are two major strategies known to date. We hypothesised that bacteria are potential “hurdles” in the life cycle of yeasts, which could influence evolution of extreme phenotypes, such as thermotolerance. We subjected a wild-type yeast, *Lachancea thermotolerans* to six species of bacteria sequentially for several generations. After coevolution, we observed that three replicate lines of yeasts grown in the presence of bacteria grew up to 37 °C whereas the controls run in parallel without bacteria could only grow poorly at 35 °C retaining the ancestral mesophilic trait. In addition to improvement of thermotolerance, our results show that the fermentative ability was also elevated, making the strains more ideal for the biofuel production process because the overall productivity and ethanol titters per unit volume of substrate consumed during the fermentation process will be elevated. Our unique method is attractive for development of thermotolerant strains or to augment the available strain development approaches for the bioethanol industry.

PROCESS DEVELOPMENT FOR THE CONTINUOUS PRODUCTION OF HETEROLOGOUS PROTEINS BY THE INDUSTRIAL YEAST, *KOMAGATAELLA PHAFFII*

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Type: Oral

The current trend in industrial biotechnology is to move from batch or fed-batch fermentations to continuous operations. The success of this transition will require the development of genetically stable production strains, the use of strong constitutive promoters, and the development of new medium formulations that allow an appropriate balance between cell growth and product formation. We identified genes that showed high expression during different steady-state conditions and explored the utility of promoters of these genes (Chr1-4_0586 and FragB_0052) in optimising the expression of two different r-proteins, human lysozyme (HuLy) and the anti-idiotypic antibody fragment, Fab-3H6, in comparison to the widely employed glyceraldehyde-3-phosphate
dehydrogenase (GAP) promoter. Our results showed that promoter strength was highly dependent on the cultivation conditions and thus constructs should be tested under a range of conditions to determine both the best-performing clone and the ideal promoter for the expression of the protein of interest. An incidental, but important, benefit of continuous production is that it facilitates the use of genome-scale metabolic models in the design of strains and cultivation media. In silico flux distributions showed that production of either protein increased the flux through aromatic amino acid biosynthesis. Tyrosine supplementation increased the productivity for both proteins, whereas tryptophan addition did not cause any significant change, and phenylalanine addition increased the expression of HuLy but decreased that of Fab-3H6. These results showed that a genome-scale metabolic model can be used to assess the metabolic burden imposed by the synthesis of a specific r-protein and that this information can be used to tailor a cultivation medium to increase production.
2-PHENYL ETHANOL AND 2-PHENYL ETHYL ACETATE PRODUCTION BY NON-CONVENTIONAL YEASTS FROM TEQUILA VINASSES

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Vinasses are a wastewater generated from distillation in Tequila production, and they are a severe environmental issue. Obtaining value added products from industrial residues in fermentative processes provides an option to treatment and reuse of these wastewaters, such as 2-phenylethanol (2-PE) and 2-phenylethylacetate (2-PEA), rose like aroma compounds used in several industrial applications. In this study, four yeasts species (W. anomalus, C. glabrata, C. utilis and C. parapsilopsis) were studied with two different chemically defined mediums to induce the production of 2PE and 2PEA by the Ehrlich pathway under two metabolic process; (i) De novo synthesis (DNS) from glucose (shikimate pathway) and (ii) the catabolic pathway (CP) (Phenylalanine degradation) respectively. Afterwards, fermentations were carried out in different tequila vinasses as a substrate. There were found differences in the production of aroma compounds in function of the chemically defined medium and the yeast species. The highest accumulation of 2-PE and 2-PEA was observed in CP medium, 242 mg/L of 2PE with Candida utilis, while the highest 2-PEA production was 665 mg/L and 689 mg/L for Candida glabrata and Wickerhamomyces anomalus respectively. In the case of tequila vinasses, the production of 2-PE was not observed, only 2-PEA was produced in lower concentrations than in DNS and CP mediums, possibly due to the low concentration of phenylalanine in vinasses and to the intracellular toxicity of 2-PE. The highest production of 2-PEA was 65 mg/L with C. glabrata. Therefore, it could be possible that production of 2-PE and 2-PEA was carried out by the De novo synthesis pathway on tequila vinasse, which must be taken into account for a fermentation process optimization.

DEVELOPMENT OF A HANSENULA POLYMORPHA STRAIN PRODUCING HYALURONIC ACID

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Type: e-Poster, Session 1

Hyaluronic Acid (HA) is a polymer formed by glucuronic acid and N-acetylglucosamine disaccharide units linked by β-1,4 and β-1,3 glycosidic bonds. This polymer has a broad application in the areas of orthopedics, ophthalmology, and dermatology. According the Grand View Research Inc., the global market for hyaluronic acid in 2016 reached a total of USD 7.2 billion being expected to reach USD 15.4 billion is expected by 2025. This increased marked demand is a consequence of advances in medical and cosmetic treatments that utilizes HA, especially in osteoporosis treatments. The synthesis of HA involves the enzyme hyaluronan synthase that catalyzes the ligation of the two precursors in the cytosol, elongates the polymer chain and release it into the
extracellular space. Thus, with few genetic modifications, it is possible to produce this polymer heterologously since the N-acetylglucosamine pathway is present in most yeasts. *Hansenula polymorpha* is a methylotrophic yeast, thermotolerant and GRAS status used as a host for recombinant protein production and bioethanol. Besides, several methods for genetic manipulation are available for this microorganism. Here, we report the genetic modifications necessary for HA production in *H. polymorpha*. Two expression plasmids pHIPZ18/hasP and pHIPH4/hasB were constructed and inserted into *H. polymorpha* strain NCYC495 by electroporation. At the present moment, positive clones are being tested for the presence of the two genes in their genome and production of HA.

**RECOMBINANT INGA LAURINA TRYPsin INHIBITOR (ILTI) PRODUCTION IN KOMAGATAELLA PHAFFII CONFIRM ITS POTENTIAL ANTI-BIOFILM EFFECT AND REVEALS AN ANTITUMORAL ACTIVITY**

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Protease inhibitors are amplified in biotechnology, ranging from the development of various drugs to their use as a bioinsecticide. However, these are found in small amounts in their natural sources, which makes their use impossible on an industrial scale. Therefore, heterologous production ends up as a method that allows the progression of proteins. The Inga laurine trypsin inhibitor (ILTI), which will be performed following protease inhibition, extracted from the insect gut, besides being from its larval laboratory in up to 84%, thus a candidate to be used as a potential bioinsecticide. Thus, this work had a heterogeneous production of ILTI in *Komagataella phaffii*, with the objective of maximizing the production of recombinant protein. For this, the gene encoding the ILTI was cloned into the pPIC9K expression vector, followed by its species in GS115 by electroporation. The reaction was run for 96 hours, adding 0.5% methanol. Analyzing as proteins in the culture of the recombinant lineage, by SDS-PAGE, the production of a protein with the next number of 20 KDa was confirmed. MALDI-TOF data confirm that the protein is, in fact, recombinant ILTI. In addition, a protein generated inhibitory assays. Thus, culture in bioreactors was performed to optimize the production of this heterologous protein. To increase its expression, it was carried out during the phase where biomass production was favored, and the feed phase was programmed to continuously supply methanol, based on specific methanol consumption and growth rate, using methanol as the source carbon. To the moment, in the data in the production of inhibitors of serine proteases in *K. phaffii*, making this study and essential to the upper process of the amplitude of the technology.
STARTER NATIVE YEASTS: HOW THEY INFLUENCE ON THE PERMANENCE OF STRAINS IN BIOETHANOL FERMENTATION?

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Fuel-ethanol fermentations are subject of huge selective pressures resulting in the growth and persistence of yeasts adapted to stress conditions. In alcoholic fermentation processes in Brazilian distilleries, native strains from feedstock input as contaminating microorganisms and usually dominate the process along the harvest period. In some cases, native strains hinder the fermentation process. Conversely, the native yeast strains selection to start the fermentation process could be an advantage if the strain selected has an interesting fermentation profile. In this study, we examined yeast population dynamics of one industrial fuel-ethanol fermentation, which started the fermentation processes with different kind of yeast strains during two successive harvest seasons (2016/2017) in order to evaluate the effect of the starter yeast strains over the permanence of native strains during the harvest season. The assessed sugar mill is located in the State of São Paulo/Brazil, where a follow-up of dynamics of yeast population was performed from April to December. Yeast strains differentiation was performed using karyotyping (PFGE). It allowed us to assess the composition of yeast population and quantify representative native strains for all harvests evaluated. In 2016, the industrial unit started its fermentation with yeast from previous harvest without selection or characterization of strains. In contrast, in 2017 it used 2 native strains from 2016 selected and characterized beforehand. We found 13 different native strains during the first harvest without the prevalence of strain. In 2017 we found just 2 yeast strains used as inoculum in the entire harvest period. Our results showed the effect of native yeast selected beforehand as starting yeast over the permanence, composition and population dynamics of yeast strains in bioethanol fermentation. This work has implications on the understanding of native yeast population in Brazilian industry and yeast selection for the bioethanol fermentation improvement.

MICROBIAL CONSORTIA STRATEGIES FOR THE PRODUCTION OF ALCOHOLS FROM LIGNOCELLULOSIC WASTES

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Biofuels is one of the renewable energy sources that can partially replace the use of fossil fuels. The recovery of lignocellulosic residues will be a key source for the development of alternative energies due to its rich sugar components. Lignocellulose is the most abundant component of biomass (mainly wood and agricultural crops), but the challenge is to hydrolyze the cellulose and hemicellulose that are the main sources of sugars. Although there are physicochemical methods that allow use of biomass in the production of fuels, a promising cheaper and sustainable alternative is the microbial fermentation to ethanol. In the present work, we propose the development of microbial consortia with the aim of fermenting the different sugars produced by the treatment of...
lignocellulose. Yeasts from our grape culture collection and from other fermentation industries were screened to characterize the fermentation capacity of glucose, cellobiose, xylose and arabinose, in order to find strains suitable for mixed cultures treatments of these substrates to produce alcohols. Consumption of sugars was analyzed by HPLC, and the production of ethanol and higher alcohols (methanol, Isobutyl, isoamyl and 2-phenylethyl alcohols) was determined by GC-FID. The best fermentation of sugars was obtained with a consortia composed by Spathaspora passalidarum, Scheffersomyces stipitis, Candida akabanensis and Saccharomyces cerevisiae. Sugars were completely consumed except arabinose for all the consortia treatments. Comparison of the mixed cultures with single strain fermentations showed the improve performance of consortia in optimizing sugars consumption. A significant increase of isoamyl and 2-phenylethyl alcohols was found by consortia treatments compared to pure cultures. Preliminary results with real forest lignocelluloses wastes are discussed.

LABORATORY EVOLUTION APPLIED TO KLUYVEROMYCES MARXIANUS AIMING AT FUEL ETHANOL PRODUCTION IN SUGARCANE-BASED BIOREFINERIES

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The natural properties of the yeast Saccharomyces cerevisiae underpin its widespread use in ethanol producing fermentations. In the case of the sugarcane-based first generation fuel ethanol biorefineries in Brazil, the following features can be highlighted: tolerance to high ethanol concentrations (above 10% volume/volume), ability to grow under complete anaerobiosis and tolerance to low pH (below 2.5) during cell recycling. However, one important problem remains in this non-aseptic thermophilic (32 to 35 °C) industrial process: contamination and the associated use of sulfuric acid and antibiotics. In order to eliminate or decrease this public health threatening and environmentally relevant practices, one alternative would be to employ high-temperature ethanol production, which would decrease the capability of contaminating microorganisms to thrive in the industrial fermentors. Several research groups have been trying to push S. cerevisiae to grow above 40 °C, but this has not been straightforward. A different approach is to start with a microorganism that naturally grows at higher temperatures and improve it to produce ethanol under anaerobic conditions, with tolerance to low pH, which maintains the possibility of performing cell recycling with acid treatment, albeit at lower loads due to the lower contamination levels. Kluyveromyces marxianus is a species with this potential. Here we describe three parallel laboratory evolution lines, based on serial transfers. Appropriate selective pressures were applied, in order to obtain mutants with higher specific growth rates (compared to the parental strains) under: 1) anaerobiosis; 2) high ethanol concentration; and 3) low pH. After a phenotypic characterization of the improved strains, demonstrating that they indeed perform better under the desired conditions, genome sequencing is currently been performed for the identification of causal mutations, which could be subsequently used for reverse engineering of the desired traits into the parental strains.
GENOTYPIC AND PHENOTYPIC CHARACTERIZATION OF KLUYVEROMYCES MARXIANUS STRAINS ISOLATED FROM TRADITIONAL MEXICAN SUBSTRATES FOR THE PRODUCTION OF BIOETHANOL BY SSF

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Lignocellulosic biomass is a promising feedstock for the production of second generation bioethanol. Although Saccharomyces cerevisiae has been the workhorse for the industrial production of bioethanol from sugar cane and corn starch, it may not be the organism of choice for the fermentation of lignocellulose-derived sugars. The conditions encountered in lignocellulosic fermentations include, in addition to high ethanol concentrations, the presence of fermentation inhibitors (organic acids and furans) and high temperature in case of the SSF (Simultaneous Saccharification and Fermentation) process configuration. In SSF the enzymatic hydrolysis of cellulose is performed together with the fermentation which requires thermostolerant ethanologenic yeasts that can operate at the temperature of cellulase preparations (40-50°C). In this work, several autochthonous Kluyveromyces marxianus strains were obtained from traditional Mexican substrates as pulque, a pre-Hispanic alcoholic beverage, and Agave fourcroydes, a plant native to Southern Mexico and used to produce henequen fibers. K. marxianus is a non-conventional thermostolerant yeast that has been reported as a promising candidate for developing SSF processes. The genotypic characterization of the seven strains obtained here by ITS (Internal Transcribed Spacer) and (GTG)5 microsatellites allowed to group the strains according to their isolation source, pulque or A. fourcroydes. Some of the A. fourcroydes isolates were further separated into distinct subgroups according to their specific origin, fermented and non-fermented cooked agave juice. All the strains could grow up to 45°C and 48-50°C for the A. fourcroydes strains. Phenotypic characterization included tolerance to exogenous ethanol, fermentation inhibitors (acetic acid, furfural and hydroxymethylfurfural) and NaCl at 30°C and 42°C (the SSF temperature). In all cases, although strains presented slight differences, the general trend was a marked decreased tolerance to ethanol, inhibitors and NaCl at 42°C compared to 30°C. All strains tolerated higher hydroxymethylfurfural than furfural concentrations at 30°C.

DECOLORIZATION OF REACTIVE BLACK 5 BY TRICHOSPORON AKIYOSHIDAINUM IMMOBILIZED IN CA-ALGINATE BEADS

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An eco-friendly treatment of industrial dyes and effluents is the biggest environmental challenge for textile industries. Numerous bio-decolorization processes have been studied, but their industrial application is still marginal due to practical and economic obstacles. In this survey, we analyzed the immobilization of *T. Akiyoshidainum* HP-2023 in Ca-alginate beads to improve its practical effectiveness. The effects of different parameters on the decolorization of Reactive Black 5 by immobilized cells of *T. akiyoshidainum* HP-2023 were investigated under aerobic conditions. The analyzed factors included alginate concentration (1, 2 and 2.75%), initial dye concentration (200, 400, 600 and 800 mg/L) and rotation speed (150, 200 and 250 r/min). Under optimal conditions, (2% sodium alginate, 200mg/l, and 200 r/min), the immobilized yeast caused to 95% decolorization in 6 h, while free, suspended cells, achieved only 50% decolorization in the same period. In addition, immobilized yeast decolorizes a wider range of initial dyes. Immobilized cells could completely decolorize 600mg/l of Reactive Black 5 within 24 h, and caused 75% decolorization of 800 mg/L of the dye in 24 h. Furthermore, results of successive cycles of decolorization showed that insolubilized beads were reusable for at least four repeated cycles keeping a high efficiency in color removal. Results of decolorization tests suggested that Ca-alginate immobilized yeast may effectively be used for decolorization of dyes present in industrial effluents.

**NON-SACCHAROMYCES YEASTS FOR ALCOHOL FERMENTATIONS: CHALLENGES AND OPPORTUNITIES**

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Although *Saccharomyces* spp. are the premier industrial microorganisms that are exploited in many biotechnologies, several diverse non-*Saccharomyces* yeast species have great potential in industrial fermentations, particularly in alcohol production. For example, in the fuel alcohol sector, several non-*Saccharomyces* yeasts are advantageous, not least due to their utilisation of pentose sugars in lignocellulosic hydrolysates for second-generation bioethanol production. Examples include *Scheffersomyces stipitis* that can ferment xylose and displays Crabtree-negative metabolic behaviour. Concerning beverage fermentations, several diverse yeast species have applications, including: *Brettanomyces* and *Saccharomycodes* spp. exploited in production of speciality “sour” and low-alcohol beers, respectively; *Methchnikowia* and *Torulaspora* spp. used in commercial wine production; and *Kluyveromyces* spp. used for distilled spirits production from cheese whey. Such yeasts are employed due to their synthesis of interesting flavour congeners that contribute desirable organoleptic characteristics in fermented beverages. Currently, in whisky production whisky from cereal substrates, commercial strains of *S. cerevisiae* dominate in distillery fermentations, mainly due to tradition and for their rapid alcohol production properties, but there is scope to employ more flavoursome yeasts. Our research focuses on evaluating the potential of non-*Saccharomyces* yeasts in Scotch whisky fermentations to impart desirable aromas and flavours in distilled spirits prior to maturation in oak wood casks. Results will be presented from trial fermentations using diverse species of yeast and subsequent distillations, including GC-MS analyses of major volatile congener chemicals together with sensory analyses of freshly distilled spirits. This presentation will highlight the opportunities of using non-*Saccharomyces* yeasts for production of distilled spirits and will discuss some of the fermentation challenges when compared with the use of more conventional distilling yeast strains. Acknowledgements: We thank colleagues at Abertay University (John Grigor and Peter Maskell) and at The Scotch Whisky Research Institute (Frances Jack, Jane Walker, Irene Baxter and Barry Harrison) for their invaluable collaboration on this project.
CHARACTERIZATION OF Cr(VI) REMOVAL FROM AQUEOUS SOLUTIONS CO-CONTAMINATED WITH Cu(II) USING AN INDIGENOUS YEAST

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Chromium(VI) compounds are known to be extremely toxic to living organisms due to their strong oxidizing nature. Biological Cr removal offers an alternative to traditional physicochemical methods and is considered as a sustainable technology of lower impact on the environment. Resistant microorganisms have been studied they may exhibit Cr-bioaccumulation or biospeciation mechanisms. The endophytic *Rhodotorula mucilaginosa* 7Apo1 from sugar cane was tested on their Cr(VI) removal and resistant mechanism when it was incubated in YM medium plus different concentrations of Cr(VI) and Cr(VI)+Cu(II). Residual Cr(VI) (1,5-Diphenylcarbazide method) and total Cr (Atomic Absoprtion Spectroscopy) were estimated. Cell observations were made by scanning electron microscopy SEM and by EDS dispersive energy spectroscopy. 7Apo1 was able to remove 100% of the 0.5 mM Cr(VI) initial concentration after 96 h of cultivation, whereas with 0.25mM Cu(II) + 0.5mM Cr(VI) the removal was reached at 48 h. Similar behavior was observed at higher concentrations [1 mM Cr(VI) and 0.25 mM Cu(II) + 1mM Cr(VI)], but not reaching the total Cr(VI) removal. Most of the total chromium was detected in the cell-free supernatants (which might be ascribed to the trivalent state) with a minimal residual proportion in the biomass digested with acid. The analysis by SEM showed changes in the general appearance and shape of cells according to the control cultures whilst EDS analysis of 7Apo1 exposed to Cr showed spectra with almost imperceptible low levels of the metal on the cell surface. The results herein presented put in evidence that the capability of the yeast 7Apo1 for Cr(VI) removal are highly influenced by the presence of low concentrations of Cu(II). Accordingly, bioreduction may be suggested as the main mechanism implicated in the removal of Cr(VI) from cultures. Furthermore, low bioaccumulation is not located on the cell surface.

FIRST ASPECTS ON ACETATE METABOLISM IN THE YEAST *DEKKERA BRUXELLENISIS*: A FEW KEYS FOR IMPROVING ETHANOL FERMENTATION

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*Dekkera bruxellensis* is continuously changing its status in fermentation processes, ranging from a contaminant or spoiling yeast to a microorganism with potential to produce metabolites of biotechnological interest. In spite of that, several major aspects of its physiology are still poorly understood. As an acetogenic yeast, minimal oxygen concentrations are able to drive glucose assimilation to oxidative metabolism, in order to produce biomass and acetate, with consequent low yield in ethanol. In the present
study, we used disulfiram (DSF) to inhibit acetaldehyde dehydrogenase (ACDH) activity to evaluate the influence of cytosolic acetate on cell metabolism. *D. bruxellensis* was more tolerant to DSF than *Saccharomyces cerevisiae* and the use of different carbon sources revealed that the former yeast might be able to export acetate (or acetyl-CoA) from mitochondria to cytoplasm. Fermentation assays showed that ACDH inhibition re-oriented yeast central metabolism to increase ethanol production and decrease biomass formation. However, glucose uptake was reduced, which ultimately represents economical loss to the fermentation process. This might be the major challenge for future metabolic engineering enterprises on this yeast.

**NITRATE STIMULATES ETHANOL PRODUCTION BY THE YEAST DEKKERA BRUXELLENSIS IN THE ABSENCE OF OXYGEN**

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In the past few years, the yeast *Dekkera bruxellensis* has gained much of attention among the so-called non-conventional yeasts for its potential in the biotechnological scenario, especially in fermentative processes. Ever since we pointed out this yeast as an important competitor to *Saccharomyces cerevisiae* in bioethanol production plants in Brazil, and some works have reported its capacity to produce ethanol. However, most of that we know so far regards to its metabolism in aerobiosis, mainly because most of the wine and beer strains cannot grow if full anaerobiosis. Hence, the present work aimed to fulfil a gap regarding the lack of information on the physiology of *Dekkera bruxellensis* growing in the complete absence of oxygen and the relationship with assimilation of nitrate as nitrogen source. The ethanol strain GDB 248 was fully capable of growing anaerobically and produce ethanol at the same level of *S. cerevisiae*. The presence of nitrate in the medium increased this capacity. The profile of gene expression helped us to figure out that even in anaerobiosis the presence of nitrate driven the yeast cells to an oxidative metabolism that ultimately incremented both biomass and ethanol production. These results finally provided the clues we seek to explain most of the success of this yeast in industrial processes from ethanol production.

**BIO-PROTECTION IN WHITE WINEMAKING: CAN WE DO WITHOUT SULPHITES?**

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Modern oenology tends to decrease sulphites doses added from harvest to bottling. The maximum dose allowed was reduced by 10 mg/L in 2009. The organic specifications (Demeter, Nature & Progrès (France), National organic program (USA)) require...
very low sulphites doses, under 100 mg/L total SO$_2$. In addition, it is known that sulphites may have negative effects on people having allergies. Hence, alternatives to sulphites have been proposed including ascorbic acid, sorbic acid or inactivated yeasts enriched with glutathione, due to their antioxidant properties. DMDC and lysozyme are also used for their antimicrobial properties. However, none of these products demonstrate the same efficacy of action as sulphites. Bio-protection is another strategy, consisting in the addition of bacteria, yeasts or a mixture of microorganisms on grape must before fermentation in order to reduce the sulphite doses. However, there is a lack of scientific data capable of proving the effectiveness of adding these yeasts on grape must. This study reports for the first time the analysis of antimicrobial and antioxidant effects of one non-
-Saccharomyces yeast, Torulaspora delbrueckii, inoculated at the beginning of the white winemaking process in two wineries as an alternative to sulphiting. The implantation of the T. delbrueckii strain was successful in both wineries and had no impact on fermentation kinetics. Adding T. delbrueckii reduced biodiversity during the pre-fermentation stages compared to sulphited controls and it also effectively limited the development of spoilage microorganisms in the same way as the addition of sulphites. T. delbrueckii could protect must and wine from oxidation but this seems to be matrix dependent.

**BIOEMULSIFIERS PROPERTIES OF YEAST SPECIES STARMERELLA BOMBICOLA ISOLATED IN BRAZILIAN ECOSYSTEMS**

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Bioemulsifiers are surface active compounds with high molecular weight that in aqueous medium form stable emulsion. These compounds are proposed as an alternative to the use of synthetic emulsifiers because they generate a lower environmental impact. The yeast Starmerella bombicola is prominent in the production of sophorolipids, a biosurfactant widely studied and with extensive biotechnological applications. In this context, we propose the evaluation of bioemulsifier properties of sophorolipids produced by strains of Starmerella bombicola from Atlantic Forest and Cerrado ecosystems in Brazil. For the development of this work, seven strains of the S. bombicola were used. These yeasts were submitted to fermentation in medium containing 100 g/l glucose, 100 g/l glycerol and 1 g/l yeast extract at 30 °C with shaking at 200 rpm for 168h. The emulsifier activity was evaluated using the emulsification index and its stability tested for 7 days. The culture supernatants were tested using toluene, hexane and sunflower oil as substrates for sophorolipid production. All strains were capable of emulsifying the oil with sunflower rates ranging from 44-54% though not remained stable. In the hexane tests the UFMG-CM-Y5447, UFMG-CM-Y5458, UFMG-CM-Y5479 and UFMG-CM-Y6419 strains had indices above 60% and remained stable. Tests with toluene also highlighted the IPM 87.1 and PST 3 strains that obtained the emulsification indexes above 60% and remained stable at the end of the test. Then, UFMG-CM-Y5479 and UFMG-CM-Y6419 strains proved to be capable of emulsifying aromatic hydrocarbons and alkane hydrocarbons, and the UFMG-CM-Y5447 and UFMG-CM-Y5458 strains only alkane hydrocarbons. The next steps of this work include the purification, quantification and chemical elucidation of the bioemulsifier properties of the biosurfactants produced by these strains.
MODEL-BASED DESIGN OF CO-CULTURES OF S. CEREVISIAE AND NON-CEREVISIAE STRAINS IN WINE FERMENTATION

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The use of co-culture processes combining S. cerevisiae strains with non-conventional or non-Saccharomyces species has the potential to produce new market tailored wines. Still, the design of mixed culture fermentations requires investigating the ecological and metabolic interactions between the intervening species under different environmental conditions. In real practice, the alcohol-tolerant strains of S. cerevisiae dominate the final stages of fermentations. In this work, we aimed at using a model-based approach to explain ecological interactions at different processing conditions to subsequently design co-cultures in such a way that two species --S. cerevisiae wine strain T73 and S. kudriavzevii CR85—could coexist at final stages of the fermentation. An experimental plan to analyse the survival capacity of S. kudriavzevii during co-culture fermentation at different temperatures and inoculation conditions showed significant exclusion effects in most experimental conditions. To model such competition, we proposed an unstructured, modified Lotka-Volterra model, which accounts for the resources competition between species, and includes a non-linear density-dependent decay which accounts for various mechanisms contributing to the exclusion (production of ethanol or other toxic metabolites, cellular aggregation, etc) observed at high microbial densities. We selected a single step modelling approach in which we combined the modified Lotka-Volterra interaction model with the corresponding secondary models that describe the role of the temperature in both individual growth and yeasts interaction. We identified model parameters through multi-experiment data fitting using the AMIGO2 toolbox. We used a cross-validation approach to assess the predictive capabilities of the proposed model, and final results show that the model can reliably explain the data. The model was then embedded into a dynamic optimization approach to obtain the processing temperature and inoculation conditions to maximize final biomass. Results illustrate that sequential inoculation, i.e. late inoculation of S. cerevisiae, is the key to improve co-existence.

MOLECULAR AND BIOCHEMICAL ASPECTS OF BRETTANOMYCES FERMENTATION EMPLOYED IN BEER INDUSTRY

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Abstract ID: 267
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The fermentative process on brewing industry changed with the employment of single yeast strains. The use of single yeast strains allowed a better fermentative control process, and thus improving beer qualities. This way, the yeasts Saccharomyces cerevisiae and Saccharomyces pastorianus have been industrially used in beer fermentations of ale and lager beers, respectively. However, spontaneously fermented beers, like Lambic and Gueuze, contains distinct bacteria and wild yeasts species. In this sense, a semi-domesticated yeast genus which is crucial component of Lambic
beers, *Brettanomyces* (Dekkera), is gaining increasingly attention on brewing industry. *Brettanomyces* display *Saccharomyces*-like features, as Crabtree effect, ethanol synthesis and tolerance to harsh environments. On the other hand, its higher β-glucosidase and esterase activities, capacity to form phenolic compounds, tetrahidropyridines, as well as ferment dextrins and metabolize cellobiose from wooden casks had made it attractive for breweries. Although *Brettanomyces* spp. is recognized by its importance in some beer styles, its molecular and biochemical features for brewing are poorly understood until now. Therefore, this review aim to approach the current molecular knowledge and the biochemistry underlying the performance of *Brettanomyces* on brewing industry.

**FIRST REPORT OF OLEAGINOUS YEASTS IN COLOMBIA: LAKES AND MUNICIPAL WASTE IN CALI AS MICROBIAL SOURCES FOR LIPID PRODUCTION**

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Microbial oil for third generation biodiesel production is a promisor alternative as biofuel. Yeasts are considered as a good lipid source, with reports of accumulation even 70% of dry weight. Brazil reported many novelty oleaginous yeasts in last years, but other countries in Latin America made less efforts. In this sense, the objective of this work was to assess the capability of lipid production in two collections in Cali, Colombia. The first collection include yeast strains associated to five municipal wastes, and the second collection include yeast strains isolated from two artificial lakes at Universidad del Valle. Each yeast strain was grown in an inductor culture media (C/N 100:1) for 72h, 150rpm, 28°C. After, we extracted total lipids and determined the lipid content through gravimetric parameters (weight of lipids / dry weight), where values equal or higher than 20% were considered as oleaginous yeasts. From 25 strains, 24% presented the character oleaginous, reaching values between 34 and 59% of lipids related to dry weight, suggesting that these isolation sources have potential to find novelty industrial strains. Financial support: This Project was funded by Universidad Santiago de Cali (C.I. 934-621118-8).

**PRODUCTION OF KILLER TOXIN FROM SACCHAROMYCES EUBAYANUS USING AGRO-INDUSTRIAL WASTE AND ITS APPLICATION AGAINST WINE SPOILAGE YEASTS**

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Juicing industry generates amounts of waste that mostly lack commercial value and produce environmental pollution in the absence of waste treatment policies. On the other hand, microbiological spoilage is a major concern in wine industry, and control tools are limited. Taking these challenges into account, agro-industrial waste coming
from ultrafiltrated apple and pear juice (WUJ) were used to grow *Saccharomyces eubayanus* and to produce its killer toxin (SeKT). A Plackett–Burman screening was performed in order to optimize SeKT production in WUJ. The “optimized WUJ medium” was characterized: 75% v/v WUJ, 0.5% m/v KH₂PO₄, 0.5% m/v MgSO₄, 0.5% m/v (NH₄)SO₄, 0.5% g/L urea, 10% v/v glycerol and 0.1 % v/v Triton X-100. SeKT produced in WUJ “optimized medium” showed antagonistic activity against the beverage spoilage yeasts *Brettanomyces bruxellensis*, *Pichia guilliermondii*, *Pichia manshurica* and *Pichia membranifaciens*. Inhibition percentage against spoilage species in control medium was 37-57%. Different inhibition percentages against spoilage species in wine environment (49-69%) was detected and preserved at least for 48 h in wine. This work reports for the first time the ability of *S. eubayanus* to produce a killer toxin with potential use as a biocontrol tool in winemaking. Producing SeKT using agro-industrial wastes as alternative medium to cultivate *Saccharomyces eubayanus* would have industrial, economic and ecological benefits.

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**EFFECT OF DIFFERENT MEDIA ON PRODUCTION, OXIDATIVE STRESS AND BIOCONTROL EFFICACY OF VISHNIACOZYMA VICTORIAE**

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*Vishniacozyma victoriae* NPCC 1263 yeast biomass, with antagonistic properties against fungal pathogens of postharvest pear fruits, was produced in a previous work using a not optimized molasses-based medium (NOM) and an optimized medium (OM). The objective of this work was evaluated biomass productions, the stress oxidative tolerance and effectiveness of the control agent obtained from both mediums. Large scale biomass productions were carried out with both conditions NOM (12.8%v/v molasses, 1g/L urea, 20°C) and OM (9%v/v molasses, 0.25%p/vKH₂PO₄, 0.25%p/vZnSO₄, 0.25 ppm Tiamine and 1g/L urea, at 13°C in a bioreactor (20L). The kinetic parameters Y x/s and μ was calculated (0.24g/g and 0.043h⁻¹ in OM medium and 0.21g/g and 0.034h⁻¹ in NOM medium, respectively) evidencing an improvement in culture parameters with the OM. Cell viability, catalasa activity and intracellular ROS production following exposure of 30 mM H₂O₂ were evaluated in yeast obtained from two mediums. The highest percentages of viability (100%) was obtained in NOM, the ROS production increased 2-fold in NOM against 5-fold in OM. CAT activity was slightly diminished by H₂O₂ exposure in cells from OM (55%) and increased from NOM (65%). On the other hand, a highest intracellular content of Trehalose (160 mg/gdw) were obtained in cells from NOM compared with those cells from OM (87 mg/gdw). Finally, the biocontrol capacity of the two biomass preparations was evaluated in wounds in fruit after 6 months of storage. In all cases the yeasts grown in NOM presented better antagonism than those grown in OM. Our findings suggest that resistance to oxidative stress could represent a mechanism by which *V. victoriae* regulates their viability and biocontrol efficacy when develops in NOM.
OLEAGINOUS YEASTS GROWING ON MICROBREWORKY SPENT LIQUIDS

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Yeasts accumulating more than 20% of their dry weight in lipids are considered oleaginous. These lipids are mainly triacylglycerides and may serve in biodiesel production, animal and human nutrition, among others. Accumulation occurs under nutrient deprivation (e.g. N) and excess C. Finding low cost C sources to grow yeasts producing these valuable lipids is one of the challenges for feasible industrial productions. In this work, lipid production of five oleaginous yeast strains from Patagonia and Antarctica, was evaluated using microbrewery effluents as sole nutrient source. Yeasts were grown on YPD and then transferred to brewery spent liquid (5 brix boil remainings) and grown seven days at 20°C, 180 rpm. Subsequently, cells were used for biomass, lipid and carotenoid analyses. Supernatants were used for Chemical Oxygen Demand (COD), N, and P analyses. Also, remaining sugars were evaluated by HPLC (dextrins, maltotriose, maltose, glucose). All yeast strains (Guehomyces pullulans M425b, Holtermaniella festucosa CRUB1358, Dioszegia patagonica ANT99, Sporobolomyces ruberrimus CRUB1640 and Rhodotorula taiwanensis CRUB1425) produced 10 to 12 g/L of biomass, excepting R. taiwanensis (5.7 g/L). Nevertheless this yeast together with D. patagonica and S. ruberrimus were the best lipid producers (3.5, 4 and 6.3 g/L respectively). These three strains also produced carotenoids, ranging 150 to 300 µg/g. As for COD removal, H. festucosa removed 73% of initial COD, followed by S. ruberrimus (63%). In consistency, both strains left few remaining sugars. R. taiwanensis removed only 29% COD and was unable to use dextrins and maltotriose and only used less than half available maltose. Total Nitrogen was removed between 52 to 71% (G. pullulans) and total P 43 to 82% (S. ruberrimus). As a conclusion S. ruberrimus CRUB1640 shows potential for its use in lipid production using brewery effluents. Future analyses will include mixed strain cultures and different brewery spent liquids.

BIOSURFACTANT PRODUCTION BY WICKERHAMOMYCES ANOMALUS CCMA 0358

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In this work, biosurfactant production by Wickerhamomyces anomalous CCMA 0358 was increased through the development of an optimized culture medium using response surface methodology. The optimized culture medium contained yeast extract (4.64 g/L), ammonium sulfate (4.22 g/L), glucose (1.39 g/L) and olive oil (10 g/L). Biosurfactant production using this medium was validated both in flasks and bioreactor, and the surface tension was reduced from 49.0 mN/m up to 31.4 mN/m and 29.3 mN/m, respectively. In both cases, the highest biosurfactant production was achieved after 24 h of growth. W. anomalous CCMA 0358 demonstrated to be a fast biosurfactant producer
(24 h) as compared to other yeast strains previously reported (144–240 h). The produced biosurfactant remained stable at high temperature (121°C), NaCl concentrations as high as 300 g/L, and pH values between 6 and 12. The crude biosurfactant allowed the recovery of 20% of crude oil from contaminated sand, being a promising candidate for application in bioremediation or in the petroleum industry.

**PRODUCTION UNDER THE AOX1 PROMOTER IN METHANOL UTILIZATION NEGATIVE PICHIA PASTORIS: AN EFFICIENT EXPRESSION SYSTEM FOR LESS INTENSIVE FERMENTATION**

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The classical Pichia pastoris (syn Komagataella spp) fed-batch process, which is used for driving protein expression under the control of the AOX1 promoter (P_{AOX1}) exhibits several drawbacks. The consumption of methanol is accompanied by high heat generation and oxygen demand leading to extensive need for aeration and cooling in up-scaled processes. All of these issues have promoted the search for alternative simple processes that still allow the high productivities of the P_{AOX1}-system. The methanol utilization phenotype of P. pastoris is determined by the presence of its alcohol oxidase genes AOX1 and AOX2. Knock-out of one or both AOX genes leads to methanol utilization slow (mut⁺) and methanol utilization negative (mut⁻) phenotypes, respectively. Ideally, the use of mut⁻ strains would circumvent or lessen the problems associated with methanol utilization while still retaining the inducibility and high expression of P_{AOX1}. Thus, we tested mut⁻ strains for secreted protein production under the control of P_{AOX1}. In this scenario a non-hazardous carbohydrate carbon source is used for biomass generation while methanol is used for induction of P_{AOX1}. Screening data as well as fed batch bioreactor cultivations show that the mut⁻ strains are capable of producing secreted recombinant proteins. Protein production is achieved when the methanol induction is parallel to or in succession of the carbohydrate feed. In the latter case, protein production does not coincide with an observable biomass increase. This represents a specific case of growth independent protein production in P. pastoris.

**GENETIC ENGINEERING OF KOMAGATAELLA PHAFFII FOR XYLONIC ACID PRODUCTION**

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Lignocellulose derived sugars can be converted to different fuels and chemicals by microorganisms. Organic acids have many applications on industry and their production from cheaper bioprocesses is desirable. Xynolic acid is an organic acid that may be employed in food, chemical, and construction industries. Few microorganisms have been engineered to produce xylonic acid by overexpression of xylose dehydrogenases either from Caulobacter crescentus or Trichoderma reesei. The yeast Komagataella
Phaffii (Pichia pastoris) is a promising microorganism for production of xylonic acid as it is tolerant to low pH and able to grow at very high cell densities. On this work, new putative xylose dehydrogenase (XDH) genes from bacteria and fungi were identified by phylogenetic analysis. Than, ten of those were chosen for genetic engineering Komagataella phaffii. Recombinant strains expressing each gene were evaluated by the ability to produce xylonic acid and the best candidate genes were chosen for further analysis. The effects of co-substrates on xylonic acid production were evaluated on fermentations with xylose/glucose and xylose/glycerol mixtures. Strains were able to produce up to 36.2 g/L of xylonic acid from 40 g/L xylose, which accounts for a yield of 0.95 g/g, under the best evaluated conditions. Finally, strains capability to produce xylonic acid on sugarcane biomass hydrolysate was evaluated. Results will be presented and discussed.

BIOPROSPECTION OF YEASTS NATURALLY ABLE TO CONVERT XYLOSE FROM SUGARCANE BAGASSE HYDROLYSATE AND PRODUCE XYLITOL

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Xylitol is a five-carbon sugar-alcohol that is being studied because of its many applications in the food and pharmaceutical industries. This compound has similar taste to sucrose, and it is only industrially produced by chemical routes, by the catalytic hydrogenation of purified xylose. However, more economic and environmentally friendly production processes have been considered. In this context, conversion of the xylose, present in sugarcane biomass hydrolysates, to xylitol, employing microorganisms appears to be a good opportunity. The chosen microorganism should be able to keep high production yield and productivity even in presence of lignocellulosic derived inhibitors. This work aimed to select wild yeasts naturally able to produce xylitol from sugarcane bagasse derived sugars. For this, 960 yeast strains were isolated and screened by the ability to grow in minimal medium supplemented with xylose (40g/L) as a sole carbon source. Then, 42 the strains that showed the highest growth rate were cultivated on xylose-supplemented medium and the metabolite production profile analyzed by HPLC. Based on these results, the six strains that most consumed xylose were chosen for fermentative kinetic comparisons in bench bioreactor, on defined medium and on sugarcane bagasse hydrolysate. Yeasts consumed all the xylose provided, with yields higher than Y = 0,83 g/g, in hydrolysates. Finally, the fermentation optimization and effects of the aeration, supplementation, and inhibitors in sugarcane bagasse hydrolysate on xylitol production will be discussed.
EVALUATION OF XYLITOL PRODUCTION BY STRAINS OF CYBERLINDNERA XYLOSILYTICA AND WICKERHAMOMYCES RABAULENSIS FROM SUGARCANE BAGASSE HEMICELLULOSIC HYDROLYSATE

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In Brazil, the sugarcane processing generates tons of sugarcane bagasse every year. It may be hydrolyzed to release the sugars from cellulosic and hemicellulosic fractions, mainly glucose and xylose, respectively. These carbohydrates can be converted into products of biotechnological interest by yeasts, such as xylitol (a natural sweetener). On the other hand, xylitol accumulation in yeasts depends on factors, which includes nutritional supplements, temperature, pH, inoculum size, substrate, and aeration. In this context, the present work aimed to evaluate some of these variables (nutrients, initial cell concentration and aeration) to obtain xylitol from sugarcane bagasse hemicellulosic hydrolysate (SBHH) by new xylitol producers strains of the species Cyberlindnera xylosilytica (05 strains) and Wickerhamomyces rabaulensis (11 strains) isolated from Brazilian ecosystems. Therefore, a screening for xylitol production was performed with these yeasts in YPX medium (60 g L⁻¹ D-xylose, 20 g L⁻¹ peptone, 10 g L⁻¹ yeast extract). C. xylosilytica (UFMG-CM-Y409 and UFMG-CM-Y309) and W. rabaulensis (UFMG-CM-Y3716 and UFMG-CM-Y3747) showed the higher amounts of xylitol. Then, the preselected strains were cultivated in SBHH, obtained by dilute acid treatment and supplemented with ammonium sulfate, rice bran extract, and yeast extract based on 2³ full factorial design. C. xylosilytica UFMG-CM-Y-409 achieved the highest xylitol titers (14.06 g L⁻¹), xylitol yield (Yxylp/s = 0.63gg⁻¹), and xylitol productivity (Qxylp = 0.20 g L⁻¹h⁻¹) utilizing the maximum level (10 g L⁻¹) of rice bran, and the minimum level (1.0 g L⁻¹) of yeast extract. In the other case, the xylitol production (22.13 g L⁻¹) was improved by the use of a lower initial cell concentration (1.0 g L⁻¹) and oxygen availability according to a 2² full factorial design analysis. These results suggest that C. xylosilytica UFMG-CM-Y-409 has a potential to be used in further biotechnological applications for xylitol production.

PHYSIOLOGICAL AND GENETIC CHARACTERIZATION OF NEWLY XYLITOL PRODUCING-YEAST STRAINS ISOLATED FROM CERRADO BIOME

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Lignocellulosic biomass, as sugarcane bagasse and straw, is a renewable raw material that could be used to produce high value-added chemicals and fuels, such as ethanol, glycolic acid, succinic acid and xylitol. Xylitol is a five-carbon polyol of economic interest in dental, pharmaceutical and food industries that can be produced by microbial xylose reduction. In this way, yeast fermentation can be an interesting alternative to replace the chemical process to produce xylitol; however high yields and productivity are
necessary to lower production costs. Considering this context, we herein investigated the potential of two newly yeast strains isolated from Brazilian Cerrado biome to produce this compound. Xylose conversion capacity by the new strains *Spathaspora* sp. JA1 and *Meyerozyma* sp. JA9 was evaluated and compared with control strains using xylose or sugarcane biomass hydrolysate in the fermentation medium. Among the tested strains, *Spathaspora* sp. JA1 was the best xylitol producer, reaching product yield as high as 0.74 g/g, which is a value similar to that obtained with the best xylitol producers described until now. Activities of enzymes related to the first steps of xylose metabolism and the hydrolysate detoxification process was determined in these yeasts. Moreover, the complete genome sequences of *Spathaspora* sp. JA1 and *Meyerozyma* sp. JA9 were obtained and annotated. Comparative genomic analysis was performed to clarify some aspects of yeast physiology.

**IDENTIFICATION AND FUNCTIONAL ANALYSIS OF NOVEL ENDOGENOUS SIGNAL PEPTIDES FOR KOMAGATAELLA PHAFFII**

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*Pichia pastoris*, reclassified as *Komagataella* sp, is considered an excellent platform for protein production. More than 600 proteins have been expressed using the *Komagataella* expression system. Most expression vectors use *Saccharomyces cerevisiae* α-factor pre-pro sequence as a secretion signal, which provides excellent secretion levels, but can be improperly processed in hyperexpression conditions. Using a native *Komagataella* sp secretion signal should overcome this problem, maintaining the right structure of the heterologous protein. *In silico* analysis identified thirteen secretion signals from the *Komagataella phaffii* secretome. These sequences and the putative α-factor of *K. phaffii* were cloned into pPIC9 carrying *Bacillus subtilis* α-amylase (*amyE*) as a reporter gene. All of them except PS9 showed a hydrolytic halo. Secretion levels were similar or superior to that of *S. cerevisiae* α-factor. All analyzed clones had only one copy of *amyE*. Amylases from four clones were purified for subsequent N-terminal sequencing. These new signals can be used as an alternative to the *S. cerevisiae* α-factor, mainly when more specific N-terminal processing is required or for better levels of secreted protein.

**UTILIZATION OF METABOLIC FLUX ANALYSIS FOR METABOLOMME DATA VALIDATION OF XYLOSE-FERMENTING YEASTS**

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Metabolic flux analysis (MFA) is used to understand how fluxes are distributed in a metabolic network given a certain substrate. It can predict growth and product
distribution based on the stoichiometric reactions within a given network by addition of measured fluxes and production rates as constraints in the mathematical model. In this study, a stoichiometric model was developed using xylose fermentation data for the yeasts *Scheffersomyces stipitis*, *Spathaspora arborariae*, and *Spathaspora passalidarum*. Those models were utilized for the first time to validate the quantification of eleven intracellular metabolites within xylose and glucose catabolic pathways. Within the investigated metabolic network, eleven fluxes rates were calculated using the metabolomics data. Among them, 80% of total metabolites were validated with a correlation above 90% when compared to the stoichiometric model. Thus confirming that MFA can be utilized for metabolome data validation. Among the measured intracellular metabolites, fructose-6-phosphate, glucose-6-phosphate, malate, and ribulose-5-phosphate were validated in all studied yeasts. Nevertheless for the metabolites phosphoenolpyruvate and pyruvate the measured concentrations could not be correlated the predicted ones. Finally, it was possible to compare metabolism within the three different xylose-fermenting yeasts showing that xylose metabolism occurs at higher fluxes rate in *S. stipitis* than *S. passalidarum* and *S. arborariae*. The fluxes rate is divided similarly between pentose phosphate pathway and glycolysis. *S. arborariae* presents 3.0 times higher demand for NADPH regeneration than observed in *S. passalidarum*. The flux rate to glycerol formation in *S. passalidarum* is inactive and this yeast looks like occur a better NADH/NAD⁺ balance, which permits efficient xylose fermentation.

**BREWING WITH THE MOTHER OF THE LAGER YEAST IN PATAGONIA**

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**Type: e-Poster, Session 6**

*Saccharomyces eubayanus* is a criotolerant yeast native to Andean Patagonia. It was formally described in 2011. Genetic studies confirmed that hybridization between this species and *S. cerevisiae* (Ale yeast) gave rise to *S. pastorianus* (Lager yeast, responsible for 96% of beer production worldwide). This discovery sparked interest in the scientific-technological sector due to the potential application of wild parental yeast as a starter culture in the brewing industry. From our laboratory we began to work to know its fermentative and organoleptic characteristics and to develop the starter cultures that could be transferred to the breweries. This labor forged a relationship between the scientific and the productive sector: prepare the industry to work with yeasts in liquid format (most of the craft breweries of Argentina only worked at that time with dry yeast), teach courses and instruct on the management and repitching of brewer’s yeasts, train on contaminant detection and perform controls in breweries. Experiments were started with brewers on a semi-pilot scale (20-50 Lts) with *S. eubayanus* to learn its behavior outside the laboratory. In May 2017, the first 100% Argentine beer was launched in Bariloche, where 5 breweries in the area presented their own styles made with the wild yeast. In conjunction with a local craft brewery, the low-alcohol beer “Wild Lager Sauvage” was presented at the Ironman 70.3 Bariloche. Currently, through an agreement with Bariloche’s local breweries association (ACAB), several beers are being made and commercialized with the Patagonian yeast at starting volumes ranging 150 to 1500 Lts. The “Proyecto Patagonia Salvaje” (Wild Patagonia Project) intends to create, in cooperation with local craft brewers, experimental beers employing *S. eubayanus* yeast, and local ingredients allowing to create new styles of
beer with the Patagonian identity, resulting in greater added value and a consequent improvement of competitiveness for the sector.

GENETIC-PHYSIOLOGICAL VARIABILITY WITHIN THE SPECIES DEKKERA BRUXELLENSESIS AND ITS POTENTIAL OF INDUSTRIAL APPLICATION

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Dekkera bruxellensis has been considered for long time a spoiler yeast in industrial processes such as winemaking, brewing and fuel-ethanol production. However, recent growing evidences in the literature are expanding this status by highlighting its biotechnological potential. In this work, we surveyed 29 isolates of D. bruxellensis from distinct industrial processes (winemaking and fuel-ethanol) for assimilation and fermentation of disaccharides of industrial relevance (sucrose, maltose, cellobiose and lactose), and their constituent monosaccharides. Isolates from both niches displayed preference for glucose, fructose, sucrose and maltose. And all C- sources were more assimilated by fuel-ethanol isolates than by wine isolates, except galactose. On the other hand, lactose was poorly assimilated by any of the strains, except for some isolates from fuel-ethanol. Wine isolates were more responsive for Glucose Catabolite Repression (GCR) than fuel-ethanol isolates. When respiration is impaired, the isolates were able to ferment glucose, sucrose and cellobiose without supplementation of casamino acids or yeast extract in the culture medium. The phenotypic diversity found within this species can contribute to explain its adaptation to the different industrial environments in which these carbon sources are present. Possible application of the GCR-insensitive isolates in processes that requires co-assimilation of different sugars is discussed.
SESSION 3:
NATURAL VARIATION
AND APPLIED OPPORTUNITIES
THE GENOMIC MAKING OF YEAST BIODIVERSITY

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Type: Main Speaker

The subphylum Saccharomycotina evolved nearly half-a-billion years ago and has radiated into more than 1000 species of budding yeasts. Here we report the preliminary results of a large-scale project sequencing and analyzing the genomes of all known species of the subphylum Saccharomycotina (y1000plus.org). We find that budding yeasts possess different trait syndromes or constellations of traits that co-vary. These trait correlations are often caused by overlapping and interacting genetic pathways. By reconstructing metabolic trait evolution across a phylogeny of 332 species, we infer that the dominant mode of yeast evolution has been one of loss, including the repeated losses of many carbon source utilization pathways and the loss of most secondary metabolism gene clusters. In some cases, gene losses have been counteracted by horizontal gene transfer. One particularly striking event involved the horizontal transfer of a complete 7-gene bacterial operon encoding a secondary metabolite into a clade of yeasts approximately 300 million years divergent from S. cerevisiae. We have also used phylogenomic footprinting and reverse genetics to characterize the evolution and function of the first known budding yeast secondary metabolite gene cluster, a pathway absent in most extant yeast species. We conclude that the budding yeast common ancestor was metabolically complex and that a full characterization of yeast traits and potential applications will require detailed study of a broad array of yeast biodiversity.
FUNCTIONAL CHARACTERIZATION OF HORIZONTALLY ACQUIRED GENES IN YEASTS USING TRANSCRIPTIONAL-TRANSLATIONAL REPORTERS AND OPTOGENETIC CONTROL

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The use of yeast as eukaryotic model organism and its wide biotechnological applications have fuelled the genome sequencing of numerous yeast strains. The extensive amount of sequencing information available has revealed, in different strains, unique genomic features acquired by Horizontal Gene Transfer (HGT). Additionally, the genes acquired by HGT have shown to play important roles in yeast adaptation to the fermentation process, improving nitrogen and carbon sources utilization. However, the functional characterization of these genes at the molecular level remains poorly attended. In this work, we performed a functional analysis of genes contained in three regions horizontally acquired, commonly known as regions A, B and C. In three different wild yeast strains, we used the luciferase reporter gene and the mCherry fluorescent protein to monitor the transcriptional and translational activity of those genes, respectively. Additionally, we overexpressed and repressed horizontally acquired genes utilizing a recently described optogenetic switch named FUN-LOV, which allows light-controlled gene expression in yeast. The results showed that depending on the growth condition, the light-induced overexpression of horizontally acquired genes can increase or reduce yeast adaptation (fitness), as measured through growth parameters (lag time, growth rate and efficiency). Similarly, the darkness-activated repression of horizontally acquired genes showed a decrease in yeast fitness, depending also on the growth condition assayed. Altogether, our results revealed molecular evidence on the contribution of horizontally acquired genes to yeast adaptation in specific culture conditions.

CONTRIBUTION OF LARGE-SCALE CHROMOSOMAL STRUCTURAL VARIATIONS TO SACCHAROMYCES CEREVISIAE GENETIC AND PHENOTYPIC DIVERSITY

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There are mounting evidences that Structural Variations (SVs) of chromosomes play a major role in phenotypic variation. However, these genetic variants are the most
difficult to identify and to interpret with respect to their functional consequences. We undertook a comprehensive mapping of natural SVs in *S. cerevisiae* using the long-read single molecule sequencing technology from Oxford Nanopore. We generated high quality end-to-end genome assemblies for a large cohort of strains selected to maximize the sampling of the species genetic diversity. We catalogued a large number of natural copy number variations and balanced rearrangements. In addition, in order to unequivocally determine the SVs contribution to phenotype variations, independently from the confounding effect of SNPs, we recapitulated targeted SVs at a base pair resolution in a controlled genetic background by CRISPR/Cas9-assisted genome editing. In parallel, we developed a powerful methodology of chromosome shuffling allowing to efficiently generating large libraries of random SVs through the concomitant induction of multiple Double Strand Breaks in repeated regions of the genome. Phenotyping of strain libraries derived from both targeted and random SVs in a wide range of environmental conditions revealed the fitness impact of SVs and their contribution to adaptation.

**A HIGH-THROUGHPUT YEAST ASSAY TO TEST THE ACTIVITY OF CYP2C9 VARIANTS**

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The field of pharmacogenomics is currently overwhelmed by the huge amount of genetic variation being discovered by new sequencing efforts. One key limitation is the lack of corresponding functional annotation of these gene variants that would allow the field to link them to clinically actionable drug interactions. We are addressing this problem in a particularly important pharmacogene: *CYP2C9*. *CYP2C9* encodes an enzyme responsible for metabolizing many different drugs including warfarin, a widely-prescribed oral anticoagulant with a narrow therapeutic window. Efforts to comprehensively characterize *CYP2C9* and other pharmacogene variants have been hindered by the low-throughput nature of classic biochemical assays. Instead, we have developed a yeast-based activity assay that can test variants at high-throughput in a pooled manner. This assay, which uses activity-based protein profiling, is able to recapitulate the activity of known variants in both individual and pooled tests. Briefly, yeast cells expressing a single *CYP2C9* variant are bound in an activity-dependent manner by a modified *CYP2C9* inhibitor and are then labeled with a fluorophore for cell sorting and sequencing. Key improvements to the assay came from yeast strain background engineering. This included yeast humanization by adding other human metabolism enzymes, screening different yeast strain backgrounds, and making targeted strain background modifications. We are in the process of testing a library of all 9,800 single amino acid variants of *CYP2C9* with our yeast-based assay. We will use this data to classify unknown variants and ultimately create a sequence-function map of *CYP2C9* variants. Our approach will lead to advances in adverse drug response prevention by providing *CYP2C9* clinical guidance for patients carrying both currently known and yet-to-be discovered alleles.
**ABSENCE OF SUBSTRATE ACTIVATION IN STARMERELLA BACILLARIS (SYN. CANDIDA ZEMPLININA) PYRUVATE DECARBOXYLASE**

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Pyruvate decarboxylase (PDC) (EC4.1.1.1) catalyze the non-oxidative decarboxylation of pyruvate, yielding acetaldehyde and carbon dioxide. PDC plays an essential role in...
the fermentation pathway ethanol production. The yeast *S. cerevisiae* has three active PDC isoenzymes (PDC1, PDC5 and PDC6) which, similar to PDCs from other yeasts, exhibit substrate activation. This work presents the first characterization of PDC in *Starmerella bacillaris* (SbPDC), a fructophilic yeast species frequently isolated from grape and wine ecosystems. PDC activity was readily detected in cell lysates from indigenous *S. bacillaris* isolates obtained from spontaneously fermenting must of Malbec (*V. vinifera*) and Isabella (*V. labrusca*) grapes. Similar optimum both pH and temperature were observed for PDCs from *S. bacillaris* and *S. cerevisiae*. However, steady state measurements of SbPDC activity showed that this enzyme does not present substrate activation but a Michaelis-Menten kinetic profile. *In silico* analyses of the recently available genome sequence of *S. bacillaris* showed the presence of a single gene encoding PDC (SbPDC). This gene has higher homology with *S. cerevisiae* PDC6 than with the PDC1 or PDC5 isoenzymes. Further characterization of the conceptually translated amino acid sequence of the SbPDC gene reveals that critical amino acids recognized at the catalytic site of *S. cerevisiae* PDCs are conserved. However, C221, H92, H225, critical residues at the regulatory site of PDCs, mediating substrate activation, are absent from the *S. bacillars* PDC enzyme. The last results are consistent with the observed kinetic behaviour of the SbPDC enzyme.

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**GENE EXPRESSION PROFILING OF SACCHAROMYCES EUBAYANUS STRAINS EXHIBITING DIFFERENCES IN VOLATILE COMPOUND PRODUCTION**

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Type: e-Poster, Session 5

Understanding the genetic and physiological diversity in natural populations is critical to untangle the genotype-phenotype relationship and identify heritable variations underlying adaptation. This is particularly important for biotechnologically relevant organisms such as *Saccharomyces eubayanus* where genomic exploration can foster the discovery of profitable biological activities. Until now only a handful of *S. eubayanus* strains have been found, primarily in Argentina and to a lesser extent in the northern hemisphere, and therefore we know relatively little about the biological and phenotypic diversity in this species. Recently, we have isolated a large set of strains in Chile and characterized their differences in terms of volatile compound production under lager beer fermentation conditions. A principal component analysis allowed us to identify two strains – LGMUSC450 (isolated in Coyhaique) and LGMUSC216 (isolated in Villarrica) – representing strains with divergent fermentation and aroma profiles. To determine the genetics and molecular mechanisms underlying these phenotypic differences, we obtained whole genome sequences combining Illumina technology with long-read sequencing from Oxford Nanopore and generated end-to-end assemblies in these two species. Moreover, we characterized their gene expression profile utilizing RNA-seq and identified a series of genes differentially expressed between both strains, providing insights into the genetics underlying physiological differences between strains. This analysis allowed us to characterize the natural genetic and physiological biodiversity of *S. eubayanus* populations in the region, thereby providing a deeper understanding of their molecular response to lager fermentation conditions.
STUDYING GENOMIC REGIONS INVOLVED IN TORC1 SIGNALING PATHWAY ACTIVATION IN SACCHAROMYCES CEREVISIAE

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Type: e-Poster, session 5

Saccharomyces cerevisiae is the main species responsible for the alcoholic fermentation in the production wine, being one of the main problems the deficiency of nitrogen in the must, which can lead to stuck or sluggish fermentations. A major challenge is to identify the genetic basis underlying the phenotypic variability in nitrogen consumption and metabolism, with emphasis on the study of TORC1 signaling pathway, given its central role in responding to nitrogen availability and influencing growth and cell metabolism. However, the mechanism by which different nitrogen sources activates TORC1 is not completely understood, with the study of allelic diversity appearing as an alternative to identify genes involved in this process. Using a recently developed microculture method, which uses the luciferase gene as a reporter, representative strains of clean lineages described in S. cerevisiae (North American ‘NA’, Sake ‘SA’, West African ‘WA’ y Wine/European ‘WE’) were phenotyped. Among them, strains SA and WE showed the greatest phenotypic differences. Subsequently, a recombinant population composed of 96 segregants derived from these two strains was phenotyped. The phenotypic data obtained were used to carry out a linkage analysis, from which several genomic regions involved in the phenotype under study were obtained, particularly in chromosomes II, VII and XI. Further analysis of these regions will allow obtaining candidate genes, which will be validated by reciprocal hemizygosity analysis.

COMPARATIVE GENOMICS OF ASTAXANTHIN-PRODUCING YEASTS

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The basidiomycetous yeast Phaffia rhodozyma (synonym Xanthophyllomyces dendrorhous) possess a set of unique characteristics of outstanding scientific interest and technological value. P. rhodozyma is the only astaxanthinogenic yeast known, and this carotenoid pigment is considered one of the most potent free reactive oxygen species scavenger. P. rhodozyma has additional photoprotective strategies such as the accumulation of mycosporine-glutaminol-glucoside, a UVB-screening compound that also has antioxidant properties. Many genetically distinct natural populations of Phaffia spp. are known worldwide, but most of the diversity is found in the Southern Hemisphere, mainly in Australasia, whereas Holarctic populations are mostly genetically uniform. The aim of this work was to evaluate the presence of genes related to photoprotection and antioxidant activities in Phaffia spp. isolates from different geographical origins. Eight Phaffia spp. genomes from South America, New Zealand,
Japan, Northern Europe, Australia and Tasmania were studied. An alignment-free method (Kr) retrieved divergence values of 0.00076 - 0.0081 between Holarctic strains, but 0.089 between Australasia strains and 0.06 - 0.084 between South America and Australasia strains. Genes involved in the synthesis of astaxanthin (idi, CrtE, FPS, crtI, crtYB, crtR, crtS/Ast, Mig1), mycosporine-glutaminol-glucoside (EEVS-like, O-methyl, ATP-grasp) and ROS scavenging enzymes (SOD1 and 2, Catalase 1, 2 and 3) were identified in the genome assemblies by genBlastG. The sixteen genes analyzed were identified in every genome of *Phaffia* spp. with eval = 0, except for CBS6938 that lacks of MGG genes. The predicted proteins were aligned with Blat to the genome reference CBS7918 and visualized with IGV 2.4.10. Multiple alignments were produced using MUSCLE and protein domains were confirmed with PFAM. Our results indicate that the *Phaffia* spp. genome is enriched in antioxidant mechanisms and that these mechanisms are conserved through *Phaffia* spp. from different geographical origins.
SESSION 4:
BIOPROSPECTION OF
EXTREMOPHILIC YEASTS
GREENLAND BLACK BLOOM FUNGI

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Abstract ID: 350
Type: Main Speaker

Research has recently highlighted the importance of ice-algal blooms in driving the darkening of the Greenland Ice Sheet (GrIS) and acceleration of its melting; however very little is known regarding the interactions between ice algae and other supraglacial microbial communities. Almost nothing is known about fungi proliferating on the surface of the GrIS. To fill this significant knowledge gap, both yeasts and filamentous fungi were isolated from black ice characterized by a dense bloom of non-cultivable ice algae Mesotaenium berggrenii and Ancylonema nordenskioeldii, from clear ice with little visible algal growth, cryoconite holes on the surface of the ice sheet, meltwater and snow cover. Isolations were performed from the South-West part of the GrIS and a combination of both in-situ and ex-situ incubation experiments were performed to characterise fungal-algal-communities and interactions taking place within the GrIS surface. In-situ incubations were performed during the 2017 ablation season at the ice camp located ~35 km from the GrIS margin. The analysis of diversity was performed both with cultivation methods and with next generation amplicon sequencing of taxonomic markers. Both cultivation experiments and fungal NGS analysis revealed a possible interaction between novel fungal species isolated from ice samples with high algal biomass and the ice algae. Although no major impact of fungal addition on ice algal physiology was apparent, short incubations indicated that fungal-algal interactions within the surface ice were beneficial and not detrimental for the ice algae. Analyses of inorganic and organic nutrients, major ions, algal abundance, ergosterol quantification, secondary metabolites, and scanning electron microscopy were performed to clarify the potential role of the fungi. Here we present the first findings on fungal/yeast biodiversity on the GrIS, suggesting their importance for the development of ice algal bloom and the consequent darkening of the GrIS surface.
CHARACTERIZATION OF HALOTOLERANT AND HALOPHILIC YEASTS ISOLATED FROM DANISH CHEESE BRINES

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The surface microbiota of cheese is highly influenced by the growth of yeasts, especially Debaryomyces hansenii. Yeasts de-acidify the cheese surface thereby enhancing the growth of smear bacteria. Further, yeasts contribute to the ripening by aroma compound formation. Surface-ripening cultures or old-young smearing are applied for surface inoculation of un-ripened cheeses, however, cheese brines could also serve as a key source with a range of halotolerant and/or halophilic yeasts. The aim of this study was to isolate, identify and characterise the technological properties of halotolerant and halophilic yeasts isolated from brines used in Danbo cheese production. Yeasts in brines from two dairies were enumerated on MYGP agar added 0.85%, 4.0%, 8.0% and 16.0% NaCl (w/v). Large variations in the yeast counts were observed among the brine samples, even within the same dairies. The highest total yeast count was 8.60 x 10⁴ CFU/ml, obtained on MYGP added 4.0% and 8.0% (w/v) NaCl. D. hansenii, Kluyveromyces lactis, Yamadazyma triangularis, Candida intermedia and Sterigmatomyces halophilus were predominant as identified by 26S rRNA gene sequencing. Yeast isolates representing the identified species were, by spot tests, further investigated for growth at different combinations of [NaCl], pH and temperature. All yeast isolates were able to grow at 0.85-10.0% (w/v) NaCl and the majority at 15.0% (w/v). Further, isolates of D. hansenii, Y. triangularis, and S. halophilus were all able to grow under conditions similar to cheese brines, i.e. 20.0% NaCl (w/v), pH 5-6, 16°C. The results indicate that cheese brines harbour a wide range of halotolerant and halophilic yeasts highly capable of growing under conditions mimicking cheese brines and -surfaces. The importance of these yeasts, especially establishment on the surface of un-ripened cheeses and potential mould inhibition, needs to be studied further.

UNDERSTANDING THE BIOCONTROL ACTIVITY OF ANTARCTIC DEBARYOMYCES HANSENII YEASTS AGAINST THE FRUIT PATHOGEN PENICILLIUM EXPANSUM

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The employment of low temperatures during postharvest storage is the main strategy to reduce losses and maintain fruit quality for several months. However, low temperature, cannot avoid the development of fungal pathogens, so synthetic fungicides are normally applied to reduce fruit decay. The consumers´ concern about harmful effects of some pesticides in human and animal health has led to the development of different alternatives. Biological control of pathogens using microbial antagonists appears as a potential alternative. In this work, 19 psychrotrophic yeasts isolated from
King George island (sub-Antarctic region) and identified as *Debaryomyces hansenii* were evaluated as biocontrol agents against *Penicillium expansum* in Red Delicious and Granny Smith apples and Williams pears stored at 0-1°C. In Red Delicious apples, all the strains significantly reduced disease incidence relative to the control, while in Granny Smith apples 9 strains resulted effective biocontrol agents. In pears, *P. expansum* could be effectively controlled only by 3 strains. These results showed that antagonistic activity was fruit and strain dependent. Different mechanisms have been proposed to be responsible of biocontrol activity by microbial antagonists. Production of soluble and volatile antifungal compounds, siderophores and chitinases, biofilm formation and inhibition of spore germination by the yeast strains of our collection were evaluated. The results showed that there is not a significant correlation between any of the activities evaluated and the biocontrol capacity, leading to the hypothesis that in this case the biocontrol activity could be related to the ability of yeasts to grow and colonize fruit wound at low temperatures. Growth curves of yeasts and pathogen in fruit wounds at 0-1°C obtained by viable cell count in case of yeast and by RealTime PCR for the pathogen, confirmed the hypothesis. The interaction between antagonist and pathogen in fruit wounds was also observed using Scan Electronic Microscopy (SEM).

**NOVEL BIODEGRADATION OF VANILLIN BY A WOOD-INHABITING ISOLATE OF CYSTOBASIDIUM SP.**

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*Cytopasidium* species are mostly found in temperate or cold environments. They have been isolated from a wide range of habitats: plants, soils, rocks, aquatic environments and indoor dust. The genus *Cytopasidium* mainly consists of species of red yeasts in the *Rhodotorula minuta* clade. These basidiomycetous yeast species are commonly found in temperate to cold regions. In the present study, two strains of *Cytopasidium sp.* were isolated from decaying wood of housing on the Faroe Islands, where the average yearly temperature ranges from 2°C to 13°C. The sequences of the two strains had two identical gaps within the ITS1-5.8S-ITS2 region and a second gap within the D1/D2 LSU unit, when aligned to those of *C. laryngis* CBS 2221, their closest match. The isolates were designated as *Cytopasidium sp.* Both isolates converted vanillin into vanillyl alcohol in the presence of oxygen. The biotransformation of vanillin into vanillyl alcohol has been documented for only a few species of fungi, but to our knowledge, it has not previously been reported for any basidiomycetous yeast species. *Rhodotorula rubra*, a distantly related basidiomycetous yeast converts vanillin into vanillic acid. In the present study, the two isolates of *Cytopasidium sp.* did not produce any trace of vanillic acid, as determined by LC-MS, 1H-NMR and GC. Oxidizing vanillin into vanillic acid should be preferred by the fungi, since it results in more chemical energy, as compared to reducing it to vanillyl alcohol. The fungus may choose this pathway to escape the toxicity of both vanillin and vanillic acid. Vanillin has antimicrobial activity, and vanillic acid is more toxic than vanillyl alcohol. Vanillin is a constituent of the lignin molecule. *Cytopasidium* species are commonly found in the phyllosphere. Their ability to utilize plant chemicals should render them successful competitors on plants and wood.
DEVELOPMENT OF *PICHIA PASTORIS (KOMAGATAELLA PHAFFII)* AS A PLATFORM FOR LACTIC ACID PRODUCTION USING CRUDE GLYCEROL AS SUBSTRATE

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Type: e-Poster, Session 1

Actic acid is the monomeric unit of polylactic (PLA), which generates biodegradable, biocompatible and bioabsorbable material for the cosmetics, medical and pharmaceutical industries. This organic acid has a market expected to reach USD 3.82 billion by 2020. Among several substrates utilized for industrial production of lactic acid, crude glycerol is the main residue during biodiesel production and last year; Brazil produced about 429,129.4 million cubic meters of this carbon source. Many microorganisms that can use glycerol as a carbon source but are inhibited by the other molecules that remain from the transesterification reaction during biodiesel synthesis. Nevertheless, our group has previously shown that the yeast *Komagataella phaffii* can utilize crude glycerol as carbon source without reducing growth rate when compared to glycerol P.A. Besides, *K. phaffii* is well known for the production of recombinant protein due to its ability to grow at high cell densities. However, this yeast is not able to naturally produce lactic acid. In a previous study, lactic acid production was demonstrated in *K. phaffii* by the introduction of a lactate dehydrogenase although with a yield of only about 40% of the theoretical maximum. Thus, this study aimed at increasing the pyruvate flow towards lactic acid production. For that, the gene encoding pyruvate decarboxylase (PDC) was deleted resulting in a yield of 65% of theoretical maximum. Nevertheless, in this new yeast strain, arabitol production increased about seven times being the major co-product during lactic acid synthesis. Thus, efforts turned into disruption of genes involved in the arabitol synthesis. The novel strain was compared in batch fermentations with the previously constructed. All the genetic modifications effects will be discussed in light of the yeast redox balance and product distribution.

LIPASE FROM ANTARCTIC YEASTS *GUEHOMYCES PULLULANS* FOR BIODIESEL PRODUCTION

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Type: e-Poster, Session 5

A need in biorefinery for biodiesel production is to find novel enzymes capable to get an affordable and efficient transesterification of triacylglycerides into methyl/ethyl esters replacing chemical processes. Psychrotolerant yeasts are known for its ability to excrete a large repertoire of enzymes, including lipases. In the search of novel enzymes for future industrial applications, we have screened 110 isolates of Antarctic yeasts collected in 2012 directly from glacier’s ice during an expedition in the Greenwich Island (Ecuador`s scientific station). Amongst the yeast producing lipases *Guehomyces pullulans* strain (CLQCA-ANT-073) showed the highest lipase activity. The induction medium for lipases was inoculated with *Guehomyces pullulans* strain and incubated at 20°C and 200 rpm for 40 days. About 268 U/L of enzyme was obtained. A of 38 kDa protein showing lipase activity was characterized by SDS-PAGE. The enzyme´s kinetics
showed optimal conditions for catalysis at pH 8.0 and temperature 40°C. The $K_M$ obtained was $3.7 \times 10^{-4} \text{M}$. These characteristics place the isolate of *Guehomyces pullulans* (CLQCA-ANT-073) as a promising cell factory for the application in future biodiesel biorefineries.
SESSION 5: EVOLUTIONARY GENOMICS AND DOMESTICATION OF YEASTS
A POPULATION GENOMICS PERSPECTIVE ON THE MULTIPLE DOMESTICATION PATHS IN SACCHAROMYCES CEREVISIAE

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Saccharomyces cerevisiae is used since the dawn of civilization to ferment a myriad of foods and beverages that range from more familiar and globally dispersed products such as wine, beer and bread to less well-known and more locally-consumed beverages. Although it is known that S. cerevisiae underwent a domestication process equivalent to those already known for plant crops and livestock, a detailed understanding of the genetic causes and phenotypic consequences of the transition from wild to domesticate has not yet been achieved. Nevertheless, several evolutionary events ranging from hybridization, chromosomal rearrangements, gene gain and loss, and horizontal gene transfers, have already been associated with these changes. Adding to the complexity of the mechanisms that promoted the genomic and phenotypic changes associated with yeast domestication, a lack of a detailed understanding of the ecological patterns of S. cerevisiae in the wild, especially its paradoxical occurrence in low-sugar natural environments like oak bark, further complicates the studies of yeast domestication. The picture emerging from population, comparative and functional genomics suggests a complex scenario with multiple transitions from wild to domesticate associated with different regions and/or different types of fermentations. Moreover, in some instances, primarily domesticated lineages appear to have underwent a secondary round of domestication, which adds another layer of complexity to the trajectories of yeast domestication. Here, recent results concerning the genomics of S. cerevisiae lineages used in cachaça, a Brazilian beverage based on the fermentation of sugar cane juice, will be presented together with an updated model of yeast domestication that implicates both primary and secondary domestication events. This work was supported by Fundação para a Ciência e a Tecnologia (Portugal) grant UID/Multi/04378/2013.
RETICULATE EVOLUTION IN THE SACCHAROMYCES GENUS

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Type: Oral

The \textit{Saccharomyces} genus has become one of the most important model genera to understand evolution. The scientific community has put a lot of effort into understanding the evolution and population structure of some of the \textit{Saccharomyces} species. Indeed, recently more than 1,000 \textit{Saccharomyces cerevisiae}, 162 \textit{Saccharomyces paradoxus}, 46 \textit{Saccharomyces uvarum} and 25 \textit{Saccharomyces eubayanus} have been sequenced. However, there are 3 additional \textit{Saccharomyces} species (\textit{Saccharomyces mikatae}, \textit{Saccharomyces kudriavzevii}, \textit{Saccharomyces arboricola}) and the recently discovered new \textit{Saccharomyces jureii} species which population structure remains fairly underexplored. Here, we studied the mitochondrial inheritance of 2,000 \textit{Saccharomyces} strains, we sequenced a representative of each available \textit{Saccharomyces} lineage at high quality, and we included additional \textit{Saccharomyces} sequences to study the population structure and gene flow within and between lineages. Together, with representatives of recently sequenced \textit{Saccharomyces} species, we were also able to detect and quantify several reticulate events between species at the nuclear, mitochondrial, and the 2-micron plasmid genomes.

ETHANOL EXPOSURE INCREASES MUTATION RATE THROUGH ERROR-PRONE POLYMERASES

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Type: Oral

Ethanol is a ubiquitous environmental stressor. In high levels, it is toxic to all forms of life. We find that even relatively low levels of ethanol causes proteotoxic and replication stress in the model eukaryote \textit{Saccharomyces cerevisiae}, and that exposure to such naturally occurring ethanol levels increases mutation rate. Ethanol-exposed cells display mutations characteristic of dysfunctional replication forks. Our data indicates that ethanol slows down replication and affects localization of Mrcl, a crucial and evolutionary conserved component of the episome. Additionally, we find that error-
prone polymerases are recruited to the replication fork in ethanol-exposed cells. Taken together, these observations suggest a model wherein ethanol causes dysfunctional replication forks and replication stress, ultimately leading to recruitment of error-prone polymerases and subsequent increased genome instability. Importantly, alcohol consumption has been linked to cancer development and global mortality in humans. Since ethanol affects evolutionary well-conserved proteins and processes, our findings could provide a route to better understanding and preventing ethanol-associated carcinogenesis.

THE ORIGIN AND EVOLUTION OF SACCHAROMYCES YEASTS FROM FAR EAST ASIA

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Type: Oral

Yeast species in the genus Saccharomyces are used worldwide as microbial agents for food and beverage fermentation. They are also commonly used as model systems in genetics, molecular biology and evolutionary biology. However, the origin and evolutionary history of Saccharomyces species in nature remain to be illuminated. Naumov proposed the Far East Asia origin hypothesis of Saccharomyces yeasts first in 1988 and we showed strong evidence supporting this hypothesis in recent years. All the currently recognized biological species of the genus have been found from Far East Asia and the genetic diversity of individual Saccharomyces species in this region is generally higher than in other regions of the world. S. eubayanus, which is the wild genetic stock of lager yeast firstly found from Patagonia, is actually native to the Tibetan Plateau with three distinct lineages exhibiting over 6% sequence divergence. A Tibetan population of S. eubayanus is more closely related to lager yeast than the Patagonian population, suggesting that the Tibetan population is the progenitor of lager yeast. Two significantly diverged native lineages of S. paradoxus coexist in China. Wild isolates of S. cerevisiae from China contribute the majority of the global variation of the species and contain the oldest lineages of the species documented so far. Our recent population genomics analysis showed more evidence supporting that China/Far East Asia is the center of origin of the both wild and domesticated populations of S. cerevisiae. The domesticated populations of the species form two major groups associated with solid- and liquid-state fermentation and appear to have originated from one or two heterozygous ancestors, which were likely formed by outcrossing between diverse wild isolates primitively for adaptation to maltose-rich niches.
A SINGLE TRANSCRIPTION FACTOR CONTROLS THE CRABTREE EFFECT IN KOMAGATAELLA PHAFFII

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Type: Oral

The Crabtree phenotype defines whether a yeast can perform simultaneous respiration and fermentation under aerobic conditions at high growth rates, a phenomenon that resembles the Warburg effect in cancer cells. It is assumed that this phenotype evolved in yeasts with the first fruit plants about 125-150 million years ago, providing Crabtree positive yeasts an evolutionary advantage of consuming glucose faster and producing ethanol to outcompete other microorganisms in sugar rich environments. Whole genome duplication, global promoter rewiring and loss of respiratory complex I are the main molecular events that contributed to the evolution of the Crabtree effect. Here we show that overexpression of a single Gal4-like transcription factor is sufficient to convert Crabtree-negative Komagataella phaffii (Pichia pastoris) into a Crabtree positive yeast. Upregulation of the glycolytic genes and a significant increase in glucose uptake rate due to the overexpression of the Gal4-like transcription factor caused an overflow metabolism, triggering both short-term and long-term Crabtree phenotypes. This indicates that a single genetic perturbation leading to overexpression of one gene may have been sufficient as a first molecular event towards respiro-fermentative metabolism in the course of yeast evolution.

THE DARWINIAN FITNESS OF WINE STRAINS OF SACCHAROMYCES CEREVISIAE IN THE GRAPE MUST ENVIRONMENT

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Saccharomyces cerevisiae have been exposed not only to natural selection in human-made environments and artificial selection for desirable traits, but also to genetic hitchhiking driven by that selection and genetic drift due to population bottlenecks and subdivisions. Strains isolated from different environments show wide phenotypic divergence (1, 2), but the extent of the impact of drift and selection on the phenotypes of wine yeast is a matter of debate. The wine-making environment is defined by the chemical content of grape must (high sugar, low nitrogen contents and low pH) as well as its microbial community, the yeasts and bacteria that compete with S. cerevisiae and whose selective impact on S. cerevisiae is unknown. In this project we used experimental evolution to study the adaptation of S. cerevisiae to grape must and a model microbial community composed of three yeast and one bacterial species. In a first step of the project we performed an asexual selection experiment starting with a large set of strains from various origins (wine, Mediterranean Oak, North American Oak,
As expected, wine strains quickly took over the population, and two wine strains were almost equally abundant at the end of the experiment (90 population doublings over 10 fermentation cycles). These two strains were later shown to have nearly equal fitness in the experimental environment. We then built populations with different amounts of standing variation using the same strains as in the asexual environment. These recombinant populations were grown for 210 generations over 24 fermentations interspersed with nine population-wide sexual cycles. The final populations are being sequenced in order to evaluate the evolution of allelic frequencies during the selection experiment.

THE EVOLUTION OF FRUCTOPHILY IN THE SACCHAROMYCOTINA

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Fructophily is a relatively rare trait that consists in the preference for fructose over other carbon sources including glucose. In yeasts, the cornerstone molecular determinant of fructophily is a specific fructose transporter (Ffz1) with a dynamic evolutionary history. We used functional comparative genomics to unravel the evolutionary history of fructophily in yeasts and found that it likely arose in a clade comprising the Wickerhamiella and Starmerella genera (W/S clade), through the horizontal acquisition of the Ffz1 transporter from filamentous fungi. We also showed recently that fructophily probably evolved in a lineage lacking the ability to conduct alcoholic fermentation, which constitutes an interesting feature in common with fructophilic bacteria. Notably, both W/S clade yeasts and fructophilic bacteria are associated with the sugar-rich flower niche. However, unlike their bacterial counterparts whose extant representatives still lack alcoholic fermentation capacity, W/S clade yeasts reinstated this pathway through the acquisition of bacterial alcohol dehydrogenase genes. We also found that the direct conversion of mannitol to fructose plays an important role in fructophily in W/S clade yeasts. This constitutes a very efficient parallel fructose consumption pathway with likely roles in redox balance and thermo resistance, which is currently under investigation. Our working model concerning the evolution and current physiological role of fructophily will be discussed.
EHAVIOR OF THE YEASTS OF GENERA SPATHASPORATA AND SCHEFFERSOMYCES AT STRESS CONDITIONS

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The dynamics of microorganisms to adapt to environmental disturbances are varied and specific to each of them. The interest in understanding these mechanisms in order to build robust strains in different industrial processes have allowed addressing the different approaches to cellular and molecular level. Currently, the search for molecular mechanisms resistant to stress conditions in yeast has revealed a potential still little characterized in wild strains, which have shown to have a high level of tolerance to inhibitors. Applying culture regimens under stress condition allowing exploits the plasticity of the microbial genome. For this reason, the behaviour of different species of two xylose-fermenting yeast genera were studied. The phenotypic changes in the production of biomass and ethanol yield were the parameters evaluated under several growing conditions. The results showed that the environmental background and the pre-exposure to acidic and thermal stress in strains of the genera Spathaspora and Scheffersomyces generates a phenotypic change in terms of parameters of growth kinetics and ethanol production. This allows exploring a natural potential for the adaptation of these yeasts in fermentative processes.

CONVERGENT ADAPTATION OF SACCHAROMYCES UVARUM TO AN ANTIMICROBIAL PRESERVATIVE IN HUMAN-DRIVEN FERMENTATIONS

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Different species can find convergent solutions to evolutionary constraints. There are examples of genetic convergence due to point mutations, gene duplications or species hybridizations but gene conversion by chromosomal rearrangements have not previously been described. In this work, we describe that two domesticated yeast species, Saccharomyces cerevisiae and Saccharomyces uvarum, acquired chromosomal rearrangements to convergently adapt to the presence of sulfite in (wine and cider) fermentation environments. We found new heterologous recombinations in fermentative strains of S. uvarum at the SSU1 locus, implicated in sulfite resistance, an antimicrobial additive used in winemaking and cider production. These are parallel events of previously described SSU1 locus recombinations in domesticated strains of S. cerevisiae. In S. uvarum, XVI VII recombination generates an overexpression of SSU1
GENOMES FROM LIMITS OF LIFE

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For long time extremophiles were terra incognita, since aggressive conditions of their ecological niches were considered as dead zones. Nowadays it is well known that these places are inhabited by a wide spectrum of microorganisms that became valuable resources in biotechnology and interesting candidates when exploring life origin. Odd microorganisms cannot be studied by comparing their genomes with model organisms (like Saccharomyces cerevisiae or Cryptococcus neoformans). Generating a good quality database of extreme life genomes is thus essential. In this work we sequenced yeast isolated from extremely acidic, and high heavy metal polluted environments. Selection of strains was based on their outstanding metal and acid tolerance. Source origin was either anthropogenic (acid rock drainage from São Domingos mine in Portugal) or natural (argentinian acidic water bodies from Río Agrio-Lago Caviahue system -RALC-). Three portuguese strains were selected along with phylogenetically related strain exclusively found in RALC. These four Goffeauzyma’s strains (G. iberica, G. metallitolerans, G. aciditolerans and G. agrionensis), collectively known as ARD ecoclade resulted in genomes ranging from 16 to 25 mbp with comparable number of putative genes predicted (mean 6.2k). Four strains belonging to an undescribed species that may represent the first report of an acidophilic yeast, were also sequenced (two Portuguese strains and two RALC’s). Assemblies resulted in 20.3 mbp average, accounting for 7k number of putative genes. Coniochaeta fadinicola and Rhodotorula mucilaginosa, more ubiquitous strains but frequently found in these two extreme environments, were sequenced and their genomes analyzed for gene prediction. Genomes quality was assessed for all ten basidiomycetous strains by comparing traditional genomic statistics and genome completeness was tested by looking for sets of eukaryotic core genes. Automatic and manually curated sets of annotations are being generated in order to explore possible genomic traits of their extreme tolerance, whether it was shaped in a natural or anthropogenic environment.
CHARACTERIZATION OF THE FERMENTATIVE PROFILE OF SACCHAROMYCES EUBAYANUS STRAINS ISOLATED IN CENTRAL AND SOUTH CHILE

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The production of beer historically involves the use of a limited panel of yeast highly adapted to industrial fermentation conditions, restricting the spectrum of organoleptic characteristic of the ferment products. The production of Lager beers is dominated by the use of cold tolerant Saccharomyces pastorianus strains, a natural hybrid between S. cerevisiae and cold tolerant S. eubayanus. Yet, the lack of S. eubayanus isolates limits the understanding of their brewing potential. Our recent fieldworks in Chile have allowed us to isolate ethanoltolerant yeasts from bark samples from Nothofagus tress in national parks ranging from Altos de Lircay National Park (VII Maule Region, Chile) to forests surrounding Karukinka National Park (XII Magallanes Region, Chile), representing overall a 2.090 km distance between sampling extremes. Among the found species, nearly a hundred isolates were identified as S. eubayanus, representing an enormous potential for the production of beer with novel organoleptic characteristics. In this work we analyze the phenotypic and fermentative profile of dozens of native Chilean strains on beer wort. The strains shown a variable fermentative profile and resistance to several stressors conditions, essential for their potential application in the industry. For example, the strains shown a variable response to different stressor, such as; exogenous ethanol, NaCl, hygromycin and high temperature (34 ºC). Additionally, the strains show a wide phenotypic diversity for the use of alternative carbon sources, such as maltose and fructose. Interestingly, the fermentative profiles are variable depending on their geographic origin directly impacting ethanol production rates, ranging from 2% v/v to 4% v/v. Our results demonstrate the high phenotypic diversity in strains obtained from different Chilean geographic regions and suggest an interesting brewing potential depending on their geographic origin.

BREAKING THE GENETIC CODE: WHY AND HOW DID YEASTS CHANGE THEIR TRANSLATION OF THE CUG CODON?

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The genetic code is the set of rules assigning codons to amino acids according to which mRNAs are translated into proteins. In the vast majority of organisms it is universal and was initially thought to be immutable, as the proteomic consequences of changing the meaning of any codon would be difficult to adapt to. Currently, a small number of codon reassignment events are known to have happened, including CUG-Leu -> CUG-Ser in a clade of yeasts known as Ser1, containing the pathogen Candida albicans. Recent work shows there are two other yeast clades, Ser2 and Ala, in which other reassignments of CUG occurred independently. These three phylogenetically close genetic code changes are, as of today, the only known examples of sense-to-sense codon reassignments in the
nuclear genomes of eukaryotes. This is hypothesised to have been caused by natural selection acting against the ancestral tRNA gene recognising CUG, leading to its eventual deletion and replacement by tRNA-Ala or tRNA-Ser genes recognising CUG. The genus Saccharomycopsis belonging to the Ser2 clade is particularly informative in the study of this transition. Its three sequenced member species all have novel CUG-Ser tRNA genes, while still retaining the ancestral CUG-Leu tRNA. This suggests that the Saccharomycopsis genus is still in the final stages of a genetic code change. Taking a closer look at the exact positions of CUG codons in the protein-coding genes of these species as well as the multiple gains and losses of other leucine-charged tRNAs in the sister Leu1 clade of yeasts provides further insights into the mechanisms governing the change. Our study contributes to a deeper understanding of the evolution of the genetic code, about which there are many hypotheses but few examples in nature to test them on.

GENOMIC STABILITY AND ADAPTATION OF BEER YEAST DURING SERIAL REPITCHING IN THE BREWERY

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Serial repitching of yeast from one fermentation to the next is a common practice for brewers to reduce the cost of purchasing a new inoculum for each new batch of beer. However, it is unclear what effect serial repitching has on the brewing capabilities of yeast, and how many repitches are possible before noticeable changes in beer profile arise due to genetic instability of the yeast. In collaboration with Postdoc Brewing, we investigated the genomic stability of ale yeast over 28 serial repitches in an industrial-scale fermenter using pooled whole genome sequencing. Comparing repitches along the time-course, we discovered two high-frequency, large-scale genomic rearrangements after just 15 repitches in the form of aneuploidy and mitotic recombination. However, we found a complete absence of high-frequency single nucleotide polymorphisms, insertions and deletions over the full extent of the experiment. We are currently testing whether the observed large-scale rearrangements affect brewing and growth characteristics, including profiling beer produced by yeast bearing each of the mutations. We are also collecting an independent replicate timecourse to determine whether similar changes arise. Together we aim to uncover general patterns by which beer yeast adapt to the brewery to shed light on how beer yeast have adapted in the past.

EVOLUTION OF A SACCHAROMYCES CEREVISIAE X EUBAYANUS HYBRID UNDER INDUSTRIAL LAGER BREWING CONDITIONS

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Mating between *Saccharomyces* species generates hybrids with novel phenotypes which may have potential for various industrial applications. However, domesticated hybrids such as *S. pastorianus* typically display extensive chromosome copy number variation and chromosome recombinations. These observations raise the question if frequent genome rearrangements are intrinsic to domestication of hybrids and if they contribute to their industrial performance. In this study, a hybrid between a haploid *S. cerevisiae* and a haploid *S. eubayanus* was evolved in six independent sequential batch reactors under conditions mimicking lager brewing for up to 418 generations and a total of 55 isolates were characterized. The evolution resulted in large phenotypic and genotypic diversity. Whole genome sequencing of the isolates revealed diverse SNPs, INDELs and loss of heterozygosities. While loss of heterozygosity was commonly observed, chromosomal translocations were extremely diverse and differed from those observed in *S. pastorianus*. Contrarily to *S. pastorianus*, only limited (segmental) aneuploidy was observed. Mutations could be linked to calcium-dependent flocculation, loss of maltotriose utilisation and loss of mitochondrial activity; three industrially-relevant traits which also occur in *S. pastorianus*. These results indicate the extensive aneuploidy observed in *S. pastorianus* is not intrinsic to domestication of a *S. cerevisiae* x *eubayanus* hybrid, and the genetic instability of hybrids does not visibly exceed that of non-hybrids. In addition, undesirable properties such as lack of maltotriose utilisation can be acquired during domestication. For industrial applications, laboratory-made hybrids should not be evolved in industrial conditions but in conditions tailored to select desired phenotypes.

**METHODS FOR THE SELECTION AND ADAPTATION OF BIOETHANOL YEAST FOR USE IN BEERS**

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The industry of wines, beers and other fermented products needs selection and production services of yeasts with special characteristics that allow to solve problems such as: (I) Long times and high costs in yeast selection process. (II) Fermentation is stopped due to the low availability of fermentable sugars. On the other hand, different amounts of complex sugars such as maltose can be found in wort beer, which reaches up to 60-70% of the available carbon sources. However all yeasts can not consume this type of sugars even though they are *S. cerevisiae* strains. Several studies have shown that the limitation of the maltose and maltotriose use is the ability to transport these sugars. Thus, different a-glycoside transporter genes has been identified in *Saccharomyces* such as MALx1 - MALx4 and MALx6, MPH2 and MPH3, ATG1. In this context, applications have been focused towards in genetic engineering technology. Nonetheless, there are still restrictive regulations on the GMO use and evolution techniques are now considered a good tool for expressing yeast phenotypes. The research group at the Laboratory LBGA at DGE / UFSCar has been developing a project of isolation and genotyping yeasts obtained from the bioethanol industry. Most of these yeasts are *S. cerevisiae* strains and they could be used in other fermentation. Because of this, selection techniques are required to isolate yeasts with technological characteristics and applicability, especially in the beer industry. Based on this, the challenge proposed in this project is focused on the development and optimization of a selection and adaptation system for yeasts that consume maltose and maltotriose, accelerating the evolution of isolated yeasts.
SYNTHETIC TWO- AND THREE-SPECIES HYBRIDISATION IN SACCHAROMYCES: NON-INTROGRESSIVE GENOME CHIMERISATION AND MITOCHONDRIAL INHERITANCE

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The biological isolation of the Saccharomyces species is ensured by two postzygotic sterility barriers: the sterility of the allodiploids (no viable spores are produced) and the F1-sterility of the allotetraploids (viable but sterile spores). By analyzing of large numbers of synthetic two-species "cevarum" (S. cerevisiae x S. uvarum), "kudvarum" (S. kudriavzevii x S. uvarum) and "cekud" (S. cerevisiae x S. kudriavzevii) hybrids, we demonstrated that the F1 sterility of the allotetraploids is due to the diploidisation of the tetraploid meiosis (resulting in sterile allodiploid spores) but can occasionally be broken down by malsegregation of autosyndetically paired chromosomes carrying MAT loci (loss of MAT heterozygosity). The resulting alloaneuploid F1 spores were nullisomic for one parental MAT-carrying chromosome and produced clones of nullisomic vegetative cells able to switch mating type and then mate with each other to form allotetraploid zygotes (nullisomic for the same MAT-carrying chromosome) producing viable F2 spores. Subsequent malsegregation of additional chromosomes and allosyndetic recombination between the chromosomes of the subgenomes reduced and chimerised the hybrid genome without introgressive backcrossing with parental species (GARMMe: Genome AutoReduction in Meiosis). The reduction of the genome size was asymmetric. In the cevarum hybrids the S. uvarum chromosomes, in the kudvarum and cekud hybrids, the S. kudvarum chromosomes were preferentially lost. The transmission of the mitochondrial genome to the hybrids was mostly uniparental and no correlation could be seen between the origin of the mtDNA and the hybrid sterility and its breakdown. As the chimerised descendants of the allotetraploids were fertile, we could hybridise them with a third species to produce three-species “cekudvarum” chimeras. True (allotriploid) three-species cekudvarum hybrids with complete genomes of three species could be obtained by rare-mating between sterile allodiploid kudvarum and haploid S. cerevisiae strains. In the individual cekudvarum strains uniparental, biparental and recombinant mitochondrial genomes were detected.

PARALLEL RESTORATION OF FERTILITY BY WHOLE-GENOME DUPLICATION IN TWO INTERSPECIES HYBRIDS OF THE ZYGOSACCHAROMYCES BAILII SPECIES COMPLEX

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Interspecies hybridization is an important evolutionary mechanism in yeasts. Many interspecies hybrids have been discovered, but most of them are asexual and can only replicate mitotically. Whole-genome duplication has been identified as a mechanism by which interspecies hybrids can regain fertility, restoring their ability to perform meiosis and sporulate. The genus Zygosaccharomyces contains numerous hybrid strains and/or
species. We investigated the genome of Zygosaccharomyces strain MT15, an isolate from Maotai-flavor Chinese liquor fermentation, identifying this interspecies hybrid as *Z. pseudobailii*. We showed that *Z. pseudobailii* regained fertility when one of its MAT loci became inactivated by a 1.5 kb deletion, causing the diploid interspecies hybrid to behave as a haploid gamete. Consequently, gametes were able to switch mating-type at their surviving MAT locus, after which mother-daughter mating occurred, producing diploid zygote cells. In addition, we also observed that one copy of the *HO* endonuclease gene, responsible for producing the dsDNA cleavage during mating type-switching, was inactivated in *Z. pseudobailii*, which altogether resembles the process for fertility restoration we previously described for *Z. parabailii*. The *Z. bailii* species complex consists of three species: *Z. bailii*, which is not a hybrid and whose 10 Mb genome is designated “A”, and the two hybrid species *Z. parabailii* (“AB” genome, 20 Mb) and *Z. pseudobailii* (“AC” genome, 20 Mb). The A, B and C subgenomes are all approximately 7-10% different from one another in nucleotide sequence and are derived from three different parental species. Despite being hybrids, *Z. pseudobailii* and *Z. parabailii* are capable of mating and sporulating. These half-sibling species, therefore, went through remarkably parallel but independent steps to regain fertility after they were formed by interspecies hybridization.

### LAGERS YEASTS AND THE DELICATE BALANCE BETWEEN ITS HYBRID GENOMES AND BREWERY’S STRESS

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Lager yeasts (*Saccharomyces pastorianus*) have been employed in brewery industry for the fermentation of Lager beers, a mass product consumed worldwide. Despite its industrial importance, poorly is known about how *S. pastorianus* deals with brewery stress (worts with high carbohydrate concentration, low pH, high hydrostatic pressure and dissolved CO₂). Additionally, the stress effects induced by brewery’s propagation systems, which employ worts with high nutrient and oxygen concentrations for cell biomass production is also little understood. In this sense, we evaluated the major stress-associated biological mechanisms that are modulated by brewery’s fermentation and propagation conditions by using a metatranscriptomic and systems biology analyses. Our data indicated that, during propagation, genes associated with DNA repair and meiosis mechanisms are overexpressed, especially those linked to homologous recombination (HR) pathway. The biological implications of meiosis and HR activation in *S. pastorianus* is discussed considering the hybrid genome of this species.
FROM SOIL TO STEIN; POPULATION GENOMICS OF WILD AND DOMESTICATED LINEAGES OF THE LAGER-BREWING ANCESTOR; SACCHAROMYCES EUBAYANUS

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Lager beer has been made with hybrids of *Saccharomyces cerevisiae* and *Saccharomyces eubayanus* for hundreds of years, but it was less than a decade ago that the wild pure stock of *S. eubayanus* was discovered in Patagonia!. We are exploring the extensive natural genetic and phenotypic diversity not found in industrial settings, through whole genome sequencing and fitness assays of almost 200 strains of *S. eubayanus* strains, isolated in the last 10 years. With this considerable collection we are investigating the population structure and demographic history of this species. Approximately 80% of known *S. eubayanus* strains have been isolated in Patagonia; these strains belong to two distinct and diverse populations, one that is limited to Northern Patagonia, and the other spanning all of Patagonia and encompassing strains from outside of it. Non-Patagonian isolates are rare, but the strains that have been found offer a unique view of the spread of admixed lineages and the origin of brewing hybrids. A handful of strains represent a Holarctic sub-population, which includes the *S. eubayanus* sub-genome of lager-brewing yeast and strains found in North Carolina and Tibet. Yet, with this limited pool of Holarctic strains we have shown that no extant strain is the closest relative of lager-brewing yeast, but rather the standing genetic variation found in this wild Holarctic lineage still persists in the two lineages of lager-brewing yeast. Through the comparison of phenotype and genotype of these diverse strains, we are gaining insight into the evolutionary process of microbial domestication and wild yeast ecology.

PHYSIOLOGICAL SIGNS OF DOMESTICATION IN SACCHAROMYCES UVARUM

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Four subpopulations of *S. uvarum* have been reported: Holartic and South America-A (SA-A) detected in both natural and fermentative environments and Australasian and South America-B (SA-B) only detected in natural environments. However, little is known about the phenotypic differences associated with either their phylogenetic origin or their ecological adaptions. We studied the response of 61 strains of *S. uvarum* included in three subpopulations (Holartic, SA-A and SA-B) to the following stress conditions: temperature (13°C to 30°C), ethanol (0 to 8% v/v) and nitrogen concentrations (20 to
300mg/L YAN) using microplate assays. The Holartic strains exhibited the greatest plasticity (ability to modify their performance in different conditions), being also the most cryotolerant. Regarding ethanol tolerance, very low plasticity was detected in populations from natural environments (independently from their genetic history), showing the lowest ethanol tolerance. Finally, strains from fermentative environments showed the highest nitrogen requirement. One strain representing each subpopulation was selected for competition assays in extreme conditions of temperature (13°C and 25°C), ethanol (0 and 8%v/v) and YAN (40 and 300 mg/L). The implantation capacity was evaluated by mtDNA-RFLP analysis. In extreme conditions (either 8% ethanol or 13°C), a significant implantation of the Holartic strain (more than 55%), versus SA-A (aprox. 25%) and SA-B (aprox. 20%) was observed. Either at 0% ethanol or at 25°C, the three strains were detected in equal proportions. At 40mg/L YAN, South American strains (both SA-A and SA-B) dominated over the Holartic strain (50% and 35% vs 15%, respectively). The SA-B strain was not able to grow at high YAN concentrations while the remaining strains showed similar implantation percentages. We evidenced different physiological adaptive mechanisms in *S. uvarum* that could be associated to its domestication, as well as a putative explanation about the absence of SA-B subpopulation in fermentative processes.

**HIDDEN IN PLAIN SIGHT: WHAT REMAINS TO BE DISCOVERED IN THE EUKARYOTIC PROTEOME?**

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The first decade of the genomic era featured a burst of novel gene characterization, at a rate substantially accelerated compared to previous norms. More recently, however, the pace of functional analysis has slowed, leaving a substantial proportion of complete proteomes uncharacterized in any species, even in otherwise well-studied model organisms. Many of the uncharacterized proteins are universally conserved, suggesting that they contribute to fundamental biological processes. Elucidating their roles would thus advance our understanding of essential biological pathways, and make an invaluable contribution to established and emerging fields such as systems biology, drug discovery, and personalized medicine. We have reviewed protein-coding gene characterization for humans and two model microbes, the fission yeast *Schizosaccharomyces pombe* and the budding yeast *Saccharomyces cerevisiae*. Using a simple yet powerful biological process-based metric, we defined both characterized and uncharacterized proteins for these three species. Combined with information about taxonomic conservation, our classification identified a set of broadly conserved unstudied proteins. For *Sz. pombe*, we further classified the conserved unstudied proteins based on combinations of orthogonal attributes determined by large-scale experimental and comparative methods (e.g. taxonomic conservation, mutant viability, peptide sequence features, localization). This multi-factorial classification can help to predict likely physiological roles for unstudied proteins, thereby identifying candidates for further investigation by researchers interested in particular biological processes, and working towards the long-term goal of building a comprehensive description of the basic workings of a eukaryotic cell.
SESSION 6: GENETIC AND METABOLIC IMPROVEMENT OF YEASTS
NEW METABOLIC REGULATION AND PHYSIOLOGICAL FUNCTIONS OF AMINO ACIDS FOUND IN YEAST AND THEIR APPLICATIONS

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Valine (Val) is widely used in animal feed, dietary supplements and pharmaceuticals. Val is commercially produced by bacterial fermentation. In Saccharomyces cerevisiae, Val biosynthesis is regulated by the Val feedback inhibition of acetohydroxyacid synthase (AHAS), which consists of the catalytic subunit Ilv2 and the regulatory subunit Ilv6. To improve the Val productivity of yeast, several Ilv6 variants were constructed by introducing amino acid substitutions based on a protein sequence comparison with the Escherichia coli AHAS regulatory subunit IlvH. Among them, the Asn86Ala, Gly89Asp and Asn104Ala variants resulted in approximately 4-fold higher intracellular Val contents compared with those in cells with the wild-type Ilv6. Asn86, Gly89 and Asn104 are located in the vicinity of a Val-binding site. The Ilv6 variants were much less sensitive to feedback inhibition by Val than the wild-type Ilv6. This approach could be a practical method for the development of yeast strains with high-level production of Val or isobutanol. Cysteine (Cys) is required for the biosynthesis of glutathione (GSH). In E. coli, Cys biosynthesis from sulfide proceeds with serine O-acetyltransferase (SAT) and OAS sulfhydrylase. SAT regulates the biosynthesis of Cys via the Cys feedback inhibition. S. cerevisiae synthesizes Cys from homocysteine through the reverse transsulfuration pathway. Interestingly, a novel mitochondrial SAT was found in the thermotolerant methylo trophic yeast Ogataea parapolymorpha, which is considered as a promising strain for high-level production of GSH. The O. parapolymorpha SAT (OpSat1) is functionally interchangeable with the E. coli SAT, despite that it displays much less enzymatic activity, with marginal feedback inhibition by Cys. The Opsat1Δ mutant showed remarkably reduced intracellular levels of Cys and GSH. Considering its key role in the regulation of the Cys biosynthesis pathway in O. parapolymorpha, OpSat1 with critical function in sulfur metabolism could be a rational target of metabolic engineering to generate yeast strains overproducing GSH.
REGULATORY MECHANISM OF ETHANOL FERMENTATION MEDIATED BY THE E3-UBIQUITIN LIGASE Rsp5

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Sugar metabolism and ethanol fermentation in the budding yeast *Saccharomyces cerevisiae* have attracted great attention because of their bearing on the ethanol production. Recently, we found that the substitution of Ala401 into Glu in the Nedd4-family E3-ubiquitin ligase Rsp5 (*rsp5*<sup>A401E</sup>) leads to decreasing total carbon dioxide emission during ethanol fermentation, suggesting that the *rsp5*<sup>A401E</sup> mutant is less efficient in ethanol production. This finding raises the possibility that Rsp5 is involved in ethanol fermentation processes. Metabolomic profiling after 24-hour fermentation showed that the *rsp5*<sup>A401E</sup> mutant cells accumulated a high level of intracellular pyruvate and produced less ethanol. Intriguingly, disruption of *ART1*, which encodes an adaptor protein for physical interaction between Rsp5 and its protein targets, repressed the transcriptional level of a minor isoform of the pyruvate decarboxylase *PDC6* and the alcohol dehydrogenase isoenzyme type *V ADH5*. Pdc6 and Adh5 play important roles in the ethanol production by catalyzing decarboxylation of pyruvate to acetaldehyde and by reducing acetaldehyde to ethanol, respectively. Based on these data, Rsp5 and its adaptor protein Art1 likely mediate ethanol fermentation through the transcriptional induction of the *PDC6* and *ADH5* genes, which is necessary for the efficient ethanol production.

PARALLEL QTL MAPPING PERFORMED ON NEARLY ISOGENIC STRAINS REVEALS THAT MITOCHONDRIAL INHERITANCE, GENE CONVERSION AND PLOIDY LEVEL STRONGLY IMPACT THE GENETIC DETERMINISM OF COMPLEX TRAITS IN SACCHAROMYCES CEREVISIAE

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Complex (quantitative) traits are resulting from the combination of thousand natural genetic variations in the nuclear genome. The genetic determinism of such traits can be investigated by studing the segregation of nuclear loci in a large meiotic progeny by achieving QTL-Mapping approaches. In the *Saccharomyces cerevisiae* yeast a non-negligible part of the phenotypic variation observed within the strains is also due to non-Mendelian heredity including gene conversion, cytoplasmic inheritance and in a broader way the ploidy level. By comparing the results of QTL mapping performed on two set of ~60 progeny clones obtained from two independent, isogenic hybrids, we fortuitously found that those phenomena may have a critical impact on the QTL detection. The phenotype investigated were related to the alcoholic fermentation of grape juice, including fermentation kinetics and metabolite production. Up to 40% of the 47 QTLs mapped show a significant interaction according
to the hybrid origin despite their isogenic nature confirmed by genome sequencing. This surprising result has different causes. First, the genetic maps of both population were compared revealing five haplotype conversions. One of them, mapped close to the gene ENO2, explained a strong background effect for the pyruvate production. Second, the two populations investigated differ for their mitochondrial inheritance and for their ploidy level. As expected ploidy level impact the fermentation kinetics resulting in scale-effect interactions. More surprisingly the effects of some QTLs mapped are opposed in the two progeny populations, suggesting a possible nucleocytoplasmic interaction. This mitotype effect was investigated in newly synthetized isogenic hybrids showing an unexpected role of the mitochondrial-DNA inheritance in the alcoholic fermentation.

**IS ANOXIA NOXIOUS? SEVERE OXYGEN LIMITATION DECREASES FITNESS AND ALTERS LIPID COMPOSITION IN YEAST**

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Type: Oral

It has been debated whether *Saccharomyces cerevisiae* can grow under complete anaerobiosis only if oleic acid and ergosterol are added to the medium, since the biosynthesis of ergosterol requires twelve O₂ (mol/mol) and each unsaturation in a fatty acid moiety requires one O₂. We performed chemostat cultivations with the *S. cerevisiae* CEN.PK113-7D strain, under the most stringent oxygen-excluded conditions. After an aerobic-anaerobic switch, when a defined medium devoid of the above mentioned anaerobic cofactors was employed, with glucose as limiting nutrient and a dilution rate of 0.1 1/h, a steady-state with growing cells was still observed. When compared to aerobic cultures, the unsaturated fatty acid fraction (C16:1 and C18:1) dropped significantly under extreme oxygen limitation from 60 to 30% and from 15 to 8%, respectively. On the other hand, C16:0 and C18:0 (saturated) fractions increased from 12 to 36% and from 3 to 6% of total fatty acids, respectively. Regarding sterol composition, extreme oxygen limitation led to an accumulation of squalene and lanosterol (4.8 and 1.3 μg/mgDCM respectively – 91% of quantified sterols), at the expense of ergosterol (0.6 μg/mgDCM – 8% of quantified sterols). Contrarily, aerobic cultures accumulated predominantly ergosterol (1.7 μg/mgDCM – 91% of quantified sterols) and very little squalene and lanosterol (summed up 0.1 μg/mgDCM – 8% of quantified sterols). Although O₂-starved cells diligently adjusted their lipid composition to incorporate moieties that are not dependent on oxygen for sustaining growth, they exhibited a pronounced decrease in cellular fitness. When anaerobic cells were exposed to ethanolic (100 g.L⁻¹) or acidic (pH 1.5) stresses, we observed a sharp decrease in cell viability, when compared to aerobic steady-state cells exposed to the same stressors. We will also discuss and compare the performance of the fuel ethanol *S. cerevisiae* PE-2 strain in identical experiments.
GENOMIC, TRANSCRIPTOMIC AND SECRETOME ANALYSIS OF RHODOSPORIDIUM FLUVIALE

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Abstract ID: 285
Type: Oral

Rhodosporidium fluviale, known as “red yeast” because of their pigment colony related to carotenoids production, is an oleaginous yeast, capable to produce and accumulate more than 20% of their dry cell weight as lipids. Besides that, species of this gender show native adaptability to a wide variety of carbon source and high tolerance to hydrolysate inhibitory compounds, representing a significant biotechnological potential.

In this work, R. fluviale was isolated from a lignin-degrading microorganism consortium that used sugarcane soil sample as inoculum. Using genomic, transcriptomic and secretomic strategies, we were able to characterize this yeast strain that is capable to growth in lignin media without other carbon source. The genome size was estimated to be 50 Mb encoding 17936 protein sequences, including more than 30 peroxidases, a key enzyme for lignin degradation, and 125 CAZymes (Carbohydrate-Active enZYmes).

For the transcriptomic and secretomic analysis, R. fluviale was cultivated in two different culture media (Kraft lignin + glucose and glucose). 1806 genes were up-regulated and 2125 genes were down-regulated in the cells grown in lignin-containing medium comparing to only glucose medium. Two enoyl-CoA hydratase/aldolase (Ech), an enzyme with protein domain related aromatic compound degradation (PF00378), were up regulated in the cells. Futhermore, five secreted proteins were obtained in the glucose-containing medium and eight secreted proteins in the medium containing lignin and glucose, using a 5% False Discovery Rate (FDR) index. These omics analyses reveal important pathways for rational use of this microorganism in biotechnological processes.

HIGH RESOLUTION ANALYSIS OF JUST-IN-TIME GENE EXPRESSION DURING FERMENTATION

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Abstract ID: 286
Type: Oral

Brewing processes have long been thought of as though they naturally divided into a small number of discrete phases in which the yeast proliferates, generates ethanol, and slowly exhaust their production capacity. As transcriptional analysis has become available, these studies followed this pattern by focusing on snapshots of transcription during each of these phases. In truth however, there is nothing natural about this division of the fermentation process. As a microorganism evolved to respond rapidly to environmental and competitive challenges and opportunities, Saccharomyces cerevisiae cells reveal themselves to be almost spectacularly well-adapted to responding to their environment at a given moment. As such, sparse sampling from the fermentation process may only reveal a limited amount of information about S. cerevisiae transcriptional status and little to no information on the dynamics of gene expression and regulation and the complex networks that control these molecular processes. We ask whether observing yeast transcriptional dynamics on the order of minutes to hours...
can display how yeast gene regulation responds while fermentation substrates are consumed and remodeled by yeast. Here, we describe experiments involving dense time sampling of yeast during the initial hours of fermentation, extraction of yeast RNA from commercial-type substrates, next gen RNA-sequencing and data analysis which display large programmatic shifts in the yeast gene regulatory system. Consistent with the fast kinetics of chemical changes undergone by the fermentation substrate, we see rapid changes in the transcriptional landscape of the yeast population. With this transcriptional data in hand we next asked if yeast gene expression can be used as high-resolution window into the complex dynamics of *S. cerevisiae* yeast metabolism and proliferation that occur during early fermentation and adaptation to an ever-changing fermentation environment. These results will be discussed.
SYNERXIA™ THRIVE, A HIGH ETHANOL YIELD SACCHAROMYCES CEREVISIAE STRAIN GENERATED BY METABOLIC ENGINEERING

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Abstract ID: 29
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The first generation (1\textsuperscript{st}G) of yeast-based ethanol production is to convert grain into fuel ethanol. The annual fuel ethanol production by 1\textsuperscript{st}G yeast is about 90 billion liters. It is estimated that about 70\% ethanol production cost is the feedstock. Since the production volume is so big, yield improvement will have massive economic impact for the whole industry. The introduction of carbon rerouting pathways has led to the development of Saccharoyces cerevisiae yeast strains to increase ethanol production yield. Our research has focused on the engineering of phosphoketolase (PKL) pathway and identification of beneficial traits for more ethanol production. The DuPont engineered yeast, SYNERXIA™ THRIVE, is robust and increase ethanol production up to 2.5\% compared to conventional yeasts in commercial plants. We will discuss metabolic engineering strategies and show performance of the SYNERXIA™ THRIVE at commercial production scale.

METABOLIC ENGINEERING FOR HYALURONIC ACID PRODUCTION IN KLUYVEROMYCES LACTIS

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Abstract ID: 38
Type: e-Poster, Session 1

Hyaluronic Acid (HA) is a multifunctional biopolymer composed by the monosaccharides Glucuronic Acid and N-Acetyl glucosamine found in all vertebrates. Biologically, the HA possess several cellular functions mainly related to recognition/protection between neighbor cells, cell adhesion and cell migration. This polymer is applied in medical and beauty industries being used for orthopedics and ophthalmological interventions and for the preparations of anti-aging skin care products. Currently, commercial HA is produced by bacteria, which at distinct levels are pathogenic to animals, and consequently results in a costly production process due to the downstream process. Therefore, this study aimed at developing a generally regarded as safe yeast, Kluyveromyces lactis, for HA production. For that, four strains were genetically modified containing different isoforms of the Hyaluronic Acid Synthases (HAS) enzymes. This enzyme is responsible for synthesizing the HA chain by joining the two molecular precursors in the cytoplasm side of the cell and simultaneously to export the biopolymer to the extracellular ambient. Transcript analysis of the HAS genes in the four different strains showed the presence of HAS gene transcript in three strains. HA production was initially observed by scanning electron microscopy where among all tested strains, only one was producing HA, whereas the remaining three strains had the same pattern as observed for the wild-type strain. After that, the only strain producing HA was grown in optimized medium tested with the different carbon sources. This resulted in different levels of HA production ranging from 0.06 g/L in Galactose to 0.39 g/L in Lactose. Future strategies and molecular bottleneck identifications will be used to increase HA production in K. lactis for developing an industrial process for HA production using this yeast as a biocatalyst.
METABOLIC ENGINEERING OF SACCHAROMYCES CEREVISIAE FOR LACTIC ACID PRODUCTION USING GLYCEROL AS A CARBON SOURCE

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Abstract ID: 245

Type: e-Poster, Session 1

Pollution caused by plastics, is becoming a subject of growing research together with an increasing awareness of their environmental damage. The possibility of using new technologies of materials, such as bioplastics, results in an interesting option. In this work we propose the genetic modification of \textit{Saccharomyces cerevisiae} to produce lactic acid, which is used as a monomer for the synthesis of the bioplastic PLA (polylactic acid), and the design of a culture medium that maximizes culture growth and product formation. PLA is one of the most attractive bioplastics used by the moment, it can be degraded under composting conditions and its production is economically more favorable compared to other bioplastics. On the other hand, the use of robust microorganisms, such as yeast, which can tolerate high concentrations of acid, would make it possible to obtain high concentrations of lactic acid through fermentation processes, avoiding the generation of toxic effluents as in the case of chemical synthesis. The use of a residue of the biodiesel industry as a substrate, such as glycerol, offers the possibility of reducing costs of production and the accumulation of this residue. As a strategy of modelling the metabolic pathways to direct the carbon flux to lactic acid production we expressed, using episomal plasmids, a L-lactate deshydrogenase of \textit{Bos taurus} in the strain of \textit{S. cerevisiae} BY4741. Currently, we are carrying away the deletion of some genes from the ethanol biosynthesis pathway (\textit{PDC1} and \textit{ADH1}). In the next place we will overexpress genes related to the glycerol catabolism. Given the importance of the medium’s composition and the culture conditions to favor a specific metabolic pathway, an experimental design will be performed to allow a statistical analysis of the results (Factorial design with central point), varying the components of the medium and its different levels of concentration.

BIOSENSOR AND GENOME-SCALE MODELING FOR CLONING OF SACCHAROMYCES CEREVISIAE GENES AFFECTING HEME AND HEMOGLOBIN PRODUCTION

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Abstract ID: 7

Type: e-Poster, Session 2

Human hemoglobin (Hb) is a major target for the development of oxygen carriers and therefore its microbial production is attractive, as it may provide a cheap and reliable source of hemoglobin. To increase the production of human hemoglobin in selected \textit{Saccharomyces cerevisiae} strains we aimed to clone genes affecting heme and hemoglobin level. We applied \textit{S. cerevisiae} Genome-scale metabolic model with
Enzymatic Constraints using Kinetics and Omics data, GECKO, to deduce fluxes and genes important for the improvement of heme production from glucose. To clone other genes affecting the hemoglobin level, we introduced a biosensor for the hemoglobin’s degradation product bilirubin in S. cerevisiae, based on a fluorescent protein from the Japanese eel, UnaG. The UnaG fluorescence was detected in HMX1 gene wild-type strain background, and its fluorescence level correlated with different heme/hemoglobin amounts.

PRODUCTION OF HUMAN EPIDERMAL GROWTH FACTOR (HEGF) IN KOMAGATAELLA PHAFFII

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Abstract ID: 17
Type: e-Poster, Session 2

The expression system based on the utilization of the yeast Komagataella phaffii has been used successfully in the production of a large variety of heterologous proteins. This yeast combines several relevant features such as easy molecular manipulation, rapid cell growth, ability to perform post-translational modifications and efficient secretion, in addition to growth at high cell densities. One protein of great interest for the biopharmaceutical and cosmetic industry is the human epidermal growth factor (hEGF). The aim of this study was to develop a system for hEGF production in K. phaffii. For protein secretion, three signal peptides (αF, SUC2 and PHO1) were tested. All three expression vectors were constructed under the control of the PGK1 promoter and used to transform K. phaffii M12-K, a strain mutant for KEX1, a gene coding for a carboxypeptidase. Recombinant clones were confirmed by colony PCR and grown in minimal medium to evaluate growth kinetics. Positive clones were selected and used in flask expression using complex media. Our results showed the presence of hEGF as confirmed by western blot (WB) using specific antibody. One clone from each system was selected for the production and purification of hEGF by molecular-exchange chromatography and RP-HPLC. From these experiments the presence of hEGF was confirmed by WB and the polypeptide was partially purified. Finally, a clone with the αF::hEGF construct was chosen to optimize the purification process with a single step using HILIC. hEGF was successfully purified. N-terminal sequencing and mass spectrometry confirmed the integrity and sequence of hEGP. The results obtained showed that K. phaffii is a promising cell factory for hEGF production.

HETEROLOGOUS EXPRESSION OF LIPASES IN KOMAGATAELLA PHAFFII UNDER THE CONTROL OF DIFFERENTS CONSTITUTIVES PROMOTERS

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Abstract ID: 69
Type: e-Poster, Session 2

The methylotrophic yeast Komagataella phaffii stands out as an expression system due to its ability of produce and secrete high levels of heterologous proteins at high cell
densities. Other features have prompted this yeast as a platform for industrial production of enzymes and biopharmaceuticals such as easy molecular manipulation (when compared to other eukaryotic expression systems), correct protein processing, folding and post-translational modifications. Heterologous gene expression is typically controlled in K. phaffii by the endogenous AOX1 promoter which tightly control by the levels of methanol, however, this alcohol toxic and flammable which makes its use dangerousness in large processes. As an alternative constitutive promoters for K. phaffii have been tested, such as those from GAP, a glycolytic gene coding for glyceraldehyde-3-phosphate dehydrogenase, PGK1 coding for 3-phosphoglycerate kinase and TEF1 which codes for an elongation factor during translation. In the work we assessed protein yield and stability when a heterologous gene was placed under the control of these three constitutive promoters. Two lipase genes were tested: the well know lipase B from Pseudozyma (Candida) antarctica, and a putative lipase from Ustilago hordei. Lipases are part of an enzyme class of great interest because of it various industrial applications. The selection of recombinant clones secreting lipase was carried out in medium containing tributirin and enzymatic assays were conducted to evaluate the levels of ester hydrolysis of the secreted lipases under each promoter. Fermentation studies will be performed to assess the optimal conditions for the lipase production.

AMYLASES EXPRESSION IN KOMAGATAELLA PHAFFII: AOX1 AND PGK PROMOTERS COMPARISON

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The methylotrophic yeast Komagataella phaffi is a wildly used model for protein heterologous expression. Its utilization is mostly associated to the methanol induced promoter from the gene that encodes the enzyme alcohol oxidase 1 (AOX1), however methanol transport and storage might be dangerous. The promoter region from the gene that encodes the enzyme 3-phosphoglycerate kinase (PGK) has being used as an effective constitutive alternative to the promoter AOX1, but its complete region is often required (about 2000 bp). Knowing that smaller promoters may facilitate cloning and enhance heterologous DNA stability, in this work we compared a smaller optimized region from PGK promoter (about 400 bp) to the promoter AOX1 for heterologous expression in K. phaffi of three amylases constructions: an alpha-amylase from Bacillus subtilis, a glucoamylase from Aspergillus awamori and a phusion of both proteins. A one-copy transformant strain was selected for each construction and then analysed for growth rate and enzyme activity in supernatant. After 44h of incubation, AOX1 promoter induced 156,2 ± 5,2 U/mL of alpha-amylase activity, while PGK promoter induced alpha-amylase activity of 108,8 ± 3,7 U/mL. The glucoamylase activity was 4,99 ± 2,08 U/mL when promoted by AOX1, and 9,54 ± 1,25 U/mL when promoted by PGK after 23h of cultivation, but the difference between them is not statistically relevant. And the phusion protein has 50,7 ± 5,2 U/mL of activity when controlled by AOX1 promoter and 121,0 ± 3,9 U/mL of activity when promoted by PGK, after 33h of incubation. Also, a qRT-PCR experiment indicated that there are about three times more amylase mRNA in all AOX1 strains than in the corresponding PGK strain. Therefore, this small version of PGK promoter was more effective than AOX1 promoter when inducing the expression of the largest amylase construction in the tested conditions.
PRODUCTION OF BOVINE CHYMOSIN BY KOMAGATAELLA PHAFFII CELLS TRANSFORMED WITH A MULTI-COPY EXPRESSION VECTOR

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Abstract ID: 122
Type: e-Poster, Session 2

Chymosin is an aspartic protease that is used in cheese industries for the clotting of milk by the cleavage of the peptide bond between Phe 105 and Met 106 in κ-casein. Its low proteolytic activity and high specificity results in desirable organoleptic properties and higher yield of cheese production. Traditionally, chymosin is extracted from calf abomasum, however, because of economic and ethical problems and the risk of contamination, the production has been progressively replaced by recombinant enzymes. In this study, the host cell used was the yeast *K. phaffii* since it can grow rapidly to high cell density and has the ability to secrete up to grams per liter of heterologous protein. Strain M12 was transformed with an integrative vector carrying bovine chymosin gene under the control of the constitutive *PGK* promoter, the auxotrophic defective marker leu2-d and sequences for targeted integration at different loci such as 5S rDNA, *PGK1* and repetitive region of chromosomes 3. Transformants were evaluated for their ability to produce chymosin by and enzymatic assay following SDS-PAGE. Three clones were obtained after 38 hours growth on complex medium with enzymatic activities of 330U/mL, 270U/mL and 100U/mL. The active form of chymosin (36 kDa) was the only one observed on supernatant.

GENETIC ENGINEERING OF SACCHAROMYCES CEREVISIAE STRAINS TOWARDS FATTY ACID SYNTHESIS

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Abstract ID: 33
Type: e-Poster, Session 3

Rhamnolipids are the most studied biosurfactants. These molecules consist in a hydrophilic part composed of the rhamnose sugar linked to the 3-(hydroxyalkanoyloxy) alkanoic acid (HAA), the hydrophobic part of the molecule. The yeast *Saccharomyces cerevisiae* has been widely used in the production of chemicals and our group has already successfully produced rhamnose in *Saccharomyces cerevisiae* by the introduction of the five enzymes responsible for the conversion of sucrose into rhamnose. Now, in this study, we aimed at increasing carbon flux from glycolysis to the fatty acyl biosynthesis in order to overproduce β-hydroxydecanoyl-ACP fatty acid, an HAA precursor. For that, three strains were constructed: 1. increased pyruvate dehydrogenase activity (PDH, Strain EMB51), to increase the conversion of pyruvate in acetyl-CoA, 2. Acetyl CoA carboxylase (ACC, Strain EMB52), that directs the acetyl-CoA into Malonyl-CoA and 3. Fatty acyl biosynthesis (Fab, Strain EMB53) genes, that leads to the β-hydroxyacyl-ACP production. Strains were evaluated in aerobic batch fermentation in which Strain EMB51 and Strain EMB52 showed no significant kinetic differences when compared to the control strain, while Strain EMB53 has a much lower growth rate. The total amount of produced fatty acids was evaluated in all constructed
strains by GC-MS analysis. It showed that the strain EMB51 increased 18% of total fatty acids while the strains EMB52 and EMB53 reduced fatty acid production in about 50%. Moreover, the EMB51 strain increased about 3 times the production of small chain fatty acids (C10, C12). While in strains EMB52 and EMB53 it was predominant fatty acids with higher length than C18. Next steps include the evaluation of the 4 strains (including a control strain, EMB50), developed, combined with the introduction of the rhamnose pathway, in order to determine which pathway combination results in higher rhamnolipid production.

COMPARATIVE METABOLOMICS AND PROTEOMICS BETWEEN SACCHAROMYCES CEREVISIAE HYBRIDS AFTER ADAPTIVE EVOLUTION IN LIGNOCELLULOSIC SUBSTRATE FOR BIOETHANOL PRODUCTION

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In the search for alternative sources to fossil fuels, biofuels, such as bioethanol, are shown highly efficient. However due to ethical issues regarding the use of foods such as sugar and corn in ethanol production, research has been carried out in search of by-products, such as lignocellulosic residues, which can be used in the production of bioethanol. Sugarcane bagasse is a promising by-product that can be used for this purpose, to be available in the plant, does not require transportation expenses, relatively be abundant and cheap. Nevertheless, lignocellulosic hydrolyzate from bagasse is constituted of non-naturally metabolizable sugars by Saccharomyces cerevisiae (employed microorganism for ethanol production), and the presence of inhibitors against microorganisms responsible for fermentation, as hydroxymethylfurfural, furfural and acetic acid formed during acid pretreatment of bagasse. The challenge of this work was to circumvent these difficulties through the use of S. cerevisiae hybrids tolerant to inhibitors found in the substrate for second-generation (2GE) ethanol production in Brazil. Such hybrids had been obtained in previous work by massal and direct crossings of mutagenized S. cerevisiae followed by adaptive evolution. These hybrids were genetically engineered with the cassette X123 containing the three genes responsible for xylose metabolism (xylose reductase, xylitol dehydrogenase and xylulokinase), and then were followed by adaptive evolution (in YPX and hydrolysate media) in search of an optimal strain for pentose and hexose simultaneous fermentation. Therefore, the objective was to obtain strains with potencial of industrial use in the production of 2GE from sugarcane bagasse. The evolved strain was compared with the original by evaluating their physiological and technological traits. Proteomic and metabolomic analysis were performed in order to better understand the metabolic basis of any improvement observed.
UNRAVELING THE POLYGENIC BASIS OF FURFURAL AND HMF TOLERANCE IN YEAST STRAINS FOR IMPROVEMENT OF 2G-BIOETHANOL PRODUCTION

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One of the major challenges society faces today is global warming, mainly driven by greenhouse gas emissions. To reduce fossil fuel consumption and related CO2 emissions in the transportation sector, second-generation (2G) bioethanol – produced from the abundantly available lignocellulosic biomass (i.e. waste products or energy crops) - is proposed as an alternative transport fuel. To obtain economically viable 2G-bioethanol, the fermentation by Saccharomyces cerevisiae of sugars present in the lignocellulosic hydrolysate requires improvement. A key factor is to enhance tolerance of the yeast for inhibitory compounds in the hydrolysate that affect the fermentation process. Here, furfural and hydroxymethylfurfural (HMF) have been identified as the most crucial inhibitors. Therefore, we improved tolerance of 2G-bioethanol yeast strains against furfural and HMF by performing whole-genome transformation and by making use of the wide biodiversity of non-conventional yeast species. Now, we are unraveling the polygenic basis of furfural and HMF tolerance by performing bio-informatics analysis, pooled-segregant whole-genome sequencing and CRISPR/Cas9 allele exchange. In this way, novel mutations in genes previously not linked to furfural or HMF tolerance have already been identified. Moreover, novel mutations in ADH1, known to convert furfural and HMF into less toxic compounds, were identified. In this way, a 2G-bioethanol yeast strain with very high furfural and HMF tolerance will be obtained, and this will significantly increase the potential of 2G-bioethanol as a promising alternative transport fuel.

IMPROVEMENT OF THERMOTOLERANCE BY WHOLE-GENOME TRANSFORMATION

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Non-conventional yeasts such as Kluyveromyces marxianus and Ogataea polymorpha are able to grow at temperatures up to 52°C and 50°C, respectively. However, the lack of a genomic toolbox, industrial use and low ethanol and inhibitor tolerance make these species less attractive as yeast cell factories. Transferring this trait to Saccharomyces cerevisiae can have a disruptive influence in producing biofuels and other bio-based compounds by lowering the capital costs, reducing contamination risk and energy costs amongst others. By using whole-genome transformation where we transform an S. cerevisiae strain with the complete gDNA of K. marxianus or O. polymorpha, we were able to obtain superior thermotolerant strains that were able to ferment rapidly at 42°C. Three of these transformants were characterized and 4-5 SNPs were identified by bio-informatics analysis in each of them. Additionally, a pooled-segregant whole-genome sequence analysis was performed by crossing one of the transformants with an inferior
strain. Six quantitative trait loci (QTLs) have been identified and are currently under investigation. Interestingly, none of these QTLs are overlapping with the SNPs introduced by whole-genome transformation. Therefore, we can conclude that these two techniques can complement each other. By introducing these new SNPs in industrial strains we might be able to reduce production costs of bio-based compounds and move away from fossil fuel-based economy.

INFLUENCE OF THE INITIAL CARBON SOURCE CONCENTRATION AND BIOREACTOR TYPE IN CAROTENOID PRODUCTION BY RHODOTORULA GLUTINIS P4M422

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Carotenoids are natural compounds with important biological properties for the benefit of human health such as anticancer, antioxidant and anti-inflammatory activities. These also have applications as colorants or supplements in the pharmaceutical, cosmetic and food industries, the demand for these compounds increases. Due to the biological and commercial relevance of this bioactive molecule is necessary and attractive to define a biotechnological process with high yields. In the present study, the influence of carbon source concentration on the carotenoids production by Rhodotorula glutinis P4M422 under submerged culture was evaluated. The highest carotenoids concentration was obtained when dextrose at 40 and 80 g/L was used (1.85 and 1.88 mg/g, respectively). However, the best productivity was achieved when dextrose at 40 g/L was added. In addition, 2 types of bioreactors (flask and aluminium can) were evaluated, and the maximum production of biomass and carotenoids was obtained using the conventional reactor (flask). Carotenoids production, biomass formation and substrate consumption were analyzed to calculate the kinetic parameters. It was determined that 40 g/L of carbon source produced the highest concentration of carotenoids and that better bioreactor to improve the production of carotenoids was the flasks.

DEVELOPMENT OF A STERILE STRAIN OF THE BRAZILIAN INDUSTRIAL YEAST SACCHAROMYCES CEREVISIAE JP1

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Industrial strains of Saccharomyces cerevisiae have been isolated directly from Brazilian distilleries in the past 20 years. The strain JP1 is a more robust one. It was isolated from Brazilian northeast and can grow at higher temperature and lower pH than the standard fermentative strains. In order to use this strain as a model for ethanol production from starch, it was imperative to adjust its metabolism to Brazilian Biosecurity Rules for Genetic Modified Organisms (GMO), which includes to block sexual reproduction and meiosis, reducing to insignificant levels the possibility of gene
horizontal transfer and sporulation. So, in this work, the gene STE5 (sterile), that is essential for matting process, and the gene IME1 (inducer of meiosis), which encodes a sporulation associated factor, were deleted from the S. cerevisiae JP1 genome by homologous recombination with an antibiotic resistance gene flanked by upstream and downstream regions from the target gene. After confirming the deletion, the antibiotics marks were recycled by Cre-loxP recombinase system. The resulting strain phenotype were analyzed, and the deletions confirmed by the absence of matting and sporulation. Now, this strain is ready to be used as a GMO standard in Brazilian ethanol industry.

**FLAVANOLS PROTECT SACCHAROMYCES CEREVISIAE FROM HEAT INJURY AND FREEZING INJURY**

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Flavan-3-ols, the main flavonoids of tea extracts, are interesting due to their antioxidant properties and other biological effects. This study was aimed at examination of the capacity of selected flavanols [(−)-epigallocatechin gallate and (+)-catechin] to protect *Saccharomyces cerevisiae* (two strains: wild-type strain BY4741 and mutant BY4741D sod1) against thermal stress promoted by a low temperature freezing at -20 °C as and by heating at a temperature of 50°C. Oxidative stress has been demonstrated to play an important role in cell death induced by both high and low temperature. The flavanols showed no toxicity to yeast cells at concentrations of up to at least 100 µM. The flavanols protected cells of both strains against heating- and freezeing-induced lethality, as assessed by colony forming assay. The catechins (50 µM) decreased the production of reactive oxygen species in temperature-stressed yeast cells, increased total antioxidant capacity of yeast cell extracts, and prevented the decrease of the level of reduced glutathione and reduced/oxidized glutathione ratio in cells exposed to thermal stress. These results demonstrate efficient antioxidant action of catechins in the yeast model, which may be of value in technological processes involving temperature stress.

**EXPERIMENTAL EVOLUTION OF PHAFFIA RHODOZYMA STRAINS FOR INCREASE PRODUCTION OF CAROTENOID PIGMENTS AND MYCOSPORINE-GLUTAMINOL-GLUCOSIDE**

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Experimental evolution has been successfully applied for increase production of relevant secondary metabolites in microorganisms. *Phaffia rhodozyma* is a red basidiomycetous yeast capable of synthesizing two compounds with high value in marine and cosmetic industry: carotenoid pigments and mycosporine-glutaminol-glucoside (MGG). Both metabolites exert protection against UV radiation and have *in vitro* and *in vivo* antioxidant activity against reactive oxygen species. The aim of this work is to obtain MGG and carotenoids hyper-producers mutants from *P. rhodozyma* by means of experimental evolution, using reactive oxygen species and UV exposure as
selective pressure. Three populations of *P. rhodozyma* strains CRUB 1149 and MUT 7918, were grown in 24-well microplates for 120 hs (11 duplications approximately). Cells were expose to subinhibitory concentrations of H$_2$O$_2$ and UV radiation. In every serial transfer to a new well, each stress was increase by 10%. Periodically, samples were taken for cryopreservation, and determination of carotenoid pigments and MGG production and compared with the parental strains. After 165 generations exposure to UV radiation, we could obtain a mutant derived from MUT 7918 that accumulates 90 mg/g dry weight of MGG, corresponding to a 3-fold increase in MGG accumulation with respect to its parental strain (29 mg/g dry weight). Populations of MUT 7918 exposed to hydrogen peroxide showed no differences in MGG accumulation with respect to the parental strain. On the contrary, from CRUB 1149, only a population evolved in the presence of hydrogen peroxide register enhanced MGG accumulation (1,5-fold increase). So far, we have been able to apply an experimental evolution strategy for increase production of MGG in *P. rhodozyma*, obtaining two hyper-producers mutants. Further studies of carotenoid pigment production and stability of strains are being carried out.

**UTILIZATION OF YEASTS AS PLATFORMS FOR PRODUCTION OF BIOSURFACTANTS AND BIOPOLYMERS**

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Over the years, utilization of tailor-made microorganisms for the production of fuels and chemicals has become a preferential option to attend environmental and economic demands worldwide. Within this context, many bio-based processes were developed and scaled-up. In our group, efforts have turned into genetically modifying yeast cells for production of the biosurfactant rhamnolipid and the biopolymer hyaluronic acid using *Saccharomyces cerevisiae* and non-conventional yeasts as biocatalysts. Rhamnolipids are glycolipid biosurfactants whose large-scale production is still in its infancy, hindered due to the pathogenicity of natural producer, high substrate and purification costs and low yields and productivities. It is composed of one or two rhamnose molecules linked to beta-hydroxy fatty acid chains. Strains of *S. cerevisiae* were modified to produce the rhamnose sugar by overexpression of five genes from *Pseudomonas aeruginosa*. Also, the effect of overexpressing different genes within fatty acid synthesis was also investigated regarding total fatty acid production and length of fatty acid chain. Altogether the resulting strains are a proof of concept for, the first time that rhamnolipids can be produced using *S. cerevisiae* as host. Hyaluronic acid (HA) is a biopolymer naturally present in all vertebrates with various functions. The increase in HA demand over the years is associated with its application in various industrial fields such mainly related to aesthetics, pharmacology, and medicine. Nowadays this polymer is obtained through animal tissue extraction or bacteria either a natural producer or genetically engineered. In all cases, downstream processes are costly to guarantee a polymer that is pathogen free. Thus *K. lactis*, a GRAS yeast already used at industrial scale was modified for HA production resulting in 0.4g/L. Batch fermentations and qPCR were utilized to investigate the main bottlenecks during HA synthesis in *K. lactis* and will be further discussed.
ANALYSIS OF IMPLANTATION DINAMYCS AND ETHANOL REDUCTION OF A LOW-ETHANOL YEAST STARTER IN REAL WINE CONDITIONS

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Lately, wine industry has been looking for disrupting methodologies to reduce alcohol content without compromising wine sensory characteristics. Increment in the alcohol concentration have been related with global warming and viticulture management strategies to obtain wines with improved polyphenolic features (e.g. sweet tannins). In previous laboratory-scale assays, we have demonstrated that the insertion of the pdc2Δ519 mutation in Saccharomyces cerevisiae wine yeast strains, can reduce the ethanol concentration up to 1.89% (v/v) without affecting the fermentation performance. This difference in alcohol production between mutant and control strains was observed in culture media and natural grape musts previously pasteurized. However, verification in real vinification conditions is still lacking. The aim of this work was to evaluate our reducing mutant yeast strain in natural grape must fermentation at pilot-scale, including an ethanol reducing commercial strain as a positive control. In order to diminish the natural microbiota of must and consequently improve the implantation of mutant strains, two minimal invasive treatments (microwave and centrifugation) were applied to fresh grape musts. Fermentation in non-treated grape must was also included as control and the implantation dynamics of the pdc2Δ519 mutant strain was monitored during the whole process. The results showed that the pdc2Δ519 mutant strain was largely dominant during the vinification with implantation percentages higher than 70% after only 24h, and reaching values over 90% with a good performance of alcoholic fermentation. However, ethanol reduction was at best 0.4% v/v with little difference between treated and non-treated grape must. This trend has been previously observed in other studies performed with natural must. Our results suggest that the inhibition of the ethanol reduction effect under natural conditions is not a consequence of a substrate competition and other interaction mechanisms may be involved.

GENERATION OF SACCHAROMYCES HYBRIDS FOR LAGER BEER FERMENTATION

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Saccharomyces pastorianus is a natural interspecies hybrid between S. cerevisiae and S. eubayanus. The success of this hybrid in the environment of a lager fermentation is due to the fact that it combines flocculation, the use of maltotriose and the aromatic profiles of S. cerevisiae with the cryotolerance, volatile compound production and use of maltose from their other parent, S. eubayanus. Currently only a limited genetic diversity is found in this hybrid - reflected in the poor amount of lager beers styles compared to the immense diversity of ale beers – mostly due to the lack of S. eubayanus isolates.
Since the original isolation of *S. eubayanus* from Nothofagus trees in the Argentinian Patagonia, new alternatives are emerging for generating new *S. pastorianus* hybrids with potential in the brewing industry. Recent efforts by the Chilean group within this proposal have allowed the isolation of hundreds of ethanol-tolerant yeasts from ten sampling sites, ranging from Altos de Lircay National Park (VII Maule Region, Chile) to forests surrounding Karukinka National Park (XII Magallanes Region, Chile), representing overall a 2.090 km distance between sampling extremes. Among the found species, a 50% of isolates belong to *S. eubayanus*. Preliminary results suggest significant differences between isolates in their fermentation profile depending on their geographic origin. In this work, we assess the sugar consumption and fermentation kinetics of Chilean native sets of *S. cerevisiae* and *S. eubayanus* under lager fermentation conditions in order to select the best candidates for the generation of artificial hybrids. Selection parameters and comparison between parents and hybrids included: sugar consumption, maximum CO₂ lost and CO₂ Vmax. These new hybrids could combine the beneficial characteristics of the original strains, fermenting over a wide range of temperatures and provide new organoleptic profiles currently nonexistent in the market, generating a new beer preferred by producers and consumers.

### GENETIC BASIS FOR ACCELERATED SULFITE TOLERANCE MEDIATED BY A SULFITE EFFLUX TRANSPORTER, SENSITIVE TO SULFITE1(SSU1)

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Sulfite is biosynthesized in yeasts during fermentation, and possesses antimicrobial and antioxidant activity, thereby enabling to long-term storage of alcoholic beverages such as beer and wine. Sulfite is transported into extracellular space via a sulfite efflux transporter, SSU1. Here we show that SeSSU1, which was derived from a *Saccharomyces eubayanus*-type genome set of a lager yeast (*S. pastorianus*), is responsible for superior SO₂ transporting activity in a lager yeast than ScSSU1 from an ale yeast (*S. cerevisiae*). This result is in clear consistent with the high contents of sulfite in lager type beers relative to ale type beers. By comparing homology models of the two SSU1 proteins based on the crystal structure of a Tellurite-resistance/Dicarboxylate Transporter (TDT) family anion transporter, SLAC1, we found that a unique Val19 residue, which is proximal to the gate Phe361 residue crucial for the catalysis in the SeSSU1 model structure, is responsible for the superior sulfite efflux activity. Moreover, ale yeasts expressing active ScSSU1 containing M19V substitution exhibited extracellular sulfite tolerance as well as high sulfite production. Surprisingly, the Val residue was also found in SSU1-R, which is a paralog that had specifically occurred in sulfite-resistant wine yeast strains (*S. cerevisiae*) e.g., EC1118. Thus, accelerating SSU1 activity is based on the common genetic basis in two different brewing yeasts, but is distinctly implemented through: 1) Translocation of SSU1 in wine yeast and, 2) Hybridization of *S. cerevisiae* with *S. eubayanus*, which has the active SeSSU1 with Val19, in lager yeast. Collectively, our date suggests that SSU1-mediated SO₂ production/tolerance is a self-reinforced adaptive trait, which is not only beneficial for stable fermentation but also for suppression of competitive microorganisms, thereby exerting ecological dominance in nature.
NEW TOOLS FOR GENOME EDITING DERIVED FROM ERYTHRITOL CATABOLISM IN YARROWIA LIPOLYTICA

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The oleaginous yeast \textit{Yarrowia lipolytica} is increasingly used as an alternative cell factory for the production of recombinant proteins. At present, several tools for genome editing have been developed but most of them suffer from drawbacks, limiting thus their utilization in practice. For instance, most of the selectable markers used are based on auxothrophy (e.g. leucine, uracyle, lysine) and thus require complementation of the culture medium or the restoration of prototrophy of the strains. Dominant markers, such as the \textit{E. coli} \textit{hph} gene, conferring resistance to hygromycin B, could also be used. However, this marker proves difficult to handle in practice due to a high level of spontaneous resistance in transformed cells. Regulated promoters such as \textit{pLIP2} and \textit{pPOX2} require the utilization of water non-miscible inducers (e.g. oleic acids, triglycerides); rendering their utilization difficult at industrial scale. Here, we report on two genes from the erythritol catabolic pathway; namely \textit{EYK1} and \textit{EYD1}, as new catabolic selectable markers. We also present novel tunable and regulated promoters derived from these two genes with applications in the field of heterologous protein production, metabolic engineering, and synthetic biology.

YEAST CELL WALL TRANSCRIPTOME

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Yeast \textit{Saccharomyces cerevisiae} cell wall is a strong, but flexible structure consisting mostly of \(\beta-1,3\)- and \(\beta-1,6\)-glucan, chitin and incorporated mannoproteins. This structure is continually remodeled as yeast cells undergo processes such as cell division, mating, meiosis and sporulation, which are therefore expected to be coupled with changes in expression of a certain subset of cell wall-related genes. Over the past decade, advances in high-throughput RNA-sequencing methods have enabled deep insight into complex yeast transcriptome. Many RNA transcripts, that are not translated into proteins and are therefore referred to as long non-coding RNAs (IncRNAs), have been found to play important functions in various cellular processes such as gene transcription, splicing and translation as well as nuclear organization, cell growth and developmental pathways. In our work we explore the cell wall transcriptome, which can be defined as the condition-specific cellular pool of mRNAs coding for proteins that are either incorporated in the cell wall or take part in biosynthesis and remodeling of the cell wall. We take advantage of high-throughput RNA-sequencing methods, as well as transcript-specific methods, to gain insight into how the cell wall transcriptome is modulated to support different life cycle phases and tolerate various environmental perturbations. The results of this work indicate that IncRNAs might regulate expression of certain cell wall-related genes.
THE AMDS GENE AS A DOMINANT RECYCLABLE MARKER FOR PICHIA PASTORIS

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Genetic manipulation of Pichia pastoris relies on a limited range of selection markers. In this context, the amdS gene from Aspergillus nidulans represents a potential tool as a dominant recyclable marker. Its selection is based on growth on acetamide as sole nitrogen source, while its counter selection relies on the production of toxic fluoroacetate from fluoroacetamide by strains carrying the marker. In order to test the amdS marker in P. pastoris, we built expression cassettes containing this gene flanked by loxP sequences together with a reporter gene or homologous sequences for gene replacement of native genes. First, a putative amidase gene was deleted from the yeast genome with an antibiotic resistance marker which was later recycled. The resulting strain, LA1, was used as well as wild-type X-33 in transformations with the amdS marker. The marker cassette and EGFP reporter gene were ligated to a plasmid derived from pPIC9 (Invitrogen), creating pAMDS. Plasmid integration was directed either to HIS4 or 3’ AOX1 locus. Colonies were analyzed by fluorescence scanning and PCR. Clones presented variable fluorescence intensities, which may indicate a variation on gene copy number. Integration sites yielded different results, the HIS4 integration site providing more fluorescent clones than the 3’ AOX1 fragment. LA1 showed no false positive results unlike X-33. Subsequently, the amdS marker was used in the deletion of ADE2 and URA5 genes. The amdS cassette was flanked by homologous sequences for complete substitution of the target coding sequences. The first transformation yielded ade2 mutants and the marker was recycled with pYRCre2, a Cre recombinase-containing plasmid. Selection of the recycled marker was performed successfully in fluoroacetamide. The next transformation replaced the URA5 gene, generating 5-FOA resistant clones and thus proving that amdS can be widely used in P. pastoris.

REAL-TIME MONITORING OF REPORTER GENES REVEALS SIGNALING BOTTLENECK IN XYLOSE-UTILIZING SACCHAROMYCES CEREVISIAE

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The fermentative capacity of Saccharomyces cerevisiae and its tight regulation for the utilization of other carbon sources than glucose have enabled this yeast to become the key organism for production of bioethanol from glucose-rich materials (such as corn starch or cane sugar). However, when broadening the substrate spectrum to non-edible substrates (such as corn stover, sugar cane bagasse or wheat straw) it was found that cell viability, process yields and productivities were affected. The apparent causes for the impaired performance were inhibitors present in lignocellulosic hydrolysates together with the inability of wild type S. cerevisiae to ferment xylose, a pentose sugar constituting up to 30% of the biomass. Although the limits have been stretched by
improving yeast robustness and enabling xylose utilization through metabolic engineering, challenges remain, especially regarding co-substrate utilization. Catabolite repression, a regulatory mechanism preventing the utilization of other carbon sources when glucose is available has been well studied in *S. cerevisiae*. However the yeast signaling response to the non-naturally fermented sugar xylose remains unclear. To resolve this matter we have created a panel of GFP-coupled reporter system representative of the major glucose signaling pathways in yeast, and investigated the fluorescent signals produced under different conditions using flow cytometry. Our results demonstrate that a significant signaling response can only be recorded when xylose is internalized and fermented, but not when it is present in the medium and not assimilated. Also the response that is observed at high xylose concentrations is similar to the signaling response to low glucose concentrations. These results as well as further results on the deletion of *IRA2*, a known PKA inactivator, in the biosensor strains, will be discussed.

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**ADAPTIVE LABORATORY EVOLUTION OF SACCHAROMYCES CEREVISIAE EXPRESSING THE OXIDATIVE WEIMBERG PATHWAY FOR XYLOSE CONVERSION TO TCA INTERMEDIATES**

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In order to compete with fossil fuel-dependent bulk chemicals production, biorefinery processes must utilize all major sugars found in the raw material. In lignocellulosic biomass up to a third of the sugars consists of xylose, a pentose sugar that the industrial workhorse *S. cerevisiae* cannot naturally use. In existing recombinant xylose-utilizing yeast, co-consumption with the most common sugar, glucose, is challenging since the current functional pathways require xylose to enter the yeast metabolism in the upper part of glycolysis, competing with the glucose flux. Several bacterial and archaeal species have been found to express, instead, oxidative xylose assimilation through the Weimberg or the Dahms pathways, which end with the products α-ketoglutarate or pyruvate and glycolaldehyde, respectively. If introduced in *S. cerevisiae*, the inferred bypass of glycolysis could enable the yeast to produce value-added bulk chemicals from these important metabolic nodes without being inhibited by hexose catabolism. The Weimberg and Dahms pathways contain five and four reactions, respectively, three of which are shared. Expression of either of these exogenous pathways in *S. cerevisiae*, xylonate, the product of the first two reactions downstream of xylose, is accumulated, inhibiting growth on glucose in the presence of xylose. This effect is alleviated by removing the second enzyme, XylC. *However*, strains expressing only the first enzyme, XylB, together with the lower pathways still accumulates xylonate through a spontaneous reaction, indicating limitations downstream in the pathway. So adaptive laboratory evolution (ALE) of a yeast strain expressing a complete Weimberg pathway was carried out by selection in media with 2 g/L glucose and 10 g/L xylose and the resulting population reached a 35% higher final cell dry weight as well as increased xylose uptake. Results that will be presented on the characterization of isolates from this population will shed light on remaining bottlenecks for eukaryotic xylose oxidation.
HIGHLIGHTING KEY METABOLIC DIFFERENCES BETWEEN YEAST SPECIES DURING THE WINEMAKING PROCESS WITH MULTI-SCALE MODELS

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Saccharomyces cerevisiae is the predominant yeast in wine fermentations because of its high fermentative capacity and ethanol resistance. However, consumers demand less alcoholic wines with more aromatic profile or higher glycerol content; rendering this species less attractive for industrial wine production. Current regulation limits the use of metabolic engineering strategies based on genomic interventions. Recent works suggest the possibility of using non-conventional Saccharomyces species to start the winemaking process. However, the rational design of new fermentations using these species requires an improved understanding of their physiological and metabolic idiosyncrasies along with models that can predict their performance. Multi-Scale mathematical modeling of the process brings the possibility of understanding the evolution of yeast metabolism in a time-varying environment. For this purpose, kinetic models describe the dynamics of extracellular compounds while an iterative flux balance analysis allows for the dynamic description of intracellular metabolic fluxes. In this work, we combine an experimental approach with genome-scale models to understand and explain the physiological and metabolic differences among S. cerevisiae T73, S. uvarum BMW58 and S. uvarum CECT12600 during wine fermentations at high temperature (25ºC). Microvinification experiments, i.e. small-scale batch cultures, mimicking wine fermentations were carried out for both species and samples collected across the fermentation period. Samples were used to characterize the dynamics of yeast physiology (cell volume, OD600, biomass and number of colony forming units) and extracellular metabolism (49 metabolites measured by high-performance liquid chromatography). The data-set used to recover the kinetic parameters, includes the most relevant sugars, organic acids, aromas and amino-acids to fermentation and final wine composition. The final models were able to reproduce the dynamics of growth and the most relevant metabolites. The combination of the experimental approach with the model highlighted relevant metabolic differences between species, namely, at the level of redox balance, amino acid metabolism and energetics.

SCREENING OF POTENTIAL DELETION STRAINS FOR HYPER-SECRETION OF YEAST CELL WALL MANNOPROTEINS USING FLO1-ENCODED MANNOPROTEIN AS A MODEL

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To date, it has been reported that the five dominant FLO genes in S. cerevisiae encode for a family of glycosylphosphatidylinositol (GPI) linked glycoproteins commonly referred to as flocculins or cell wall mannoproteins. The adhesion phenotypes that are
associated with individual FLO genes have been extensively researched and well characterized. However, far less is understood about the cellular metabolic routes that lead to their biochemical synthesis and incorporation into the yeast cell wall. Additionally our fairly limited current understanding of the fine molecular architecture of these mannoproteins predominantly relies on data generated from in silico predictive research studies. In this research study, a genetic engineering approach was employed to constitutively overexpress the FLO1-encoded mannoprotein in the wild types BY4741 laboratory S. cerevisiae strains and in mutant BY4741 strains bearing a disruptive deletion in either their KNR4 or GPI7 cell wall biogenesis related genes. As such the effect of the gene deletions on the intensity of the flocculation phenotype will shed light on the contribution of the deleted gene products in biochemical processing of FLO1 mannoproteins. It is envisioned that the transgenic yeast strains overexpressing FLO1 mannoproteins will provide a viable alternative for the large-scale isolation and purification of the intact mannoprotein especially if it were to be released into the growth medium by FLO1 overexpressing deletion transgenic strains. This glycoprotein reservoir can be utilised in the structural analysis of FLO1 mannoproteins. Preliminary data have shown that in relation to the transgenic wild-type BY4741-F1P strain decreased flocculation intensity was observed in the BY4741DKNR4-F1P strain. BY4741DGPI7-F1P displayed a flocculation intensity that was similar to the BY4741-F1P strain. Interestingly, a higher protein concentration was observed in the spent growth medium of the transgenic BY4741-DKNR4-F1P strain when compared to the untransformed DKNR4 thereby seemingly suggesting the possibility of the Flo1 mannoprotein being released extracellularly.
SPONSORED TALKS
THE SYNERGY OF RATIONAL DESIGN, NATURAL GENETIC VARIATION AND EVOLUTIONARY ENGINEERING IN A HIGH THROUGHPUT BIOLOGICAL FOUNDRY FOR INDUSTRIAL YEAST STRAIN IMPROVEMENT

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Ginkgo Bioworks is the organism company. We design custom microbes (including yeast strains) for customers across multiple markets. In biological engineering, living organisms are the factories that build new products. Designing the best organisms requires a different sort of factory, one where we combine state of the art methods in genetics, synthetic and systems biology with the best tools in automation and software to do biological engineering at scale. Bioworks1 was the world’s first organism foundry, where engineers are prototyping thousands of biological designs. We have since built Bioworks 2 and 3 that greatly increases our capacity to breed, phenotype and genotype microbes in high throughput at increasingly lower costs. We will describe the suite of transcriptomic, proteomic, and metabolomic pipelines developed in our foundries, and how they can be employed to characterize the natural genetic variation in diverse strains to select those with improved performance and yield in industrial fermentations. In some cases, we go further and identify the genetic determinants underlying the desired traits using the principles of quantitative trait mapping. Finally, we will discuss the power of generating further diversity from natural genetic variation by leveraging a high-throughput breeding pipeline and/or directed evolution approaches, in context of a few examples. This enables us to rapidly generate improved yeast strains for a variety of applications in industries based on fermentation, including distilling, brewing, wine making, and baking.
FROM GRAPE MUST TO WINE: TOWARDS A BETTER KNOWLEDGE OF YEASTS ACHIEVEMENTS

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Sponsored talks

Fermentation of grape must, a crucial step of winemaking, consists in the conversion of sugars into ethanol, CO2 and numerous other metabolites by yeasts. Most of the fermentations are performed by selected yeasts, essentially from *Saccharomyces cerevisiae* species, inoculated under dried active forms. Such strains have been selected for their specific properties and are well characterized. They contribute greatly to the aromatic balance of wines. Since many years, knowledge about wine yeasts steadily increased thanks to numerous scientific studies and the gain in the understanding of their metabolism is huge. Consumption and requirements in nutrients (sugars, lipids, nitrogen, sulfur, vitamins, minerals), synthesis and production of biomass and metabolites (ethanol, glycerol, acids, alcohols, esters, sulfur compounds) have been characterized. Current work combining different levels of study, from physiologic and metabolomic to transcriptomic and genetic, provides us a global overview and allows us to deepen our understanding. Furthermore, following the market trends and consumers preferences, demand for new wine yeast strains combining different properties of interest or adapted to winemaking conditions and global climate change, is continuously increasing. To meet those requirements, new methods based on the generated knowledge can be implemented such as clonal selection, metabolic engineering or hybridization. Some of those new wine yeasts and the science behind them will be presented.
SESSION 7: YEASTS IN BIOREFINERIES
IMPROVING THE SUGARCANE-BASED BIOREFINERY VIA MODULATION OF FREE-ENERGY CONSERVATION IN SACCHAROMYCES CEREVISIAE

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Industrial bioprocesses carried out under anaerobiosis present some advantages over their aerobic counterparts: cost savings with air compression, air filtration and mechanical agitation; cost savings with heat removal and improved carbon utilisation in the final product. The sugarcane-based biorefinery is a remarkable example of such a process, in which the current major commercial product is fuel ethanol. The main biochemical conversion involved is the fermentation of sucrose into ethanol, which Saccharomyces cerevisiae uses as a means to conserve free-energy: 4 moles of ATP are synthesised from each mole of sucrose fermented. By manipulating the native sucrose utilization pathway, via metabolic engineering and laboratory evolution, two different improvements are proposed for this biorefinery. In the first case, the native extracellular sucrose hydrolysis pathway was substituted by active transport and intracellular hydrolysis, rendering a yeast strain that conserves only 3 moles of ATP per mole of sucrose utilized, with a concomitant 10% higher ethanol yield than the reference strain. In a second example, the native pathway was substituted by passive transport and sucrose phosphorolysis, rendering a yeast strain with an 8% higher free-energy conservation, when compared to the native 4 ATP/sucrose yield. In the latter case, a 25% increase was expected from theoretical calculations and the possible reasons for the lower-than-aimed increase achieved will be discussed. A yeast chassis with a higher ATP yield on sucrose would be a valuable platform for the production of metabolites that require energy for their export from the cells, such as lactic acid, under anaerobic conditions.
IMPROVEMENT OF SACCHAROMYCES CEREVISIAE BY HYBRIDIZATION FOR INCREASED TOLERANCE TOWARDS INHIBITORS FROM SECOND-GENERATION ETHANOL SUBSTRATE

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Second-generation biofuels produced from cheap and abundant lignocellulosic biomass, has been viewed as one plausible solution to the "food versus fuel" problem. Sugarcane bagasse is an abundant source of lignocellulosic biomass in Brazil and is generally recognized as a very promising feedstock for lignocellulosic ethanol production. Nevertheless, inhibitors such as furfural, HMF and carboxylic acids are formed during acid thermochemical pretreatment of lignocellulosic biomass, which has negative effect on fermentative microorganisms, Saccharomyces cerevisiae. Second-generation ethanol (2GE) in Brazil has the possibility to use a novel substrate, prepared as a blend of sugarcane bagasse hydrolysate and cane molasses. Molasses supplements the nutritional deficiencies of bagasse hydrolysate, contributing with minerals, amino acids and vitamins. However, molasses also contains additional inhibitors, such as HMF, sulfite, and toxic concentration of some minerals, which affect S. cerevisiae fermentation performance. The goal of this work was to generate tolerant derivatives of S. cerevisiae industrial strains that are able to cope with inhibitors present in hydrolysate and molasses, by means of sexual hybridization and adaptive evolution. Isolates showed good fermentation properties compared to reference strains, showing that hybridization and adaptive evolution of Brazilian industrial strains (PE-2, CAT-1 and SA-1) was good strategy to develop new tolerant strains for 2GE production. To better utilize all sugars present in hydrolysate, a cassette containing genes responsible for xylose fermentation (XR, XD and XK) was integrated using CRISPR/Cas9 into the genome of derivative haploid which had the highest tolerance to inhibitors hydrolysate. Fermentation studies demonstrated that this engineered strain was able to metabolize xylose into ethanol. Finally, the haploid was analyzed by QTL mapping to identify the genetic basis of hydrolysate tolerance. Although the causative gene(s) were not identified in this work, a number of QTL peaks were identified that will serve as the starting point for future fine-mapping studies.

BIOCHEMICAL BASIS OF PROLIFERATION OF AUREOBASIDIUM ON WOOD: NOVEL ENZYMES OF THE GENUS

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Aureobasidium pullulans is a dark yeast-like microorganism known to colonize weathered wood surfaces. Biochemical basis for this property lays in ability of this species to produce enzymes causing complete hydrolysis of wood hemicelluloses. The Aureobasidium strains are also cellulase positive, although do not grow on crystalline cellulose. The two major wood hemicelluloses are xylan and glucomannan. The constituents of the wood deteriorating enzymes systems of Aureobasidium are
xylanolytic and mannanolytic glycoside hydrolases. We report here that *Aureobasidium* strains, similarly as some wood-degrading fungi, produce a recently identified esterase, classified in carbohydrate esterase family CE15 (CAZY), called glucuronoyl esterase (GE). Physiological function of GE is cleavage of ester cross-links between 4-O-methyl-D-glucuronic acid of xylan and lignin alcohols. Such enzyme could contribute significantly to proliferation of the species on wood surfaces. Several lines of evidence show that the enzyme plays an important role in plant cell wall degradation. Its addition to a commercial cellulosolytic enzyme system from Novozymes considerably enhanced the amount of liberated reducing sugars. The enzyme reduced molecular mass of isolated lignin-carbohydrate complexes and released large xylan fragments. Expression of fungal GE altered plant cell wall composition and architecture, improved extractability of xylan from plant biomass and enhanced enzymatic cellulose digestibility. These examples of catalytic activity of these relatively new carbohydrate esterases suggest their biotechnological potential in saccharification of transgenic plants, saccharification of lignocellulosic biomass and as agents for lignin removal. The need of pursuing studies of GE in yeast and yeast-like microorganisms is obvious. This work was supported by Scientific Grant Agency under the contract No. 2/0016/18.

**COMPARATIVE PROTEOMICS OF NON-MODEL YEASTS TO LOW pH IN HYDROLYSATE CULTIVATIONS**

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Biological production of platform chemicals and fuel precursors from lignocellulose represents an attractive alternative to the current petroleum-based routes. However, bioconversion of realistic feedstocks presents considerable challenges for the biological chassis. Stressors including pH, osmolarity, temperature, substrate toxicity, or product inhibition, often compromise the overall microbial performance, especially in model organisms. In this vein, non-model microorganisms that exhibit beneficial phenotypes for industrial applications can provide a solution to some of these challenges. To that end, we present a comparative proteomics study of three low-pH tolerant yeasts *Pichia kudriavzevii*, *Zygosaccharomyces parabailii*, and *Dekkera bruxellensis*. Strains were cultivated on corn stover hydrolysate under anaerobic conditions at pH 5.5 and 3.0 to examine the tolerance mechanisms and identify candidates for further metabolic engineering. Comparing pH 3.0 to pH 5.5, sets of 73, 74, and 158 proteins were differentially expressed within *P. kudriavzevii*, *Z. parabailii*, and *D. bruxellensis*, respectively. Moreover, at the lowest pH value, up to 25 proteins were uniquely expressed in *P. kudriavzevii*, whereas 31 proteins were uniquely expressed in the case of *Z. parabailii* and *D. bruxellensis*. This pool of proteins will enable a functional annotation and a Gene Ontology term enrichment analysis to highlight the unique and common features across this multi-strain comparison.
OPTIMIZING GENERAL ROBUSTNESS OF YEAST STRAINS FOR CORN-ETHANOL INDUSTRY USING GENETICS & GENOMICS TOOLS
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To improve the robustness of our industrial yeast strains for the corn-ethanol industry, we first needed to understand the complexity of stresses present during ethanol fermentation. Previous internal work and from literature have shed light on the complexity of stresses the yeast encounter during corn fermentation such as high temperature, pH, osmotic, weak acids and high ethanol concentration stresses. All of these stresses negatively impact the viability, specific rates of fermentation of the yeast and decrease the total amount of ethanol produced at the ethanol plant. Because of the complexity of multi-stress tolerance, it is impossible to improve the general robustness of strains by rational design, so we decided to use unbiased screening of mutants to overcome this obstacle. We decided to leverage commercially available systematic mutant collections to study two types of genetic changes: i) single gene deletion and ii) overexpression using plasmids. We measured the fitness of nearly every single gene deletion and amplification in the \textit{S. cerevisiae} genome using pooled competitions of thousands of mutants under selection followed by barcode sequencing. Several mutants were found to be interesting candidates for commercial bioethanol production. We showed that the efficiency of ethanol production on an industrial scale is increased by using yeasts that are tolerant to multi-stresses.

TOWARDS AN EFFICIENT AND COMPETITIVE INDUSTRIAL PROCESS FOR CELLULOSIC ETHANOL IN BIOREFINERY
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Cellulosic ethanol has been developed since the last two decades in response to energy needs and global warning about fossil fuels use. The main constrains for cellulosic ethanol production have been the recalcitrance of lignocellulosic materials; the high cost of cellulase enzymes; the time required to hydrolyze cellulose; the rather low yield of glucose vs biomass weight; and the finding of proper yeast strains able to ferment under rough conditions. A number of different approaches have been assayed to fulfill all these conditions, but very few have succeeded. In our research we have developed a new system, which takes as a principle the so-called Simultaneous Saccharification and Fermentation (SSF); the new approach combines pretreatments, increase of biomass charge in the fermenters, the optimal cellulose enzyme dosing and the semi-continuous biomass fed/fermented product harvest cycles. We have named the whole process as SSFSCC (Simultaneous Saccharification and Fermentation in Semi Continuous Culture). Moreover, we have selected a single \textit{Saccharomyces cerevisiae} yeast strain among 4 industrial strains for its ability of adaptation, its high tolerance to ethanol and dominance while in cofermentation if compared to other yeast strains. As a result, we have been able to obtain a daily production of cellulosic ethanol ranging from 8.36 to 10.79% v/v, which is highly competitive with sugar cane ethanol and other first
generation sources. The theoretical ethanol yield obtained by the yeast strain (CLQCA-INT-005) was 96.72%, corresponding to 0.49 g ethanol/g glucose. This method presents several advantages namely: high ethanol concentration for more efficient distillation processes, lower costs for enzymes use, highly controllable fermentation process, and significant production time reduction. This whole technology is currently as pending patent status.
EFFECT OF SYMBIOTIC INTERACTION BETWEEN A FRUCTOOLIGOSACCHARIDE AND PROBIOTIC ON THE KINETIC FERMENTATION AND CHEMICAL PROFILE OF MAIZE BLENDED RICE BEVERAGES

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There is an important demand for the development of new non-dairy probiotic beverages in the functional food market. This work aimed to develop new fermented beverages from maize and rice. Lactobacillus plantarum CCMA 0743, Torulaspora delbrueckii CCMA 0235, and the commercial probiotic Lactobacillus acidophilus LACA 4, were used as a mixed starter culture. Two prebiotic concentrations, 20 and 50 g/L fructooligosaccharide (FOS) were tested. The growth of L. acidophilus LACA 4 was favored by 50 g/L FOS and after refrigerated storage at 4 °C for 28 days, its population remained above 10⁷ CFU/mL. Lactic and acetic acids were the main organic acids detected, at around 3.7 and 0.5 g/L, respectively. Ethanol was present at less than 5 g/L in non-alcoholic beverages. Fifty-five volatile compounds including acids, alcohols, aldehydes, esters, ketones, pyrazines and others, were detected. The sensorial analysis demonstrated that more than 50% of consumers liked slightly or liked extremely the beverages (scores from 6-9). Therefore, potential symbiotic cereal beverages were successfully obtained using a mix of lactic acid bacteria and yeast as a starter culture. This is an important step in the commercial production of alternative beverages from common food substrates for consumers.

KLUYVEROMYCES MARXIANUS SLP1, A VERSATILE YEAST TO USE IN AGRO-INDUSTRIAL WASTE BIOREFINERIES

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The non-Saccharomyces yeast Kluyveromyces marxianus SLP1 has advantageous potentials for to be used in agro-industrial waste biorefineries, it not only for its ability to produce ethanol from different sugars, but also for its versatility to produce metabolites of high added value even in stressful conditions. In our lab, we had demonstrated that K. marxianus SLP1 show outstanding capacities to produce first and second generation ethanol in comparison with the commercial yeast Saccharomyces cerevisiae ethanol red (ERD). Besides, K. marxianus SLP1 can produce high-value compounds as esters (ethyl acetate, isopentyl acetate, isoamyl acetate, phenyl acetate, etc.), higher alcohols (isobutanol, 1-propanol, amyl alcohols, etc.) and aldehydes as co-products during the alcoholic fermentation. K. marxianus SLP1 had demonstrated its versatility to growth and produce metabolites in different fermentative processes: batch, continuous, fed-batch, etc. As well as, K. marxianus SLP1 produce enzymes as
fructanases and in consequence, it can be used in simultaneous saccharification and fermentation (SSF) of fructans for ethanol production. Since *K. marxianus* is a thermotolerant yeast, it had been evaluated to direct the second-generation ethanol production from different lignocellulosic wastes. Our studies also had shown that *K. marxianus* SLP1 could ferment with non-detoxified diluted acid or autohydrolysis pretreated corn cob, wheat straw, agave bagasse and sugarcane bagasse to produce ethanol, xylitol, and high-value compounds. *K. marxianus* SLP1 possess considerate inhibitors tolerance especially to furfural and HMF, and it presents a better physiological response to furan derivatives stress than *S. cerevisiae* ERD. *K. marxianus* SLP1 can ferment vinasses to produce several metabolites; this waste is very aggressive to the environment because of its high content of toxic and recalcitrant organic matter. In conclusion, all the experimental evidence had demonstrated that the yeast *K. marxianus* SLP1 is a promising alternative to be used in agro-industrial waste biorefineries.

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**EFFECT OF THE CHITOSAN OBTAINED BY LACTOBACILLUS FERMENTATION ON BIOETHANOL YEAST STRAINS**

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Chitosan is a versatile biopolymer widely used in agriculture, food, medical, and pharmaceutical industries. It is obtained from chitin deacetylation derived from crustacean shells and fungal mycelia. In spite of the potential antimicrobial activity of chitosan, it has not been explored in the bioethanol industry. *Dekkera bruxellensis* is an opportunistic yeast of great interest in the wine industry and in the ethanolic fermentation process in Brazil. In this work, chitosan was first obtained from shrimp waste (head and shell) by a bioconversion process using *Lactobacillus plantarum* in MRS media under optimized concentrations of residue, bacterial inoculum and glucose. Deacetylation process was made by the autoclave method using sodium hydroxide 45%. Following the antimicrobial effect of the natural chitosan obtained compared to a commercial chitosan (both diluted in 2% acetic acid solution) was evaluated against a strain of *D. bruxellensis* (Db) and an industrial strain (PE-2) of *Saccharomyces cerevisiae* (Sc). This experiment was carried out at the Plate reader Infinite 200 PRO equipment in a 96-well cell culture plate with YPD medium and chitosan concentrations ranging from 50 mg/L to 500 mg/L, at 30°C, 24 hours. The natural chitosan was effective to reduce the maximal specific growth rate (µ) and to increase the lag phase at the concentration of 500 mg/L for Db. With the commercial chitosan, 100 mg/L was effective but it did not increase the lag phase. An effect only on the rate of Sc was observed with both chitosans. The extension of the lag phase in 4 hours by the natural chitosan is relevant to control the growth of Db in the ethanolic fermentation. Further experiments are demanded to increase the solubility of natural chitosan in order to achieve better results with lower concentrations.
ENGINEERING YEAST CHASSIS FOR A SUGARCANE-BASED 1.5G FUEL ETHANOL BIOREFINERY

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Sugarcane-based raw materials can be used for the production of both first-generation (1G) and second-generation (2G) fuel ethanol. However, deconstructing the recalcitrant lignocellulose structure entails a high operational cost that has prevented the 2G technologies from becoming a commercial reality. One of the most attractive ways of overcoming the high cost in 2G technologies in Brazil is to retrofit them in the well-established 1G sugarcane ethanol industries. This retrofitted ‘1.5G’ biorefinery would require yeast strains that can utilize the different sugars present in the combined 1G+2G stream as well as tolerate the inhibitors and stress factors inherent to both 1G and 2G processes. Here we propose a strategy whereby a single engineered Saccharomyces cerevisiae strain can be used to ferment the sugars present in sucrose-rich media (sugarcane juice and/or molasses) as well as those sugars released during a partial hydrolysis of the cellulosic fraction of lignocellulosic biomass (this combined sugar stream would consist mainly of sucrose and cellobiose, together with a small amount of monosaccharides). To achieve simultaneous consumption of both the disaccharides and to increase the ethanol yield on sugars, we propose active transport of sucrose and cellobiose followed by their hydrolysis intracellularly. We surmise that this strategy would confer an advantage to yeast, as less monosaccharides would be present in the extracellular environment for other contaminating microorganisms. Moreover, the amount of β-glucosidase required during the enzymatic hydrolysis of cellulosic biomass will be reduced, as partial hydrolysis of the feedstock is sufficient. In this work, we intend to address the opportunities and challenges of a 1.5G sugarcane-based biorefinery for ethanol production, in terms of yeast physiology and metabolic engineering.

KLUYVEROMYCES MARXIANUS STRAIN SELECTION FOR CO-PRODUCTION OF FUEL ETHANOL AND VIVABLE BIOMASS FROM CHEESE WHEY

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Agroindustrial by-products and residues can be transformed into valuable compounds in biorefineries. Here we present a new concept: production of fuel ethanol and viable yeast from cheese whey. For this purpose, a microorganism must be employed that is capable of converting lactose into ethanol with a good compromise among yield, rate and titer. Besides, it should also maintain high viability after this conversion, in order to be used for downstream applications. Kluyveromyces marxianus, a respiro-fermentative and generally regarded as safe yeast species, has been explored separately.
as an ethanol producer and as viable bioactive microorganism. We started our study with a selection involving thirty *K. marxianus* strains, which were screened on plates for the capacity to grow on cheese whey (7.5 g lactose/L) under aerobic and anaerobic conditions, at 5% ethanol concentration and also at pH 2.5 (achieved by H2SO4 addition). *K. marxianus* strains Km9 (an isolate from a fermented milk in Argentina) and NCYC1429 displayed good growth properties compared to the other strains and were further evaluated in a miniaturized first generation fuel ethanol process that had been previously benchmarked with *Saccharomyces cerevisiae* on sugarcane molasses. The system included five consecutive fermentation rounds with cell recycling and acid treatment between two consecutive fermentations. Both *K. marxianus* strains produced ethanol with high yields (≈40 g of ethanol produced on 100 g of lactose) and rates, and maintained cell viability higher than 90%, even after the five fermentation cycles. We are currently investigating the bioactive properties of these two strains, aiming at evaluating the possibility of using them as an ingredient in functional foods.

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**YEAST STRAINS FOR FUELS AND CHEMICALS PRODUCTION: BIOPROSPECTING, PHYSIOLOGY AND GENETIC IMPROVEMENT**

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Lignocellulosic biomass, which is rich in pentose and hexose sugars, is an inexpensive and abundant substrate that can be used for production of fuels and chemicals. However high yields and productivity are necessary to lower production costs. In this context, yeasts can be interesting alternatives to produce polyols, organic acids and bioethanol. In this study, the potential of wild and recombinant yeast strains to produce xylitol, xylonic acid and ethanol was investigated. Initially, newly isolated and publicly known yeast strains were physiologically and genetically characterized. A collection of non-*Saccharomyces* strains was screened by the ability to metabolize xylose in different experimental conditions. Then, fermentative performances of selected strains were compared with *Scheftersomyces stipitis*, *Spathaspora passalidarum*, *S. arborariae* in minimal medium supplemented with glucose/xylose and sugarcane biomass hydrolysate. In addition, the complete genome sequence of newly identified strains of *Spathaspora* sp. and *Meyerozyma* sp. was obtained and annotated. Comparative genomic analysis revealed the enrichment of genes related to membrane transport proteins, oxidoreductase activities and cellulose catabolic process in these strains. In parallel, the yeast *Komagataella phaffii* (previously *Pichia pastoris*), which is able to grow at very high cell densities, was engineered to produce xylonic acid. For this, new putative xylose dehydrogenase (XDH) genes from bacteria and fungi were identified by phylogenetic analysis and ten of those were chosen for expression in *K. phaffii*. Recombinant strains expressing each gene were evaluated by the ability to produce xylonic acid and the best candidate genes were chosen for further analysis. The effects of co-substrates on xylonic acid production were evaluated in different fermentation setups. Strains were able to produce xylonic acid with yield up to 0.95 g/g, under the best evaluated conditions. Results will be presented and discussed.
IDENTIFICATION AND EXPRESSION OF HETEROLOGOUS XYLOSE TRANSPORTERS IN SACCHAROMYCES CEREVISIAE

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Utilization of microorganisms to obtain fuels and chemicals from renewable feedstocks are important due to socio-economic and environmental issues. In this context, the yeast *Saccharomyces cerevisiae* has been engineered and employed in several bioprocesses. However, xylose transport is considered one of the major bottlenecks in this yeast, once the high affinity of yeast endogenous glucose transporters inhibits the uptake of this pentose to the intracellular environment, hindering the utilization of the second most abundant sugar in lignocellulosic biomass. In this work, new xylose transporters were identified and expressed in *S. cerevisiae*. Initially, the mutated hxt11 transporter, which presented higher affinity for xylose than glucose, was used in comparative genetic analysis for the selection of putative genes coding for transporters with high xylose affinity. Then, three new genes were synthesized, cloned and constitutively expressed in *S. cerevisiae* EBY.VW4000 (xylose transport null strain) for validation of xylose transporter activity. Xylose transport activity was analysed in the engineered strains through measurements of intracellular sugar accumulation and by evaluation of yeast ability to ferment xylose. For this, yeast was also engineered with xylose metabolic pathway composed by xylose reductase, xylitol dehydrogenase and xylulokinase. Our results demonstrate that one new efficient xylose transporter was identified and successfully improved sugar utilization in *S. cerevisiae*. In addition, transport affinity to other monosaccharaides and disaccharides was evaluated. Results will be presented and discussed.

EFFECTS OF HYDROXYCINNAMIC ACID AND VOLATILE PHENOLS ON THE GROWTH OF SACCHAROMYCES CEREVISIAE

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One of the main characteristics of the yeast *Dekkera bruxellensis*, an important contaminant of ethanolic fermentations, is the ability to produce volatile phenols such as 4-vinylphenol (4-VP) and 4-ethylphenol (4-EP) from p-coumaric present in wine grapes due to the action of the enzymes hydroxycinnamate decarboxylase and viniphenol reductase. Hydroxycinnamic acids such as p-coumaric acid are also present in molasses and in sugarcane juice and, thus, volatile phenols could be synthesized during ethanolic fermentation and may act as inhibitors of *Saccharomyces cerevisiae* growth. This work evaluated the effects of p-coumaric acid, 4-VP and 4-EP on the growth of an industrial strain of *S. cerevisiae* (PE-2) in semi-synthetic medium. The experiments were carried out in a 96-well cell culture plate filled with YPD broth, the yeast inoculum (10^6 cells/mL) and the phenolic compounds. The plate was incubated at 30°C, 24 hours, with shaking, in a Plate Reader Infinite 200 PRO equipment (TECAN), with absorbance readings at 600 nm every 15 minutes. The concentrations ranged from 4 to 20, 2 to 10
and 3 to 15 mg/L of p-coumaric acid, 4-VP and 4-EP, respectively. The choice for these concentrations was based on p-coumaric concentration found in sugarcane juice and molasses, and in the concentrations of volatile phenols produced by strains of *D. bruxellensis* in these sugarcane musts in a simulated fuel ethanol process in a previous experiment. There was no inhibitory effect of p-coumaric acid and volatile phenols on the maximum specific growth rate and no extension of lag phase of *S. cerevisiae* within the concentration range studied. Further experiments are in progress to evaluate the effects of 4-EP in YPD with low pH, high ethanol concentrations and high initial sugar concentrations in order to verify whether the interaction among these factors could impact the growth of *S. cerevisiae*. Support: Fapesp (2016/20680-4).

**EFFECT OF FURFURAL ON THE PHYSIOLOGY AND METABOLISM OF KLUYVEROMYCES MARXIANUS SLP1 DURING THE PRODUCTION OF ETHANOL**

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Lignocellulosic ethanol is considered a promising alternative in the use of renewable fuels. Furfural is one of the main compounds formed in the hydrolysis process of the lignocellulosic biomass that generates greater cellular toxicity. However, the mechanism of assimilation of furfural and its association with physiological damage caused in response to stress in yeasts is not known with clarity. In this work, we evaluated the physiological and metabolic changes in the yeast *Kluyveromyces marxianus* strain SLP1 caused by furfural during the production of ethanol. The experiments were carried out in mini-bioreactors with 150 mL of a chemically defined medium using 40 g/L of glucose as a carbon source (control condition) and added with 1 g/L of furfural (stress condition). Physiological alterations were monitored using flow cytometry technique with different fluorochromes. Metabolic changes were evaluated measuring organic acids, acetaldehyde, glycerol, furfuryl alcohol, and ethanol production using liquid and gas chromatography. Under the stress condition, several physiological and metabolic changes were observed. The cell population decreased 38%, 40% of the population decreased its size, 26% of the cells showed membrane damage, 22% of the cells presented an increase in the number of vacuoles, 40% presented damages in mitochondria, 38% of the cells showed depolarization of the membrane and 59% of the population presented accumulation of reactive oxygen species. On the other hand, *K. marxianus* SLP1 produced glycerol, acetic acid, formic acid, acetaldehyde and furfuryl alcohol as a product of the assimilation of furfural. The final result was a 50% decrease in ethanol production. In conclusion, the physiological and metabolic changes observed allow determining the physiological state of *K. marxianus* SLP1 under furfural inhibition conditions, as well as the identification of the different metabolites generated in response to stress and its possible association with the assimilation metabolism of furfural.
INFLUENCE OF THE CULTURE CONDITIONS ON THE ACCUMULATION OF INTRACELLULAR LIPIDS FOR THE PRODUCTION OF SECOND GENERATION BIODIESEL BY THE OLEAGINOUS YEAST R. GRAMINIS S1 / 2R

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The incorporation of biodiesel into the energy matrix in Uruguay has generated environmental and economic benefits. Biodiesel is generated by transesterification of triglycerides from different sources, mainly oils from oleaginous crops, with short-chain alcohols. However, concern has been raised about diverting farmland or food crops to biofuel production in detriment of the food supply. In this sense, the development of processes that use alternative raw materials such as microbial oils produced from waste or industrial by-products is relevant. In a previous work, our group selected, an oleaginous yeast, identified as Rhodotorula graminis, capable of accumulating intracellular lipids in amounts greater than 40% of its dry weight, when it was cultivated in batch with glucose as a carbon source. The selected yeast could also grow and accumulate lipids using crude glycerin (by-product in biodiesel production) instead of glucose. The aim of this work was to optimize the production conditions of the selected strain in the presence of crude glycerin as carbon and energy source, in order to maximize the accumulation of intracellular lipids to be transformed into biodiesel. A factorial design was applied to study the main factors that affected the growth and lipid accumulation and a central composite design was adopted to derive a statistical model for optimizing the composition of the medium. After optimization lipid production resulted 1,3 times higher. In batch conditions in a 3L fermentor, yields were similar to those obtained in a small scale. However, in fed batch cultures the obtained biomass and lipids were 3,4 times higher. In those conditions the amount of biodiesel obtained from the intracellular lipids was 12g/L of culture medium.

EFFECT OF TEMPERATURE ON GROWTH AND LIPID PRODUCTION OF THE COLD-ADAPTED RHODOTORULA GLUTINIS STRAINS

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Cold-adapted oleaginous yeasts are excellent lipid-producers and they are considered as a potential source of triglycerides (TAG) for the biodiesel synthesis. The lipogenesis is influenced by temperature. This work evaluated the effect of different temperatures on the growth and lipids accumulation by two strains of Rhodotorula glutinins (R4 and
EVALUATION OF LIPID BIOSYNTHESIS ABILITY BY RHODOTORULA SPP.

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Numerous Rhodotorula spp. were characterized as oleaginous yeasts. Under certain culture conditions, they can accumulate neutral lipids, mainly triglycerides (TAG), in a high proportion of dry biomass (20-70%). Microbial TAG can be used as raw material for biodiesel synthesis and they are attractive for biofuels industry. The capability to synthesize lipids among 8 strains of Rhodotorula spp was investigated and the most efficient strain was selected for biotechnological purposes. R. toruloides (Y-6985, Y17092, Y 6987, Y1588 and Y-1091), R. glutinis (Y-34 and R4) and R. mucilaginosa (RCL11) were cultured under nitrogen limiting conditions and excess of carbon source. Yarrowia lypolitica (Y-323) was included as a positive control. Lipid accumulation was evaluated comparatively in the nitrogen-limited glucose-based GMY medium. Yeasts were cultured at 25 °C for 120 h, with agitation (250 rpm). Lipid bodies were observed by fluorescence microscopy with the lipophilic dye Nile red in all strains but the number, size, and shape of lipid bodies as well as, intensity and persistence of fluorescence were different for each strain. Growth cell, production and accumulation of lipids were determined. Yeasts accumulated between 19-38% (w/w) of lipids. R. glutinis R4 showed the higher growth (17g/l) and lipid accumulation (38%). The composition of the fatty acids determined by GC-FID showed a profile similar to vegetable oils and suitable for use as a raw material in the synthetic of biodiesel, with 61% of oleic acid. These results indicate that the lipids produced by R. glutinis R4 could be used as raw material for the synthesis of biodiesel and other biotechnological applications. R. glutinis R4
accumulates significant amounts of lipids and could be studied for commercial purposes because of its high potential for the synthesis of lipids of industrial interest.

A SET OF HAPLOID SACCHAROMYCES CEREVISIAE STRAINS BASED ON NATURAL STRAIN ISOLATES TO STUDY GENETIC FACTORS IMPERATIVE TO SECOND GENERATION BIOFUEL PRODUCTION

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The yeast *Saccharomyces cerevisiae* is an important workhorse regularly chosen for the production of heterologous proteins. During consolidated bioprocessing, this strain is required to produce sufficient titers of recombinant cellulases namely exoglucanases (CBH), endoglucanases (EG) and β-glucosidases (BGL) for the efficient hydrolysis of lignocellulosic substrates to fermentable sugars. Since recombinant protein secretion profiles vary highly among different natural strain backgrounds, careful selection of robust strains with optimal secretion profiles are of crucial importance. Here, we construct and screen sets of haploid derivatives, isolated from natural strain isolates YI13, FINI and YI59, available for research towards improved general cellulolytic secretion. This report details a novel approach that combines secretion profiles of strains and their phenotypic responses to stresses for the development of a screen for isolation of strains with distinct secretory capacities. A clear distinction was observed between the YI13 haploid derivatives and industrial and laboratory counterparts, Ethanol Red and S288c respectively. By using sub-lethal concentrations of the secretion stressor tunicamycin and cell wall stressor Congo Red, YI13 haploid derivative strains also demonstrated interesting tolerance profiles related to their heterologous secretion profiles. Although model *S. cerevisiae* strains are preferentially used as hosts in bioengineering, some natural strain isolates do have specific requisite properties. Preliminary results show that the natural strain isolates and haploid derivatives represent new tools for genetic studies and breeding approaches on heterologous protein secretion. This study reports different properties of enzyme secretion and secretion stress between haploid strains and diploid engineered strains and provides guidelines for selection of host strains.

COLOMBIAN NATIVE SACCHAROMYCES CEREVISIAE ABLE TO METABOLIZE XYLOSE

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Lignocellulosic biomass is a renewable and abundant natural resource that may be used to produce fuel ethanol, an important liquid combustible, as well as a building block to the biochemical industry. After glucose, xylose is the second more abundant sugar in
the lignocellulosic biomass. Despite that various yeasts are able to metabolize this pentose sugar, including recombinant strains of *Saccharomyces cerevisiae*, their productivities and yields are considerably low to be advantageous to industrial bioethanol production. *S. cerevisiae* is excellent for hexose fermentation, and the most used microorganism in the ethanol industrial production. However, scientific literature has generally reported that *S. cerevisiae* is not able to metabolize pentoses, such as xylose from lignocellulosic hydrolysates, in view of its low expression of xylose-metabolizing genes. A new yeast strain, isolated by our research group in the Colombian territory, and identified as *S. cerevisiae*, was found to be able to grow in xylose. By evaluating its growth kinetics, we observed that this strain grows in xylose as the only carbon source in microaerobic conditions and produces alcohol. We confirmed the presence of the putative gene *XYL2* (*YLR070C*) in this strain, that codes for xylitol dehydrogenase activity. Currently, we are engineering the native strain to improve its ability to ferment xylose.

**RECURRENT YEAST STRAINS: ARE THEY ASSOCIATED WITH FLOCCULATION THROUGH HARVEST SEASONS IN BIOETHANOL INDUSTRY?**

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In bioethanol fermentation processes native strains from feedstock input as contaminating microorganisms and usually replace the starter yeast along the harvest season period. In this research, we examined yeast population dynamic of a fermentation process in a Brazilian sugarcane mill during three consecutive harvest seasons (2014-2016) in order to evaluate the recurrent native strains during different harvests. The assessed industrial unit is located in the State of São Paulo/Brazil, herein designated Unit A. Collections were made from April to December, in intervals of around 30 days. Yeast colonies were selected based on their cell morphology. We isolated 120 colonies with different biotypes from 32 different samples stemming from industrial fermented during three successive harvests and submitted to DNA extraction. Strain differentiation was performed using karyotyping (PFGE -Pulsed Field Gel Electrophoresis). It allowed us to assess the composition of yeast population during the bioethanol fermentation process along the harvest period and quantify representative native strains for all harvests evaluated. We found 32 different native strains of which 17 were exclusive related to 2014 harvest season period followed by 3 exclusive from 2015 and just 1 yeast strain was exclusive from 2016 harvest period. In addition, we discovered 9 recurrent yeast strains. We also analyzed the flocculation performance of strains which showed a heavy flocculation profile associated with recurrent strains in successive harvest seasons. These findings show the recurrent yeast strains in bioethanol fermentation as result of introduction, selection and maintenance of native strains probably associated from feedstock in alcoholic fermentation processes in Brazilian distilleries. Thus, feedstock can be considered a determinant factor in yeast population selection of fermenters. This work has implications on the understanding of native yeast population in Brazilian industry since the use of selected native yeast to start fuel-ethanol fermentation processes has been a trend.
EFFECT OF CHITOSAN ON YEASTS AND BACTERIUM IN FUEL ETHANOL FERMENTATION

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Chitosan is known to be efficient in controlling non-Saccharomyces and bacteria contamination in the wine industry, eliminating or promoting significant reduction of contaminant populations. However, it was not tested yet in the conditions of fuel ethanol fermentation against wild yeasts or bacteria. To test the effect of chitosan on this fermentation, we simulated a fermentation contaminated with both Dekkera bruxellensis and Lactobacillus fermentum, important contaminants of the fuel ethanol fermentation, in sugarcane juice, in a cell recycling batch process with six fermentative cycles carried out by an industrial Saccharomyces cerevisiae strain (PE-2). Initially we determined the effect of chitosan in concentrations ranging from 10 to 200 mg/L on the growth of each microorganism isolatedly in assays in sugarcane juice. The concentration of 100 mg/L caused significant decrease in the growth of both contaminants without great effect on S. cerevisiae. Chitosan was then added at this concentration in the acidic solution (pH 2.0) in which the cells are treated between the fermentative cycles and the effects on the microorganism number and on the fermentation parameters were evaluated. Compared to a fermentation without chitosan addition, we observed that the inhibitory effect of chitosan was progressive but small along the fermentative cycles on the viability of both D. bruxellensis and S. cerevisiae and no effect on L. fermentum. A decrease on the ethanol production and an increase in the concentration of residual sugar in the must were observed with chitosan, with no significant effect on ethanol yield. The expected selective effect of chitosan on the viability of D. bruxellensis in relation to S. cerevisiae was not observed to occur when it was added to the acidic solution in fermentation conditions. Further experiments are required to observe the effect of the addition of chitosan on the sugarcane juice during fermentation. Support: Fapesp (2014/17794-2).

A CYBERLINDNERA SATURNUS WILD-TYPE STRAIN IS ABLE TO PRODUCE SINGLE CELL PROTEIN IN SUGARCANE BAGASSE HEMICELLULOSIC HYDROLYSATE

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Cyberlindnera saturnus (synonymous of Williopsis saturnus) was first described as potential S-stereoselective for oxidation of racemic secondary alcohols. Most recently, its ability to produce xylitol from sugarcane bagasse hemicellulosic hydrolysate (SBHH) fermentation was described. A wild-type strain of C. saturnus (GenBank accession number KP257575) was isolated in association to the gut of a xylophagous beetle Veturius transversus, and identified as xylitol producer, but with low yield. In this work, its potential for single cell protein (SCP) production was investigated. The yeast was reactivated in Sabouraud Agar (glucose 40 g.L⁻¹, yeast extract 10 g.L⁻¹, agar 20 g.L⁻¹)
for 48h at 30ºC. After this time, a loopful was cultured in YPD broth (yeast extract 10 g.L⁻¹, peptone 20 g.L⁻¹, glucose 20 g.L⁻¹) for pre-inoculum preparation (72h, 150 rpm, 30 ºC). The cell suspension was centrifuged (3500 g, 40 min, 4ºC), and the sediment was inoculated (0.39 g.L⁻¹ dry weight) in SBHH supplemented with 10 g.L⁻¹ of yeast extract (final total reducing sugar concentration [TRS]= 61.64 g.L⁻¹). The assay reached a total of 144h and was performed in triplicate. Cell growth was monitored by absorbance at 600 nm (OD600), protein concentration was evaluated by Biuret method and [TRS] was evaluated using DNS method. The yeast has consumed 85.6% of the total reducing sugar present in the SBHH. Final biomass produced was 8.76 g.L-1 and protein concentration was 4.04 g.L⁻¹ (46.2 percent of total). The final biomass yield was 0.165 g.g⁻¹. Despite low biomass yield, the percentual of protein indicates that this strain is promising for SCP production using SBHH as substrate, with potential for use in biorefineries. Subsequent efforts must be employed to stablish optimal bioprocess to increase the biomass yield. Acknowledgements: Financial support by CAPES and CNPq.

CHARACTERIZATION OF NATIVE SACCHAROMYCES CEREVISIAE STRAINS FOR BIOETHANOL PRODUCTION

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The production of bioethanol based on the fermentation of sugar from renewable sources is a friendly environmental alternative that promotes reducing the consumption of fossil fuels. Saccharomyces cerevisiae is a yeast widely used in fermentation processes, it has proved to be more robust than bacteria, being more tolerant to ethanol and inhibitors present in hydrolysates of lignocellulosic materials. The fruticulture, the main economic activity of Río Negro and Neuquén provinces (North Patagonia), produces abundant raw material wastes rich in lignocellulose and sugar that could be utilized for bioethanol production. The aim was to study the behavior of S. cerevisiae strains from different origins under stress conditions encountered during the production of bioethanol. In a first step, the ethanol tolerance of sixty S. cerevisiae strains from wine (Argentina), apple chicha (Chile) and Toddy (India) was analyzed in microplates assays containing 0-15% (v/v) ethanol. OD data were fitted to Gompertz model and kinetic parameters (both μₘₐₓ and lag) were calculated. All strains grew until 12% (v/v) of ethanol. Twelve strains -3 from each origin- showing the shortest lag value and the highest μₘₐₓ (media values: 11.83 ± 0.89 h and 0.15 ± 0.01 h⁻¹, respectively) were then evaluated in their ability to grow in different stress conditions including temperature (25°C-45°C), pH (2 to 5), glucose (2 to 300 g/L), Na₂SO₄ (0 to 50 g/L) and acetic acid (0 to 8 g/L) concentrations. As a general rule, all the strains grew at pH 3, 4 and 5, and at temperatures below 40°C. Glucose and Na₂SO₄ concentrations did not affect the growth of strains. Conversely, concentrations of acetic acid higher than 3 g/L negatively affected the development of the yeasts. The tolerance of selected strains to different stress factors make it possible to think about their potential application in bioethanol production using regional industry wastes.
BASIDIOMYCETOUS YEASTS CULTURED ON LIGNOCELLULOSIC BIOMASS OF CARDOON: A POSSIBLE EXTRA-SOURCE OF OLEOCHEMICALS?

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Due to their ability to accumulate high amounts of intracellular triacylglycerols (TAGs), oleaginous yeasts are well-known producers of lipids. Lignocellulosic biomass feedstocks have been recently studied as cheap C sources (after physical removal of lignin + enzymatic hydrolysis) for developing sustainable processes (biorefineries) for producing high amounts of TAGs. Lipid yields depend on the yeast strain, culture conditions, carbon and nitrogen sources, C/N molar ratio, temperature, etc. Accordingly, the lipogenic aptitude inside the yeast world is still far from being fully explored. As the result of a large-scale screening survey carried out on over 700 strains cultured on a mixture of C3, C5, C6 and C12 carbohydrates, the basidiomycetous yeasts Leucosporidium creatinivorum DBVPG 4794, Naganishia adeliensis DBVPG 5195 and Solicoccozyma terricola DBVPG 5870 were selected. Their ability to accumulate high amounts of intracellular TAGs was evaluated using the lignocellulosic biomass of cardoon (Cynara cardunculus L.) as C source. S. terricola grown at 20°C in shake-flasks produced 13.2 g lipids/l (yield = 28.9%, close to the maximum theoretical value = 31.6%), which exhibited a fatty acid profile comparable with that of commercial palm oil. On the contrary, L. creatinivorum and N. adeliensis gave the following results (at 20 and 25°C, respectively): maximum lipid production = 10.5 and 7.2 g/l; yield = 22.8 and 13.6%. After scaling-up in 5l bioreactor L. creatinivorum produced 12.8 g/l of high-oleic (80.8% of total fatty acids) lipids. A predictive evaluation of the physical properties of the biodiesel potentially obtainable from the above oils was performed: an excellent agreement with reference values reported by the EU standards (EN 14214) was found. This suggests that high-oleic TAGs produced by basidiomycetous yeasts from lignocellulosic biomass of cardoon could be considered as an extra-source of oleochemicals and biofuels.
SESSION 8 A:
YEASTS IN FOOD AND BEVERAGE:

TRADITIONAL FERMENTATIONS
IMPROVEMENT OF THE QUALITY OF TRADITIONAL FERMENTED FOODS

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Yeasts play a significant role in the majority of traditional fermented food and beverages. For traditional fermented food yeasts are predominantly introduced spontaneously from raw materials, ingredients or process equipment, or by backslopping. Starter cultures are used especially for food and beverages produced under more industrialized conditions. The biodiversity of fermented food and beverages is often not investigated in depth, and successions taken place at both species and strain levels are often overlooked. However, detailed knowledge on the microbiota and its interactions is required for improvement of product quality and for development of suitable starter cultures. Due to the harsh environments of fermented products i.e. low pH, high osmotic pressure, antimicrobials etc., fermented food and beverages are ecosystems of academic interest. Natural selections occur leading to new species and strains with specific traits. Due to the complex microbiota, microbial interactions can be studied encountering intra- and interspecies communication. Yeasts are not only important for the organoleptic quality of fermented products, they additionally have an impact on the shelf-life and nutritional value, in some cases even providing host-beneficial effects. The presentation will give an update on current knowledge supported by examples on previous and on-going research on yeasts in fermented food and beverages - hidden identities, small talk and gut feeling will be in focus.
STRUCTURAL, PHYSIOLOGICAL AND REGULATORY ANALYSIS OF MALTOSE TRANSPORTER GENES IN SACCHAROMYCES EUBAYANUS CBS12357T

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Saccharomyces pastorianus lager brewing yeasts are domesticated hybrids of Saccharomyces cerevisiae and cold-tolerant Saccharomyces eubayanus. To better understand the contribution of both parental genomes to maltose metabolism in brewing wort, this study focuses on maltose transport in the S. eubayanus type strain CBS12357T/FM1318T. To obtain complete sequences of the MAL loci of this strain, a near-complete genome assembly was generated using the Oxford Nanopore Technology MinION sequencing platform. Except for CHRXII, all sixteen chromosomes were assembled as single contigs. Four loci harboring putative maltose transporter genes (SeMALT1-4), located in subtelomeric regions of CHRII, CHRV, CHRXIII and CHRXVI, were completely resolved. The near-identical loci on CHRV and CHRXVI strongly resembled canonical S. cerevisiae MAL loci, while those on CHRII and CHRXIII showed different structures suggestive of gene loss. Functionality of the SeMALT1-4-encoded transporters was confirmed by their ability to restore growth on maltose, but not on maltotriose, of a maltose-transport-deficient S. cerevisiae strain. Simultaneous CRISPR-Cas9-assisted deletion of SeMALT2 and SeMALT4, which shared 99.7% sequence identity, eliminated growth of S. eubayanus CBS12357T on maltose. Transcriptome analysis of S. eubayanus CBS12357T established that, in maltose-grown cultures, SeMALT2 and SeMALT4 were expressed at much higher levels than SeMALT1 and SeMALT3, thus resolving the apparent discrepancy between heterologous expression and deletion studies. These results represent a first genomic and physiological characterization of maltose transport in S. eubayanus CBS12357T and provides a valuable resource for further industrial exploitation of this yeast. This project was funded by the Seventh Framework Program of the European Union in the frame of the SP3 people support for training and career development of researchers (Marie Curie), Networks for Initial Training (PIITN-GA-2013 ITN-2013-606795) YeastCell (https://yeastcell.eu/), our industrial partner Heineken and the BE-Basic R&D Program (http://www.be-basic.org/), which was granted an FES subsidy from the Dutch Ministry (EL&I).

VIABILITY AND PHYSIOLOGICAL RESPONSES OF YEASTS EXPOSED TO STRESS CONDITIONS OCCURRING IN WEST AFRICAN FERMENTED CEREAL DOUGHS

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In this study twelve yeast strains, all isolated from fermented cereal doughs in Benin, were investigated for viability and physiological responses when exposed to stress conditions mimicking those in fermented cereal doughs. The study included three strains each of Saccharomyces cerevisiae, Candida glabrata, Kluyveromyces marxianus.
and *Pichia kudriavzevii*. Yeast strains were exposed to individual stress conditions of cereal doughs, i.e. at pH 3.4, ethanol 3% (w/v), lactic acid (285 mM) and acetic acid (150 mM), respectively as well as to combinations of these stress conditions. Growth and single cell viability were studied by flow cytometry using SYTO13 and propidium iodine. Further, intracellular pH (pH$_i$) and micro-colony development were studied by fluorescence microscopy using propidium iodine and carboxyfluorescein diacetate succinimidyl ester (CFDA-se). Exposure to ethanol reduced the specific growth rate significantly for all strains of *S. cerevisiae* and *C. glabrata* as well as for one strain of *K. marxianus* but had no effect on *P. kudriavzevii*. Further, significantly reduced specific growth rates were observed for all strains when exposed to lactic acid and no strains grew when exposed to acetic acid or the combined stress conditions. Flow cytometric viability staining confirmed these results. Exposed to the combined stress conditions, the most resistant yeast was *S. cerevisiae* followed by *P. kudriavzevii*, whereas *C. glabrata* and *K. marxianus* were more sensitive. When transferred to optimal growth conditions (MYGP, pH 5.6) after exposure to the combined stress conditions, 45% of the single cells of *S. cerevisiae* could recover their pH$_i$ and proliferate, which was not the case for *K. marxianus*. This study provides information on yeast heterogeneity when exposed to stress conditions in fermented cereal dough and demonstrate that the *S. cerevisiae* strains studied are particularly relevant for development of starter cultures.

**POTENTIALITY OF YEAST STRAINS ISOLATED FROM BEER STARTERS AND BREWING FERMENTATION RESIDUES TO BE USED AS PROBIOTICS**

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The aim of this work was to study the potential probiotic properties of yeast strains isolated from brewer’s starters. Beer is the most popular alcoholic beverage worldwide, and the third-most popular drink overall after water and tea. During brewing wort fermentation, yeast biomass increases largely, obtaining a high nutritional by-product, today considered waste. From twelve brewer’s starters, we selected M6 starter and its two constituent strains, *Saccharomyces cerevisiae* and *Pichia kudriavzevii*, which showed the highest resistance to gastrointestinal (GI) conditions in vitro. The consortium and its isolates proved to be capable to bind aflatoxin B$_1$ (AFB$_1$), a potent carcinogenic compound naturally produced by *Aspergillus flavus* and *Aspergillus parasiticus* on agricultural substrates, such as corn. The highest toxin binding capability in vitro was observed when the isolates were grown in brewer’s wort (80 %), while YPD broth cultures (yeast extract 10 g/l, peptone 20 g/l, dextrose 20 g/l) only bound 20% or less of the initial AFB$_1$ concentration (300 ppb) (detected by Aflatoxin competitive direct ELISA test Veratox®, NeogenCorporation, USA) De-adsorption of AFB$_1$ during in vitro GI passage was also evaluated in brewing fermentation residues (BFR), and no more than 26% of the initial mycotoxin concentration (300 ppb) was detected in supernatants, remaining more than 50% of toxin strongly bound to BFR. Finally, we observed a decrease of the cytotoxic effect of AFB$_1$ (500 ppb) on HepG2 cells to levels similar to those to the negative controls (HepG2 cells in Dulbecco´s modified Eagle´s medium - DMEM - without AFB$_1$). It is remarkable that yeasts grown in brewer’s wort showed better potential probiotic properties than the yeasts grown in a nutritive balanced
designed microbiological medium, like YPD broth. These results suggest the brewery’s wastes potential as a high-value nutritional and probiotic by-product.

**FREEZE TOLERANCE AND BAKING POTENTIAL OF THE PSYCHROTOLERANT YEASTS SACCHAROMYCES EUBAYANUS AND SACCHAROMYCES JUREI**

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*Saccharomyces cerevisiae* is synonymous with baker’s yeast, but its capacity for dough proofing (fermentation) is compromised by its sensitivity to the low and freezing temperatures that are often used in modern bakeries. Screening trials, including representatives of all 8 known *Saccharomyces* species, showed that *S. cerevisiae* was, generally, the most sensitive member of the genus with respect to cold and freezing conditions. We hypothesized therefore that the superior cold tolerance of the non-*S. cerevisiae* yeast would enable their use as frozen-dough baking strains. The different yeast species were incorporated into doughs, flash frozen and kept in a frozen state for 2 weeks. During the subsequent proofing stage, dough development was lower in doughs that had been frozen, relative to fresh doughs. This reduction in fermentation performance was however most pronounced with *S. cerevisiae*. The psychrotolerant yeasts *S. eubayanus* and *S. jurei*, in particular, had a strong capacity for post-freeze proofing in terms of dough development and duration of lag phase prior to fermentation. The superior proofing power of these two species resulted in breads that were significantly larger, softer and less dense than those involving *S. cerevisiae*. A sensory panel could distinguish the *S. cerevisiae* and non-*S. cerevisiae* breads based on their physical properties, but aroma and taste were unaffected by the species employed. *Saccharomyces eubayanus* and *S. jurei* therefore exhibit good potential for use in frozen-dough baking. This would represent the first technological application of the latter species.

**BREWING PROPERTIES OF SACCHAROMYCES EUBAYANUS SUB-POPULATIONS**

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Type: Oral

The discovery in Patagonia of the missing close relative of Lager-brewing yeasts, *Saccharomyces eubayanus*, solved a mystery that had puzzled scientists and brewers for decades. In recent years additional strains were found in North America, Asia and New Zealand, as well as in Patagonia and genomic analyses showed the existence of two main populations of this yeast. In this work we focused in *S. eubayanus* sub-populations, which are geographically structured. From previous work where 60 strains were tested we selected at least two strains for each sub-population and tried them in micro-fermentations (150 ml). Strain selection criteria were attenuation and fermentation rate, and 10 selected strains were tested for sugar consumption,
production of glycerol and ethanol, production of phenols and sensory analysis. *S. eubayanus* strains, showed an average attenuation of 65% due to an efficient consumption of maltose, with the exception of a geographically distant strain (Tierra del Fuego). None of the strains were able to consume maltorose. All the strains produced phenolic off flavors (+pof) above the sensory threshold, a clear trait for non-domesticated strains. Genomic analysis confirmed the presence of active *PAD1* and *FDC1* genes. Although these compounds are undesirable in typical Lager beers, the different concentrations produce a variety of flavors that could be used for product differentiation. To date attempts to generate novel Lager hybrids mostly used *S. eubayanus* CRUB1568\(^T\) as parental. This work represents the first screening of brewing performance of a large set of isolates of *S. eubayanus* yielding new interesting candidates for future hybridization programs aiming at creating new lager yeast for industrial innovation.
SESSION 8 B: YEASTS IN FOOD AND BEVERAGE:
INDUSTRIAL FERMENTATIONS
HOW DO YEASTS MANAGE THE COMPLEX NITROGEN RESOURCE DURING WINE FERMENTATION?

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Type: Main Speaker

A current challenge for the wine industry, in the view of the extensive competition in the worldwide market, is to best meet the consumer expectations regarding the sensory profile of the product while ensuring an efficient fermentation. To fulfill these objectives, understanding how the yeasts manage the nutrient resources and how their metabolism operates is essential. In grape must, nitrogen is provided as a mixture of diverse N-containing molecules. During wine fermentation, this scarce nitrogen resource plays a central role, as its utilization affects the growth and the fermentative activity of yeasts, and the production of flavor compounds. Despite an extensive knowledge of the network involved in the nitrogen and aroma metabolisms, little was known on the management of such a complex nitrogen source by yeasts and its intracellular fate. The aim of this project was to comprehensively explore the consumption by yeasts and the further redistribution, particularly for the cell growth and the synthesis of volatile compounds, of the available nitrogen when present as a composite mixture. We demonstrated that the different nitrogen sources of grape must were sequentially consumed by S. cerevisiae, through an order likely controlled through the NCR and SPS regulatory systems and the kinetic characteristics of N-compounds transporters. Then, using an strategy based on the reconciliation of data from stable isotope tracer experiments, we elucidated the fate of consumed N-sources within the cells, highlighting the substantial contribution of their catabolism. Another important finding was that the precursors required for the neo-syntheses, including the formation of aromas, mainly originated from sugars. Finally, we showed that the yeast species from the enological consortium displayed specific traits regarding the use of N-compounds. Overall, this study provides new insights on the role of nitrogen during wine fermentation, useful for better control the process and the sensory quality of wines.
CAN A THERMAL SHOCK PRODUCE STUCK OR SLUGGISH FERMENTATION IN OENOLOGICAL CONDITIONS?

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Problematic fermentations are one of the main problems affecting winemaking industry leading in many cases to important economic losses in part due to a decrease in the quality of the wine. Several factors have been described to be the reason for stuck and sluggish fermentations, being exposure to extreme temperatures among them. During early fermentation stages, high metabolic activity of yeast usually leads to an increase in musts temperature. This increase can be favored by the nutrition of the musts with nitrogenous sources, which is a widely used oenological practice. On the other hand, sudden drops in temperature may occur during the first days of autumn influencing fermentation development. The objective of this study was to identify thermal conditions leading to problematic fermentations, focusing on the impact of abrupt increase/decrease of temperature on fermentation kinetics and yeast viability. Saccharomyces cerevisiae strains used in this study were: T73 and PDM (commercial yeasts) and SBB11 (isolated from Mendoza). Fermentations were conducted at 25°C using synthetic must. The impact of heat shock at 36°C and 40°C was assessed increasing must temperature during 16 hours on the third day of fermentation, together with must nutrition with diammonium phosphate. In contrast, cold shock was assessed by decreasing must temperatures (1.5°C, 8°C and 10°C) during 16 hours on days 2, 6, 10 and 14 of alcoholic fermentation. Fermentation kinetic was monitored through density measurements whereas cell viability/vitality was evaluated with flow cytometry. Heat shock affected fermentation kinetics with different intensity depending on the temperature or yeast strain evaluated. None of the conditions evaluated lead to a stuck fermentation, although sluggish fermentation was observed for heat shock assays. Native strain SBB11 showed to be the most sensitive to heat shock. Moreover, no effect of cold shock on alcoholic fermentation performance was observed for all the conditions assessed.

DOMINANCE OF S. CEREVISIAE OVER S. KUDRIAVZEEVII IN FERMENTATION IS A STRAIN DEPENDENT CONTACT MEDIATED RESPONSE RELATED TO ACCELERATION OF NUTRIENT CONSUMPTION

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Fermented foods are the result of complex interactions among microorganisms and the environment they inhabit. The knowledge related to these ecosystems gained in the last decades led to important innovation in the selection of new starters organisms able to meet current market demands. In fact, this has been a principal focus for wine industry
during the last decades. However, the presence of the extremely well adapted *Saccharomyces cerevisiae* normally implies that alternative yeasts of industrial interest are outcompeted from the fermentation. Classically, Make-Accumulate-Consume strategy by means of the *crabtree effect* has been considered the main *S. cerevisiae*’s competition strategy. On the other hand, relatively closely related yeasts present their own respiro-fermentative metabolism as well as high ethanol tolerance, and yet, they are also displaced from the culture. What is more, a variety of specific *S. cerevisiae*’s mechanisms have been recently described depending on the competitor organism, evidencing the high complexity of this trait. They may be classified according to two parameters, the necessity or not of cell-to-cell contact/proximity, and the existence of a triggered response mediated by the identification of a competitor versus a constitutive mechanism. In the present work we describe a competition case of a *S. cerevisiae* wine strain and a naturally isolated *S. kudriavzevii* in which there was a contact mediated response that released an extensive regulation of gene expression, with accelerated nutrient uptake, that efficiently inhibited *S. kudriavzevii* growth. Furthermore, we report the lack of response of an oak bark *S. cerevisiae* isolate under the same co-culture conditions. Thus, we hypothesize that competitive traits are strain specific, and recently evolved in wine strains.

**HANSENIASPORA VINEAE, A FRUIT EPIPHYTE YEAST THAT IS ADAPTED TO WINE FERMENTATION NICHES**

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Non-*Saccharomyces* yeasts are dominant in grape berry skins during maturity and, at this stage, the apiculate group of the genus *Hanseniaspora* is considered the main group of microorganisms. After grapes are crushed and juice fermentation starts in the winery, there is an extreme change in the environmental conditions and yeasts with higher fermentation capacity will dominate this niche. Within the genus *Hanseniaspora*, species *H. vineae* shows highest fermentation capacity, comparable with that of *Saccharomyces cerevisiae*. This fact explains the difficulties found in isolating this species before fermentation starts due to its low population in the berry. At winery level we have obtained positive contributions with *H. vineae* T02/5AF, in white and red wine production. The high fermentation capacity also might explain the intense contribution of this species to flavour, increasing sensory complexity of wine. In this work, we explore the *H. vineae* genome in order to understand the different adaptations of this species compared to the rest of the genus and to *S. cerevisiae* wine yeasts. Twelve genes responsible for alcoholic fermentation were analysed. Among them, the four key enzymes in this pathway showed higher homology in protein sequences with *S. cerevisiae*, compared to other five grape associated species of *Hanseniaspora*. In addition, increased diversity was found in the secondary fermentation pathways responsible for flavour compounds of *H. vineae* compared to five wine *S. cerevisiae* strains. Results by GCMS analysis of the flavour phenotype showed an increase in acetates, benzenoids, 3-methyl-1-propanol and 3-methyl-1-butanol, while a decreased formation is detected of medium chain fatty acids, ethyl esters and higher alcohols such as 1-propanol. Discussion of olfactory aroma values will help to explain the sensory evaluation impact of wines produced by *H. vineae* compared to conventional vinifications.
SULFATE TRANSPORT MUTANTS EFFECT HYDROGEN SULFIDE PRODUCTION DURING ALCOHOLIC FERMENTATION

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Hydrogen sulfide is the most prominent volatile sulfur compound (VSC) in wine that causes unfavourable reductive aromas; with H₂S levels directly related to yeast metabolism in response to nitrogen and sulfur availability. In grape juice, sulfate is the most abundant inorganic sulfur compound, which is taken up by yeast through two high-affinity sulfate transporters, Sul1p and Sul2p and a low affinity transporter, Soa1p. Sulfate contributes to H₂S production, through the reduction of sulfate (SO₄²⁻) to sulfite (SO₃²⁻) and sulfide (S²⁻) via the Sulfur Assimilation Pathway (SAP). The SAP is central to the production of the essential sulfur containing amino acids, methionine and cysteine, as well as the antioxidant, glutathione. Yeast strains with limited H₂S production and the ability to preserve desirable thiol derived sensorial characteristics are highly sought after in winemaking. We report on the isolation of six wine yeast strains that produce limited or no H₂S during fermentation. These strains are derived from commercial wine yeast Lalvin EC1118 (E178, E211, E212, E214) and Maurivin Distinction® (D13, D25) on YPD agar plates containing the toxic sulfate analogues potassium dichromate and sodium selenate after treatment with ethyl methanesulfate. The strains were evaluated in 100 mL juice and synthetic grape juice or 10 L juice fermentations and shown to yield no or reduced H₂S and SO₂. Sequencing of the sulfate transporter genes (SUL1, SUL2 and SOA1) revealed four identical non-synonymous mutations in the SUL1 gene in all strains except D25. D13 had an additional mutation, whilst E211 and E214 each had an extra (non-identical) mutation in the SUL2 gene. These findings allude to the importance of Sul1p in H₂S production and raise the possibility of the mutations being the basis for strain improvement.

[†GAR+] PRIONS HARDLY EXPLAIN EITHER YEAST ADAPTATION TO, OR SLUGGISH WINE FERMENTATION

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[†GAR+] prion-like elements partially relieve yeast carbon catabolite repression (CCR) and have been suggested to affect ethanol content of wines. However, experimental proofs for these statements are inconsistent. This issue is addressed in this work, first, by selecting [†GAR+] variants from several genetic backgrounds. These strains have been characterized for prion specific phenotypes, including CCR features, rate of prion isolation, penetrance, and heritability; as well as fermentation performance and the yield of major fermentation metabolites, including ethanol, glycerol, and acetic acid. Some [†GAR+] prion-related phenotypic traits are clearly depending on the genetic
background. For example, prion isolation rates range from below 0.01 to 0.5, penetrance from 1% to 50%. In general, CCR is affected only when using glucosamine as repressor, but rarely for 2-deoxy-glucose. Concerning fermentation performance, the impact of the prion-like elements ranges from slight to apparently null. Fermentation yields, under both aerobic and anaerobic conditions, are also largely unaffected by the prion state, notably for ethanol and glycerol. The impact on acetic acid production is clearer, but it does not follow a single pattern, it is increased for some backgrounds, and reduced for others. In summary, prion-like elements do not seem to be of practical use to modulate fermentation output; while the features described here would also rule out prions as an explanation for sluggish or stuck fermentation. Funding: MINECO through grant AGL2015-63629-R, and PhD training contracts for AJR and AM, is acknowledged.

THE WINE YEAST MICROBIAL ECOSYSTEM: FROM MOLECULAR MECHANISMS OF INTERACTIONS BETWEEN SPECIES TO SYNTHETIC ECOLGY

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In many food production systems, species-diverse microbial ecosystems transform a raw material into a final product. In the case of wine production, numerous yeast species are present in the initial juice, and it is well established that several of these species can contribute significantly, in desirable or undesirable ways, to the fermentation process and/or the final characteristics of the product. Our current understanding of the species and population dynamics within such food production ecosystems is limited, but for the rather generic observation that the final stages of alcoholic fermentation tend to be dominated by Saccharomyces species. Indeed, and due to the complexity of microbial ecological interactions, it remains largely impossible to predict the growth patterns and population dynamics of any given microbial ecosystem, even if the initial state of this system, including species and cell numbers, is known. A predictive capability would significantly improve the ability of producers of fermented foodstuff to manage the process successfully, and to better exploit the technological potential of any given natural ecosystem. Here we present new insights into the basic functioning of the wine ecosystem, and propose ways on how to improve ecosystem control. The data reveal some of the molecular and physiological mechanisms of interactions between individual yeast species, including the importance of physical contact between cells of different species and the impact of FLO gene expression. Through the investigation of constructed consortia of yeast species, antagonistic and synergistic processes that are different from those observed in one-on-one experiments become apparent. Taken together the data suggest that wine ecosystem dynamics are predictable and, to some degree, controllable. Finally, we show that synthetic ecology-based approaches may provide a tool to develop purposefully designed ecosystems. We suggest that such ecosystem-based approaches can reduce the need for technological interventions.
SELECTION OF INDIGENOUS SACCHAROMYCES CEREVISIAE ISOLATED FROM CACHAÇA FERMENTATION WITH POTENTIAL FOR BREWING

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The Brazilian specialty beer market is very promising and has been expanding rapidly across the country due to a high demand for different beers in sensory quality. Cachaça yeasts are exposed to high osmotic pressure and alcohol concentration, variations in temperature and competition with other contaminants microorganisms from the fermentation process. Therefore, the search for yeast strains in the spontaneous fermentation of cachaca production has great relevance due to the adverse environment. We selected 965 Saccharomyces cerevisiae strains isolated from vats of cachaca fermentation to test their ability to be used in brewing. In a preliminary screening, the yeast strains were tested in relation to their ability to ferment maltose (6%) at 10 and 20 °C, and H₂S production. A hundred and sixty-five yeast strains were able to ferment maltose at both temperatures and did not produce H₂S. These yeast strains were tested for resistance to different ethanol concentrations (8, 10, 12, 15 and 20 %), pH (2.5, 3.0 and 4.0), glucose (20, 25 and 30 %) and temperatures (10, 16, 25, 30, 37 and 42 °C). We verified 47 yeast strains that were able to grow in 20% ethanol, in contrast to commercial yeast that grew in ethanol concentration up to 10%. Ninety-seven yeast strains grew at a concentration of 30% glucose and 94 strains grew at a pH of 2.5; these results were similar to those found in commercial brewing yeast strains. In the temperature of 10 °C, 55 strains had the same or better growth than the commercial strains and 105 strains grew at a temperature of 30 °C. With these results, it can be inferred that the vats of cachaca fermentation are favorable environments to the isolation of yeast strains with appropriate characteristics to withstand the stress conditions imposed by the beer fermentation process.

SELECTION OF NATIVE YEASTS FOR LOW-CARB CRAFT BEER PRODUCTION

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Over the past years, the interest in crafts beers have increased worldwide, and Uruguay is not an exception. Craft beers have experienced a significant increase in sales and the number of local brands doesn’t seem to stop growing. Considering this expanding market, it becomes interesting to develop products with its own identity in an increasingly competitive market. Flavor and aroma of beer will depend on the interaction of various compounds generated in the production process. However, many of these substances are produced during the fermentation stage and depend mainly on the selected yeast. Our study proposes the selection of native yeast for craft brewing processes, particularly for beers reduced in carbohydrates. For yeast’s isolation, raw materials, such as cereals (fresh and malted) and hops, and native plants and fruits, were sampled. The material was enriched in a maltose culture liquid media, incubated...
and plated in the selective and differential solid culture medium ChromAgarPure cultures of microorganisms with different morphologies were obtained. We also isolated yeast from grape must obtained from the 2018 harvest. The characterization of the isolated yeasts consisted of fermentation capacity in a maltose liquid medium and an α-amylase activity screening. This enzyme is responsible for the degradation of starch during the mashing stage, thus a yeast with this enzymatic activity could degrade residual starch and therefore produce a wort with higher fermentable sugar content and less calories in the final beer. We obtained 23 yeasts strains with one of these two characteristics of interest, and therefore with the potential to be used in mixed cultures. The next steps of this work include the scale up of these strains at real craft brewing level to confirm their fermentation capacities, α-amylase activity and the sensory evaluation of the obtained beer.

LARGE SCALE SULFITE RESPONSE STUDY REVEALS SPECIFIC ADAPTATION OF B. BRUXELLENSIS POPULATIONS THROUGH HUMAN-RELATED SELECTION

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The yeast species Brettanomyces bruxellensis is associated with important economic losses due to red wine spoilage. The most common method to prevent and/or control B. bruxellensis spoilage in winemaking is the addition of sulfur dioxide into must and wine. However, recently, it was reported that some B. bruxellensis strains could be tolerant to commonly used doses of SO2. In this work, B. bruxellensis response to increasing SO2 concentrations (from 0 to 0.6 mg.L-1 of molecular SO2 was assessed on 145 isolates representative of the genetic diversity of the species, and from different fermentation niches (roughly 70 % from grape wine fermentation environment, and 30% from beer, ethanol, tequila, kombucha, etc.). Three behaviors in terms of lag phase, maximal growth rate and maximal population were defined: sensitive, tolerant and resistant strains. The relationship between SO2 tolerance/resistance and genotyping using microsatellite markers was confirmed with high reliability (90%). The detection of both resistant and tolerant growth profiles suggests that B. bruxellensis strains have developed multiple strategies to cope with SO2 present in wine. Pairwise competition experiments using genetic transformants of sensible and tolerant B. bruxellensis strains were performed in presence of sulfites. A contrasted relative fitness of the tolerant strain at high SO2 concentration (0.4 and 0.6 mg/L) confirmed the previously stated hypothesis that tolerant strains could have a relative selective advantage in the presence of SO2 compared to sulfite sensitive ones, thus suggesting a specific adaptation to the main antimicrobial used in winemaking. This work contributes to a deeper understanding of this wine spoilage microorganism in means of genetic and phenotypic diversity and sheds light on putative evolutionary strategies for adaptation to human related environment of this non-conventional model yeast species.
CHARACTERIZATION OF VOLATILE COMPOUND PRODUCTION IN NATIVE ISOLATES OF SACCHAROMYCES EUBAYANUS

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A current goal in modern genetics is to determine the molecular changes and the different sources of heritable variation underlying niche adaptation in nature. In this context, exploring natural populations provides a way to understand genome evolution and adaptation mechanisms. Since the recent isolation of S. eubayanus, the study of this species has gained particular attention for their potential utilization in the brewing industry. Within the Chilean territory, we have isolated hundreds of ethanol-tolerant yeasts, including S. eubayanus, from ten sampling sites, ranging from Altos de Lircay National Park (VII Maule Region, Chile) to forests surrounding Karukinka National Park (XII Magallanes Region, Chile), representing overall a 2.090 km distance between sampling extremes. This new species, has been characterized by the production of esters, higher alcohols and other volatile compounds at low temperature, which bring fruit and floral flavors to brews. In this work, we looked for the preliminary population structure of the Chilean strains utilizing the highly polymorphic marker GDH1. Moreover, we characterize in a subset of these strains their fermentation profile and production of volatile compounds, such as esters and higher alcohols, in order to correlate the geographic distribution and brewing potential. Our preliminary results suggest extensive genetic and phenotypic differences between Chilean Patagonian strains with possible biotechnological and industrial applications.

MICROBIOTA SUCCESSION DURING THE COMMERCIAL PRODUCTION OF PULQUE

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Pulque is an alcoholic beverage of pre-Hispanic origin, produced by the fermentation of the sap from different Agave species. It is considered one of the oldest and most traditional in Mexican beverages. It is produced at artisan and commercial levels, through a semi-continuous process. Sap fermentation is induced by addition of a mixed inoculum of bacteria and yeasts. Fermentation time is variable and depends on several factors, which determine the product quality. Although pulque has been subject of numerous microbial studies, to date the behavior of the various microbial groups that participate in its fermentation has not been established. The aims of this work were to elucidate both, the microbial succession (by dependent and independent culture techniques), and the main biochemical changes (by HPLC, GC, GC-MS) during pulque commercial production in Tlaxcala. From production process, 19 samples were obtained; after their microbial counts, 395 strains were isolated (151 bacteria and 244
Yeasts were identified by polyphasic taxonomy. The dominant species in each stage were identified by DGGE. The microbial count of bacteria varied from $10^4$ to $10^{12}$, and of yeasts from $10^5$ to $10^{11}$ CFU x mL$^{-1}$. Out of the total number of isolates more than 50% was dominated by *S. cerevisiae*, *Cl. lusitanae*, *Leuconostoc* spp. and *L. mesenteroides*. For DGGE the highest yeast diversity was detected in sap, and at the beginning of the third fermentation, while for LAB it was uniform throughout the process. Volatile compounds, phenylethyl alcohol, 2,3-butanediol, organic acids, and esters remain constant throughout the fermentation; ethanol concentration was within the limits specified by official regulation. It was demonstrated by the two techniques used, that in commercial pulque the diversity of species was similar, which may be due that the process at the site of the sample has been carried out for a long time.

**VIRULENCE LEVELS AND MECHANISMS IN YEASTS SPECIES PRESENT IN THE FOOD CHAIN**

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Yeasts have an impeccably good record compared to other microorganisms like viruses, bacteria and some filamentous fungi in terms of food safety. Compared with other microbial groups, yeasts are not seen as aggressive pathogens. However, the greater frequency of individuals at risk (persons with weakened health and immune systems including cancer, AIDS and hospitalized patients, and those undergoing treatment with immunosuppressive drugs, broad-spectrum antibiotics and radio-chemotherapies) has led to increase the reporting cases of infections by food yeast species. Hence, the main goal of this project is to understand the mechanisms responsible for the virulence of food-related yeasts on humans, and to provide tools and recommendations to prevent the release into the food chain of yeasts strains that may pose a risk to the population. Thus, we centered our study in yeast species isolated in the food chain that have been described more frequently in human infections like e.g. *D. hansenii* (*Candida famata*), *K. marxianus* (*Candida kefyr*), *S. cerevisiae*, and *Wickerhamomyces* (*Pichia*) *anomalous* (*Candida pelliculosa*). The first step was the physiological evaluation of characteristics associated with yeast virulence as growth at different temperatures (between 28 and 42ºC), phospholipase production, pseudohyphal growth and invasive growth. Strains isolated form different sources either food or clinical environments, including well-characterized strains regarding *S. cerevisiae* pathogenicity. Then, we used *Galleria mellonella* as a model hosts to check the pathogenicity and finally we sequence 16 strains of *S. cerevisiae* isolated from hospitals with different virulence levels.

**INSIGHT INTO THE FUNGICIDAL ACTION FROM CANDIDA INTERMEDIA LAMAP1790 AGAINST THE WINE-SPOILAGE YEAST BRETTANOMYCES BRUXELLENSIS**

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**Brettanomyces bruxellensis** has been described as a principal spoilage-yeast in the wine industry. To avoid its growth, the grape must is commonly supplemented with SO2. However, the use of this compound has been questioned for the potential health problems that entails its consumption. Our laboratory has previously described the production of antimicrobial peptides (AMPs) secreted by the strain *Candida intermedia* LAMAP1790. These peptides are present in the supernatant of saturated cultures media and affects the proliferation of *B. bruxellensis*, without affect the growth of *Saccharomyces cerevisiae*. Thus, the objective of this work was study the cellular damage produced in *B. bruxellensis* after the exposure of antifungal supernatant from *C. intermedia* LAMAP1790, using fluorescence microscopy. To evaluate changes in the yeast morphology we marked the cell wall using Calcofluor-White (CW) stain. Additionally, to determine the membrane permeability and redox imbalance, we use propidium iodide (PI) and 6-carboxy-2',7'-dichlorodihydrofluorescein (C400) respectively. The treatment was done exposing 3 x105 cells of *B. bruxellensis* to 1 mL of saturated supernatant from *C. intermedia* during 12 h and 24 h. After the exposure we observed that no exist significative changes in the morphology and fluorescence of *B. bruxellensis* treated with CW. However, the exposure to supernatant produce a significative accumulation of Reactive Oxygen Species (ROS) in *B. bruxellensis*, which increase proportionally with the time exposure. Additionally, we observed a small increase in the cells permeability correlated with the accumulation of ROS in *B. bruxellensis* stained with PI. These results have correlation with one of the mechanisms described to AMPs secreted in other species of Fungi kingdom.  

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**IDENTIFICATION OF YEASTS IN CHICKPEA FERMENTATIONS**

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Chickpea liquid starter is produced by the fermentation of chickpea seeds and used as a leavening agent for chickpea bread production in some Mediterranean and Balkan countries. Chickpea bread is commonly known in the Aegean and Thrace and also some parts of the Middle Anatolia and Mediterranean Regions of Turkey. Chickpea liquid starter is produced traditionally by the fermentation of coarsely ground chickpea seeds with hot water in a warm place for 16-18 hours. At the end of the chickpea fermentation, chickpea liquid starter is mixed with flour and hot water and kept in a warm place for a few hours. Then chickpea dough is used as the leavening agent in chickpea bread production. In the present study, a total of 216 presumptive yeast colonies were isolated from 12 chickpea liquid starter and dough samples that were collected from different bakeries at two different times. All of the presumptive yeast isolates were grown in the YPD medium for 24-36 hours and subjected to DNA extraction after lyticase treatment. 5.8S ITS rRNA region of the genomic DNA of 205 isolates were amplified using primers ITS1 and ITS4 and subsequently digested by the restriction endonucleases HaeIII, Hhal and Hinfl. Yeasts sharing identical restriction patterns were classified into groups and 1 or 2 representative cultures of each group was chosen for sequence analysis of the D1/D2 domains of the 26S rRNA gene. Resultant sequences were compared with nucleotide sequences of the closest relatives deposited at the database of National Center for Biotechnology Information (NCBI). Identified strains belong to the 5 genus including *Saccharomyces* spp., *Candida* spp., *Meyerozyma* spp., *Pichia* spp. and...
Cryptococcus spp. Totally 52 yeast isolates were identified at species level (≥400 bp, 99% identity) as Saccharomyces cerevisiae (24), Candida parapsilosis (20), Meyerozyma guilliermondii (5), Pichia membranifaciens (2) and Cryptococcus albidosimilis (1).

WINE YEAST AND THEIR APPLICATIONS IN BIOTECHNOLOGY

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The UNCUYO Facultad de Ciencias Agrarias has a native yeasts’ collection from Mendoza. During the development of different research’s works, it has increased in the number of individuals wich were characterized as Saccharomyces spp. During winemaking, the alcoholic fermentations can be stopped for different reasons, so it is important have microorganisms be able to solving specific problems; such as ferment highly sugary musts, and consequently resist high concentrations of alcohol, or restart stopped fermentations with fructose in a higher concentration than glucose. These microorganisms has continuously being evaluated from a technological and qualitative point of view in order to identify those yeasts so that being used in order to specific winemaking. The aim of this work had been the evaluation of different resistance by yeasts against specific stress situations, such as resistance to 15% ethanol, preference for fructose and growth in breeding ground with 300 g / L of glucose. We worked with 313 strains from five Departments: Luján de Cuyo, Rivadavia, Junín, Maipú and San Martín. First, all the strains of the study were verified in their viability and purity, and then were placed again under maintenance conditions at -20ºC. 16% of the strains were able to grow in alcoholized broths, while all of them grew in fructose broths; their preference for glucose was showed by only 10% of the individuals studied. Finally and with response variability, all grew in strongly osmotic broths. In the yeast´s collection there are strains of interest to be used according to specific winemaking objectives. The maintenance of microbial crops protects biodiversity while offering useful tools to solve everyday problems in the industry.

THE ROLE OF YEASTS IN THE FERMENTATION OF “TUBA”, A FERMENTED MEXICAN BEVERAGE

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Tuba is a Mexican beverage made from coconut palm (Cocos nucifera), primarily consumed in coastal regions of the states of Colima and Jalisco. Tuba is prepared using the sap from the inflorescence of coconut palms collected in 12-hour periods. In some countries in Africa, the palm sap is used to obtain an alcoholic beverage called “palm wine”, where the yeasts are responsible for producing ethanol and other volatile compounds through the consumption of sugars. Some yeast species like Candida tropicalis, Saccharomyces cerevisiae and Pichia kudriavzevii are present in Tuba; however the role they play during fermentation is unknown. The aim of this work was
to elucidate the contribution of *C. tropicalis*, *S. cerevisiae* and *P. kudriavzevii* to Tuba composition. Independent fermentations of sterile coconut palm sap using *C. tropicalis*, *S. cerevisiae* and *P. kudriavzevii* was developed. The fermentations were performed in 500 mL flasks with 200 mL of non-fermented coconut palm sap at 15°Bx, 30°C and without agitation during 12-hour to simulate natural fermentation conditions. Sugars, ethanol and volatile compounds were evaluated during fermentation by HPLC and GC. The results displayed the production mainly of ethanol and acetaldehyde by *S. cerevisiae*, isobutanol and amyl alcohol by *C. tropicalis* and ethyl acetate by *P. kudriavzevii*. The three yeast evaluated showed different roles in Tuba fermentation, on the one hand *S. cerevisiae* and *C. tropicalis* in the higher alcohols and ethanol production, and on the other hand *P. kudriavzevii* in ester production. The native yeasts play important roles in Tuba fermentation producing different volatile compounds that contribute to the aromatic profile. The yeasts evaluated can be used for future directed fermentations in order to modify specific product characteristics.

**BIOCONTROL OF ZYGOSACCHAROMYCES ROUXII IN WINEMAKING OPTIMIZATION OF PHYSICOCHEMICAL FACTORS**

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Wine is the product of complex microbial interactions in grape-must. Some of them generate detrimental characteristics in wine causing economic losses, such as *Zygosaccharomyces rouxii*. Traditionally, SO₂ is used in winemaking to control microbial proliferation. Due to toxic and allergenic effects on human health, and consumer’s preferences on natural and healthy food, SO₂ should be reduced. An SO₂ alternative is biocontrol, there are yeast able to biosupress other yeast through different mechanisms: toxic compounds secretion, limiting substrates competence and/or cell-cell contact. Statistical experimental designs could be used to state relevant physicochemical factors and optimize them for biocontrol. We had advances on positive-flavor-attributes of the biocontrolling yeasts, biocontrol nature and relevant participating physicochemical factors. Aim: To determine optimal conditions to biocontrol *Z. rouxii* and reduce SO₂ in winemaking. Non-*Saccharomyces* native yeasts (IBT-UNSJ) *Zygosaccharomyces rouxii* BZr6 (Spoilage yeast) and *Wickerhamomyces anomalus* BWa156 (Biocontroller) were used. Box-Behnken experimental design was used to work-out response surfaces and optimize three relevant factor for biocontrolling BZr6 in grape must (pre-sterilized) fermentation: reducing sugars concentration (23, 24.5, 26°Bx), pH (3.7, 4.0, 4.2) and molecular SO₂ (0.0, 0.25, 0.5ppm). As response, viable BZr6 population was quantified (CFU/mL). A significant quadratic model was found, R:0.99 and R²:0.99, errors were normal, homoscedastic and independent. Was found total biosuppression over BZr6 population by BWa156 in all sugar range evaluated: (Sugar:23, pH:3.7, SO₂:0), (Sugar:24.5, pH:4.2, SO₂:0.5), (Sugar:26, pH:4.2, SO₂:0.25). Nevertheless, at SO₂=0, was found one point of total biosuppression over BZr6: (23°Bx, pH:3.7). Due to sought SO₂ reduction, the better model values were validated under SO₂ absence and the higher possible reduction over *Z. rouxii*. The points validated satisfied the model prediction. Through response surface methodology were found conditions that predict satisfactorily the biocontrol of microorganisms in grape-must.
STUDY OF THE EFFECT OF THERMAL PASTEURIZATION AND HIGH HYDROSTATIC PRESSURE IN THE VIABILITY OF ZYGOSACCHAROMYCES ROUXII POPULATION IN CONCENTRATED GRAPE JUICES

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The process of juice concentration involves the elimination of water until a concentration of sugars and osmotic pressure capable of inhibiting the microbial development is reached. However, these products are not free of microbiological spoilage problems, being Zygosaccharomyces rouxii the most frequent spoilage microorganism. Many efforts have been done in the industry to avoid yeast spoilage using a non-chemical approach, where pasteurization and high hydrostatic pressure (HHP) could be suitable technologies. The aim of this work was to find the conditions of thermal pasteurization and HHP necessary to reach the inactivation of Z. rouxii and the extension of the shelf-life of concentrated grape juice. Different Z. rouxii were inoculated in concentrated grape juice at 10^7 CFU/g. The samples were submitted to pasteurization using different temperature (70, 75, 80 and 85 °C) and times (5, 10, 20, 30, 45, 60, 75 and 85 seconds); and to HHP using different pressures (300, 400, 500 and 600 MPa) and times (2, 3 and 5 minutes). Pasteurization at 70 °C was not lethal for Z. rouxii in any of the times evaluated, while a reduction of 7 logarithms was obtained at 75 and 80°C for 90 seconds, and at 85°C for 5 seconds. The application of HHP at 300 MPa was not lethal for Z. rouxii in none of the evaluated conditions. A significant reduction in viable populations of Z. rouxii was observed at 400 MPa, although a slowly recovery of the cells was observed, resulting in spoilage after 14 days of storage at 28 °C. Treatments with pressures over 500 MPa resulted in a significant reduction in the population and an extension of the shelf life of the stored product was observed. Our studies suggest that both technologies; HHP and pasteurization; could be applied to increase the microbial stability of concentrated grape juices.

INHIBITORY EFFECT OF EPIPHYTIC WINE GRAPE YEASTS ON ALTERNARIA ALTERNATA GROWTH

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Prevention of growth of mycotogenic fungi is the most effective strategy to control the presence of mycotoxins on foods. Alternaria is the main component of wine grape mycobiota from different winemaking regions in Argentina and worldwide. During our previous studies, Alternaria alternata strains isolated from Malbec wine grapes have demonstrated the in vitro ability to produce alternariol (AOH), alternariol monomethyl ether (AME) and tenuazonic acid (TA) and exhibited pathogenicity and TA production
on wine grapes. During the present study, the inhibitory effect of 14 epiphytic wine grape yeasts on mycelial growth of three toxicogenic and pathogenic A. alternata strains (5.5, 7.5 and 25.1) in a synthetic nutrient (SN) media similar to grape composition at three different temperatures (15, 25 and 30 °C) was evaluated. All Metschnikowia spp. yeast strains (6) completely inhibited A. alternata growth at all tested temperatures. Meanwhile, the growth inhibition exerted by Starmerella bacillaris yeast strains (3) was variable upon assayed conditions. All yeast strains inhibited A. alternata 5.5 growth at 25 °C and 30 °C, one of them inhibited A. alternata 7.5 and 25.1 growth at all temperatures and the other two inhibited A. alternata 7.5 and 25.1 growth only at 30 °C. Finally, only one of the Hanseniaspora uvarum yeast strains (5) evaluated showed an inhibitory effect on the three A. alternata strains growth but only at 30 °C. In conclusion, among the epiphytic wine grape yeasts evaluated, Metschnikowia spp. yeast strains seems to be promising for A. alternata biological control.

**OPTIMIZATION OF FERMENTATION-RELEVANT FACTORS: A STRATEGY TO REDUCE ETHANOL IN RED WINE BY SEQUENTIAL CULTURE OF HANSENIASSPORA UVARUM/ SACCHAROMYCES CEREVISIAE NATIVE YEASTS**

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Current consumer preferences are determined by well-structured, full-bodied wines with a rich flavor and with reduced alcohol levels. One of the strategies to obtain wines with reduced ethanol content is sequential inoculation of non-Saccharomyces and Saccharomyces cerevisiae yeasts. However, different factors could affect the production of metabolites like ethanol, glycerol and acetic acid by inoculated yeasts. In order to obtain low alcohol wines without quality loss, the aims of our study were: i) to optimize the fermentative factors to improve the performance of non-Saccharomyces yeasts prior S. cerevisiae inoculation to reduce final ethanol in wines; ii) to validate the optimized factors in grape must; and iii) to assess sensory quality of the wines obtained. Hanseniaspora uvarum BHu9 and S. cerevisiae BSc114 previously selected were used. A Box-Behnken experimental design was employed to assess the effects of three independent factors (fermentation temperature, time of permanence and initial inoculum size of the BHu9 population at the beginning of the process, prior to inoculation with BSc114). The optimal conditions to obtain lowest ethanol levels were: temperature of 25°C, initial inoculum size of 5x10^6 cells/mL and a time of permanence of 48h 37min of Bhu9. Then BSc114 yeast strain was inoculated at a concentration of 2x10^6 cells/mL to finish the process. After optimization, the three factors assayed were validated at lab-scale in grape must cv Malbec. Single culture fermentations of BSc114 was used as control. Wines obtained using sequential culture registered ethanol levels significantly lower than control treatment (p<0.05) and were associated with higher aromatic complexity characterized by the presence of red fruit aromas. Whereas wines obtained with BSc114 (control) were described by parameters linked with high ethanol levels such as hotness, bitterness, astringency. In conclusion the strategy proposed would be a useful tool to face the new challenges of current enology.
PREVENTION OF WINE AND CONCENTRATE GRAPE JUICE YEAST SPOILAGE USING PREDICTIVE MODELS AVAILABLE IN MOBILE APPLICATIONS

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Grape juice and its by-products are an important part of the food industry in the world. Argentina grape production is mainly industrialized, where wine and concentrated grape juice are the two major types of commercial products. Both substrates could be spoiled by yeasts. *Dekkera bruxellensis* is associated with phenolic flavours that have a negative impact on the organoleptic characteristics of wines, whereas *Zygosaccharomyces rouxii* is the most frequent spoilage microorganism in concentrated grape juices. The prevention of contamination is the best way to avoid microbial problems and maintain the quality of the products. Previously, two mathematical models were developed in our Laboratory to predict the growth of *D. bruxellensis* in wines and *Z. rouxii* in concentrate grape juices. The predictive models were built considering the variables of the food that could be measured and modified in the industry, such as pH, concentration of ethanol and SO₂ in wines; and pH and concentration of sugars in the concentrated grape juices. The mathematical models have been successfully validated in wines and grape juices concentrated naturally contaminated. In an effort to provide useful and practical tools to winemakers and producers, mobile applications were developed to use easily access to this models. Using the App the producers could know which of these variable combinations may be inhibitory for the growth of the spoilage yeasts and estimate the shelf-life of the product under the storage and shipping temperature conditions until 60 days. Moreover, accurate control strategy to prevent spoilage could be designed, avoiding the economic losses associated with rejection of the products.

COMPARATIVE GENOMICS OF SAKE YEAST STRAINS OF SACCHAROMYCES CEREVISIAE

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Sake is a Japanese traditional alcoholic beverage and is fermented from steamed rice by concerted action of filamentous fungus *Aspergillus oryzae* and yeast. “Sake yeast” is collective term of *S. cerevisiae* strains well suited for sake brewing. Compared with other industrial and laboratory strains, they possess interesting characteristics: high ethanol productivity, good growth and fermentation at a low temperature under 15°C and superior taste and flavor of final products. As a whole, such features are common to contemporary sake yeast strains, K7 and the related ones in practical use, because of their probable phylogenetic closeness. However, under a same condition, each strain shows individual fermentation and metabolic profiles. The genetic differences, phylogenetics, individuality in brewing characteristics, and those relationships among the strains are therefore intriguing problems. Accordingly, we performed
comprehensive genome resequencing of sake yeast and the related strains, using common sake yeast strain K7 as the reference. Several tens of sake yeast strains including both of practically used and historical ones, several shochu (Japanese spirits) and Japanese wine strains were applied to sequencing with genome sequencers (Illumina HiSeqs) and subsequently mapped to the reference genome by the BWA, followed by SNP calling using the Samtools. A NJ-tree based on genome wide homologies indicated all the sake and shochu strains were determinately located within “the sake clade”, which was suggested by previous genome-wide studies, in S. cerevisiae phylogenetic systematics. Additionally, contemporary sake yeast strains that are commonly used and including K7, formed a monophyletic group within the sake cluster. Accordingly, relatively small number of variations should define their identity such as brewing characteristics. Alternatively, although SNPs distributions of the monophyletic common strains were very similar, zygosity of SNPs were not identical each other. It would suggest lineage-independent loss-of-heterozygosity events largely contributed to strain differentiations from a common ancestor.

OXYGEN SEQUESTRATION BY METSCHNIKOWIA PULCHERRIMA TRIGERS A SPECIES-SPECIFIC TRANSCRIPTIONAL RESPONSE IN SACCHAROMYCES CEREVISIAE

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Despite relatively low initial cell numbers in grape must, S. cerevisiae is usually dominant by the end of the fermentation process. The dominance of S. cerevisiae has often been associated to its high sugar consumption rates, quick depletion of key nutrients, and ethanol production. To decipher the response of yeast cells to the presence of other yeast species, we judged interesting analysing yeast transcription profiles after short contact times, before changes induced in the medium composition by the metabolic activity of yeast cells would mask more specific responses. First, we analysed the interaction with Torulaspora delbrueckii, and found a remarkable transcriptional reprograming for both yeast strains in the presence of each other, concerning the glycolysis pathway. Gene overexpression in S. cerevisiae takes place in as soon as 2 h, while it is detected in T. delbrueckii after 12 h. Later, we focused in the response of S. cerevisiae to 3 different non-Saccharomyces species (T. delbrueckii, Hanseniaspora uvarum, and Candida sake). Results point to some common features in the way S. cerevisiae modifies its transcriptome in front of other yeast species, namely activation of glucose and nitrogen metabolism, being the later specific for aerobic conditions. Finally, we show how the specific requirements of a non-Saccharomyces yeast strains like Metschnikowia pulcherrima modulate the response of S. cerevisiae. Although the common response described previously can still be observed, in this case S. cerevisiae shows also the up-regulation of a particular group of genes related to anoxic conditions. By knowing how yeast respond to each other, we can anticipate the specific requirements of the different strains under co-cultivation conditions and define the necessities that must be fulfilled to improve the fermentation process.

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STUDY OF THE PROBIOTIC PROPERTIES OF YEASTS ISOLATED FROM KEFIR

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The term probiotic describes live microorganisms that are supplemented to the diet to improve the health of those who consume them. Lactic acid bacteria and bifidobacteria are the most frequently used in the field of probiotics, although some yeasts also have beneficial properties, being the genus *Saccharomyces* the most documented. Kefir is a fermented beverage that is obtained by milk fermentation with kefir grains where coexists in sympbiotic association lactic acid bacteria and yeasts. There are numerous reports about the beneficial health properties attributed to the consumption of kefir. Our aim was to study of the probiotic potential of the yeasts from kefir. Among 35 strains analyzed, 20 showed a survival greater than 50% after being subjected for 2 h and 37°C at pH 2.5 and hight bile resistance. Viable *K. marxianus* CIDCA 8154 was also recovered after passage of gastrointestinal tract of both BALB/c mice and hamsters confirming the behavior observed in vitro. Selected strains showed adherence to intestinal epithelial cell line Caco-2 cells. Different kefir yeast strains inhibited the activation of innate immure response triggered on Caco-2 cells with flagellin (FliC), both in a reporter system (Caco-2 ccl20: luciferase), as on the mRNA expression of CCL20, CXCL-8 and CXCL-2. We analyzed the anti-inflammatory capacity of the strain *K. marxianus* CIDCA 8154 in different models, demostrating reduction of intracellular levels of reactive oxygen species upon pretreatment of the epithelial cells with yeast. Likewise, *K. marxianus* CIDCA 8154 administered orally protects from histopathological damage and rise of serum IL-6 triggered by TNBS in an acute model of colitis. Results indicate that kefir yeasts are able to modulate the intestinal epithelial inflammatory response that could explain some of the benefical properties of kefir and lead to formulation of novel functional food for patients with an increased gastrointestinal inflammatory status.

SELECTION OF ENVIRONMENTAL SACCHAROMYCES CEREVISIAE STRAINS FOR THE PRODUCTION OF CACHAÇA

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Brazil has a rich market of fermented and distilled beverages and we can highlight the cachaça (an alcoholic beverage derived from sugarcane). Several factors may influence the quality of alcoholic beverages, however, the yeast identity and the fermentation conditions are determinant for the sensorial improvement of the beverage. Among the different species occurring during the sugarcane fermentation, *Saccharomyces cerevisiae* is predominant in the traditional cachaça fermentation. Thus, this work aims at the selection of an environmental strain of *Saccharomyces cerevisiae* that may be used in the cachaça fermentation to provide improvements in the sensorial
characteristics of the product. In this study, we selected, to test their resistance to the fermentation process, 74 strains of \textit{S. cerevisiae} isolated from tree bark, lichen, and decayed wood in Brazil. Among these strains, 49 did not produce H$_2$S, and these \textit{S. cerevisiae} strains were submitted to stress tests with different conditions, varying the values of concentration of pH (2.8, 3.0, 3.2), sucrose (10, 15, 20%), ethanol (8, 10, 12, 15%) and temperature (10, 16, 25, 30, 37, 42°C). The traditional cachaça fermentation process presents the following standard stress conditions in their 24h-cycles of fermentation: pH: 3.0-3.5; sucrose: 14-16%; ethanol: 8-10%; temperature: up to 42°C. All strains were able to grow at the stress conditions tested. All strains developed in medium with 8 and 10% of ethanol, while with at 12 and 15% of ethanol, only 2 and 5 strains, respectively, were able to grow. All strains were able to grow at 25, 30, 37 and 42 ° C. However, these strains did not grow at 10 and 16° C. Therefore, it is possible to claim that the tested \textit{S. cerevisiae} strains are promising alternatives for the fermentation of cachaça, which may result in a beverage with differentiated flavor.

**IDENTIFICATION OF A NOVEL ALLELE CAUSING A DEFECTIVE SPINDLE ASSEMBLY CHECKPOINT IN ETHYL CAPROATE HIGH-PRODUCING SAKE-YEAST STRAIN K1801 AND ITS APPLICATION**

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Ethyl caproate (EtCap), a major flavor component in high-quality Japanese alcoholic beverage sakes, such as \textit{Daiginjo-shu}, which is produced from highly polished rice (less than a 50% polishing ratio), is synthesized from ethanol and caproic acid by sake yeast (\textit{Saccharomyces cerevisiae}). The cerulenin-resistant sake yeast K1801 with high EtCap-producing ability has been used widely for the high-quality sake brewing. However, we recently revealed that this strain had a defective spindle assembly checkpoint (SAC). The genetic stability of sake yeast is thought to be important for the maintenance of both the fermentation properties of sake yeast and quality of the final product, sake. Therefore, the isolation of a spontaneous mutant from K1801, having both the excellent brewing properties of K1801 and normal checkpoint integrity, has been desired. In this work, as the first step for this breeding, we sought to identify the mutation causing the SAC defect in K1801. To identify the mutation causing this defect, we first searched for sake yeasts with a SAC-defect like K1801 and found that K13 had such a defect. Then, we searched for a common SNP in only K1801 and K13 by examining 15 checkpoint-related genes in 23 sake yeasts, and found 1 mutation, R48P of Cdc55, the PP2A regulatory B subunit that is important for the SAC. Furthermore, we confirmed that the Cdc55-R48P mutation was responsible for the SAC-defect in K1801 by molecular genetic analyses. Cdc55-R48P is semi-dominant mutation in the SAC function. Morphological analysis indicated that this mutation caused a high cell morphological variation. But this mutation did not affect the excellent brewing properties of K1801. Thus, this mutation is a target for breeding of a new risk-free industrial yeasts with normal checkpoint integrity.
**PICHIA KUDRIAVZEVII IN MIXED CULTURES: AN ALTERNATIVE FOR MALOLACTIC FERMENTATION IN BIOLOGICAL WINE DEACIDIFICATION?**

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In the Comahue Region, placed in the Argentinian north Patagonia 40°SL, grape musts present high malic acid contents (3 to 6 g/L). While Malolactic Fermentation (MLF) is a routine practice for wine deacidification, it is a process difficult to control at the industrial scale. Therefore, the aim of this study was to evaluate the competence of a native *Pichia kudriavzevii/Saccharomyces cerevisiae* mixed culture for L(-)-Malic acid consumption during Alcoholic Fermentation (AF). *P. kudriavzevii* proved its ability to metabolize this substrate as the only carbon source, reason why we proposed it for wine deacidification. Wine vinifications were carried out with Malbec musts at a pilot scale (200L) during 2015 to 2017 vintages. Wines were elaborated with conducted AFS either with a native *S. cerevisiae* starter (ScF8) or with ScF8 and *P. kudriavzevii* simultaneous mixed starter (1/100 ratio) (CoC). Young wines were sulphited to inhibit MLF. Physicochemical and aromatic quality was determined once bottled, by means of conventional (INV) and chromatographic (HPLC y GC-FID) methods. Sensorial quality was evaluated by qualitative assays and trained panel tasting, and subjected to consumer preference polls. All the obtained wines were considered normal, dry and qualified as very good in the tasting, but the CoC presented significantly lesser malic acid content and higher esters content than ScF8 wines. Sensorial analysis evidenced statistical differences between CoC and ScF8 products, with a fruitier aroma and consumer preference for CoC wine. CoC validation in real environment for three consecutive years presents this mixed starter as an alternative for FML in Patagonian wine deacidification.

**MANDELATE IS AN INTERMEDIATE IN BENZENOIDS SYNTHESIS BY HANSENIA SPORA VINEAE**

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Volatile compounds can impact differently on wine aroma, among them, benzenoids and phenylpropanoids usually are associated to positive aromatic characteristics. These molecules are well described as secondary plant metabolites and they arrive to wines from grapes. However, yeasts are also able to produce them throughout the routes that involve aromatic amino acid conversion. In particular, *Hanseniaspora vineae* produces higher amount of volatile benzenoids than *Saccharomyces* strains, contributing positively to wine aroma and texture. The metabolic route for volatile benzenoids by yeast remains unknown, but the mandelate pathway has been proposed instead of the
phenylalanine ammonium lyase pathway (PAL), found in plants and some Basidiomycetes. The available knowledge about genome sequence of H. vineae made possible to propose several enzymes as involved in a putative mandelate pathway, but PAL homologous sequences were not found. For that reason H. vineae is a good model to study this metabolic route. In this study we propose an experimental design to determine the presence of mandelate pathway intermediates in phenylalanine to benzoates biosynthesis. To our knowledge, this is the first experimental approach designed to confirm the mandelate pathway either in plants or in yeast. The analysis of intra and extracellular metabolites at 5 and 12 days of H. vineae fermentation was performed by gas chromatography-mass spectrometry. 13C-labelled phenylalanine was used to follow intermediates in mandelate pathway and the benzenoids produced. The labeled intermediates mandelic and phenylacetic acid were detected at 5 days in the intracellular medium. However, at day 12 only phenylacetic acid was detected. Benzyl alcohol, one of the main products, was found to accumulate in the extracellular medium during the fermentation and at 5 days it was found inside of cells. Quantification of labelled compounds and a blocking method of pyruvate decarboxylase are discussed as a first approach to elucidate the final pathways utilized by H. vineae.

IMPACT OF COMPLEX YEAST NUTRIENT PRODUCTS ON SELECTED SACCHAROMYCES AND NON-SACCHAROMYCES YEASTS

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During wine fermentation, nitrogen, in the form of yeast assimilable nitrogen (YAN), is often one of the growth limiting resources. Nitrogen levels in grape must can vary significantly in concentration, while the yeasts nitrogen requirement can be either relatively high or low, or it can be for a specific nitrogen compound. In many commercial fermentations, inorganic nitrogen is added in the form of ammonium salts, usually di-ammonium phosphate (DAP). Alternative nitrogen sources can also be added in the form of complex yeast nutrient. These can contain inorganic, as well as undefined organic nitrogen components. These yeast nutrients are generally produced and marketed with the wine yeast Saccharomyces cerevisiae in mind. With the increasing use of non-Saccharomyces yeasts to produce more complex wines, it is important to know how these complex nutrients affect the growth of non-Saccharomyces yeast. This will enable the wine producer to optimize the influence of specific non-Saccharomyces inoculums. This study therefore investigated the impact of different categories of commercial nutrients on commercial non-Saccharomyces yeasts in laboratory-scale trials in synthetic and real grape must. A commercial S. cerevisiae wine yeast was used as a reference and for co-inoculations. Results showed that the non-Saccharomyces yeasts investigated, Torulaspora delbrueckii and Metschnikowia pulcherrima, reacted differently to the various categories of yeast nutrients in synthetic grape must. The trials in the more complex real grape juice also showed differences in growth rate and cell numbers, but was not an extrapolation of the synthetic must trials. These preliminary results indicate that judicious use of complex yeast nutrients could be a way to support the presence of specific non-Saccharomyces yeast to enable them to participate more fully in wine fermentation.
A STUDY OF THE RESPIRO-FERMENTATIVE METABOLISM AMONG SPECIES OF SACCHAROMYCES IN MICROVINIFICATION CONDITIONS

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In this work, we focused on the first metabolic data obtained from several wine fermentations performed at 25°C with a set of Saccharomyces yeast strains. The aim of the study was to find evidences of the different metabolism strategies used by alternative species of S. uvarum and S. kudriavzevii compared to a commercial S. cerevisiae wine strain (T73, Lallemand, Montreal) to perform alcoholic fermentation at 25°C in natural white must of Merseguera. In very well controlled bioreactor - mimicking winemaking conditions - fermentations were monitored and the main fermentative by-products, gases yields and biomass parameters registered to identify signs of metabolism strategies that could explain their oenological performance in those conditions. On the one hand, the results obtained suggest that at 25°C, the S. cerevisiae wine strain mainly directs carbon flux through ethanol production, a way to outcompete with other microorganism and to maintain the NADH/NAD+ redox balance. On the other hand the higher glycerol, succinic acid, 2,3-butanediol and 2-phenyl ethanol yield of S. uvarum strains are signs that exists other sinks of the carbon flux in this species. Moreover, since pathways involved in the formation of former by-products are responsible for NADH re-oxidation, these results also suggest other mechanisms of balancing redox homeostasis in the cell. Finally, under the same conditions, even if the S. kudriavzevii strain showed important shortcomings in growth resulting in high levels of acetic acid and few by-products production, the glycerol and 2,3-butanediol yields were still higher than in the S. cerevisiae strain. These last results underlined the cryotolerant character of the S. kudriavzevii species and its bad fermentative properties at high temperature (25°C), but also supported the hypothesis of other redox balancing strategies in cold-adapted species like S. uvarum under fermentative conditions.

CO-CULTIVATION OF PROBIOTIC YEAST AND LACTIC ACID BACTERIA TO PRODUCE FERMENTED MAIZE-BASED BEVERAGES

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Cereal-based fermented beverages are non-dairy products which are considered possible carriers for probiotic strains and alternatives for use by vegans and lactose-intolerant consumers. In general, bacteria strains are the most common probiotic microorganism used by industries for probiotic products elaboration. In the present work, the commercial probiotic, Lactobacillus paracasei LBC-81, was used single and in co-culture with the potential probiotic yeasts, Saccharomyces cerevisiae CCMA 0731, S. cerevisiae CCMA 0732, and Pichia kluyveri CCMA 0615, to ferment a maize-based substrate. The yeast strains were considered potential probiotics due their tolerance to
low pH and bile salts, high percentages of hydrophobicity, autoaggregation, coaggregation with *E. coli*, antioxidant activity and adhesion to Caco-2 cells. In addition, they were phytase producer, important for nutrient availability in plant-based foods. The strains showed viability higher than 6 log CFU/mL, as recommended for probiotic products, except for *P. kluyveri* which decreased during fermentation and storage time. A reduction in pH value, from approximately 7 to 4, was observed. This decrease was due organic acid production, which did not affect the microbial viability. Lactic and acetic acids were the main organic acids produced during fermentation, and they decreased over 28 days of storage (0.5 and 0.1 g/L for lactic and acetic acids, respectively). Ethanol was detected in the *S. cerevisiae* assays; however, the content was 5 g/L (0.5 % w/v) as required to characterized as non-alcoholic beverages. Seventy volatile compounds were detected, including acids, alcohols, aldehydes, esters, ketones, and other compounds. Sensory analysis showed score of 5.93 – 4.57, respectively for appearance and taste. This is an important result, considering that the beverage had no flavoring additive and lacked a sweet taste. Therefore, probiotic beverages were successfully obtained by maize fermentation inoculated with co-culture of *S. cerevisiae* (CCMA 0731 or CCMA 0732) and *L. paracasei* LBC-8.

**SULFITE TOLERANCE CONFERRED BY DOMINANT FZF1 MUTATIONS IN BREWING YEAST**

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Sulfite is biosynthesized in yeasts during fermentation, and possesses anti-microorganism and antioxidant activity, thereby enabling long-term storage of alcoholic beverages such as beer and wine. Sulfite is transported into extracellular space via a sulfite efflux transporter, Ssu1p. Therefore, Ssu1p has an important role for sulfite tolerance. Fzf1p (Five zinc finger-type C₂H₂ transcription factor), the only known positive regulator for *SSU1* so far, is also known to be crucial for sulfite tolerance. Among five zinc finger domains, the fourth and fifth domains are thought to be dispensable for DNA binding. Casalone et al (1994, Yeast) reported that a H180D mutant, in which His180 was replaced by Asp residue in the fourth zinc finger, affects the structure, thereby conferring sulfite tolerance. Similarly, replacement of Cys57 by Tyr was shown to confer sulfite tolerance (C57Y; Park et al., 1999, Curr Genet). HereIn this study, we show that sulfite tolerant brewing yeasts were obtained through the mutagenesis followed by enrichment culture, and most mutants were found to harbor non-synonymous mutations in the endogenous *FZF1* gene. Some mutations were novel, but located in the vicinity of known mutations (H180D or C57Y), and another mutation was present at a Cys residue constituting a C₂H₂ domain. Moreover, *SSU1* gene expression was significantly increased in yeast expressing these mutated *FZF1* genes, showing that these *FZF1* mutations enhance sulfite tolerance by up-regulation of Ssu1p. Finally we tested the combinational effect of *SSU1* with the *FZF1* on sulfite tolerance (See the presentation by Ono et al. in ISSY34). Combination of active mutations in *SSU1* and *FZF1* showed synergistically higher sulfite tolerance than the case with only one of those active genes. These findings provide a genetic framework for enhancement of sulfite tolerance through the quantitative regulation of *SSU1* in non-sulfite producing yeasts.
INVESTIGATION OF YEAST GENES REQUIRED FOR THE MODULATION OF COLOUR IN MODEL RED WINE

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Colour development in red wines is a dynamic process, involving extraction of grape-skin anthocyanins and conversion into stable pigmented polymers. Yeast play a role, producing metabolites, hydrolytic enzymes which have been implicated in colour stabilisation and decolourisation, via pigment adsorption onto the yeast surface. We investigated the genetic basis of colour modulation during fermentation, using a chemically defined grape juice medium (CDGJM) with added grape skin anthocyanins. Two quantitative trait loci (QTLs) were identified in a population of 96 F₂ segregants resulting from a cross between two selfed-diploid strains derived from Enoferm M₂ and Zymaflore F₁. OYE2 (YHR179W), ROF1 (YHR177W) and HPF1 (YOL155C) from chromosomes 8 and 15 respectively, were deleted in the selfed-diploids, M₂ ho::Hph and F₁ ho::Nat haploids, and the re-constructed F₁ hybrid (M₂ ho::Hph x F₁ ho::Nat). The strains were fermented in 100 mL CDGJM and the colour of the model wines and yeast biomass visually measured. We show that yeast mutants lacking Oye2p (F₁∆oye2 and M₂∆oye2) produce significantly lighter coloured wines; the degree to which varied between spore progeny. The observed phenotype was independent of pigment adsorption onto yeast cells. The data related to ∆rof1 and ∆hpf1 is less clear, highlighting the genetic complexity associated with colour development. Further research is needed investigate the mechanism behind wine decolourisation in ∆oye2, and whether over-expression would improve colour stability, an attribute associated with wine quality.

QTL MAPPING OF NATURAL ALLELIC VARIATIONS OF SACCHAROMYCES CEREVISIAE IMPACTING STUCK FERMENTATIONS CAUSED BY HIGH TEMPERATURE IN WINE

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Thermo-tolerance of the fermenting yeast Saccharomyces cerevisiae is a complex and quantitative trait of relevant interest for many industrial processes including bioethanol production and winemaking. The natural genetics variations impacting thermo-tolerance were partially elucidated in laboratory conditions by measuring growth related phenotypes. In contrast the genes involved in temperature resistance during the stationary phase remain unknown. In this study three QTLs explaining the major part of phenotypic discrepancy within two wine starters were identified. Those QTL were detected by using a selective genotyping strategy carried out on a backcrossed population previously obtained. The precision of mapping allows the identification to
identify two causative genes VHS1 and OYE2 harbouring non sense SNP. Reciprocal Hemyzygous Assay validated the effect of these allelic variations and demonstrated their interaction with the fermentation temperature. The molecular function of the protein identified would strengthen the presumption that Programed Cell Death and ROS metabolism may play a crucial role in the resistance of fermenting yeast to harsh conditions including the high temperature.

MANNOPROTEIN CONTENT AND VOLATILE MOLECULE PROFILES OF TREBBIANO WINES OBTAINED BY S. CEREVISIAE AND S. BAYANUS STRAINS

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The production of volatile compounds is become one of the major technological character for yeast selection. In fact, although the wine flavour is the sum of varietal, pre-fermentative, fermentative and post-fermentative flavours, volatiles from fermentation dominate wine flavour, since yeasts affect the quality of the grape prior to harvest and, during fermentation, metabolising grape sugars and other components into alcohols, esters, organic acids and aldehydes. Among the new technological features, also the production of mannoprotein has gained interest. In this perspective, main aim of this work was to characterize 8 strains of S. cerevisiae and 2 strains of S. bayanus for their volatile molecule profiles and the release of mannoproteins in trebbiano wines. The strains were inoculated in Trebbiano must and incubated at 15°C at the end of fermentation the wines were evaluated by GC/MS/SPME for their volatile profiles and mannoprotein content by FTIR. The strains, inoculated at level of 4.9 and 6.3 log cfu/ml but only the strains L318 and 12233X6167 were able to reach values of 7.5 log cfu/ml. The volatile molecule profiles were characterized by a great amount of alcohols and in any case, the profiles obtained can be considered as a strain fingerprinting. According to the principal component analysis, the strains L288, L234 and L318 were characterized by the presence of propanoic acid, butanol, octanoic acid and 3 methyl pentanol while the strain 12233_35G2 was characterized by the presence of decanoic acid ethyl ester, eptanoic acid ethyl ester, acetic acid 2 phenetyl ester. Regarding mannoproteins, the strain12233_6167 produced 104 mg/l in trebbiano wine. The data permitted to select the strains endowed with the best volatile molecule profiles for Trebbiano wine and able to release the major content of mannoproteins. Moreover, the good potential of the infra-red spectroscopy was demonstrated for mannoprotein evaluation.

BOOSTING YEAST BASED FLAVOUR PRODUCTION BY IN SITU PRODUCT RECOVERY

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Due to a steadily increasing consumer’s demand for natural flavour and fragrance compounds to be used in foods, beverages and cosmetics, we have been investigating
biological and technical aspects of their bioproduction for about two decades now. To become economically viable, bioprocesses need high productivities and product titers. In yeast fermentations, product toxicity often becomes the final bottleneck during bioprocess development. Most flavour and fragrance compounds, like other small hydrophobic molecules, which tend to interact with cellular membranes - turn out to be toxic to the producing microbes at industrially relevant titers. Unfortunately, yeasts - in comparison to other microbes - often show a rather low resilience against elevated concentrations of these compounds making in situ product recovery (ISPR) mandatory.

In this work, different ISPR techniques used in yeast based flavour production processes are compared. Examples include the fedbatch production of rose-like 2-phenylethanol and 2-phenylethylacetate with *Saccharomyces cerevisiae* and *Kluyveromyces marxianus* coupled to ISPR techniques such as organic phase extraction and the membrane-based organophilic pervaporation. Results from the bioconversion of monoterpenes, such as the flavour molecules linalool and limonene, with other microbes in bioreactors coupled to in situ adsorption, are also summarized. In all cases, the use of ISPR increased final product titers x-fold compared to the conventional process regime. The pros and cons of the different ISPR methods are discussed.

NEW INSIGHTS IN YEAST-YEAST INTERACTIONS DURING ALCOHOLIC FERMENTATION OF GRAPE MUST: A FLOW CYTOMETRY APPROACH

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Alcoholic fermentation (AF) is the main step for winemaking, mainly performed by the yeast *Saccharomyces cerevisiae* (*Sc*) But other wine yeasts called non-*Saccharomyces* may contribute to AF and improve the wine aroma complexity. The recurrent problem with the use of these non-*Saccharomyces* yeasts is their trend to die off prematurely during AF, leading to a lack of their interesting aromatic properties searched in the desired wine. This phenomenon appears to be mainly due to interactions with *S. cerevisiae*. These interactions are most of the time negatives but remain unclear because of the species and strain specific response. Among the non-*Saccharomyces* yeasts, *Lachancea thermotolerans* (*Lt*) is a wine yeast naturally found in grape must and well known as a great L-lactic acid producer and an aromatic molecules enhancer, but its behavior during AF can be completely different in co-fermentation with *S. cerevisiae* in function of strain used. Thus, *Sc/ Lt* couple was used to unravel interactions between these two species during AF. Thanks to a modified *Sc* strain expressing a GFP, both yeast physiology was monitored by flow cytometry in pure (PF) and sequential fermentations of grape must with (SF+) or without (SF-) cell-cell contact. *Sc* present a better vitality and membrane integrity as well as less oxidative stress in SF+ and SF- compared to PF despite an increase of lipid accumulation in SF+. *Lt* present less vitality and membrane integrity as well as more oxidative and lipid stress in SF+ and SF- compared to PF. Moreover, L-lactic acid is only produced by *Lt* in SF- at 6.0 g.L⁻¹ instead of 1.5 g.L⁻¹ for other fermentations. In this way, multiparametric flow cytometry is a powerful tool to monitor yeast physiology during AF allowing new insights about interactions occurring during AF between *S. cerevisiae* and *L. thermotolerans*.
**Studying the Effect of Amino Acid Assimilation in the Phenotype of P-Coumaric Acid Adaptation in Brettanomyces Bruxellensis**

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Brettanomyces bruxellensis is the main agent of aromatic spoilage in wine, due to the production of phenolic derivatives from the hydroxycinnamic acids, a molecule with antimicrobial activity. Thus, microorganisms that grow in presence of these hydroxycinnamic acids must have efficient processes of adaptation. Previous studies have shown that two Chilean B. bruxellensis strains, denominated LAMAP1359 and LAMAP2480, present different response to p-coumaric acid, but the molecular mechanism that underlie this phenomenon is unknown. Data from our laboratory showed that B. bruxellensis LAMAP2480 (with high tolerance to p-coumaric acid) have several genes associated with use of nitrogen sources. Transcriptomic analyses have shown that p-coumaric acid induce the expression of genes that encode proteins related
with the efflux of toxic compounds that could be related to the intracellular nitrogen levels. Based on the evidence, we hypothesized resistance to \( p \)-coumaric acid of \( B. \) \textit{bruxellensis} is positively correlated with amino acid assimilation, especially those with aromatic nature. Thus, our purpose is to study the effect of two different sources of nitrogen over \( B. \) \textit{bruxellensis} L1359 and L2480 in the response to \( p \)-coumaric acid. To achieve this goal, we propose defined the nitrogen consume of both strain to determine specific amino acid assimilation profile during lag phase for each. The results will help to find strategies for inhibition of the production of the unwanted phenolic derivates. Acknowledgements: 081871GM Postdoc-USACH and Fortalecimiento USACH USA1799_GM181622 grants.

**EXPRESSION OF SOME GENES PROBABLY INVOLVED IN THE PRODUCTION OF ESTERS IN \textit{KLUYVEROMYCES MARXIANUS} DURING FERMENTATION UNDER DIFFERENT CONDITIONS**

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Introduction: Esters are indispensable volatile compounds in alcoholic beverages. It is important to know the conditions that favor the production of esters and the genes involved in their biosynthesis by yeasts. Little is known about these genes in \textit{Kluyveromyces marxianus}. Main objective: Evaluation of the effect of temperature, micro-aeration and C/N ratio on the volatile compounds production and the expression of possible genes involved in the synthesis pathways of esters. Methodology: The fermentations were carried out in 3 L reactors with 1.5 L of chemically defined medium; fructose was used as carbon source at 110 g/L with a \( K. \) \textit{marxianus} yeast strain isolated from a fermentation of agave juice. Stirring was 200 rpm and pH 4.5. The study variables were temperature, C/N ratio and micro-aeration. Results: the maximum specific production of ethyl acetate (0.22 mg/g) was observed at low temperature (15°C), without micro-aeration and C/N ratio of 71 this results is related to an increase (4x) in the expression of the gene \textit{HSP70} (heat shock protein). However, in these conditions no great variations were observed in the expression of the \textit{ATF2}, \textit{EHT1} and \textit{EAT1} genes, previously reported as involved in the production of esters. This phase of overproduction of esters was observed in the first 24 hours of fermentation corresponding to a possible stage of adaptation of the yeast at low temperature. The lower production of ethyl acetate was observed with micro-aeration and correspond to a repression of \textit{EAT1} gene expression. Conclusion: Even when an overproduction of ethyl acetate was observed during the fermentation at 15°C, no significant changes were observed in the expression levels of the genes reported as being directly involved in the production of esters; therefore, it is possible that there are other genes involved in the production of these compounds not yet known.
APPLE BAGASSE CHARACTERIZATION AS A SUBSTRATE FOR WINE YEAST BIOMASS PRODUCTION

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In the Comahue Region, pear and apple production constitute one of the main economical activities. 40% of harvested apples are destined to concentrated or squeezed juice production. In this study, apple bagasse obtained as a juice industrial waste was characterized for its potential use as an alternative substrate to sugar cane molasses or sugar beet molasses for native wine yeasts biomass propagation. Apple bagasse was characterized by batches, in reducing sugar content (RS), assimilable nitrogen (AN), humidity, pH and total polyphenols according to AOAC techniques. At the same time, different culture media were studied using the Experimental Statistical Design methodology. Growth parameters of two Patagonian wine yeasts Saccharomyces cerevisiae F8 and Pichia kudriavzevii P15 were analysed with Placket-Burman designs comparing apple bagasse and cane molasses (control) as carbon source. Results obtained in the physicochemical analysis indicate that apple bagasse presents a high quality as a potential substrate for biomass propagation, with values similar to those reported for the same apple variety in the region. When apple bagasse was used as carbon source in a culture medium, results showed that this substrate constitutes an improved alternative for yeast biomass production than cane molasses, since it does not require nutritional supplementation to obtain maximum microorganism growth. Apple bagasse generated during regional juice production is presented as a quality alternative for yeast biomass production, with the advantage that it also solves the problem of bagasse disposal without generating environmental liabilities, improving the profitability of the producing companies and contributing to regional productive diversification.

CRAFT BEER: INCREASING FLAVOR DIVERSITY USING MIXED CULTURE FERMENTATION WITH NON CONVENTIONAL YEASTS

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Non-Saccharomyces yeasts represent a valuable group of microorganism useful for their application in the brewing industry. According to recent research, non-Saccharomyces yeasts present a great potential in the production of desirable aromatic compounds for the production of quality craft beers. In this work we studied the compatibility, and interaction capacity, of non-conventional yeasts under mixed culture conditions to increase the sensory complexity of craft beers. The Saccharomyces and non-Saccharomyces yeasts used were isolated from raw materials of the Uruguayan brewing and wine industries. Pure and mixed culture fermentations in a brewing wort medium,
designed specifically for this purpose, were evaluated for their fermentation capacity, production of aromatic compounds, and sensometric evaluation using an expert panel. Four strains were studied in these experiments: *Pichia anomala* (Pa), *Zygoascus meyerae* (Zm), and *Saccharomyces cerevisiae* (Sc 00/30 and Sc 00/35, both wine strains with β-glucosidase activity). Based on previous studies where the physicochemical characterization was performed, the following combinations of mixed cultures were evaluated: Pa:Zm; Pa:Sc 00/30; Pa:Sc 00/35; Zm:Sc 00/30; Z.:Sc 00/35. Sequential inoculation were performed after 24 hours, except for the Pa:Zm assay, where a co-inoculation was conducted. Results showed good compatibility between strains when colony formed units were followed in a differential culture media. GC-MS analyses showed for mixed cultures that non-*Saccharomyces* strains contributed to the final products with a higher concentration of 4-vinyl-guaiacol (Odour Active Value, OAV> 10), and lower concentration of higher alcohols and medium chain fatty acids, whereas the strains of *Saccharomyces* contributed increasing acetate esters and the monoterpenes myrcene and limonene (fruit / floral notes) with OAVs> 1. Spicy and cloves aromas in mixed cultures were highlighted by the tasting panel. These results are promising for the production of wheat craft beers and their blends.

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**REDUCTION OF ACETIC ACID PRODUCTION DURING WINE FERMENTATION BY SACCHAROMYCES CEREVISIAE X SACCHAROMYCES KUDRIAVZEVII HYBRIDS USING ADAPTIVE EVOLUTION UNDER LIPIDS LIMITATION**

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*S. cerevisiae* x *S. kudriavzevii* hybrids are typically used for white wine fermentation because of their cryotolerance. One group of these hybrids presenting a unique ability to release thiols varietal aromas produces as well unacceptable amounts of acetic acid under specific conditions, which is detrimental for wine organoleptic quality. The objective of this work is to reduce this acetic acid production through an adaptive evolution strategy. A first comparison of the different strains for their production of acetic acid revealed the presence of two groups of strains, further called High (HAP) and Low (LAP) acetic acid producers. When comparing the genomes of the different strains, two genetic groups corresponding to the level of acetic acid production were revealed. HAP strains have lost copies of the region C, while LAP present a different balance of chromosome copy number of each species and both of these modifications may contribute to differences in acetic acid production. Thanks to a Box Behnken experimental design aiming to study the impact of environmental conditions on acetic acid production, we showed that lipids modulate acetic acid and thiols production for both LAP and HAP *S. cerevisiae* X *S. kudriavzevii* hybrids during wine fermentation. Based on this conclusion, we used an adaptive evolution strategy based on a long-term batch culture under lipids and oxygen limitation in order to reduce the acetic acid production of those hybrids. After 250 generations, we selected several evolved strains able to release lower amounts of acetic acid in wine while keeping high thiols liberation ability. The genome of these evolved strains will be sequenced in order to identify the mutations involved in the phenotype.
GENERATION OF SACCHAROMYCES CEREVISIAE HYBRIDS AS A TOOL TO IMPROVE THE OENOLOGICAL PROPERTIES OF A WINE YEASTS COLLECTION

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The alcoholic fermentation for winemaking is mainly carried out by Saccharomyces cerevisiae. The physiological properties of these yeasts influence the final characteristics of the produced wine. Although several commercial strains are offered in the market, there is a continuous search for novel yeas starters better adapted to different regions, oenological practices or with some special feature. Current winemaking practices favor the harvest of very mature grapes with high sugar concentration, which leads to more alcoholic wines. Thus, it would be convenient to have a yeast strain which combines a resistance to high osmotic conditions (300 g/L of fermentable sugars) and high ethanol concentration (15% v/v). From our autochthonous characterized wine yeasts collection, we considered 46 strains which possesses one of these properties and could act as parental strains to generate hybrids capable of tolerating both stress conditions. After a phenotypic re-evaluation, 10 parental strains were selected and induced to sporulation. Considering that native strains are homothallic and no genetic markers are available, the crosses between parental strains were performed with the random spore method. A total of 17 independent crosses were performed, and 207 potentials hybrid were isolated. All generated individuals were phenotypically evaluated for both high osmotic and high ethanol tolerance. So far, 3 hybrid strains and two homozygous cultures from a tetrad dissection have shown improved phenotypes for both characteristics with respect to their parental strains. Furthermore, lab scale fermentations under simulated wine conditions showed promising results for some of these strains as compared to their parental strains. Our results demonstrate that it is possible to improve yeast oenological properties of a wine yeasts collection using simple and traditional breeding techniques.

OXIDATIVE STRESS RESISTANCE OF YEASTS WITH BIOLOGICAL CONTROL POTENTIAL

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A current demand for agriculture is the distribution of safe and agrochemical-free food. In this sense, the introduction of biological control agents is an alternative for protection of fruits and vegetables. The effectiveness of the use of these agents is based on the efficiency of inhibition of the pathogen and resistance to different environmental stresses, such as oxidative stress. The yeasts Torulaspora globosa (strain 5S55) and Trichosporon asahii (strain 3S44) showed potential in the control of the fungus Alternaria alternata, causing black rot in tomatoes, in previous in vitro tests. The objective of this study was to determine the resistance of these two yeasts strains to oxidative stress conditions. A viable cell count test was performed on liquid YEPD medium with concentrations of 0, 10, 20, 30 and 40 mM H2O2 in quintuplicates with an
initial yeast inoculum of 106 cells/mL and incubation under agitation at 150 rpm at 25 °C for 96 hours. Every 12 hours, the yeast cell viability was verified by counting cells stained with methylene blue. The 5S55 strain presented a reduction of live cells at different concentrations, reaching mortality of 78, 77, 81 and 78% at 10, 20, 30 and 40 mM respectively, at 96 hours. The 3S44 strain presented for all concentrations de H2O2 an increase of living cells over time and less than 1% of mortality at the end of the 96 hours. Thus, the results indicate a great resistance by the 3S44 strain and low resistance to 5S55 to situations of oxidative stress. For the 5S55, strategies such as pre-adaptation to stress and development of formulations of biological product with the addition of anti-stress compounds are necessary to intensify their natural resistance.

LAGER YEAST WITH COMPROMIZED CHITIN SYNTHESIS ACTIVITY IMPROVES BEER CLARITY

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Clarity of lager beer is one of the important qualities that consumers are concerned about. Beer haze particles are known to comprise polyphenols and proteins derived from plant-origin ingredients. However, we found that the haze particles also contain yeast cell wall components such as mannoproteins. Thus, we explored the possibility that lager yeast strains with more rigid cell wall structure could result in less amount of haze in the beer fermented with such strains. To this end, we isolated a mutant lager strain resistant to a cell wall-perturbing agent Congo red (CR), and investigated the genetic alterations underlying the CR resistance by whole genome sequencing. It is known that there is a correlation between the amount of chitin in the cell wall and CR sensitivity of yeast. In our study, the parental lager strain was found to contain three distinct cerevisiae-type (Sc) CHS6 (CHitin Synthase-related 6) alleles, two of which have nonsense mutation(s) in the open reading frame (ORF), leaving only one allele functional. On the other hand, the isolated CR-resistant clone contained inactive alleles only. As the same eubayanus-type CHS6 alleles shared by both parental and mutant strains, appeared to contribute to a very weak chitin synthase-activating function, CR resistance of the mutant strain was attributable to the overall compromised activity of CHS6 gene products. When wild-type ScCHS6 was introduced into the mutant strain, CR sensitivity was restored. The CR resistant mutant cells exhibited less chitin production, less amount of protein release into the medium, and reduction of beer turbidity when used for brewing. However, all those traits were counteracted by reintroduction of a functional ScCHS6 gene into the mutant clone. It is of great interest whether the frequent nonsense mutations found in ScCHS6 ORF in the parental lager strain resulted from domestication of lager yeast in its history.
OENOLOGICAL PHENOMES OF LACHANCEA THERMOTOLERANS REFLECT PATTERNS OF DOMESTICATION AND ALLOPATRIC DIFFERENTIATION

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The yeast Lachancea thermotolerans (ex. Kluyveromyces thermotolerans) can positively contribute to the acidity and aroma profile of fermented beverages such as wine. This common constituent of the grape/wine microbiota also occupies a range of natural habitats worldwide. The differentiation in the L. thermotolerans population is driven by geographic determination and ecological niche, as revealed by our recently-developed 14-microsatellite method. The natural isolates are grouped based on their geographic origin, whereas clustering of anthropic (in particular oenological) isolates suggests domestication events within the species. To determine whether, and to what extent, the strains differ in oenological traits and harbour signatures of domestication and/or local divergence, 94 previously genotyped strains were characterised in Vitis vinifera cv. Chardonnay fermentations. The extensive dataset comprised microbial growth and fermentation kinetics parameters, production of primary and secondary metabolites and the resultant (de)acidification, i.e. 114 measured/derived parameters for triplicate fermentations. The common oenological features of L. thermotolerans strains were their glucophilicity, relatively extensive fermentation ability (7.3% v/v ethanol), low production of acetic acid and formation of lactic acid. A seven-fold variation was observed in concentration of lactate, significantly affecting the pH of the wines, which ranged between 3.16 - 3.81. An untargeted analysis of volatile compounds revealed that 58 out of 90 volatiles were affected at an L. thermotolerans strain level. Linear discriminant analysis performed using the obtained metabolic dataset showed the separation of L. thermotolerans genetic groups driven by distinct fermentation performance and production of (non)-volatile metabolites. Together, these results provided a population-wide insight into the extent of phenotypic variability in oenologically-relevant traits in L. thermotolerans, whilst adding support for the occurrence of domestication events and allopatric differentiation within this remarkable yeast species.

INFLUENCE OF BRETTANOMYCES CLAUSSENII AND BRETTANOMYCES BRUXELLENSES IN BIOFLAVORING OF BEER

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In 1904, the Danish scientist Hjelte Claussen isolated a novel yeast species from beer, which he named Brettanomyces. Since then, the unique character given to the final
product has generated a controversial debate. In many breweries and specially wineries, *Brettanomyces* has gained the reputation of being a spoilage yeast. However, such yeast possesses several traits like tolerance to high ethanol and low pH and production of unique flavors which are attractive for beer production. The flavor contribution of such species could be controlled with a deeper understanding in the development of secondary metabolites over time. Therefore, two different *Brettanomyces* species, *bruxellensis* and *clausenni*, have been studied for its potential for beer bio-flavoring. Strains were used for re-fermentation of a lager base beer, and the changes in the flavor profile were monitored. Remarkable differences were found in ester, volatile phenols, organic acids and sugar profiles of the final beer. Those findings can contribute to the understanding of the performance of *Brettanomyces* species, giving new insights on the application for flavor development in beer.

**PHENOTYPIC AND GENETIC ANALYSIS OF NEW BREWING HYBRIDS**

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*Saccharomyces* hybrids are utilized frequently in industry, such as the lager producing hybrid *S. pastorianus*. They are desirable as they have the potential to combine beneficial traits from each parental species. As they are usually sexually sterile, most hybrids are considered evolutionary dead-ends. Hybrid sterility inhibits the potential for strain improvement through breeding. In this project we utilized genetic techniques to create de novo hybrids which have overcome the sterility barrier. In addition, we also introduced fertility to existing lager producing hybrids. In nature, particularly plant species such as wheat, hybrid sterility is overcome through genome duplication and higher levels of ploidy. Utilizing this principle tetraploid *de novo* hybrids were created, which no longer exhibited post zygotic sterility. These hybrids underwent multiple rounds of mating, creating vast genetic diversity. Populations were placed under selective pressures, with individuals pooled for sequencing. Using our advanced quantitative genetic approaches, we identified the genetic variants contributing to phenotypes of interest, for example high ethanol concentration and temperature. Knowledge of these variants allow us to produce genetically improved strains beneficial to various industrial processes. In the second part of the project fertility was introduced into the two of *S. pastorianus*. The allotriploid Saaz type and the allotetraploid Frohberg class. Both classes are infertile. Mating of Saaz strains with haploid *S. cerevisiae* isolates creates tetraploid individuals, which were capable of producing diploid offspring. Mass sporulation lead to the isolation of rare viable spores of Frohberg strains. Mating type PCR revealed individuals possessing a specific mating type. Mating between individuals and other diploid maters, gave isolates with viable offspring. The introduction of fertility to existing industrial hybrids will unleash the potential for strain improvement through breeding, the introduction of new characteristics, as well as allowing quantitative trait genetics to be performed in existing hybrids.
THE FARMHOUSE ALE YEAST STRAINS AS POTENTIAL CHASSIS FOR INTRA AND INTERSPECIES YEAST HYBRID DESIGN

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The development of yeast hybrids is crucial for brewing industries, allowing the design of strains with improved brewing phenotypes. A major brewer’s yeast phenotype that can be target in such hybridization programs is the fermentation fitness and tolerance to fermentation stresses. From all brewer’s yeast chassis considered to improve fermentation fitness in hybrids, the farmhouse ale yeast strains are the most promising. Thus, the purpose of this work was to evaluate how adapted are three different Wallonian farmhouse ale yeast strains (WLN I, II and III) to fermentation stresses in comparison to different yeast strains, like SafAle™ US-05 (american ale yeast), SafLager™ W34/70 (lager yeast), Voss Kveik from The Yeast Bay™ (a norwegian farmhouse ale yeast) CAT-1 (a bioethanol producing yeast), and BY4741 (laboratory yeast strain). In addition, we also evaluated the chronological life span (CLS) of all yeast strains tested. Our data indicated that WLN I, II and III strains appear to be remarkably resistant to ethanol, osmotic and oxidative stress when compared to other yeast strains. WLN I and III present high tolerance to osmotic and H₂O₂- induced oxidative stresses and high concentrations of ethanol. With respect to osmotic stress, both WLN III and CAT-1 strains showed low sensitivity to 1.0 M KCl. Compared to US-05, all Wallonians strains were more resistant to hyperosmotic stress at 0.7 M, 1.0 M and 1.5 M KCl. Regarding oxidative stress, WLN I display resistance towards H₂O₂ (500 mM). Aging curves for both Kveik and WLN II were similar to the BY4741. Both WLN I and III strains present substantially extended CLS, with the maximum survival rate ate day 15. Finally, these results lead to the potential use of Wallonian yeasts chassis in intra and interspecies hybridization programs to improve yeast strains for brewing industry.

DETECTION AND IDENTIFICATION OF WILD YEAST FROM ARGENTINEAN CRAFT BEERS

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In brewing, the yeast chosen to conduct the fermentation is one of the key factors that influence the flavor profile. Yeasts that are deliberately not under the control of the brewer are called “wild yeasts”. The growth of these yeasts in the wort or beer can cause defects that include the formation of phenolic compounds, esters, higher alcohols, excess carbonation and super-attenuation. Wild yeasts can be divided into two large groups: those belonging to the genus Saccharomyces and those that do not, the former present the greatest risk, given their physiological and morphological similarity to the inoculated yeast. The aim of this work was to assess the incidence of contaminated bottled craft beers with wild yeasts and the identity of the contaminants. In total 90 craft beers were analyzed sensorially, physicochemically and microbiologically. The
Lin’s copper sulphate culture and in a few cases Schwarz Differential Media were used to detect and isolate wild yeasts. Isolates were molecularly identified by sequencing the ITS region of the ribosomal DNA. The growth of wild yeasts was registered in 41.1% of the beers, although only 20% showed sensory defects associated with contamination with wild yeasts (phenols). Almost 50% of the isolates belonged to *Saccharomyces cerevisiae* and the remaining identified species included *Wickerhamomyces anomalus, Candida parapsilopsis, Candida pararugosa, Clavispora lusitaniae, Pichia membranaefaciens* and *Trichosporon insectorum*, among others. The yeasts isolated in this work (n=57) constitute the first collection of craft beer spoilers yeasts from Argentina. The study of these microorganisms is of great importance to understand the origin of the contaminations and the most efficient way to approach this problem in order to improve the quality of Argentinean craft beers.

**THE "STEW IN THE BREW": CHARACTERIZING THE DYNAMIC MICROBIAL MIXTURES RESPONSIBLE FOR LAMBIC BEERS**

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For Belgian lambic beers, yeast and bacteria resident in the brewery environment serve as the "spontaneously" inoculating microbes of an open vat of cooling wort. After cooling, the wort is transferred into oak casks and undergoes fermentation and aging over the course of one to several years. During this time a succession of several different microbial communities, each consisting of various types of yeast and bacteria, occurs. While we know the general outlines of this succession from previous studies, many have relied on culturing of the microbes over the course of fermentation. More recently, genetic and sequencing techniques have been used to characterize the microbial progression of lambic beer samples, but these still are limited by ambiguity since they are performed on a homogenized mixture of cells. We are now employing high-throughput sequencing techniques that allow "deconvolution" of individual microbial genomes in a mixed sample without any need to culture the microbes (either individually or as a group), and even without any a priori knowledge of the species or strains that may be present in the sample. Using this method, we are able to explore and characterize the members of the various microbial communities that appear and disappear during a lambic fermentation, allowing unprecedented detail about the genomic sequence of each microbe present—even those at very low abundance, and even for previously unknown or un-sequenced organisms. The technique also allows us to detect the presence of interspecific hybrids as well as other genome features difficult to detect by normal deep-sequencing techniques. Studying and characterizing the lambic microbial communities in as much detail as possible will help us glean new insights about this beer style, and we may possibly identify new microbes that may be commercially important.
OTHER TOPICS
EFFECT OF TEMPERATURE AND WATER ACTIVITY ON THE INTERACTION OF A STRAIN OF METSCHNIKOWIA PULCHERRIMA WITH ALTERNARIA ALTERNATA

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The presence of Alternaria spp, mainly A. alternata, has been observed during postharvest in table grapes cv. Red Globe from Mendoza, Argentina. One of the strategies to reduce postharvest losses and the use of SO₂ could be to use biological control agents during this stage. Epiphytic yeasts were isolated from grapes harvested in Argentinean vineyards. The strains were identified by molecular methods. The effect of a strain of Metschnikowia pulcherrima on lag phase and growth rate of Alternaria alternata was evaluated in a 3% must agar based medium under different conditions of water activity (0.88, 0.991 and 0.995 aw) and temperature (0, 4, 15 and 28 °C), these conditions were selected considering the water activity of grapes during the maturation stages and the range of temperature during phenological cycle and postharvest of table grapes. The growth rate and the lag phase were influenced by all the interactions evaluated (p< 0,05). Studies are in progress to evaluate these strains in vivo under postharvest conditions.

POSSIBLE MECHANISMS OF ACTION OF NATIVE YEASTS AGAINST OPPORTUNISTIC SPOILAGE FUNGI ISOLATED FROM MATURE OLIVE FRUITS

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Some genera of fungi can affect the quality of mature olive fruits. Aspergillus is one of the opportunistic genera that caused rot and showed potential to produce mycotoxins in olive fruits and derivate products. Yeasts metabolism have presented several advantages as biocontrol agents and different modes of action against fungal pathogens. Elucidation of the possible antifungal mechanisms of yeasts could be used for optimization, formulation, and application of a biocontrol agent. The objective of the work was to determine possible mechanisms of action of native yeasts against A. niger, an opportunistic fungal pathogen of mature olive fruits. Thirty native yeasts from vitivinicultural (6) and olivicultural (7) environments were employed in the tests since they had shown antagonistic activity at in vitro and on mature olive fruits against two opportunistic fungi belonging to A. niger species (BoAn1 and BoAn2). Four mechanisms of action were evaluated in vitro: a- production of volatile metabolites (V), b- antifungal killer activity (K), c-siderophore production (S), d- hydrolytic enzymes secretion: chitinase (Q) and laminarinase (L). Eleven yeasts presented at least two of the modes of action assayed. The killer activity and volatile metabolites production were the main mechanisms involved in the biocontrol activity of the native yeasts against A. niger. Wickerhamomyces anomalus Bo128, isolated from healthy olive fruit surfaces, presented all the antifungal mechanisms assayed against A. niger BoAn1 and BoA2. W.
anomalus Bo128 presented different mechanisms of action against potential mycotoxigenic Aspergillus. The use of this olivicultural yeast that possesses several antifungal mechanisms to inhibit A. niger reduces the potential risk of pathogen resistance and could reduce the contamination by mycotoxins produced by Aspergillus in olive fruits. This research is the first report demonstrating the putative modes of action of olivicultural yeasts against opportunistic spoilage fungi of olive fruits.

COMPETITION BETWEEN NATIVE ANTAGONIST YEASTS AND PENICILLIUM EXPANSUM, TABLE GRAPES PATHOGEN UNDER POSTHARVEST CONDITIONS

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Table grapes are preserved in cold storage chambers for its subsequent commercialization, but this environment is favorable for the development of pathogenic fungi such as Penicillium expansum. As an alternative to the use of synthetic fungicides that affect the quality of the grape, the environment and human health, biological antifungal agents are proposed for the control of P. expansum. Through the study of the mechanisms of competition for nutrients between pathogens and antagonists, it is possible to determine the coexistence or competitive exclusion among them, depending on the level of partition or overlap of nutritional resources. In the same way, the competence can be evaluated by measuring the ability of yeasts to produce micronutrient chelating compounds such as iron. With the objective to evaluate the competition for nutrients between antagonistic and pathogenic microorganisms, the following were determined: Niche superposition index (NOI), niche size (NS) and siderophore production at 0±1°C. We used 15 yeasts isolated from fermenting musts and grapes stored in cold storage, all of them biosuppressors of P. expansum PSS6: Rhodotorula glutinis (Rg 4, 14, 19, 56), Cryptococcus magnus (Cm 16, 23), Aureobasidium pullulans (Ap 13, 77, 88) and Metschnikowia pulcherrima (Mp 22, 36, 43, 45, 46, 53). Results suggest that there would be competitive exclusion between Rg4, Rg14, Rg19, Cm23, Rg56, Ap77 and PSS6. These yeasts could affect the development of the pathogenic fungus by decreasing the availability of nutritive resources. A. pullulans Ap13 could be proposed as an effective antagonist against PSS6, being the only yeast capable to compete for carbon and nitrogen nutrients and to produce siderophores under refrigerated chamber conditions. The study of mechanisms of action of yeasts based on the microbial interactions of competition for substrate is a relevant contribution, since there are no reports on this specific pathosystem in low temperature postharvest conditions.
POSSIBLE ANTIFUNGAL ACTION MECHANISMS OF VITICULTURE YEASTS AGAINST *BOTRYTIS CINEREA* ISOLATED FROM LETTUCE. SAN JUAN, ARGENTINA

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The use of naturally occurring yeasts as biocontrol agents has attracted special interest because of their special attributes, nevertheless it has been recognized that the efficacy and consistent performance of such agents are affected by different factors. Elucidation of the action mechanisms could be useful for formulation and optimization of a biocontrol inoculum. In order to select a biocontrol agent for controlling gray mold in lettuce, 16 indigenous viticulture yeasts (15 *S. cerevisiae* y 1 *S. pombe*) were tested accordingly to inhibitory activity on the mycelial growth of *Botrytis cinerea* B1 (from Lettuce) *in vitro* for detecting the potential production of diffusible metabolites. We evaluated the capacity competition for nutrients (Niche Overlap Index) between yeasts and B11.

Materials and Methods: 1-*In vitro* yeast-pathogen direct interaction: The potential biocontrol yeasts were co-cultured with the pathogen on Petri dishes containing Czapeck-Yeast extract-Agar, to test antagonistic activity, pH4.6. Niche Overlap Index (NOI): Fungal mycelium discs (9mm) and yeast aliquots (20 μL, 10\(^6\) cells/mL) were inoculated on separate plates. Each plate contained one carbon source (10 mM), YNB with 20 g/L Agar, pH 5.5. The assayed carbon sources (14) are present in lettuce and represent the size of the niche. Results showed that all the evaluated yeasts significantly reduced the growth from 22 to 52%, in comparison with the untreated control (100%). Nine *Saccharomyces*-fungus interactions showed NOI values between 0.92 and 1. These yeasts also inhibited *B. cinerea in vitro*. These results suggest that the yeasts assayed were able to inhibit *B. cinerea* and successfully assimilate a wide variety of carbon sources, making these nutrients unavailable to fungi and allowing rapidly proliferation of yeasts (competitive exclusion). Further experiments should be conducted to determine the efficacy of these yeasts under commercial lettuce production.

RIBOSOME ASSOCIATED PROTEOME ANALYSIS DURING QUIESCENCE AND TRANSLATIONAL REACTIVATION AFTER A NUTRIENT STIMULI IN *SACCHAROMYCES CEREVISIAE*

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*Saccharomyces cerevisiae* enters quiescence when nutrients are consumed and re-enters cell cycle when nutrients are once again available. Changes in cell cycle are modulated through variations in cellular proteome at a given time; how translational regulation is achieved is our research goal. To characterize the translational machinery in monosomal or polysomal fractions of stationary phase (SP) wild type cells or after 30 or 60 minutes of fresh media addition we used nano-LC MS/MS and protein abundance estimations using exponentially modified protein abundance index (emPAI). In SP global
translation is inactivated, after 30 min of fresh media addition global translation is still not fully recovered, while after 60 min polysome profiles are similar to an exponential phase. Proteomic analysis showed several proteins other than those involved in translation and protein folding interacting with ribosomes. Monosomes -but not polysomes- in all conditions present a subset of proteins involved in protein degradation. Enrichment analysis pointed to 30 min not as an intermediate condition but as a regulatory one, highly enriched in proteins involved with isoleucine and aromatic amino acid processes. We found a subset of small and large ribosomal subunits present only in the monosome in SP, such as Rpl31B and Rps0B. Protein abundance analyses showed that Stm1, a protein required for proper translation during stress, and that chaperones like Ssb1 and Ssb2 are associated to monosomes in SP. Moreover, polysomes in SP have translation elongation factors and the association of these factors diminishes along the stimuli. Hsp26 is associated to monosomes no matter the nutrient condition and is not present in polysomes. Taken together, our results shed light into translation control regulation by nutrient signalling and suggest how quiescent yeast cells can modify the translational machinery through ribosomal core modifications and/or auxiliary proteins factors association.

**EXPRESSION OF THE PEPTIDE CP-THIONIN II IN KU-70 STRAIN OF PICHIA PASTORIS**

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In the last few decades, the demand for new and efficient molecules with antimicrobial activity has been expanded due to the microbial growing resistance to antibiotics. Thus, charges caused by the extension of hospitalization consequently reflect in increasing treatment value and consumption of antibiotics. Therefore, antimicrobial peptides (AMPs) have been emerging as interesting molecules for the development of new drugs, on account of having potential therapeutic and biotechnological applications. The defensins are peptides found in plants with four to five disulfide bonds, which interact with specific membrane components to trigger intracellular signaling cascades that hinder pathogen growth. AMPs can also inhibit the action of insect digestive proteins. Cp-thionin II is a defensin purified from cowpea (Vigna unguiculata) tissues and has significant activity against *S. aureus*, *E. coli*, and phytopathogenic *Pseudomonas syringae*. The aim of this study was the heterologous production of the defensin Cp-thionin in the yeast *Pichia pastoris*. The Cp-thionin II gene was amplified by PCR and cloned into the expression vector pPICZα-A. The linearized recombinant vector was inserted into the yeast *P. pastoris* strain Ku70 by electroporation. Sequencing analysis showed that the recombinant expression vector was constructed correctly, and PCR analysis showed that the recombinant vector was successfully integrated into the yeast genome. The gene expression induction lasted 48 hours with YPD Broth and 80 hours with UAB Broth with 1% of methanol at 28°C with agitation of 200rpm. After SDS PAGE analysis on 15% (w/v) agarose gel, it was possible to visualize that the Cp-thionin II was secreted with a peptide with an approximate mass of 5kDa. It was able to conclude that the peptide was expressed successfully. Besides the expression of the peptide, there are expectations of purifying the molecule for the further antimicrobial activity evaluation.
RCN2 AND MYO3 ARE MULTICOPY SUPPRESSORS OF VPS13 MUTATION

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Sodium dodecyl sulphate (SDS) is a widely used compound, important for cosmetic industry, agriculture, medicine and biotechnology. It helps to dissolve, extract or disperse various compounds. SDS is toxic and in excess kills cells. In low amounts it is cell wall and plasma membrane damaging agent and causes SDS stress in yeasts. Increasing cell wall hydrophobicity or lowering unsaturation level of fatty acids in plasma membrane increase tolerance of yeast cells to SDS. On the other hand, mutants with disrupted cell wall integrity or calcium homeostasis show SDS-hypersensitivity. We have found that Saccharomyces cerevisiae vps13Δ mutant is disturbed in the actin cytoskeleton organization and endosomal/vacuolar protein transport and is hypersensitive to SDS. In vps13Δ mutant the cell wall integrity signaling pathway is not activated indicating that cell integrity is not affected. To learn more about a role of Vps13 in SDS stress, we screen genomic library for multicopy suppressors. Five suppressor genes were isolated, including RCN2 which encodes negative regulator of calcineurin, a phosphatase central to calcium signaling, and fragment of MYO3 gene encoding N-terminal part of myosin, an actin cytoskeleton protein which binds calmodulin, a calcium-binding regulatory protein. Binding of calmodulin by Myo3-N was essential for suppression. We observed increased activity of calcineurin in vps13Δ cells and it was diminished when RCN2 or MYO3-N were overexpressed. This points to the connection between Vps13 and calcium signaling and shows that SDS-hypersensitivity could be overcome by changing signaling pathways. With this knowledge we tested inhibitors of calcineurin and we screen the library of drugs for those which restore growth on SDS-containing plates. We identified six hits which are analyzed further. These findings have medical and biotechnological implications. This work was supported by National Science Center Poland, grant UMO-2015/19/B/NZ3/01515 to TZ.

TRANSCRIPTOME ANALYSIS OF KOMAGATAELLA PHAFFII IN THE PRESENCE OF INHIBITORS FROM LIGNOCELLULOSIC HYDROLYSATE

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In a global context of search for technological innovations that value sustainability and sustainability, the use of lignocellulosic biomass for the production of compounds with high added value has been called attention of industries and scientists. The yeast Komagataella phaffii (Pichia pastoris) has been extensively used in the production of heterologous proteins, and increasingly applied in the industry, at the production of biopharmaceuticals (mainly peptides) and other compounds of interest. Given the potential of K. phaffii, recombinant strains of this yeast for the production of chemical compounds from renewable sources has been increasing significantly in recent years. In spite of all this, when compared to other eukaryotic systems, knowledge concerning
physiology and availability of tool for genetic manipulation of this yeast are still scarce. Additionally, there are yet still no records of studies regarding the development of resistant K. phaffii strains in the presence of lignocellulosic hydrolysates, either physiologically and genetically. One of the main challenges for the use of lignocellulosic biomass as raw material in bioprocess is the presence of inhibitors in the hydrolysate. Inhibitors grouped as organic acids (acetic acid, levulinic acid, etc.), furaldehydes (furfural, hydroxymethylfurfural – HMF) and phenolics (derived from lignin breakdown) are capable of completely inhibiting microbial metabolism, reducing rates and yields of bioprocesses. Previously, we evaluated K. phaffii physiologically, and it’s transcriptome in the presence of different inhibitory compounds present in biomass hydrolysate from sugarcane bagasse. The isolated inhibitors acetic acid and furaldehydes, along with the hydrolysate itself affected the consume of K. phaffii reducing its growth, consume of substrate and formation of products. Through the evaluation of transcriptomic data, genes potentially capable of increasing yeast’s tolerance to the inhibitors were identified.

IDENTIFICATION OF THE TRANSCRIPTION START SITES OF THE DBPAD GENE IN THE CONTAMINATING YEAST BRETTANOMYCES BRUXELLENSIS LAMAP2480

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Yeasts belonging to the genus Brettanomyces are considered the main contaminating microorganism in the wine industry due to the production of volatile phenols, formed from hydroxycinnamic acids (HCA) (p-coumaric, ferulic and caffeic acids). The generation of these compounds by Brettanomyces spp. involves the sequential action of two enzymes, a phenylacrylic acid decarboxylase (PAD) and a vinylphenol reductase. The phenolic compounds derived from p-coumaric acid such as 4-vinylphenol and 4-ethylphenol, have been described as the main contributors of aromas associated with stable, wet mouse, etc., which decreases the aromatic quality of the wine, generating significant losses in the wine industry. The gene involved in the production of 4-vinylphenol from p-coumaric acid has been identified in B. bruxellensis LAMAP2480 as DbPAD, which codes for a PAD enzyme. Recent studies have indicated the presence of a smaller open reading frame called DbPAD2, which also encodes an enzyme with the same activity. The aim of this work was to identify the transcription start sites (TSS) belonging to the DbPAD gene, with the purpose of describing a mechanism of transcriptional regulation. The identification of the TSS was carried out by the generation of cDNA from the transcripts of the DbPAD gene, subsequent circularization and inverse amplification. The obtained product was cloned and sequenced. Subsequently, a prediction of the sites of transcriptional regulation in the promoter region was carried out to evaluate the effect of the presence of the binding sites of the selected transcriptional factors in the expression of the gene. In this way, this study allows us to continue with the development of methodologies to determine the biological variables involved in the expression and regulation of the DbPAD gene, with the objective of minimizing the impact caused by volatile phenols in the wine industry.
HETEROLOGOUS EXPRESSION AND BIOCHEMICAL CHARACTERIZATION OF A MICROBIAL EXPANSIN IN KOMAGATAELLA PHAFFII

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Currently, biotechnological processes for energy production and renewable chemicals are under development as an alternative to fossil fuels. Lignocellulosic biomass presents great potential for the generation of biofuels, for example second generation ethanol, and other bio products as organic acids. Therefore, the identification and characterization of new proteins that act in the deconstruction of the biomass are strongly desired. Cellulases, hemicellulases and proteins with accessory activity are essential in the hydrolysis process. Several studies have reported synergistic effects between cellulases and expansins, which are non-hydrolytic proteins capable of loosening the cell wall of plants. Expansins are capable of disrupt the hydrogen bonds between the polysaccharides (cellulose and hemicellulose), facilitating the access of enzymes that degrade the biomass. As a result, the enzymatic activity increases and releases a greater amount of sugars. In order to produce high yields of these enzymes and reduce costs, the optimization of recombinant expression systems in microorganisms is highly desired. In this work, gene sequences from thermophilic fungus that encodes for two putative expansins were obtained for heterologous expression in Komagataella phaffii X33. These gene sequences were optimized and cloned into expression vector pGAPZB, which has as main features: the constitutive promoter GAP, antibiotic selection mark (zeocin) and histidine tail. In addition, the expansin has a native secretion signal. After genetic transformation in K. phaffii, positive clones that had the expression vector integrated into the yeast genome were confirmed by PCR. These recombinant clones were grown in Erlenmeyer flasks and the production of the heterologous protein was evaluated by SDS-PAGE and western blot techniques. Expression in K. phaffii allowed the production of the recombinant expansin in bioreactors, subsequently protein purification and biochemically characterization. The results will be presented and discussed.

RNA-SEQ BASED TRANSCRIPTOME ANALYSIS REVEALS UV-REGULATED GENE EXPRESSION IN A NATIVE ISOLATE OF AUREOBASIDIUM PULLULANS FROM PATAGONIA

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Ultraviolet radiation (UVR) is an important harmful factor for biosphere, inducing several stress responses in organisms. The exposure to UVR-B (280–315 nm) can cause direct and indirect cellular damage. For organisms living in extreme conditions, daily exposure to UVR-B (‘UV’ from now on) acts as an environmental pressure, selecting strategies to improve their performances against UV-detrimental effects, such as the production of ROS. It is known that several species of fungi are able to produce
mycosporines, a biotechnologically important molecule with antioxidant and UV sunscreen activities. We predicted a gene cluster potentially responsible for mycosporine biosynthesis in the genome of Aureobasidium pullulans. This cluster is similar to that reported in the genome of the mycosporinogenic yeast Phaffia rhodozyma. The aim of this work was to characterize global changes in gene expression induced by UV exposure in a native isolate of A. pullulans from Patagonia through a RNA-seq-based transcriptomic approach. We compared RNA-seq results for cultures grown at 20 °C in two conditions: constant darkness and with a 5-min UV pulse. Differential expression analysis of the resulting transcriptomic data showed that 2% of the genes changed their expression as a result of UV exposure, of which about 70% represented upregulated genes. Even though a 5 min UV pulse was not sufficient to trigger a massive de-regulation, our analysis support that UV participates in photostimulated processes. As expected, among upregulated genes, we found those belonging to the mycosporine cluster. Surprisingly, we were also able to detect an UV-mediated induction of circadian genes. This finding triggers new questions about the effect of UV in endogenous rhythms in fungi.

THE KILLER YEAST WICKERHAMOMYCES ANOMALUS CF20 IMPAIRS THE GROWTH OF CANDIDA SPP.

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Killer yeasts are able to produce proteins or glycoproteins with antimicrobial activity known as killer toxins, and they can be used as biocontrol agents against pathogens yeasts. In previous studies, we demonstrated the inhibitory activity of the cell-free supernatant (CFS) produced by Wickerhamomyces anomalus Cf20 against wine-relevant yeasts. In this study, we evaluated the cytotoxic effects of KTCf20, the killer toxin present in CFS, over two clinical isolates of pathogenic strains of Candida spp. Growth inhibition of C. albicans and C. tropicalis in CFS (killer activity 2x10^4 aU/ml), was studied at different temperatures (20 and 30 °C) and NaCl concentrations (0, 1 and 3%). OD_{600} and cell viability were measured to calculate inhibition. Fluorescence microscopy (FM) of pathogenic strains was performed after incubation for 2 and 4 h at 20 °C in CFS+NaCl 1% and live/dead staining using SYTO9 and PI as fluorescent probes. Transmission electron microscopy (TEM) was also performed to evaluate the cytotoxic effects of CFS on Candida cells. In every experiment, heat-inactivated CFS (100 °C, 15 min) was used as control. CFS produced a growth inhibition of 74 and 80%, and 1-2 log cfu/ml on C. albicans and C. tropicalis, respectively, at 20 °C and NaCl 1%. Its inhibitory activity decreased at 30 °C. Fluorescence microscopy, TEM and viability results showed that CFS has a fungistatic and fungicidal effect at a 2-h treatment on C. albicans 78 and C. tropicalis FBUNT3, respectively. This study reveals the inhibitory potential of CFS against pathogenic Candida spp. strains and their differential resistance to the antifungal effect, both mechanisms worthy of further study.
EFFECTS OF BIOFUNGICIDE YEASTS ON FUNGAL CONIDIA GERMINATION AND ON GERMINAL TUBE LENGTH OF *BOTRYTIS CINEREA* OF TABLE GRAPE

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San Juan, Argentina produces 90% of the total volume of table grapes in the country. It has been reported that *B. cinerea* is one of the main phytopathogenic fungi that affects the quality of the table grape of San Juan. In the search for sustainable options for the management of fungal diseases, the use of antifungal microorganisms arises as a control method. Some yeasts species have been reported as biocontrol agents of phytopathogenic microorganisms in fruit. The antagonistic microorganisms can exert their control over the pathogen by several mechanisms of action. One of the main modes of action of antagonistic yeasts against fungal pathogens is the competition for nutrients (carbohydrates, nitrogen, oxygen) and space. Objective: to evaluate the competition for nutrients through inhibiting the germination of conidia and elongation of the germinal tube of *B. cinerea* in medium with low of nutrients in vitro. Eighteen curative yeasts (13 *Saccharomyces*, 5 non-*Saccharomyces*) and two *B. cinerea* strains (B15 and B24) were used. In excavated slides were placed 25µL of a yeast suspension (10⁶ UFC/mL), 25µL of fungal conidia (10⁴ conidia/mL) and 100µL of grape must diluted at 1% vol/vol (low nutrient). The slide was placed inside a sterile petri dish with filter paper moistened with sterile distilled water (80-90%RH). Plates were incubated at 25°C for 12-24 hours. The yeasts *S. cerevisiae* (BSc60, BSc102) and *H. vinae* (BHv86) decreased the conidia germination and significantly reduced the length of the germ tube of the phytopathogenic fungi B15. Yeasts *S. chevalieri* (BSc26), *S. cerevisiae* (BSc60, BSc112, BSc206) and *H. vinae* (BHv86) were those that presented significant inhibitory activity (77-22%) against strain B24. The competition for nutrients through inhibiting the conidia germination and elongation of the germinal tube could be one of the possible mechanisms involved in the reduction of gray rot in grapes.

IDENTIFICATION AND FUNCTIONAL ANALYSIS OF NITRATED PROTEINS IN THE YEAST *SACCHAROMYCES CEREVISIAE*

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Reactive nitrogen species (RNS), such as nitric oxide (NO), function as a signaling molecule in many kinds of organisms. RNS modify amino acid residues on the protein. Peroxynitrite, which is one of RNS with the highest reactivity and is generated via the reaction of NO with superoxide anion, leads to protein tyrosine nitration (PTN). PTN has been studied as a maker for inflammation and/or nitrosative/oxidative stress, however, many researches recently showed that PTN contributes to a signaling system because PTN alters the function of proteins. In yeast, which is a model organism for higher eukaryote and pathogenic yeast/fungi, the RNS-mediated signal system has not been fully understood, even though a few reports showed PTN. First, we treated cells
with nitrite under the acidic pH conditions (pH 4.0 to 6.0) to prepare the nitrated protein sample, because the nitrous acid generated by the protonation of nitrite at the acidic pH can be converted into NO by reduction. The nitrite treatment at pH 4.0 generated highly nitrated protein sample, accompanied with an increased level of NO in the nitrite dose-dependent manner. The growth defect and decrease in cell viability were also observed under the same conditions. These results suggest that PTN generated by NO and/or other RNS derived from NO affects cell growth and induces cell death. Now, we try to identify the nitrated proteins from the sample prepared by the conditions optimized above, by the screening methods of nitrated proteins, based on the method reported previously with the modification.

**POP2 AND CCR4 HAVE DISTINCT ROLES IN THE TRANSLATIONAL REPRESSION OF LRG1 mRNA**

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Pop2 and Ccr4 are the major subunits of the Ccr4-Not complex involved in mRNA poly(A) tail shortening in *Saccharomyces cerevisiae*. We have previously shown that both Pop2 and Ccr4 negatively regulate the expression of LRG1 mRNA, encoding GTPase-activating protein for Rho1. LRG1 deletion suppresses the temperature-sensitive growth defect of the *pop2Δ* and *ccr4Δ* mutants. We have also shown that the slow growth of the *pop2Δ* and *ccr4Δ* is repressed by deleting another gene, *PBP1*, encoding poly(A)-binding protein (Pab1)-binding protein 1; however, the underlying mechanism is still unclear. In this study, we investigated how *pop2Δ*, *ccr4Δ*, and *pbp1Δ* mutations influence the length of poly(A) tail and *LRG1* mRNA and protein levels during long-term cultivation. During log-phase, *ccr4Δ* mutant cells have maintained longer *LRG1* poly(A) tail and its mRNA level was higher than those in wild-type (WT) cells. Unexpectedly, Lrg1 protein levels in *ccr4Δ* were comparable with that in WT. In the case of *pop2Δ*, both the mRNA and protein levels were increased significantly in log-phase. During stationary-phase, *LRG1* poly(A) tail length was still longer in *ccr4Δ*. This time, both the *pop2Δ* and *ccr4Δ* mutant cells have maintained increased levels of mRNA and protein compared to WT. The loss of *PBP1* reduced the *LRG1* mRNA and protein levels of both the *pop2Δ* and *ccr4Δ* mutant cells in the stationary-phase. Our results suggest that Pop2 and Ccr4 have distinct roles in regulating *LRG1* mRNA and protein levels depending on growth phase and that Pbp1 is involved in the Ccr4-Not complex-mediated regulation of mRNA stability and translation efficiency.

**FROM THE LAB TO THE CRAFT: BREWING TECHNOLOGY TRANSFER EXPERIENCES IN ARGENTINA**

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The discovery in 2011 of Saccharomyces eubayanus, the mother of the Lager yeast, had many scientific and technological implicancies, and opened the possibility to actively
work together with the growing craft brewing industry in Argentina, particularly in Bariloche. Initially, our main goal was to work with the brewers and help them get prepared to work with this special yeast, available only in liquid format; but that goal expanded as it was clear that they lacked crucial knowledge on yeasts and their management in the brewery. The first approach to the industry was through a series of theoretical and practical courses that included basic brewing science and some with focus on yeast and fermentation. Currently we have an itinerant event of courses that goes through the country called *Ciencia & Cerveza* (Science & Beer). At the request of brewers and hop producers, high-level technological services from our Institute (IPATEC) were developed and made available. They included quality controls, yeast conservation and propagation; microbiological, sensory and physicochemical analysis, among others. Provision of different yeasts to Argentinean brewers was an important tool for product differentiation given only dry yeast is still available in our country. Another strong point of transference includes assessing producers in management and re-pitching of yeasts, and helping brewers build their own QC labs. From this interaction we developed a smartphone app (MicroBrew.AR) that simplify the task of assessing under the microscope the quality and quantity of harvested yeast and pitching calculations. The interaction with the craft brewing industry become a clear synergy and multiple funding programs were obtained together, multiple analytical tools become available for the brewers and many scientists are being trained in different aspects of brewing science. In overall, we want to communicate our unique case of academic and technological outreach activities related to brewing science, in particular related to yeasts.

**STUDY OF HUMAN CONGENITAL DISORDERS OF GLYCOSYLATION USING FISSION YEASTS AS A MODEL ORGANISM**

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*N*-glycosylation is one of the most frequent post-translational modifications of proteins that enter the secretory pathway. It is catalyzed by the oligosaccharyltransferase (OST), which transfers the pre-built glycan Glc₃Man₉GlcNAc₂ from a Dolichol-PP derivative to the sequon NXS/T of proteins entering the endoplasmic reticulum (ER). Glycans play a key role in protein folding and in the quality control of glycoprotein folding. Mutations in genes involved in the Dolichol-PP-glycan biosynthetic pathway or in the transfer reaction produce protein hypoglycosylation (sequons normally occupied with a glycan are empty) thus producing folding defects and Congenital Disorders of Glycosylation (CDG) Type I. Defects in the *N*-glycan processing that is produced after the transfer reaction do not produce protein hypoglycosylation but aberrant glycan structures present in CDG Type II. The fission yeast *Schizosaccharomyces pombe* shares with mammalian cells all the mechanisms of Dolichol-PP-glycan biosynthesis, glycan transfer and the initial steps of *N*-glycan processing. Moreover, a large amount of genetic and biochemical tools make this yeast an ideal organism to model the molecular basis of CDG type I and some CDG type II. We constructed 16 yeast mutants that synthesize all possible combinations of Dolichol-PP-glycan structures to be transferred to proteins in the ER. (Glc₀⁻³Man₀⁻⁹GlcNAc₂), mimicking the defects produced in CDG Type I. Results of protein hypoglycosylation in the mutants, analyzed both by flow cytometry of a
fluorescent biosensor and by western blot, showed the relative involvement of each Glc and Man residue in the transfer efficiency by OST. The usefulness of yeast as model organism to study basic cell glycobiology processes will be discussed.

**TETRAD ANALYSIS WITHOUT TETRAD DISSECTION: MEIOTIC RECOMBINATION AND GENOMIC DIVERSITY IN KOMAGATAELLA PHAFFII (PICHIA PASTORIS)**

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*Komagataella phaffii* is one of the yeast species that were formerly known as *Pichia pastoris*, and is widely used in the pharmaceutical and biotechnology industries. However, almost all laboratory work on *K. phaffii* has been done on strains derived from a single natural isolate, CBS7435. There is little information about genetic diversity or the genetic properties of *K. phaffii*. Genetic analysis is difficult because, although *K. phaffii* makes asci with four spores, the asci are hard to dissect, and the spores are small and tend to clump together. Here, we analyzed the meiotic recombination landscape in a cross between auxotrophically marked strains derived from two natural isolates of *K. phaffii* that differ at 48,000 nucleotide sites. We conducted tetrad analysis by making use of the property that haploids of this species do not mate in rich media, which enabled us to isolate and sequence the four types of haploid cell that are present in the colony that forms when a tetratype ascus germinates. We found that approximately 25 crossovers occur per meiosis, four times fewer than in *Saccharomyces cerevisiae*. Recombination is suppressed, and natural genetic diversity is low, in a region around centromeres that is much larger than the centromeres themselves. Our method of tetrad analysis without tetrad dissection will be applicable to other species whose spores do not mate spontaneously after germination.

**MOLECULAR TYPING OF BRETTANOMYCES BREWING YEASTS USING RAM-PCR**

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Abstract ID: 364
Type: e-Poster, Session 6

The yeast *Brettanomyces* is often considered as a major contaminant in the wine and beer industries because of its production of phenolic off-flavors. Recently, few species of this genus, especially *B. bruxellensis* and *B. anomalus*, have been used in beers to enlarge the pool of aromatic compounds produced by the traditional *Saccharomyces cerevisiae* starter used for decades in breweries. The characterization of *Brettanomyces* species is therefore crucial to identify the strains showing interesting technological traits. However, to our knowledge, no method is currently available to evaluate the genetic diversity of the genus *Brettanomyces*. The aim of this study was to develop and optimize a new, rapid and reliable method based on the Random Amplification of Microsatellites (RAM)-PCR technique for the molecular typing of *Brettanomyces*.
brewing yeasts. Fourteen (14) PCR primers were tested for their discriminatory potential of the *Brettanomyces* species *B. bruxellensis* and *B. anomalus*. The six (6) primers that allowed a clear distinction between these two species were used during the optimization of the RAM-PCR reaction. A sample of ten (10) isolates, over the twenty-four (24) *Brettanomyces* isolated from beer, red wine, kombucha, sourdough and commercial starters were used during the optimization step. The best fingerprinting profiles were obtained when the annealing step performed at 33°C for the primers selected. The RAM-PCR method will be validated on the remaining fourteen (14) isolates, in order to propose the most efficient primer for the molecular typing of *Brettanomyces* species. This PCR-based typing method will then be useful for brewers and yeast suppliers for the rapid identification at the species and strain levels of various *Brettanomyces* isolates, without the need for sequencing. Moreover, the RAM-PCR method developed could allow further phylogenetic analysis useful for the prediction of the brewing potential of *Brettanomyces*.

**IMPROVEMENT OF YEAST PHENOTYPE OF INDUSTRIAL INTEREST THROUGH ADAPTIVE EVOLUTION**

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*S. cerevisiae* can go through different adverse conditions for its growth and development, conditioning the success of the process to which its function is associated. Currently there is an increasingly accentuated trend, in which consumers have increased their demand towards minimally processed products and without the use of additives to preserve their quality, which is why biocontrol has emerged as a replacement for chemical preservatives used in food processing. It has been detailed that the antagonism of yeasts on other microorganisms is mainly due to the secretion of antibacterial compounds such as killer toxins and the production of organic acids. Our laboratory has previously described the antimicrobial effect of *S. cerevisiae* LAMAP1039 against *E. coli* ATCC25922, *S. Typhimurium* ATCC14028 and *L. monocytogenes* ATCC13932. Given these antecedents, the use of adaptive evolution is suggested as a strategy to generate new genetic variants and, therefore, improved interest phenotypes. The objective of this work was to improve yeast phenotypes of industrial interest through adaptive evolution through the use of abiotic factors such as hydrogen peroxide (H₂O₂). For this, the antimicrobial capacity of the supernatant of an original culture of *S. cerevisiae* LAMAP1039 and those evolved at different concentrations of H₂O₂ against *S. Typhimurium*, *E. coli* and *L. monocytogenes* was evaluated, carrying out a plate count of the microorganisms. The results showed that subjecting the strain to 50 [mM] of H₂O₂ in the adaptation medium was sufficient to achieve inhibiting a logarithmic cycle the growth of the three pathogens tested, compared to the initial load of bacteria. In this way, the use of adaptive evolution through abiotic factors in the environment emerges as a strategy to improve phenotypes of industrial interest.
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ON BREWING YEASTS
The common brewer's yeast Saccharomyces cerevisiae is used in a broad range of industrial applications, from the production of beer, wine and bread to biofuels and pharmaceuticals. Interestingly, there are hundreds of different industrial yeast strains, but their origins and specific characteristics are largely unknown. We combined large-scale phenotyping with genome sequencing to track the genealogy and evolution of today's industrial yeasts. Using this knowledge allowed us to set up large-scale breeding programs to generate superior variants that increase production efficiency and expand the range of yeast-derived products and aroma's, allowing more efficient beer fermentation, production of superior beers and the creation of novel products.
BEER, BIOFUELS, AND BEYOND: YEAST BIODIVERSITY IN THE AGE OF GENOMICS

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Yeasts are diverse unicellular fungi with diverse applications. The genus *Saccharomyces* forms the backbone of the current fermented beverage and biofuels industries, yet its biogeographical and genetic diversity have only recently come into focus. Perhaps the most significant recent discovery was the identification of *Saccharomyces eubayanus* as the cold-tolerant parent of hybrid lager-brewing yeasts (*Saccharomyces cerevisiae* x *S. eubayanus*). Here we present an update on the genomic, phenotypic, and biogeographical diversity of *S. eubayanus* and begin to characterize the genetic basis of traits that this species contributed to lager yeasts. Specifically, we report candidate genes for cold tolerance and maltotriose utilization, two of the most important traits for lager brewing. We also discuss the development and application of a new technology for making higher order synthetic hybrids that include DNA from as many as six species. New discoveries of yeast biodiversity and new tools in yeast synthetic biology promise to accelerate innovation in both traditional and new industries.
The yeast *Saccharomyces cerevisiae* is best known for its use in wine, beer and bread-making. However, for millions of years, and long before man exploited this microbe for the fermentation of foods and beverages, *S. cerevisiae* thrived in natural environments in several regions of the world. Yet, the natural history of *S. cerevisiae* is so elusive and difficult to investigate that initially scientists thought that this yeast only existed in association with humans. However, like its closest relative *S. paradoxus*, *S. cerevisiae* can be found in natural systems little affected by human activities. How were these populations “captured” by humans? How do the “wild” populations differ from the domesticated ones? Was there a single or multiple domestication events? What were the transformations that *S. cerevisiae* endured during the domestication process? How can this new knowledge be used to improve and diversify the beers we make? This fascinating story of man’s closest microbe friend is now being unfolded, especially thanks to our ability to investigate the complete genome sequence of representatives of different populations. An overview of the main recent discoveries and of the most puzzling questions will be presented. This work was supported by Fundação para a Ciência e a Tecnologia (Portugal) grant UID/Multi/04378/2013
IMPORTANCE OF ZINC TO BREWING YEAST AND FERMENTATION

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The availability of key minerals in malt wort plays important roles in dictating brewing yeast fermentation performance. In particular, magnesium, calcium and zinc levels influence alcohol production, yeast stress tolerance and beer flavour. This presentation will focus on the effect of zinc on brewing yeast cells in terms of zinc uptake, fermentation performance and flavour congener formation. Experiments using malt wort with variable supplements of zinc salts have been conducted in both laboratory and pilot plant fermentors to reproduce lager beer fermentations. Zinc was taken up rapidly and completely from wort by yeast during fermentation. In addition, the zinc levels required for optimal fermentation ranged from 0.5 to 1.0 ppm. Zinc-deficient wort can lead to sluggish beer fermentations, but using pitching yeast that has been pre-enriched with zinc can benefit fermentation progress. Beer flavour congeners produced by yeast were affected only at high zinc levels (~10 ppm), resulting in elevated concentrations of higher alcohols and some esters (ethyl caproate and isoamyl acetate). It is concluded that brewing strategies to control wort zinc bioavailability, including Zn-supplementation (to both wort and yeast), are important in dictating brewing yeast fermentation performance and product quality.

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PHENOTYPIC HETEROGENEITY AND BREWING YEAST POPULATIONS

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Harvesting yeast at the end of a brewing fermentation and using it to re-inoculate a subsequent fermentation (serial repitching) is a common practice which is largely unique to the brewing industry. Despite its prevalence, this process can come at a cost; reusing yeast can negatively impact product and process consistency. In particular, fermentation time can vary considerably and acceptable completion times are often measured to the nearest day. In some cases, this variation is explained by differences in raw materials or yeast health, but the precise make-up of the yeast population is rarely considered to be an attributing factor.

In this paper we will discuss some of the simple methods recommended by the American Society of Brewing Chemists (ASBC) that can be implemented by brewers of all sizes to assess the quality of a yeast culture. Furthermore, using these tools we demonstrate that pitching yeast populations rarely comprise identical individuals. In this study we elucidate the degree of phenotypic heterogeneity by determining resistance to key stress factors via a series of physiological assays based on growth, appearance and cellular characteristics. The data presented indicates considerable variation within brewing yeast populations, the extent of which differs according to strain and stress factor. This work seeks to expand brewers understanding of the concept of 'yeast quality' and indicates that the 'fitness to ferment' potential of a culture should be defined at both the cellular and population level.
REUSE OF YEAST IS A COMMON BREWERY PRACTICE, BUT MAY EVENTUALLY LEAD TO MUTATION ACCUMULATION THAT CHANGES CHARACTERISTICS OF THE YEAST AND THE BEER, FOR POSITIVE OR NEGATIVE. WE USED WHOLE GENOME SEQUENCING TO FOLLOW THE GENETIC CHANGES THAT OCCURRED AFTER 29 SERIAL REPITCHES OF THE AMERICAN ALE STRAIN WY1056 BY POSTDOC BREWING. COMPARISON OF SAMPLES TAKEN ALONG THE TIME-COURSE SHOWED TWO MUTATIONS THAT BECAME DETECTABLE IN THE POPULATION AFTER 15 REPITCHES AND ROSE TO HIGH FREQUENCY BY THE LAST PITCH: AN INCREASE IN THE NUMBER OF COPIES OF CHROMOSOME V, AND A CHANGE IN THE ALLELIC REPRESENTATION ON ONE ARM OF CHROMOSOME VIII. WE HAVE BREWED BEER WITH THE ANCESTRAL STRAIN AND THE DERIVED MUTANTS, AND MEASURED DIFFERENCES BY MASS SPECTROMETRY AND SENSORY ANALYSIS. OUR RESULTS ALLOW US TO LOOK AT EVOLUTION IN THE BREWERY AT HIGH RESOLUTION, TO SELECT NEW STRAINS, AND TO MAKE RECOMMENDATIONS FOR THE PRACTICAL LIMITS OF SERIAL REPITCHING.
SACCHAROMYCES EUBAYANUS IN THE BREWING INDUSTRY

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Saccharomyces eubayanus was formally described in 2011 from forest samples near Bariloche in Patagonia, Argentina, and was demonstrated to be the missing parent of the Lager brewing yeast. Rapidly, the industry started to show interest in using this unique wild yeast for brewing purposes. First studies showed limited brewing features due to lack of assimilation of maltotriose, high fusel alcohol production, low flocculation and mostly due to the synthesis of phenolic compounds typical of wild yeasts. Clearly this yeast needed to be tamed before enabling the production of a commercially viable beer. Many failed in this quest and lost interest or invested in the development of genetically improved strains for example through rare-mating or hybridization techniques. In 2016 the first commercial brew at the industrial scale was lunched and in July of 2018 the first Craft beer brewed by Patagonian brewers was lunched under the project “Patagonia Salvaje”. This presentation summarizes the odyssey of a wild yeast into the beer industry and the journey of a group of young yeast scientists into the amazing world of brewing technology and science.
NON-TRADITIONAL BREWING YEAST AND FLAVOR PRODUCTION

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Never have brewers been so excited about yeast and its possibilities than now. The strain choices available in slants, liquid, or dry forms are endless. With the advances of DNA technology and quick screening methods, we are now able to know, in a relatively short time, a plethora of information about each strain; their impact on flavor, resistance to stress, fermentation behavior etc. Brewers have access to these tools to create unique beers for the educated craft beer consumers. Based on this information, brewers are now able to blend different strains, even strains from non-brewing applications, to achieve certain flavours or traits. Bio-flavoring with non-Saccharomyces strains is one way of added complexity to beer using co- or sequential fermentation with regular beer strains – something that has been done in wine for many years, but is still very discrete in brewing. Whereas non-Saccharomyces strains may contribute to flavors (even sometimes off-flavors), they are usually not good fermenters. While hybrids have been naturally occurring in nature - the best example is the lager yeast Saccharomyces pastorianus - breeding brewing strains to combine desired characteristics is relatively new. New possibilities of combining flavors, creating new/hybrid beer styles, is very much possible using this technology. Finally, GMO technology opens the door to even more opportunities... and all the debates! From functionality to flavor improvement, anything is possible. This presentation will take you through a journey of yeast and the flavors they produce.
DEMYSTIFYING ACTIVE DRY YEAST USE FOR BEER PRODUCTION

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Active Dry Yeast (ADY) is a widely known and used solution for beer production. The first Ale ADY was produced in 1990 and the first Lager ADY was successfully produced in 1998. Since then, apart from the continuous improvement of this technical solution, the use of ADY has been studied at International Research Institutes and Universities worldwide. At the start, people were skeptical regarding the usage of ADY. It is now widely recognized as a reliable technology of choice; offering great amount of flexibility and options related to technical and sensorial characteristics and applications; and applicable to the development of main stream brands or specialties. The purpose of the presentation is to “demystify” ADY and to highlight some of its recently identified properties with results provided by internationally recognized laboratories. The lecture will first explain what is active dry yeast, how it is produced, what are the quality standards, how it should be used and it will also highlight advantages of this product form. Secondly, it will highlight more specifically the impact of the implementation conditions of ADY on the performance and characteristics of ADY both in terms of performance and flavour components.
POLYGENIC ANALYSIS AND TARGETED IMPROVEMENT OF FLAVOR PRODUCTION IN BREWER’S YEAST

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Flavor production is arguably one of the most important characteristics of brewer's yeast for commercial beer brewing. The yeast *Saccharomyces cerevisiae* is able to produce a balanced profile of flavor compounds that is attractive in beer and other alcoholic beverages. Most traits of organisms are not determined by a single gene, but by a combination of interacting genes. This is also true for flavor production in yeast. Such traits are called polygenic or complex traits. Nowadays powerful technologies are available to identify all genes that are responsible for a polygenic trait as well as the mutations in these genes that are responsible for quantitative deviations in the trait. Polygenic analysis involves screening of a yeast strain collection for strains with a superior trait of interest compared to the industrial yeast strain that is targeted for improvement, generating haploid derivatives with a similar trait of interest as the most superior and the reference industrial strain, crossing the two haploid strains to form a hybrid diploid and generating haploid segregants from the hybrid diploid. The genomic DNA of a pool of about 30-40 superior haploid segregants is then extracted and completely sequenced, as well as the two parent haploid strains in order to identify all SNPs between the two strains. The SNPs are used as genetic markers in the mapping of the QTLs (Quantitative Trait Loci) harboring causative genes responsible for the difference in the trait of interest between the two parent strains. The identification of the causative gene(s) in the QTLs is then achieved by Reciprocal Hemizygosity Analysis and allele exchange between the parent strains. Finally, all (major) causative genes are then exchanged into the industrial target strain by genome editing using the CRISPR/Cas9 technology to improve the trait of interest. We will show two examples of such analysis in which we have identified novel genes involved in high rose flavor (phenylethyl acetate) and low solvent-like flavor (ethyl acetate) production, which allows specific improvement of these properties in brewing strains.
Over the last decades, the global beer industry has grown to reach a volume of 193 billion liters. Lager beer accounts for 89% of this volume, making it the most-produced fermented beverage. The microbial work horse of lager fermentation is Saccharomyces pastorianus, a natural hybrid of Saccharomyces cerevisiae and Saccharomyces eubayanus that has been domesticated in Europe since the late Middle Ages. Isolation and characterization of S. eubayanus provided a strong impetus for research on S. pastorianus lager brewing yeasts. Access to S. eubayanus strains stimulated vigorous research into de novo generation of hybrids between S. cerevisiae and S. eubayanus in the laboratory. This approach has the potential to increase our understanding of the domestication process of lager brewing strains and, moreover, to strongly increase the genetic and phenotypic variety of lager yeast strains available to the brewing industry. Recent advances in sequencing technology and genome editing techniques helped to get further insight in metabolism of brewing Saccharomyces species. In this paper, I will present some our recent researches aiming at understanding the origin of brewing relevant characteristics and improving them in lager yeasts.
INTERSPECIES YEAST HYBRIDS: BREWING ABILITY AND GENOME STABILITY

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Constructed lager yeast hybrids (S. cerevisiae x S. eubayanus) have the potential to increase greatly the phenotypic diversity within the lager yeast group, thereby helping to satisfy a growing demand for beers with novel sensorial properties. Here, different strategies for natural lager yeast design are presented. Firstly, parent choice has a direct influence on hybrid performance, with alcohol yield, flocculation and flavour profile being subject to change depending on the S. cerevisiae strain chosen. The majority of studies have focused on the S. eubayanus type strain as the second parent, and there is considerable potential for hybrid differentiation as a result of S. eubayanus strain type. We show also that the lager yeast phenotype can be created with alternative hybrid combinations, with psychrophilic species such as S. mikatae successfully acting as substitutes for S. eubayanus. Mating strategies determine the ploidy of the resultant hybrids. Generally, higher ploidy has been associated with higher fermentation rate and yield. Tetraploid strains have the added advantage that they can sporulate, introducing diversity through meiosis and allowing for the selection of spore clones with beneficial properties such as the absence of POF character. We show also that de novo hybrids are amenable to change via adaptive evolution. Exposure to high ethanol levels (10% v/v) resulted in variants with faster fermentation rates, increased ethanol tolerance and no negative impact on flavour volatiles. Adaptation was associated generally with loss of S. eubayanus chromosomes and gain in S. cerevisiae chromosomes. Non-synonymous mutations in certain genes (IRA2, HSP150 and MMN4) arose in independent cultures and for IRA2 in both subgenomes, suggesting a role in adaptation. The recent proliferation in publically available genome sequences for industrial yeasts offers an additional strategy for strain design. We show how hybrids with desired characteristics may be created through in silico screening of genomes of potential parents. We demonstrate this approach with a naturally occurring kveik yeast hybrid (S. cerevisiae x S. uvarum).
SACCHAROMYCES AND NON-SACCHAROMYCES BREWING STRAINS - CHARACTERIZATION APPROACHES FOR THE BREWER

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The volume and market share loss for classical beer types such as pils beer and has been declining for several years but the overall beer market remains almost unchanged as a result of the increasing interest in beer specialties. Due to the high biodiversity, the diversity of the strains and the different flavor profiles, reliable and practical information regarding the characteristics of individual brewing strains is required to help brewers to find the right strain for their brewing purposes. This paper presents a comparison of available TUM (Technical University of Munich) brewing yeast strains. The strains were screened for genetic and phenotypic characteristics. After confirming the genetic distinctiveness by using species specific real-time polymerase chain reaction systems (RT-PCR systems) and strain typing methods based on a PCR-capillary electrophoresis (e.g. IGS2-314 rDNA PCR for *Saccharomyces cerevisiae* and *pastorianus* strain typing and GTG₅-PCR for Non-*Saccharomyces* species strain typing), the strains were tested regarding phenotypic characteristics under controlled and identical fermentation conditions in standardized small-scale brewing trials. Besides the fermentation performance, flocculation behavior, sugar metabolism and other phenotypic characteristics, the main focus is on the flavor and aroma profile of each investigated TUM yeast strain. The results of this study enable brewers an easy selection of the appropriate brewing yeast strain for a distinct specialty beer and the appropriate flavor combination with special hop varieties and malt types.
"BEYOND BEER" - FERMENTATION IN HIGH-END GASTRONOMY AND HOW TO HANDLE AND CONTROL MULTIPLE MICROBES IN A NON-LAB ENVIRONMENT

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The use of yeasts and other microbes in food production goes back millennia but in recent years some of the most highly acclaimed restaurants have re-discovered and expanded on fermentation techniques as part of their culinary profile. White Labs is a known producer of microbial starter cultures for fermented alcoholic beverages since more than 23 years. Its subsidiary, White Labs Copenhagen, has in collaboration with top chefs (former restaurant noma, restaurant Amass/Broaden and Build) and the talk-of-the-town distillery Empirical Spirits, broken ground for a 1,400 m2 multi-fermentation space in Copenhagen, Denmark, to open early 2019. The facility will be housing a brewery, a distillery, as well as a restaurant, all connected to the heart of the space - a fermentation lab and starter culture manufacturing facility, supplying each of the culinary artists with cultures for their dishes, beers or spirits. This lab will literally break down the walls of conventional food microbiology and embrace the diversity of the microbes used in food and beverage production. Focusing on hands-on techniques and detection of food safety risks, the approach is a model for any restaurant to roll out a safe and diverse fermentation program with minimal laboratory infrastructure. This talk will present a full circle approach to sustainable food and beverage production through fermentation. It will show a project and space built to inspire anyone to re-discover fermentation as a tool for flavor creation, up-cycling of waste streams, as well as environmentally conscious enjoyment of food and beverages.