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POSTER ABSTRACT BOOK

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Genomics and Hybrids

P1

Saccharomyces Hybrids: Generation and Analysis

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Saccharomyces interspecific hybrids, both artificial and natural, have significant potential in industry. This is because they can inherit and combine beneficial traits from each parental species. Breeding between species allows for mixing of the gene pool and under selective pressure, gives rise to individuals with beneficial gene combinations. However most hybrids are seen as evolutionary dead-ends. This is because they are usually sexually sterile, inhibiting the potential for strain improvement through breeding.

The *Saccharomyces* sensu stricto clade consists of seven closely related species, many of which have been domesticated through use in various processes such as baking, biofuel production, wine making and brewing. In addition to the seven species, hybrids are frequently isolated and have been used in some fermentation processes. These include *S. pastorianus*, the producer of lager beer, which is a hybrid between *S. cerevisiae* and *S. eubayanus*; and *S. cerevisiae* x *S. kudriavzevii* hybrids which are used in some wine fermentations.

In nature, particularly in plant species such as wheat, hybrid sterility has been overcome through genome duplication and higher ploidy. Here we have utilised this principle to generate tetraploid *Saccharomyces* hybrids, which give rise to viable offspring. As a result of putting these hybrids through multiple rounds of mating we have generated large populations with vast diversity. Using our advanced quantitative genetic approaches we can identify the genetic variants contributing to phenotypes of interest. Knowledge of these variants will allow us to produce genetically improved strains beneficial to various industrial processes.

P2

Intraspecific hybridization to select wine yeasts low-H_2S producers

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In winemaking, the yeast strain selection, carried out within strains belonging to the species *Saccharomyces cerevisiae*, is a fundamental step to identify innovative and important oenological traits to be introduced . In this context, the production of organic wines requires starter strains low-H_2S producer. The availability of traditional genetic techniques, such as intraspecific hybridization, easily provide the construction of new yeast strains with the specific characteristics required by the fermentation industry.

In this study, 20 novel intraspecific yeast hybrids were obtained, resulting from two parental strains, both chosen on the basis of distinctive phenotypic characteristics: the first is an aromatic selected native strain, the second is low-H_2S producer. The genetic analysis of all hybrids revealed great genotypic variability. Within all, the new strains *G4* and *I4*, were selected and tested for the expression of key genes, such as *MET5* and *MET10*, involved in the metabolic pathway for H_2S production. Moreover, pure and mix fermentations with *Metschnikowia pulcherrima* were carried, and compared with a commercial starter strain low-H_2S producer. The fermentations were carried out in pilot scale and the kinetic growth, the principal enological characters and the aromatic compounds were evaluated. Preliminary results, showed that both hybrids in pure and in mix fermentation exhibited satisfactory fermentation performance. In particular, the new strains showed reduced sulfur dioxide and increased aromatic compounds production, suggesting than a possible use as improved native starter strains.

P3

Customized lager yeast strains created through a fertile allotetraploid intermediate

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Interspecific hybridization has proven to be a potentially valuable technique for generating de novo lager yeast strains that possess diverse and improved traits compared to their parent strains. To further enhance the value of hybridization for strain development, it is desirable to combine phenotypic traits from more than two parent strains, as well as remove unwanted traits from hybrids. One such trait, which has limited the industrial use of de novo lager yeast hybrids, is their inherent tendency to produce phenolic off-flavours; an undesirable characteristic inherited from the Saccharomyces eubayanus parent. Trait removal and the addition of traits from a third strain could be achieved through sporulation and meiotic recombination or further mating. However, interspecies hybrids tend to be sterile, which prevents this approach. Here we generated a set of five hybrids from three different parent strains, two of which contained DNA from all three parent strains. These hybrids were constructed with fertile allotetraploid intermediates, which were capable of efficient sporulation. The ability to produce phenolic off-flavours was successfully removed from one of the hybrids, Hybrid T2, through meiotic segregation. The potential application of these strains in industrial fermentations was demonstrated in wort fermentations, which revealed that the meiotic segregant Hybrid T2 not only didn't produce any phenolic off-flavours, but also reached the highest ethanol concentration and consumed the most maltotriose.

P4

Predicting gene expression levels from transcription factor binding events through machine learning

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Binding of transcription factors (TFs) to gene promoter regions is one of the main drivers of gene regulation, but the exact mechanism and effects are not well understood. As regulation of gene expression is one of the major determinants of cellular phenotype a deeper knowledge of gene regulation is important for understanding cellular behavior and improved metabolic engineering strategies. Many TFs are only active in certain conditions and some TF binding events show no apparent effect on gene expression, making the effects difficult to decipher and to understand. Using the recently developed ChIP-exo method one can identify the binding sites and strengths for a TF with a higher resolution and with less background than previously possible. Machine learning approaches have been used in many applications to decipher patterns in large datasets, like speech recognition or predicting cancer treatment outcomes. The large datasets generated by ChIP-exo could also provide a strong basis for a machine learning model linking TF binding events to RNA expression levels. The successful construction of an expression-predictor should then provide valuable insight into the complex interplay of different TFs binding to the same promoter. In our project we collected ChIP-exo data from Saccharomyces cerevisiae for a number of different TFs in four different metabolic conditions. Using the machine learning method Random Forests we are generating a gene expression predictor that can then be used to reliably predict expression levels for altered promoters in metabolic engineering projects.

P5

Misassembly detection and improvement of a lager beer yeast genome draft using BAC-end sequencing and physical mapping

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Next-generation sequencing technologies have allowed genome sequence acquisition of many strains of *Saccharomyces pastorianus*, the main microorganism used in the fermentation of lager beer. Nevertheless, most of the publicly available genome assemblies of this species are reported as draft. This could be due to the difficulty to assembly the whole genome from the short reads obtained with these technologies, and the complexity of the yeast genome. *S. pastorianus* is a hybrid between *Saccharomyces cerevisiae* and *Saccharomyces eubayanus*, the above means that it possesses copies of both parental genomes. Recently, a draft genome sequence of a lager beer yeast was reported; however, no efforts to asses and improve the quality of the genome assembly were made. We challenged this genome assembly with two types of experimental evidence: the BAC-end sequences and the physical map of a BAC-based genomic library of the same yeast. This allowed us to detect misassemblies, to determinate the order of the remaining scaffolds and to identify potential sequences overlaps between them. Many of the genome sequence scaffolds were consensus of different parental homologous chromosomes regions. Also, we were able to identify homologous translocation between different parental type chromosomes. These translocations together with the different chromosome copy number may play a confounding role during the assembly process. The sequence cloning strategy was advantageous compared to the whole genome shotgun assembly because it allowed us to obtain information from individual DNA molecules and to selected BAC clones as templates for the gap closing of the previous determined scaffolds order.

P6

Exploring the potential of Saccharomyces eubayanus as a parent for new hybrid strains for cider and wine production

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Yeast cryotolerance is advantageous for the production of alcoholic beverages. Cider is usually fermented at low temperatures, and these conditions improve the aromatic complexity of white wine. However, it is necessary a yeast strain that retains metabolic activity at low temperatures. Here we crossed the cryotolerant *S. eubayanus* with a *S. cerevisiae* wine strain and assessed the suitability of the hybrids for low-temperature cider and wine fermentation.
Marked phenotypic differences were observed among the hybrid strains generated. All strains outperformed *S. cerevisiae* in cider fermentations, and the best one performed similarly to *S. eubayanus* with the advantage of eliminating unpleasant sulphurous volatiles, a characteristic of *S. eubayanus* fermentations.
Grape must presented a more challenging environment due to higher sugar concentrations. Nevertheless, one hybrid that exhibited the highest potential for wine fermentation was dried and tested for fermentation of Sauvignon blanc and Macabeau musts. We obtained highly viable active dry yeast, which was able to efficiently ferment the grape musts with the advantage of producing higher glycerol content than the *S. cerevisiae* parent and up to 0.5% less ethanol. An expert panel rated the wines as complex and exotic, showing that this approach can be employed for reducing alcohol content in wines and to increase the aromatic diversity. The genome sequences of the hybrid strains revealed a high level of variation among hybrids, particularly in the inheritance of S. cerevisiae chromosomes. *S. eubayanus* proved to have potential as parent strain to improve low-temperature fermentation performance without compromising product quality.

P7

Saccharomyces bayanus Saccardo is the intraspecies hybrid in accordance with genetic hybridization analysis

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Different word laboratories are currently involved in large-scale project on origin of European larger yeasts *Saccharomyces pastorianus* Hansen (syn. *S. carlsbergensis* Hansen). According to numerous molecular data, *S. pastorianus* is an allopolyploid containing genomes of the traditional yeast *S. cerevisiae* Meyen ex Hansen and recently discovered *S. eubayanus* Sampaio et al. In nature, *S. eubayanus* is documented in Argentina, North America, and China, however, the yeast has not been found in Europe yet. Great attention is also paid to the origin of recombinant yeasts *S. bayanus* var. *bayanus* isolated from human fermentations. They contain partial genomes of *S. eubayanus* and *S. bayanus* var. *uvarum* (Hansen) G. Naumov, and some *S. cerevisiae* subtelomeric sequences. The yeast *S. bayanus* var. *uvarum* is associated with certain types of wines: Val de Loire's white, Sauternes, Alsatian and Cider (France), Tokaj (Hungary, Slovakia), Amarone (Italy), Txakoli (North Spain) and others. In order to assess the degree of genetic relatedness of *S. eubayanus*, *S. bayanus* var. *bayanus* and *S. bayanus* var. *uvarum*, we have conducted genetic hybridization analysis of these taxa. Based on ascospore viability and meiotic recombination of control parental markers of the hybrids, we have found that there is no complete interspecies post-zygotic isolation between the yeasts *S. eubayanus*, *S. bayanus* var. *bayanus* and *S. bayanus* var. *uvarum.* The genetic data obtained prove the belonging of the all three taxa to the same species. Taxonomic status of the yeast *S. eubayanus* is discussed.

P8

Genome-scale metabolic model of the oleaginous yeast *Rhodosporidium toruloides*

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Rhodosporidium toruloides is basidiomycetous oleaginous yeast that can naturally accumulate lipids to more than 50% of their cell mass. *R. toruloides* is resistant to inhibitors in lignocellulose hydrolysate, can convert xylose to lipids and form carotenoids as a value-added byproduct. *R. toruloides* is also the best known producer of lipids from glycerol feedstocks. This makes *R. toruloides* a promising organism for conversion of inedible substrates into lipids for both biofuels and food applications.

Generation of a genome scale metabolic model (GEM) of *R. toruloides* would provide a deeper understanding of oleaginicity and facilitate the design of genetic engineering strategies. We have used the RAVEN toolbox to create a first draft GEM of *R. toruloides* based on protein homology to *Saccharomyces cerevisiae* model yeast 7.6 and to *Yarrowia lipolitica* model iYali4.01. Unlike *S. cerevisiae*, *R. toruloides* contains a gene encoding ATP:citrate lyase, which is the main source of acetyl-CoA for lipid synthesis. In addition, a mitochondrial beta-oxidation pathway provides additional source of acetyl-CoA in this yeast. Unlike *Y. lipolytica*, *R. toruloides* expresses a cytoplasmic malic enzyme that provides an alternative to NADPH generation through the pentose-phosphate pathway.

The model was used to simulate lipid production in *R. toruloides* on both glucose and xylose substrates. The reaction of phosphorolytic cleavage of xylulose-5-P by phosphoketolase with generation of acetyl phosphate and glyceraldehyde-3-phosphate was shown to enable efficient conversion of xylose into lipids. Proteome data will be integrated with model to understand regulation of lipid metabolism in the yeast *R. toruloides*.

P9

Comparing the physiology of *Kluyveromyces marxianus* under bioreactor conditions

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The yeast *Kluyveromyces marxianus* exhibits valuable features for industrial applications. Despite the increasing interest in this organism for biotechnological applications, little is known about its physiology and genetics, in part becauselarge physiological variability among *K. marxianus* strains.We are investigating the behaviour of two *K. marxianus* strains, CBS 397 and CBS 6556, during growth in bioreactors, which allowsaccuratecontrol and monitoring of growth paramaters.These strains display significant difference in growth rates, biomass yield and production of ethanol. This may be due to differences in the capacity of strains to transport sugars and work in our group already established that genetic variation in lac permease genes accoungs for different efficiencies of lactose uptake. It is also interesting to note that galactose appears to be transported via different transporters in these two strains.Now we are studying a family of putative hexose transporters to explore whether differences in expression or function of these proteins can explain variation that we see during growth at different concentations of glucose and galactose. These transporters are homologous to the known glucose transporters, Rag1p and Hgt1p, but expression patterns suggest alternative functions in some cases. The function of these proteins can also be studied by heterologous expression or construction of targeted mutants and our latest findings in this area will be reported. Increading our understanding of the mechanisms that underly variability will enhance our capacity to exploit multiple strains for cell factory applications.

P10

The mt-DNA inheritance affects crucial industrial traits both for inter and intraspecific *Saccharomyces* hybrids

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Inter and intra specific hybridization within the *Saccharomyces* genus is a routine way for improving traits of industrial interest. After mating, the fusion of parental mitochondria creates a single mitochondrial organelle in the zygote that allows the rapid mixing of mt-DNAs (mitochondrial genome). After several mitotic divisions, the daughter cells tend to conserve only one parental mt-DNA inheritance (homoplasmy). Stable recombined mt-DNA can be also isolated. This phenomenon offers the possibility to study the effect of mt-DNA inheritance in a nuclear isogenic context. Here, we explored the effect of mt-DNA inheritance at the intra and interspecific level in both fermentation and respiration conditions. Firstly, we demonstrated a strong effect of mt-DNA inheritance in various synthetic hybrids *S. cerevisiae* x *S. uvarum*. By comparing isogenic strains for several phenotypes – alcoholic fermentation, respiratory growth rate, oxygen consumption assay, cytochrome content and dehydration tolerance –, we clearly demonstrated that mt-DNA of *S. uvarum* was less adapted than *S. cerevisiae* one for respiration as well as dehydration tolerance.

In a second time, we investigated the phenotypic impact of recombined mt-DNA molecules in *S. cerevisiae F1*-hybrids. By constructing 60 isogenic hybrids, we found that recombined mt-DNA genomes are frequently found (19/60) and may generates *rho+*, stable strains. The phenotypic impact of the both parental and recombined mt-DNA was measured for fermentation and respirations traits. Surprisingly, we found that the mt-DNA inheritance could also affect drastically the fermentation performances (acetic acid production and fermentation rate) and strongly interacts with the agitation level of the culture.

P11

Comparative genomics of industrial wine yeast strains.

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Saccharomyces cerevisiae has been used to obtain different fermentation products such as wine, beer, ethanol and bread, for several hundred years. This domestication reshaped the genomic structure of yeast strains by selecting several genetic variations leading to its phenotypic specialization. In this work, we sequenced 59 genomes of wine related *S. cerevisiae* using (Illumina-PE, 300bp, average coverage=30x). We built a SNP-calling pipeline on the local galaxy server by using two variant calling softwares (Samtools & Freebayes) allowing the detection of reliable SNPs (>170000). A SNP based phylogenetic analysis structured the wine yeast population in different subgroups. In comparison to beer strains, most of the wine strains form a distinct clade confirming the population structure of *S. cerevisiae*. A gene ontology analysis of SNP variants falling in coding sequences showed that most of them are functionally associated with stress related processes. This suggests that genetic variation in coding genes might be helpful for yeasts in their adaptation. In a second time, we validated the efficiency of our pipeline for detecting heterozygous loci using synthetic F1-hybrids and their relative parental strains. Within a set of 35 commercial starters, we detected an uneven loss and gain pattern of heterozygosity. Some strains were found to be highly heterozygous, while other are fully homozygous with very few heterozygous loci. Interestingly, the integration/omission of heterozygous SNP shaped the population structure of some strains revealing the difficulty to resolve the position of intraspecific F1-hybrids by basic phylogenetic analyses.

P12

Evolutionary restoration of fertility in an interspecies hybrid yeast, by whole-genome duplication after a failed mating-type switch

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Many interspecies hybrids have been discovered in yeasts, but most of these hybrids are asexual and can only replicate mitotically. Whole-genome duplication has been proposed as a mechanism by which interspecies hybrids can regain fertility, restoring their ability to perform meiosis and sporulate. Here, we show that this process occurred naturally during the evolution of *Zygosaccharomyces parabailii*, an interspecies hybrid that was formed by mating between two parents that differed by 7% in genome sequence and by many interchromosomal rearrangements. Surprisingly, *Z. parabailii* has a full sexual cycle and is genetically haploid. It goes through mating-type switching and auto-diploidization, followed by immediate sporulation. We identified the key evolutionary event that enabled *Z. parabailii* to regain fertility, which was breakage of one of the two homeologous copies of the mating-type (MAT) locus in the hybrid, resulting in a chromosomal rearrangement and irreparable damage to one MAT locus. This rearrangement was caused by HO endonuclease, which normally functions in mating-type switching. With one copy of MAT inactivated, the interspecies hybrid now behaves as a haploid. Our results provide the first demonstration that MAT locus damage is a naturally occurring evolutionary mechanism for whole-genome duplication and restoration of fertility to interspecies hybrids. The events that occurred in *Z. parabailii* strongly resemble those postulated to have occurred to cause ancient whole-genome duplication in an ancestor of *Saccharomyces cerevisiae*.

P13

Exploiting Diversity and Biotechnological Uses of Hybrids

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Hybrids among yeast species and strains are common and are known to be important in many industrial situations including various fermentations used to produce alcoholic beverages. Interspecies hybridization does not only increase the genetic variations in natural yeast populations, it is also an important mechanism for the origin of novel lineages and adaptation to new environments. However, hybrids are difficult to work with, since they are sterile - precluding any genetic analysis and removing the ability to use breeding for strain improvement. In this study, we aim to overcome the hybrid sterility by generating allotetraploid (4n) strains, which can undergo meiosis and produce viable diploid (2n) hybrid spores. We created novel types of hybrids containing different mitochondria to be tested for traits of industrial interests. Specifically, we created interspecific hybrids using a cryotolerant (*S. kudriavzevii*) and a thermotolerant (*S. cerevisiae*) yeast strains. Moreover, we also used the newly discovered *Saccharomyces sensu stricto* species '*S. jurei*' to create interspecific hybrids with *S. cerevisiae*. We showed that upon meiosis the spore viability of the engineered *S. cerevisiae*/*S. jurei* tetraploids was 80-90% and of *S. cerevisiae*/*S. kudriavzevii* tetraploids was 80%. The fitness of the progeny with randomly assorted traits is tested in different environments and in the presence of different stressors to select the best performing hybrids. Ultimately, the genome of improved strains will be sequenced and QTLs underpinning specific traits will be uncovered.

P14

A novel pathway for the endocytic regulation of Jen1 lactate transporter of Saccharomyces cerevisiae

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Eukaryotic cells internalize and turnover or recycle their plasma membrane proteins in response to physiological or stress signals imposed by a changing environment. The α-arrestins connect environmental signaling pathways to the endocytosis of specific plasma membrane transporters or receptors. The Saccharomyces cerevisiae lactate transporter Jen1p has been used as a model membrane cargo for elucidating aspects of the mechanisms that control the endocytic turnover of specific transporters in response to the presence of glucose. Here, we discover a novel pathway of Jen1p endocytosis and provide further insights on how particular α-arrestins mediate the ubiquitylation of transporters under distinct physiological conditions.

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P15

Phenotypic microarray: a high-throughput screening tool for evaluation of novel hybrid yeast strains for desirable biotechnological traits.

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Advances to molecular biology and breeding techniques have enabled the capacity to create extremely large libraries of hybrid yeasts from existing species. As such, screening novel yeasts for fermentation-specific phenotypes can present a technical problem, as many traditional assays are simply not viable for analysis of large numbers of strains. Therefore high throughput screening methods are required. Here we present the use of a phenotypic microarray (PM) as a high-throughput screening tool for evaluation of hybrid yeasts. The PM effectively conducts 'micro-fermentations' (ca. 100µL) in 96-well plates and can simultaneously run up to 50 plates (4800 fermentations) at once.

In this study the metabolic response of hybrid yeast strains was measured against key performance indicators established from analysis of a commercial brewing strain, including utilisation of sugars and stress resistance. Tolerance to stress factors is an essential characteristic in industrial strains and is particularly important in brewing since the production of many beers is often performed using higher gravity (HG) fermentations with elevated osmotic pressures. Similarly, production of biofuels (along with other fermentation-based biotechnology processes) requires operation at high gravity. Improvements in stress tolerance can also allow yeast cultures to be re-used extensively, reducing costs. Finally, the system allows novel phenotypes to be investigated including the ability of hybrid yeast strains to utilise pentose sugars which can be desirable within a variety of applications. The application of this technology to hybrid yeast breeding projects may lead to the identification of strains with potential for use at an industrial scale.

P16

Enhancement of protein production via the strong *DIT1* terminator and two RNA-binding proteins in *Saccharomyces cerevisiae*

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Post-transcriptional upregulation is an effective way to increase the expression of transgenes and thus maximize the yields of target chemicals from metabolically engineered organisms. Refractory elements in the 3′ untranslated region (UTR) that increase mRNA half-life might be available. In *Saccharomyces cerevisiae*, several terminator regions have shown activity in increasing the production of proteins by upstream coding genes (1); among these terminators the *DIT1* terminator has the highest activity (2). Here, we found in *Saccharomyces cerevisiae* that two resident *trans*-acting RNA-binding proteins (Nab6p and Pap1p) enhance the activity of the *DIT1* terminator through the *cis* element GUUCG/U within the 3′-UTR (3). These two RNA-binding proteins could upregulate a battery of cell-wall-related genes. Mutagenesis of the *DIT1* terminator improved its activity by a maximum of 500% of that of the standard *PGK1* terminator. Further understanding and improvement of this system will facilitate inexpensive and stable production of complicated organism-derived drugs worldwide.

(1) Yamanishi et al. (2013) *ACS Synth Biol*, **2**, 337-347; (2) Ito et al. (2013) *J Biotechnol*, **168**, 486-492; (3) Ito et al. (2016) *Sci Rep*, **6**, 36997.

P17

Finding homes for orphan genes in yeast.

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The significant number of genome sequencing efforts within the budding yeasts (phylum *Ascomycota*, sub-phylum *Saccharomycotina*) is a reflection of the importance of these organisms in numerous aspects of human society ranging from opportunistic fungal infections to biotechnological applications and the production of alcoholic beverages. The number of yeast species with sequenced genomes is approaching complete coverage at the genus level. However, the wealth of yeast genome data has lead to an accumulation of hypothetical genes without assigned functions – so-called "orphan genes". There is currently no sustained effort at systematic identification of orphan gene function from yeast genome data. One promising approach to assigning functions to orphan genes is through integration of genomic data with growth phenotypes for larger set of yeast species and look for correlations between the presence or absence of a particular orphan gene and a specific phenotype. This approach was used to identify genes required for the utilization of cyclic imides as a nitrogen source. The ability to utilize circular imides like succinimide and glutarimide as nitrogen sources is relatively rare among budding yeasts. The genomes of three species capable of utilizing cyclic imides as nitrogen sources were compared to six species unable to utilize cyclic imides. The survey revealed a hypothetical gene that was found exclusively in cyclic imide-utilizing species. The gene was named *CIH1* for putative cyclic imide hydrolase 1. Heterologous expression of the *CIH1* gene in *Saccharomyces cerevisiae* enabled this yeast to use both succinimide and glutarimide as its only source of nitrogen.

P18

Saccharomyces cerevisiae x *Saccharomyces bayanus* wine hybrids

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Direct mating is one of the methods used to improve wine yeasts. Interspecies hybrids between two *Saccharomyces cerevisiae* strains and a *Saccharomyces bayanus* strain have been obtained, stabilized and then analysed using molecular tools. Moreover some phenotype traits are been evaluated. First, a preliminarly study of parental strains is been performed. An evaluation of the best sporulation media and the subsequent tetrad analysis is carried out to characterize the phenotypic diversity between them. Hybrids are been obtained using direct mating, stabilized and then confirmed with RAPD and RFPL analyses. Hybrids are been also characterized and inoculated in synthetic must. We analysed the fermentative behavior and using HPLC the fermentation catabolites. Using HS-SPME-GC-MS, a comparison between volatile profile of parental strains and hybrids is been made. All the hybrids obtained shown different molecular profiles with the parental strains and they have some peculiar phenotypes. One of the major interesting traits is the incapacity to produce H_2S, some of the hybrids obtained have this characteristic even if they lose the capacity to grow in glycerol medium, so they are respiratory deficient strain, and for this reason are not useful for wine industry. Direct mating remains one of the most suitable way to obtain no-OGM hybrids.

P19

Transcriptomic response to temperature stress during mixed yeasts fermentation

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Mixed inoculum fermentations are currently being studied for improvement/modification of aroma profiles of fermented beverages, however, little is known about the effect of yeast-yeast interactions concerning temperature tolerance. For such purpose, we set-up a minifermentation membrane-based system to study the effect of the mixed culture with mezcal strains *Saccharomyces cerevisiae* LCBG-Sc3Y8 (Sc3Y8) and *Kluyveromyces marxianus* LCBG-Km1Y9 (Km1Y9) on temperature response by the *S. cerevisiae* strain, fermenting a grape-type semi-synthetic medium (M3) at 30 and 40 °C. As indicated by its transcriptome at 48 h of culture, using a probability greater than 2 (z-score), the repressed (485 y 301) and expressed (305 y 485) genes were identified at 30 °C and 40 °C, respectively. These groups of genes were plotted through Venn diagrams and 81 widely regulated transcripts were selected to further analysis. In general, all the regulated genes can be classified according to their function as involved in the re-arrangement of membranes and cytoskeleton, stress response, sugars utilisation, cell division, nucleic acid metabolism, mitochondrial activity, and regulation of amino acids and proteins. A heat map considering the expression of these 81 genes grouped in these selected functions showed that these genes are very active in the pure cultures at 30 °C but decay in the mixed culture at 30°C and in the pure cultures of Sc3Y8 at 40°C. However, in the mixed culture at 40 °C, the strain recovers the same expression profile (highly active genes) suggesting a positive effect of the presence of Km1Y9 yeast at 40 °C.

P20

Exploiting budding yeast natural variation in non-coding regions for industrial processes

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Differences in transcript abundance are frequently proposed as a source of phenotypic diversity between individuals, however, until now, little molecular evidence has been provided. Previously, we examined Allele Specific Expression (ASE) in F1 hybrids and found a wide set of genes exhibiting differences in transcript abundance. Part of this variation in expression levels could be explained by differences in transcription factors binding to polymorphic cis-regulations and to differences in trans-activation depending on the allelic form of the TF. Analysis on highly expressed alleles on each background suggested GPD1 and ADH3 as candidate transcripts underlying phenotypes of industrial interest between different genetic backgrounds, such as: sugar consumption together with ethanol and glycerol production. To estimate allelic transcript abundance and protein levels under different genetic backgrounds we performed opto-genetic and reporter allele fusions analysis under small and medium-scale fermentation conditions. Differences at different levels were found, where some alleles produced high ethanol levels while others high glycerol levels and confirming that coding and non-coding regions explained the observed phenotypic differences. Together, we provide a set of allelic variants which can be useful for a series of industrial processes.

P21

The growth and adaptation of yeasts *Kluyveromyces marxianus* in the presence of acetate.

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The metabolically active cells produce a wide array of metabolites that can inhibit their growth. The acetate is a widely-known preservative, and it is also produced by the yeast cells during their growth. *Kluyveromyces marxianus* is a promising yeast strain that could be employed in the biotechnological processes, but the knowledge of its stress physiology is scarce. During the poorly aerated fermentations with *K. marxianus*, an accumulation of acetate coinciding with a reduction in growth rate. Here, we investigate the acetate tolerance of *K.marxianus* and the possible mechanism of adaptation.
It was observed that acetate inhibits growth in a pH dependent manner and has pronounced effects if yeast is grown on lactose or galactose. *K.marxianus DSM 5422* populations are heterogeneous regarding tolerance to acetate. Acetate resistance is maintained only if acetate is present in the media or if the culture has reached end of active growth phase. In contrary to non-adapted cells, the acetate tolerant cells can simultaneously metabolize lactose and acetic acid. Also, it was found that the intracellular pH value in the tolerant yeast cells is by 0.4 lower than in non-adapted cells. The negative effect of acetic acid varies in different *K. marxianus* strains when lactose is used as carbon source. The correlation between β-galactosidase location and acetate tolerance was studied in the five *K. marxianus* strains.

P22

ROLE OF TRANSCRIPTION ACTIVATOR CAT8 IN REGULATION OF XYLOSE ALCOHOLIC FERMENTATION IN THE NON-CONVENTIONAL YEASTS *Ogataea (Hansenula) polymorpha* and *Scheffersomyces (Pichia) stipitis*

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Cat8 transcription activator is involved in regulation of gluconeogenesis, respiration and catabolism of alternative carbon sources in *Saccharomyces cerevisiae*. Cat8 exerts transcriptional activation of its target genes by binding to carbon source-responsive elements in their regulatory promoters. It was shown that knock out of *CAT8* activated glucose alcoholic fermentation in *S. cerevisiae* and non-conventional yeast *Pichia guilliermondii*.

We have shown that the deletion of the *CAT8* gene contributes to improved xylose alcoholic fermentation of *Ogataea (Hansenula) polymorpha* in the wild-type strain and the advanced ethanol producer form xylose. The *cat8Δ* strains isolated from the best available ethanol producer of *O. polymorpha* accumulated up to 12.5 g of ethanol/L at 45°C after 3 day of xylose fermentation, which is the highest ethanol titer for high-temperature xylose fermentation. Inversely, strain of *O. polymorpha* with overexpression of *CAT8* accumulated less ethanol from xylose relative to the parental wild-type strain. Deletion or overexpression of *CAT8* did not have effects on glucose alcoholic fermentation. We also aimed to construct strains with deletion of *CAT8* gene on the background of xylose-fermenting yeast *Scheffersomyces (Pichia) stipites* and to study the effects of such genetic modification on xylose and glucose alcoholic fermentation. Deletion *cat8Δ* mutants have been isolated and the characteristics of growth and xylose and glucose alcoholic fermentation were analyzed. Perspectives of the manipulation with transcription factors for construction of the advanced ethanol producers from lignocellulosic feedstocks will be discussed.

P23

NETWORK of TRANSCRIPTIONAL REGULATORS of BIOETHANOL STRESS RESPONSE in *SACCHAROMYCES CEREVISIAE*.

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S*accharomyces cerevisiae* is a major microorganism used for bioethanol production in which glucose as a carbon source is assimilated to produce ethanol. Some by-products present during fermentation are potent inhibitors, causing growth arrest and reducing ethanol productivity of cells. Here, we focus on the involvement of a sub-family of transcriptional regulators called zinc cluster proteins in regulation of genes involved in stress tolerance, particularly to high ethanol stress and in the presence of inhibitors during bioethanol production. The interplay among a less-known regulator Znf1 and well-known transcriptional regulators of stress responses was examined via bioinformatics approach to uncover hidden interactions among these regulators. We found that many stress-responsive genes are co-regulated. Deletion of one regulatory gene affected gene expression and metabolite production. This analysis will be important for future engineering and construction of robust *S. cerevisiae* strains for effective bioethanol production.

P24

STUDYING THE ROLE OF CYTOSOLIC TRANSKETOLASE AND TRANSALDOLASE IN XYLOSE METABOLISM AND FERMENTATION IN THE YEAST OGATAEA POLYMORPHA

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Lignocellulosic biomass represents important renewable resources that can be utilized for bioconversion to ethanol. The pentose-sugar xylose is the second-most abundant monosaccharide in lignocellilosic hydrolysates. Ogataea polymorpha is one of the most thermotolerant xylose-fermenting yeast species, however, with low efficiency of xylose alcoholic fermentation in the wild-type strains. Several metabolic engineering approaches have been successfully developed to improve ethanol production from xylose in O. polymorpha. However, the functional role of two key enzymes involved in the non-oxidative part of pentose phosphate pathway, namely transketolase and transaldolase, in xylose metabolism and alcoholic fermentation in O. polymorpha remained unclear.
The methylotrophic yeast O. polymorpha contains both cytosolic transaldolase (gene TAL1) and transketolase (gene TKL1) and their peroxisomal counterparts (genes DAS1 and TAL2, respectively). The deficiency or overexpression of these four genes was examined regarding their roles in xylose utilization and fermentation. The tal1Δ, tal2Δ and das1Δ mutants were constructed by gene disruption technique. The conditionally knockout tkl1Δ mutant was constructed by replacing the endogenous promoter of TKL1 gene by regulated YNR1 promoter of nitrate reductase, repressed by ammonium sulfate as nitrogen source. A significant decrease in xylose-fermenting ability and totally blocked growth on xylose was observed in tkl1Δ and tal1Δ mutants. Overexpression of DAS1 gene in tkl1Δ mutant led to restoration of growth on xylose, however, only after prolonged lag phase. Xylose utilization in tal1Δ strain was easily restored by overexpression of TAL2 gene. Moreover, overexpression of each of the mentioned genes resulted in improvement of ethanol production from xylose.

P25

Absolute quantification of protein and mRNA abundances to understand translation efficiency in yeast

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Protein synthesis is the most energy consuming process in a proliferating cell, and understanding what controls protein abundances and how this impacts changes in metabolic fluxes is a key question in biology and biotechnology. We quantified mRNA and protein levels in Saccharomyces cerevisiae under ten environmental conditions. Linear correlation across all proteins predicted only up to 46% of the final protein abundances based on corresponding mRNAs. However, a very good condition-dependent correlation was identified for individual proteins pointing to constant translation efficiency across the ten conditions. We further measured fractional lysine labeling incorporation rates to estimate protein turnover rates in the reference condition. In combination with mRNA and protein levels, we modeled translation efficiencies, which were found to vary more than 400-fold. Non-linear regression analysis detected that mRNA abundance and translation elongation are the dominant factors controlling protein synthesis rate, explaining 61% and 15% of its variance, while protein turnover rates played a minor role under the studied environmental conditions. However, they markedly contribute to overall cellular energy metabolism, responsible for 20% of total ATP synthesis. Mitochondrial fluxes were detected to be the only major exception for flux control being at the posttranscriptional level. The present dataset represents a crucial expansion to the current resources for future studies on yeast physiology and biological engineering.

P26

IMPROVEMENT OF XYLOSE UTILIZATION DURING HIGH-TEMPERATURE XYLOSE ALCOHOLIC FERMENTATION IN THE YEAST *OGATAEA (HANSENULA) POLYMORPHA* BY ENGINEERING OF THE HEXOSE TRANSPORTER HXT1

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For the last three decades biofuels produced from renewable feedstocks have received much attention because of their potential to replace conventional fossil fuels. A major issue in the conversion of saccharified lignocellulosic biomass into biofuel is the utilization of xylose, since lignocellulosic feedstocks contain a significant amount of this pentose sugar.

The ability of the thermotolerant methylotrophic yeast *Ogataea polymorpha* to ferment xylose has made this yeast species a promising organism for high-temperature alcoholic fermentation. Although *O. polymorpha* recombinant strains metabolize xylose more efficiently, uptake, and therefore consumption of xylose, is strongly inhibited by glucose, due to glucose catabolite repression. The low-affinity transport system is shared between glucose and xylose for sugar transport in *O. polymorpha*.

Recently, the first functional hexose transporter Hxt1 was identified in *O. polymorpha*. To increase the specific xylose uptake rate the modified Hxt1 was engineered by substitution of asparagine to alanine at position 358. Furthermore, N-terminal lysine residues of Hxt1 predicted to be the target of ubiquitination were replaced for arginine residues. The modified versions of Hxt1 were overexpressed in *hxt1Δ* mutant and the efficiency of xylose and glucose co-utilization during high-temperature fermentation was studied. The mutagenesis of Hxt1 resulted in simultaneous utilization of both sugars during fermentation in obtained recombinant strains.

P27

Aromas' metabolic fluxes prediction in oenological fermentation

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Aroma and flavor profiles are the most important characteristics defining the sensory properties of wines. Wine aroma involves a wide variety of volatile compounds, some of them being secondary products synthesized by yeasts during the fermentation. These molecules include, among others, aldehydes, higher alcohols, medium-chain fatty acids (MCFAs), long-chain fatty acids, ethyl fatty acid esters, acetate esters and volatile sulfur compounds. They are produced through a complex and dynamic metabolic process during fermentation, and many factors, such as grape variety, the nature of precursors (mainly amino acids), yeast strain, must treatments, and fermentation conditions, have been reported to control their production. Better understanding how they are produced is therefore of great importance. Flux metabolic modeling is a tool of choice for achieving a comprehensive overview of the production of aroma by yeast during fermentation and its regulation. However, the aroma metabolic network is not described in all the available metabolic models. In order to fill this gap, we developed a constraint based model of the yeast fermentation metabolism comprising all the reactions of synthesis and degradation of amino-acids as well as the most important reactions of aromatic compounds synthesis. Thank to this extended model we studied in silico the relation-ships between amino-acid consumption and aroma production. The efficiency of the different nitrogen sources present in grape juice to increase the production of fermentative aroma compounds was also assessed.

P28

COMPREHENSIVE TWO-DIMENSIONAL GAS CHROMATOGRAPHY WITH TIME OF FLIGHT MASS SPECTROMETRY (GCXGC-TOF-MS) APPLIED TO WINE YEAST ALCOHOLIC FERMENTATION VOLATILE COMPOUND ANALYSIS

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Comprehensive two-dimensional gas chromatography (GCxGC) has emerged as a powerful tool capable of increasing the peak capacity of a single chromatographic analysis. In GCxGC, compounds from a single sample are separated by two different retention mechanisms, allowing the discrimination of individual components that would normally co-elute in a single dimensional separation. This approach is particularly adapted for the study of volatile compounds in complex matrices, and has found numerous applications in petrochemical, fragrance, food industries, etc.
Here, we used GCxGC coupled with a time of flight mass spectrometer (TOF-MS) to analyse Merlot grape juice fermented with two different *Saccharomyces cerevisiae* strains (namely SB and GN). Extraction was achieved by head space solid-phase microextraction. Optimization of chromatographic column polarity (1D) and (2D) was a crucial step in order to maximize the separation of volatiles compounds. Using R tool, a data analysis pipeline was developed to automate the processing of GCXGC-TOF mass spectra. Finally, thanks to this approach, more than 600 compounds were reproducibly detected. Statistical analysis of the overall chromatographic data revealed differences between the two yeast strains. The interest of GCxGC-TOF-MS for yeast metabolic profiling and identification of volatile compounds in wine will be discussed.

P29

Elimination of sucrose transport and hydrolysis in *Saccharomyces cerevisiae*: a platform strain for engineering sucrose metabolism

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Many relevant options to improve efficacy and kinetics of sucrose metabolism in Saccharomyces cerevisiae and, thereby, the economics of sucrose-based processes, remain to be investigated. An essential first step is to identify all native sucrose-hydrolysing enzymes and sucrose transporters in this yeast, including those that can be activated by suppressor mutations in sucrose-negative strains. A strain in which all known sucrose-transporter genes (*MAL11, MAL21, MAL31, MPH2, MPH3*) were deleted did not grow on sucrose after 2 months of incubation. In contrast, a strain with deletions in genes encoding sucrose-hydrolysing enzymes (*SUC2, MAL12, MAL22, MAL32*) still grew on sucrose. Its specific growth rate increased from 0.08 h^{-1} to 0.25 h^{-1} after sequential batch cultivation. This increase was accompanied by a 3-fold increase of in vitro sucrose-hydrolysis and isomaltase activities, as well as by a 3- to 5-fold upregulation of the isomaltase-encoding genes *IMA1* and *IMA5*. One-step Cas9-mediated deletion of all isomaltase-encoding genes (*IMA1-5*) completely abolished sucrose hydrolysis. Even after 2 months of incubation, the resulting strain did not grow on sucrose. This sucrose-negative strain can be used as a platform to test metabolic engineering strategies and for fundamental studies into sucrose hydrolysis or transport.

P30

Towards product formation in near-zero growth rate fermentations of Saccharomyces cerevisiae under Nitrogen limited aerobic conditions

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The yeast *Saccharomyces cerevisiae* is an established microbial host for production of bio-chemical compounds. The current fermentation processes for these products are typically two-phasic, consisting of a growth phase and a separate production phase. Uncoupling of growth and product formation could prevent loss of feedstock to biomass production. Microbial physiology at near zero-growth rates can be studied in retentostats, which are continuous-cultivation systems with full biomass retention.

In previous research the physiology of *S. cerevisiae* in carbon restricted retentostats has been studied both under aerobic and anaerobic conditions. However, if the aim is to maximize the flux from substrate to product, carbon excess conditions might me more favorable. In that case the growth should be restricted through limited supply of another nutrient. Here we chose for nitrogen and developed to this end a protocol for nitrogen restricted retentostat cultivation. Using this protocol cultivations were carried out with a non-transformed CEN.PK 113-7D strain. In these cultivations the main product produced was ethanol. It was indeed observed that the biomass specific rate of ethanol production was higher under these conditions than in C-limited anaerobic retentostats. An important question is whether limited nitrogen supply is the proper way to keep *S. cerevisiae* in a non- or extremely slow growing state for prolonged periods of time. Therefore this study also focuses on the quantitative physiology and robustness of *S. cerevisiae* under nitrogen limited near zero growth conditions, whereby we determined cell viability, biomass composition, metaboilic flux distributions and applied quantitative metabolomics.

P31

Interspecies exploration of the make-up and expression of the glycolytic and fermentative pathways within the *Saccharomyces* genus

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Saccharomyces cerevisiae's ability to convert sugars into ethanol at fast rates has played an important role in its domestication. Over the past decades its glycolytic and ethanolic fermentation pathways have been extensively studied. Next to *S. cerevisiae*, other species from the *Saccharomyces* genus play an important role in our society, for example the cold-tolerant species *Saccharomyces eubayanus* and *Saccharomyces kudriavzevii*. However, despite their important role in wine and beer fermentations, little is known about the genetic make-up and expression of glycolytic and fermentative pathways in these species.

In this study the diversity of the glycolytic and fermentative pathways within the genus *Saccharomyces* is explored, using *S. cerevisiae, S. eubayanus* and *S. kudriavzevii* as paradigms. The glycolytic orthologues show high conservation at the DNA level for coding sequences, and for most regulatory elements within the promoter regions. Cultivation in aerobic bioreactors in chemically defined medium, combined with transcriptome analysis identified the dominant paralogues in *S. eubayanus* and *S. kudriavzevii* and revealed the expression levels of glycolytic genes in their native context. Finally, exploration of orthogonality of *S. kudriavzevii* and *S. eubayanus* promoters by monitoring their expression and context-dependency upon transplantation in *S. cerevisiae* revealed that the intensity and regulation of gene expression is very similar to that of their *S. cerevisiae orthologues*. Beyond insight in the orthogonality of glycolytic and fermentative promoters, this study shows that the promoters of *S. kudriavzevii* and *S. eubayanus* are attractive alternatives for gene expression in *S. cerevisiae*, thereby expanding *S. cerevisiae* strain construction toolbox.

P32

Molecular engineering of the red, basidiomycetous yeast *Rhodosporidium toruloides*

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The basidiomycetous yeast, *Rhodosporidium toruloides* is a red, oleaginous yeast that can accumulate lipids to more than 50% of its cell mass. Moreover, it can produce substantial amounts of the ω3-fatty acid linolenic acid. Therefore, this yeast is interesting for biodiesel production. Besides lipid those yeasts produce pigments such as β-carotenes that give them their characteristic colour.

However, tools for molecular manipulation of this yeast have just recently been developed and *Agrobacterium*-mediated transformation is most commonly used which is time-consuming and requires large plasmids both for meeting the requirements of both the bacterial host and the yeast.

In this study, we transformed *R. toruloides* using PEG and heat shock, based on a modified protocol from (Tsai et al 2016). We reached a transformation efficiency in the same range as the protocol described in the article but with lower DNA-concentration and shorter recovery time. Both random integration and homologous recombination were tested. We integrated genes expressing different desaturases and elongases with the aim to increase the amount of unsaturated fatty acids produced by this yeast. The impact of expressing the genes on lipid formation and FA-desaturation was tested.

KEYWORDS: Oleaginous yeasts, *Rhodosporidium toruloides*, lipids, transformation,

References: Tsai, YY., Ohashi, T., Kanazawa, T., Polburee, P., Misaki., Limtong, S. and Fuyiyama, K (2016) Development of a sufficient and effective procedure for transformation of an oleaginous yeast, *Rhodosporidium toruloides* DMKU3-TK16. Curr Genet DOI 10.1007/s00294-016-0629-8

P33

SWITCH: a flexible CRISPR tool for genetic engineering and metabolic flux control for cell factory construction in *Saccharomyces cerevisiae*.

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In the last years, the CRISPR/Cas9 technology has developed into a versatile tool for biotechnology. The technology has been applied to engineer cell factories in different organisms, including the budding yeast *Saccharomyces cerevisiae*.

We present SWITCH, a CRISPR based system that combines the genetic-engineering and gene-regulatory properties of Cas9/dCas9. Highly efficient gRNAs are crucial for SWITCH marker less recombination events. For this, we developed a screening technique, TAPE, which can be used to address the gRNA protospacer efficiency. As proof of principle, we established and optimized a yeast based cell factory for production of the plant metabolite naringenin. The results demonstrated that SWITCH was successfully allowing the strain to alternate between a genetic engineering state and a pathway control state.

We envision that with SWITCH the cell factory construction time can be greatly diminished and that the system can easily be adopted and improved by other researchers.

P34

Increase in the consumption of non-fermentable sugars by *Saccharomyces cerevisiae* through the strategy of genome shuffling

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Biotechnology strategies for the solution of technological problems have included the manipulation of microorganisms for the production of compounds in the chemical, pharmaceutical, food and biofuel industries. The classical methodologies of genetic engineering for modulation of specific metabolic pathways have demonstrated limitations, which is why techniques that affect cellular metabolism in a more global way are required. The objective of this work is to increase the consumption of xylose in a native *Saccharomyces cerevisiae* strain using genome shuffling. The yeasts subjected to the recombination of their complete genomes were: a native *S. cerevisiae* strain, a recombinant laboratory *S. cerevisiae* strain for the *xyl1* and *xyl2* genes, and *Scheffersomyces stipitis*. Three rounds of protoplast fusion were performed and selection of the colonies was performed in the YPX 2% medium. Larger colonies were inoculated in 2% YPX medium and incubated for 48 hours at 35 ° C. The xylose consumption was estimated and the colonies with the highest consumption were selected in each round to initiate a new round of protoplast fusion. Finally, yeasts were obtained which reached xylose consumption between 12 and 66% compared to parental strains that consumed up to 2% xylose in 48 hours. These results demonstrate the great advantages of this strategy in the improvement of strains for multiple industrial applications; however, the selection method used to obtain the desired phenotype is very important.

P35

Development of an autonomous replicating plasmid for *Starmerella bombicola*

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The non-convectional yeast *Starmerella bombicola* is known for the commercial production of bio-surfactant sophorolipids. Beside this, the yeast is also being used for the production of new-to-nature molecules. The production of the latter by modified *Starmerella bombicola* strains is however in some cases less efficient and requires further strain optimization. Unfortunately, state-of-the-art metabolic engineering tools for this yeast are scarce. The availability of an autonomous replicating plasmid in *Starmerella bombicola* would increase the speed of further genetic engineering. It would for example allow combinatorial fine-tuning of genes involved in the biosynthesis, catabolic or anabolic pathways of industrially relevant molecules, so more productive strains can be obtained.

For the development of an autonomous replicating plasmid at least a selection marker and an autonomic replicating sequence (ARS) are needed. For a successful segregation of the plasmid, also a centromeric sequence (CEN) is wanted. Because both ARS and CEN sequences aren't described for *Starmerella bombicola*, chromatin immunoprecipitation sequencing (ChIP-Seq) is being performed to find these regions.

P36

Cpf1, a new CRISPR-Cas endonuclease for genome editing in *Saccharomyces cerevisiae*

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The yeast *Saccharomyces cerevisiae* is a widely used cell factory. Due to tremendous progress in synthetic biology and metabolic engineering, its product portfolio is expanding rapidly from food products (e.g. wine, bread) to rocket fuels. However, successful application in biotechnological processes, e.g., the production of non-native products and resistance against harsh industrial conditions, requires extensive genome engineering. The advent of tools based on Clustered Regularly Interspaced Palindromic Repeats (CRISPR) and associated proteins (CRISPR-Cas), has tremendously accelerated the process of strain construction by enabling efficient, targeted edition of multiple genetic loci simultaneously.
Several types of bacterial CRISPR-based systems have been identified, the so-far most popular and best characterised being the class two, type II Cas9 endonuclease. However, the intensive ongoing exploration of the bacterial kingdom for CRISPR-based systems is expected to deliver attractive alternatives to Cas9. Cpf1, a recently discovered class two, type V CRISPR-Cas endonuclease, presents several distinct features that are particularly attractive for targeted genome editing. For instance Cpf1 targets a different PAM sequence than Cas9, and contrary to Cas9 it does not require a tracrRNA for DNA binding and editing, and matures the crRNA array itself, without requirement of an external RNase. Cpf1 has been shown to edit DNA in mammalian cells. We evaluated the ability of Cpf1 from *Franciscella novicida* to edit *S. cerevisiae* genome in a targeted manner. Our results show that Cpf1 holds a strong potential as new tool to simultaneously edit single and multiple targeted loci in S. cerevisiae.

P37

Development and application of a molecular toolkit for the biosurfactant chassis organism *Starmerella bombicola*

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Starmerella bombicola is a non-conventional though industrially relevant yeast, because of its high sophorolipid (SL) production. Due to its inherent highly efficient acetyl-CoA and activated glucose machinery, its high resistance to high amounts of hydrophobic compounds, high osmotolerance and efficient uptake/secretion mechanisms, this yeast has potential as chassis organism for the production of other industrially relevant (glyco)lipid compounds. However, the lack of molecular tools for this non-conventional yeast has been blocking its exploitation. Therefore, in this presentation we describe the development and validation of an extensive molecular toolkit for this yeast, consisting of an RT-qPCR platform, a Multi Reaction Monitoring (MRM) assay and a reporter system based on fluorescent proteins (FPs).

The developed RT-qPCR and MRM assays were validated as tools by respectively evaluating gene and protein expression in an engineered though inefficient glucolipid producing *S. bombicola* strain. After thorough evaluation and subsequent reengineering, its glucolipids production efficiency was dramatically increased, up to commercially relevant levels.

The developed reporter system allows to evaluate promotor activity and to develop a promotor library, which will be indispensable for further advanced metabolic engineering of *S. bombicola*.
This powerful toolset will enable us to optimize and generate engineered *S. bombicola* strains far beyond the scope of SL production. It enables a thorough and fundamental analysis of specific parts needed to design and optimize novel pathways.

P38

RNA stability engineering as a new synthetic biology tool in *Saccharomyces cerevisiae*

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Saccharomyces cerevisiae is a fast-growing microorganism which is often used as production host in industrial biotechnology. Lately, it is receiving more and more interest to produce chemically complex compounds from renewable resources. Thanks to efforts in the field of synthetic biology, integrating new pathways has become less challenging. Yet, simply implementing the pathway of interest is by far not enough to obtain an efficient production host. A major cause of this problem is the fact that all pathway steps need to be aligned with each other. Currently used fine-regulating tools are focusing on increasing the amount of transcription and result in a high metabolic burden onto the host and hence in strain instability and non-optimal production rates. Therefore, a novel strategy of controlling gene expression will be evaluated, i.e. RNA stability engineering. The stability of specific transcripts will be enhanced by adapting various regions of the transcript. This will in turn lead to lower mRNA turnover rates and decrease the need for high transcription rates and the enormous metabolic burden associated with it. Hence, by using RNA stability engineering as a new synthetic biology tool in *Saccharomyces cerevisiae*, a more efficient and stable production of interesting molecules is targeted.

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Brettanomyces bruxellensis intra-species phenotypic diversity and carbohydrate preferences. Consequences on adaptation to wine and spoilage ability.

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Wine environmental conditions are unfavourable for the growth of many microorganisms. However the yeast species *Brettanomyces bruxellensis,* which causes off-flavors described as sweaty animal, leather and medicinal due to the production of volatile phenols, is highly adapted to the winemaking process. It has been reported to be able to grow on limited carbon and nitrogen sources, at low pH and resists to high sulphur dioxide and ethanol concentrations. As a result, it can be detected at almost every stage of winemaking, from alcoholic fermentation to bottling, which makes it one of the most dreaded wine spoilage microorganisms. This is an important issue in the wine industry as “Brett” spoilage leads to economic losses due to the rejection by the consumers.

Several studies revealed a high level of genetic diversity at intra-species level. Furthermore, a comparison of genome assemblies revealed the coexistence of diploid and triploid populations. A large collection of strains isolated from wine and others fermented beverages was first characterized on a genotypic level using microsatellite markers. We then evaluated the phenotypic diversity of our collection on a subset of 50 strains regarding the ethyl-phenol production ability and the carbohydrate needs. Seventeen different carbohydrates were tested as sole carbon sources. Growth parameters (kinetics, yields) and behavior in the presence of carbohydrates mixtures were then studied with a subset of strains. Preferences in carbohydrate sources were determined and the consequence on wine spoilage ability examined.

P40

Anti-yeast ultrashort peptide: potential novel food preservative?

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Antimicrobial peptides are essential components of the immune system with promising applications to combat drug-resistant microbes, involved in clinical infections and food poisoning. However, their use as food preservatives is facing several issues, such as cost of synthesis, cytotoxicity, resistance to digestives enzymes, etc. Thus, the identification of key determinants involved in AMPs antimicrobial and cytotoxic activity, such as cationicity and amphipathicity, has enabled the design of optimized peptides.
In this study, the antifungal activity of an amidated ultrashort peptide (USP), Orn-Orn-Trp-Trp-NH2 (O3TR-NH2), and an O3TR-based lipopeptide was determined. The two peptides showed growth inhibition and killing of several yeast contaminants of beverages, such as S. cerevisiae or Z. bailii. O3TR-NH2 showed heat-stability up to 100°C for 1 h and pH resistance in the range 3-10, but was sensitive to cations increase. The addition of the lauric acid chain tail significantly increased its antifungal activity as well as its resistance in salt solutions and to proteolytic digestion. Some characteristics of the mode of action of these peptides, including changes in structural conformation and membrane permeabilization, were highlighted. Peptides were tested for their cytotoxicity towards human colonic cell lines and haemolytic activity in vitro. O3TR-NH2 did not show haemolytic or cytotoxic activity when applied at the concentrations that exhibit antifungal potency. Furthermore, the use of O3TR-NH2 delayed yeast growth in a challenge test performed in different commercial beverages.

P40

Deciphering The Molecular Mechanisms Underlying Robustness To Protein Overproduction.

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Why are proteins harmful when expressed in excess? Here, we systematically investigate the impact of genetic variation and the environment on protein overproduction costs in Saccharomyces cerevisiae. By integrating genome-wide genetic interaction and environmental stress screens, we identified three main mechanisms buffering the fitness costs of protein burden. Overproduction of unneeded proteins occupies ribosomes which could better be used for the translation of native proteins (ribosome occupancy) and wastes cellular resources (energy conservation). In addition, according to what we term the chaperone overload hypothesis, the cost of unneeded protein production is also strongly determined by the protein folding machinery. When protein folding capacity is compromised, protein overproduction disrupts the global balance of proteostasis and leads to the accumulation of aggregated proteins. Our work demonstrates the existence of multiple key genes that buffer protein overload and thereby may facilitate major changes in genomic expression during evolution.

P41

Mitochondrial development during brewery yeast handling

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Until recently the role of yeast mitochondria in brewing fermentations has been poorly understood. Primarily because mitochondrial function is associated with cellular respiration, which is repressed during fermentation due to a combination of the Crabtree effect and predominantly anaerobic conditions. However, current understanding is that mitochondria are essential for cellular function and fermentation performance, due to a number of important characteristics including their role in nuclear DNA signalling, Acetyl-CoA synthesis and lipid generation. This is supported by the fact that cells lacking in mitochondrial function, termed respiratory deficient or 'petites', do not ferment as well as wild-type cells. Fermentations conducted using cultures comprising an abnormal number of petites are typically slow with a poor conversion of sugar to alcohol. These yeast cultures display reduced growth, abnormal flocculation, and poor VDK reduction, while the final product is often characterised by irregular flavour profiles. Generation of the petite phenotype is complex and if a critical number of mitochondria are either defective (Rho-) or absent (Rho0) the individual cell will become respiratory incompetent. Conversely, it can be considered that a certain number of mitochondria should be present and able to function correctly for a cell to function as normal. We aim to determine the effect of process conditions on mitochondria number within cells, with the primary goal of establishing some of the key causes behind petite generation. It is anticipated that by furthering our understanding of the petite mutation it will be possible to reduce or mitigate their impact on brewery fermentations.

P42

Exploring of yeast biodiversity for lignocellulosic bioethanol traits

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Robust yeast strains with high inhibitor, temperature and osmotic tolerance remain a critical requirement for the sustainable production of lignocellulosic bioethanol. In this work, grape marc was selected as an extreme environment to search for innately robust yeast because of its limited nutrients, exposure to solar radiation, temperature fluctuations and high ethanol content. Four hundred new yeast strains, mainly belonging to *Saccharomyces cerevisiae* species, gave high ethanol yields at 40 °C when inoculated in minimal media at high sugar concentrations. Moreover, the isolates showed variable inhibitor-tolerance in defined broth supplemented with increasing levels of single inhibitors or a cocktail of inhibitory compounds. Both fermentative abilities and inhibitor resistance were greater than those exhibited by industrial and commercial *S. cerevisiae* benchmark yeast. The isolate *S. cerevisiae* Fm17, exhibited the most promising phenotype. The strain was then evaluated to ferment liquor from steam-exploded sugarcane bagasse, rich in weak acids, furans and aldehydes. The selected yeast produced high alcohol levels with an ethanol yield equal to 89% of the theoretical.This work demonstrated that yeast with high multiple stress tolerance can be obtained from unconventional ecological niches, such as grape marc. The selected yeast represents a promising platform to develop robust engineered strains suitable for the one-step processing of biomass into ethanol.

P43

Role of transporters mediating active uptake of glycerol in non-conventional yeast species in the osmotolerance

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Glycerol is the main osmoprotectant in most yeast species and is accumulated and produced at high quantities especially under hyperosmotic conditions. *Saccharomyces cerevisiae* possesses two systems for glycerol transport. First of them is Fps1, a plasma-membrane channel that is required for a quick release of glycerol. Second transporter, Stl1, mediates active uptake of glycerol in symport with protons and is repressed and inactivated by glucose in *S. cerevisiae*. On the other hand, the osmotolerant yeast species such as *Zygosaccharomyces rouxii* or *Debaryomyces hansenii* are distinguished by a more efficient glycerol uptake system, which helps them to reach necessary intracellular concentration with relatively low glycerol production. The regulation of glycerol transport in the wine-making yeast species (e.g. *S. kudriavzevii*, *S. bayanus*; *Dekkera bruxellensis*), which are exposed both to hyperosmotic stress and high-sugare presence during the fermentation processes, is probably more complex.
We have identified putative orthologues of the *S. cerevisiae STL1* in genomes of *S. kudriavzevii* (*SkSTL1*), *S. bayanus* (*SbSTL1*) and in the genome of *D. bruxellensis* (*DbSTL1* and *DbSTL2*). All *STL* genes were cloned and expressed in a series of *S. cerevisiae* mutant strains lacking different combinations of genes involved in osmotolerance. The presence of all these proteins improves growth of *S. cerevisiae hog1Δ stl1Δ* upon osmotic stress (except *DbStl1*) and upon conditions where glycerol was used as a source of carbon.

P44

The alteration of copper metabolism improves stress tolerance in *Saccharomyces cerevisiae*

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The yeast *Saccharomyces cerevisiae* is one of the most employed microorganisms in the biotechnological production of various industrial products, such as fine and bulk chemicals, pharmaceuticals, and biofuels. During these processes, yeasts encounter a variety of stresses that are generally associated with the growing conditions, the starting substrate(s) and the final product(s) or byproduct(s). The development of more tolerant strains is therefore indispensable for the improvement of the production, yield and productivity of fermentative processes.
Oxidative stress is a common hallmark of cell exposure to environmental stresses. Yeast cells counteract oxidative stress through a general cellular rewiring, centered on the activation of specific enzymes (e.g. superoxide dismutase) and the synthesis of antioxidant molecules. Oxidative stress resistance is also linked to metal ions homeostasis, among which copper plays a key role. This metal is the cofactor of the yeast cytosolic Cu/Zn superoxide dismutase (Sod1) and it is contained in copper metallochaperone and metallothioneins, which all have antioxidant properties.
We found that a *S. cerevisia*e strain engineered for the production of the antioxidant L-ascorbic acid (L-AA) shows a higher stress tolerance and, interestingly, a lower SOD activity compared with the wild type strain, even in permissive conditions; this might suggest that ascorbic acid may generate a cellular rewiring influencing the intracellular copper availability.
Here we present our data on copper metabolism engineering to increase yeast stress tolerance, demonstrating that an alteration in the copper uptake improved tolerance to oxidative stress both in L-AA and wild type cells.

P45

Autophagy is required for sulphur dioxide tolerance in *S. cerevisiae*

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Sulphur dioxide is used at several stages in winemaking, mainly as antioxidants and as antimicrobial, even before the onset of fermentation. Sulphites are also used as preservatives in many canned and processed foods. Its usefulness during wine fermentation depends on the relatively high tolerance to sulphites of wine strains of *Saccharomyces cerevisiae*. Sulphite tolerance in wine yeast strains has been related to mutations that boost transcription of *SSU1*, the gene coding for a plasma membrane sulphite pump required for efficient sulphite efflux. We used competition of bar-coded Yeast Knockout (YKO) collections to better understand microbial sulphite resistance, and sulphite cellular targets. Our results point to some of the cell functions that are more specifically required to survive sulphite-induced stress. For example, we found increased sulphite sensitivity for most KO strains involving genes required for autophagy, so pointing to this process as a key one in the detoxification of sulphite, probably through recycling of damaged proteins. In addition, some deletion strains, gained some competitiveness in the presence of sulphite (usually strains that were already impaired under standard growth conditions). These late functions are probably targets of sulphite (i.e. specifically sensitive to it). Acknowledgments: MINECO(AGL2015-63629-R, RTC-2014-2186-2) EU(7FP-IRSES-GA612441); Junta de Andalucía(P10-AGR6544)

P46

Production of volatile phenols by strains of *Dekkera bruxellensis* in alcoholic fermentation conditions for fuel ethanol production

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Dekkera bruxellensis is an important yeast contaminating wine fermentations causing undesirable alterations in the sensorial quality of the wine. This is mainly attributed to the phenolic compounds and their products metabolized by the yeast *D. bruxellensis*, especially vinylphenols and ethylphenols. Considering that this yeast is also a contaminant in alcoholic fermentation for fuel ethanol production, an investigation about its capacity of vinylphenol and ethylphenol production from the substrates commonly found in Brazilian distilleries may help to understand the role of this yeast in the fermentative process. In this work we analysed if three strains of *D. bruxellensis*, isolated from the ethanolic industry, were able to produce these substances from sugarcane juice and molasses simulating an industrial batch process with cell recycle, in flask scale, at 30^{o}C. The analysis of ethylphenols and vinylphenols was performed by HPLC. Ethanol production, sugar consumption, pH variation and yeast growth were monitored. Comparing to an industrial strain of *Saccharomyces cerevisiae* (PE-2), the production of vinylphenols is quite similar and low but it was higher in molasses than in sugarcane juice. Regarding the ethylphenols, the amount produced by the strains of *D. bruxellensis* was high (up to 10 mg/L), much superior in molasses and there was not a considerable production by S. cerevisiae. The strains of *D. bruxellensis* varied greatly in their overall production of volatile phenols. These results must be considered in the light of the effect of this production over *S. cerevisiae* cell viability and the consequences to the ethanol yield.
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P47

Optimizing a protocol for protoplast fusion of *Saccharomyces cerevisiae* with highly osmophilic yeasts

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Today, *Saccharomyces cerevisiae* enjoys increasing popularity as a production organism in industrial (‘white') biotechnology due to its GRAS classification, its rapid growth, its eukaryotic character and its insensitivity to phage contamination. In spite of all these advantages, various challenges still need to be overcome to allow the transition of baker's yeast from the old workhorse to a modern cell factory, fulfilling the requirements for next generation bioprocesses. Highly desired for such a modern yeast factory is the fact that fast and efficient state-of-the-art engineering possibilities can be combined with high tolerance towards process related stress conditions. Unfortunately, the former is mostly only possible in stress-sensitive laboratory strains, while the latter is specific for industrial yeast strains which are more difficult to engineer. We therefore aim to develop a method to transfer strain robustness properties to a less robust *S. cerevisiae* laboratory strain. A first step hereto is the development of a protoplast fusion method. The method will initially be tested to transfer resistance against high osmotic pressures, found in highly osmophilic yeasts.

P48

The wine spoilage yeast species *Brettanomyces bruxellensis*: a diploid-triploid complex associated with contrasted sensibility to sulphites

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The yeast *Brettanomyces bruxellensis* is the first cause of microbial spoilage in wine. Brett contamination results in the alteration of wine aromas (unpleasant barnyard smell). Sulphite remains the most widely used antimicrobial preservative in wineries, but some *B. bruxellensis* isolates could be tolerant to such treatments. *B. bruxellensis* is also associated with other industrial fermentations such as beer, kombucha (fermented tea), bioethanol, etc. In those last cases, the desirability/undesirability of this yeast is still unclear and debated. Previous studies revealed that phenotypic characteristics are strain-dependent and that Brett strains are highly diverse at the genetic level with the coexistence of diploid and triploid populations of hybrid origins (allotriploids). We genotyped a large population of Brett isolates (>1400) from 29 countries and eight substrates using microsatellite markers. Our results confirm that *B. bruxellensis* species is composed of strains with different ploidy levels (more than 50 % of isolates are triploid). *B. bruxellensis* population is structured according to ploidy level and substrate (wine, beer, tequila/bioethanol, kombucha groups). Interestingly, those different groups display contrasting behavior regarding sulphite treatments, with one major wine group being tolerant to sulphites at concentration usually used by winemakers. A molecular test, allowing predicting the tolerance ability to sulphite treatments of *B. bruxellensis* isolated from wine is in development, and could be a useful tool for winemakers to adapt their practices to better control Brett contamination. In addition, the relationship between the different polyploid/hybrid groups and other traits relevant for industrial fermentations are currently studied and will be discussed.

P49

Evolution of a laboratory-made hybrid under industrial lager-brewing conditions.

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The brewing yeast *Saccharomyces pastorianus*, a hybrid of *S.cerevisiae* and *S.eubayanus* used for the production of lager-type beer , has a highly complex aneuploid genome which possesses large diversity in chromosome copy number and displays numerous chromosomal crossovers. Prolonged domestication subjected *S. pastorianus* to the selective environment of brewing, which unequivocally had a strong impact on the evolution of this complex genome. In order to study the impact of domestication, a laboratory-made S.cerevisiae x *S.eubayanus* hybrid was cultivated on brewer's wort in repeated sequential batches under conditions mimicking lager-fermentations for up to 484 generations. A total of 55 single colony isolates from 6 independent evolution lines were characterized phenotypically and genotypically. Several industrially-relevant phenotypes were obtained which are characteristic for modern day *S.pastorianus* strains but were not expressed by the laboratory-made hybrid. Interestingly, a high degree of diversity was observed between the isolate's phenotypes of which some are undesired for brewing yeasts. The variety of phenotypes was also reflected in the genomes; various chromosomal reorganisations, changes in chromosome copy number and loss of heterozygousy were observed. These 55 sequenced genomes form the first large dataset of strains derived from one ancestral hybrid and should yield useful insights into evolution and genome plasticity of polyploid genomes. Such improved understanding would contribute to the further development of aneuploid strains in industrial applications.

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P50

Analysis of genome-Wide Transcriptome of Thermotolerant *Saccharomycopsis fibuligera*

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Amylolytic yeast *Saccharomyces fibuligera* MBY1320, which was isolated from Nuruk, exhibits a raw starch-degrading activity. Its ability to directly digest raw starch is a technologically interesting trait and property in fermentation industry. The *S. fibuligera* MBY1944 which has notably higher tolerance to temperature was obtained by adaptive evolution of parent strain *S. fibuligera* MBY1320. Transcripts of the two strains cultivated at the same temperature were analyzed. A total 174,887,872 reads was assembled into 6,289 transcripts. Comparison of transcriptome revealed that 479 were differentially expressed genes, of which 257 were up-regulated, 222 were down-regulated for an evolved thermotolerant MBY1944. This study has uncovered transcriptome of *S. fibuligera*, which could help gain a better understanding of the regulatory circuit related with thermotolerance.

P51

Synthetic ecology of yeast and microalgae: Engineered ecosystems to evolve mutualistic relationships for the bioremediation of winery wastewater

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Most natural and many industrial microbial ecosystems are characterised by a large number of species whose interactions are not well understood. For these reasons, the population dynamics within such ecosystems remain largely unpredictable, and industrial processes frequently rely on inoculation with single dominant species to better control processes. Synthetic ecology provides an opportunity to better understand and control species interactions, while engineered symbiotic co-cultures may allow the assembly of novel functional and metabolic capabilities. Such systems also facilitate the development of models to predict community behaviour under environmentally variable conditions. Here we use a synthetic ecology approach to establish stable synthetic mutualisms between *Saccharomyces cerevisiae* and winery wastewater microalgae, *Parachlorella beijerickii* or *Chlorella sorokiniana.* The strongly selective (obligatory) conditions are based on the reciprocal exchange of carbon and nitrogen. The stability of the associations in response to changes in environmental parameters such as temperature and pH were evaluated over time, as these are key parameters which contribute to the variable nature of winery wastewater. The data show that the combination of the two species under semi-selective experimental growth conditions led to increased biomass production for each species when compared to single species cultures, and to increased resistance to environmetal stresses. We suggest that such engineered mutualisms may be the first step in developing a stable multi-species ecosystem with enhanced productivity for biological winery wastewater treatment, while also providing novel insight into the evolution of mutualistic interactions between phylogenetically distant species such as yeast and microalgae.

P52

Characterization and Modification of Industrial Ale Yeast strains

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Different strains of a species as starter cultures like brewer's yeast have a great impact on the characteristics of fermented products. The characterization and improvement of industrial yeast strains for better process or product characteristics is an ongoing process. Evolutionary engineering is a powerful tool to modify and improve industrial strain traits, even without knowing the genetic network. Adaptation, selection, mutation, hybridization and genome shuffling are methods which can lead to a strain with better fermentation characteristics than the original strain. Therefore different ale yeast strains were chosen to characterize and modify their fermentation performances and genetic background with different methods. Their modifications, the "new" strains, also have to be clearly analyzed and characterized. Different molecular fingerprint–methods based on PCR and restriction analysis were tested to characterize the genetic background in this project. Here, the identification of modifications by such molecular techniques is also desirable. Furthermore the stability of the phenotypic and genetic changes of the fermentation characteristics should also be analyzed/monitored using such fingerprints. Detailed analysis of the genome by next generation sequencing should also be carried out. This may help to understand the genetic network behind the modifications of the phenotypes and the fermentation characteristics, respectively. The poster presents the current work for the characterization and modification of ale yeast strains.

P53

Improvement ethanol tolerance of S. uvarum yeasts using hybrids and adaptive evolution

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New wine yeast starters of non-cerevisiae strains like S. uvarum, can contribute to solve some problems of the wineries. This specie exhibit good fermentative capability at low temperatures, producing wines with lower alcohol and higher glycerol amounts and good aromatic profiles, of great interest for the wine industry. As an example of this application we selected a strain namely Velluto BMV58 commercialized by Lallemand. Despite that, Velluto BMV58 is a good candidate to solve the current winemaking demands, this strain cannot compete with S. cerevisiae at industrial level due to S. uvarum strains are less ethanol tolerance. So, new biotechnological approaches are required to improve ethanol tolerance. In the present work, we will apply different techniques such as adaptive evolution and hybridization to improve the ethanol tolerance, besides a genomic characterization has been done .

Acknowledgments

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P54

The sole introduction of two single point mutations establishes glycerol utilization in *Saccharomyces cerevisiae* CEN.PK derivatives

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Background: Glycerol is an abundant by-product of biodiesel production and has several advantages as a substrate in biotechnological applications. Unfortunately, the popular production host *Saccharomyces cerevisiae* can barely metabolize glycerol by nature.

Results: In this study, two evolved derivatives of the strain CEN.PK113-1A were created that were able to grow in synthetic glycerol medium (strain PW-1 and PW-2). Their growth performances on glycerol were compared with that of a previously published evolved CEN.PK113-7D derivative JL1. As JL1 showed a higher maximum specific growth rate on glycerol, its genomic DNA was subjected to whole-genome resequencing. Two point mutations in the coding sequences of the genes *UBR2* and *GUT1* were identified to be crucial for growth in synthetic glycerol medium and subsequently verified by reverse engineering of the wild-type strain CEN.PK113-7D. The growth rate of the resulting reverse-engineered strain was 0.130 h^{-1}. Sanger sequencing of the *GUT1* and *UBR2* alleles of the above-mentioned evolved strains PW-1 and PW-2 also revealed one single-point mutation in these two genes, and both mutations were demonstrated to be also crucial and sufficient for obtaining a maximum specific growth rate on glycerol of ~0.120 h^{-1}.

Conclusions: The current work confirmed the importance of *UBR2* and *GUT1* as targets for establishing glycerol utilization in strains of the CEN.PK family. In addition, it shows that a growth rate on glycerol of 0.130 h^{-1} can be established in reverse-engineered CEN.PK strains by solely replacing a single amino acid in the coding sequences of both Ubr2 and Gut1.

P55

Improving the fermentation inhibitor tolerance of the oleaginous yeast *Metschnikowia pulcherrima* through adaptive evolution results in evolved strains with increased lipid accumulation.

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The oleaginous yeast *Metschnikowia pulcherrima* has potential interest within industrial biotechnology because it has been shown to produce a microbial oil with properties to that of palm oil, from low-cost second generation feedstocks. Whilst *M. pulcherrima* can tolerate some concentrations of common fermentation inhibitors present within hydrolysed lignocellulosic feedstocks, inhibitor presence reduces both overall biomass and lipid accumulation.

In order to improve productivity in the presence of fermentation inhibitors despite the limited genetic tools available for this organism, a strategy of adaptive laboratory evolution (ALE) was used. Here, separate lineages were evolved to media containing either formic acid, or a inhibitor cocktail (formic acid, acetic acid, 5-HMF and furfural). Within the formic acid evolution strategy, initial batch concentrations of 0.6 g/L were doubled to 1.2 g/L within 1080 hours of culture time. Resulting evolved cell lines from both strategies displayed significantly increased growth rates when assayed within inhibitory conditions, as well as reduced lag times of 10-15 hours versus 40 hours for the progenitor strain, indicating both strategies had developed tolerant strains.

Despite similar growth rate phenotypes, the evolved strains displayed significantly different lipid accumulation profiles. The formic acid evolved strains displayed reduced lipid accumulation compared to the progenitor (between 13-17% vs 22.1% lipid by dry weight), but strains evolved under pressure from all four inhibitors had increased lipid production, with one strain now accumulating 41%.

With an evolved strain now displaying increased inhibitor tolerance and, unexpectedly, increased lipid accumulation, the biotechnological potential of this organism has been significantly improved.

P56

Metabolic changes during compensatory evolution following genetic perturbation in *Saccharomyces cerevisiae*

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Metabolite production patterns are important traits in yeasts employed for the production of alcoholic beverages and for industrial biotechnology. Therefore selecting or constructing yeast strains with various novel metabolite production patterns is of great interest.
Here we ask whether compensatory evolution, the process by which genotypes with harmful mutations improve their fitness by accumulating rescuing mutations, can generate novel metabolic behaviors. To address this issue, we monitored the production rates of glycerol, acetic acid and ethanol (overflow metabolites) of slow-growing *Saccharomyces cerevisiae* strains carrying gene deletions and their corresponding evolved lines showing fitness gain after evolution (Szamecz et al. 2014). Our results demonstrate that, in most gene deletion strains, the overall metabolic similarity to the wild-type state increases during compensatory evolution. The extent of metabolic pattern changes correlated with the extent of fitness compensation. However, in at least one gene deletion (*Δatp12*), compensatory evolution led to a novel metabolic state by increasing glycerol production above the wild-type level.
As the evolved strain showed growth rates similar to that of the wild-type, we conclude that compensatory evolution can drive the population to a new a high-fitness peak with alternative overflow metabolic production pattern. These results have implications for bioengineering, where genetic modifications with detrimental side effects are common and hence there should be ample opportunity for compensatory evolution.

P57

Various aspects of improving lactic acid production in *Saccharomyces cerevisiae*

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For decades, lactic acid has been used in the food industry, especially as a food preservative. Nowadays, it has gained attention in industrial biotechnology as a monomer for polylactide production. These biopolymers, besides their high biodegradable properties, are also cost-effective alternatives to petroleum-based plastics.

As a working organism for microbial lactic acid production, a pyruvate decarboxylase-negative yeast *Saccharomyces cerevisiae* has been used. The strain was engineered in such way that the ethanol metabolic pathway was replaced with the lactic acid metabolic pathway. The yeast has been cultivated in synthetic medium with a high initial carbon source concentration, accumulating the desired product and thereby lowering the pH value significantly below 3, thereby easing purification.

Nevertheless, the major challenge of such fermentations at low external pH values is the decreased yeast viability and interrupted intracellular pH (pHi) homeostasis, especially at high lactic acid concentrations.

Adaptive laboratory evolution strategies are used as a powerful tool for further improvement of yeast growth characteristics as well as its adaption to harsh and low pH conditions. This is very important for the recovery of pure lactic acid from the culture broth, where the addition of acid can be eliminated and therefore the formation of unnecessary salts in the culture broth can be avoided. Furthermore, using Fluorescence-activated cell sorting device and sorting the population of yeast cells, which can maintain their intracellular pH homeostasis during production phase (yeast cells with higher pHi), could be also beneficial in enhancing the final yield of lactic acid.

P58

Screening yeast species for bio-surfactant production from waste biomass materials

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There is great interest in exploiting biomass waste from the agrifood chain in order to address the increasing problem of waste disposal, and to enhance the sustainability of food production. A large proportion of such waste is currently disposed of by anaerobic digestion which has the potential to provide an energy return. There has also been much research to convert biomass to biofuels such as bioethanol. However, this is still difficult to achieve economically due to the high cost of technology and capital equipment, and the relatively low value of ethanol as a product.

This presentation describes research which is evaluating the feasibility of converting biomass substrates into other more valuable products. A specific example is given which concerns the production of natural bio-surfactants. A number of yeast are known to produce detergents and in this study a set of 576 yeasts from the National Collection of Yeast Cultures have been screened using a novel rapid throughput methodology using only optical imaging. Results from this initial screen were then used to reduce the number of yeast candidates to a smaller subset which could be studied in greater detail. The quantification of the surfactants of this sub-set was carried out using NMR analysis.

The results are considered in relation to the global demand for such products.

P59

A comparative study of lignocellulosic ethanol productivity by Kluyveromyces marxianus and Saccharomyces cerevisiae.

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This study investigated the lignocellulosic ethanol productivity of four yeast strains Kluyveromyces marxianus (SLP1 and OFF1) and Saccharomyces cerevisiae (RED and AR5) from sugarcane bagasse (SCB) and wheat straw (WS) hydrolysates. The composition analyses of enzymatic saccharification for diluted acid-pretreated SCB and WS showed that HMF, furfural, vanillin, acetic acid and other yeasts growth inhibitors were present in the hydrolysates at moderate concentration. The four yeast strains were able to produce ethanol in both hydrolysates; however, the highest volumetric ethanol productivity (Qp, g ethanol L- 1 h-1) and ethanol yield (Yp/s, g ethanol g-1 glucose) on SCB were showed by K. marxianus SLP1 with 0.292 g L-1h-1 and 0.389 g g-1, respectively. Similar results were obtained for WS, K. marxianus SLP1 showed the highest ethanol productivities, Qp of 0.300 g L-1 h-1 and a Yp/s of 0.273 g g-1. In contrast, the lowest productivities were observed for S. cerevisiae AR5 and K. marxianus OFF1 on SCB and WS, respectively. This results proved that K. marxianus SLP1 was more suitable for ethanol production from SCB and WS hydrolysates and this yeast has shown potential for obtain lignocellulosic ethanol under conditions of inhibition.

P60

Computing a yeast Tree of Life to underpin bioindustrial exploration

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Phylogenetics both underpins strain identification and acts as a framework on which to view and exploit academically- and bioindustrially-relevant trait information. The UK National Collection of Yeast Cultures (NCYC) consists of over 4000 diverse strains, ideal for beginning to construct a yeast Tree of Life that can be used as such a framework. A current NCYC genome sequencing program further provides the raw material for tree estimation.

There are several different approaches to phylogenetic analysis including new techniques which use next-generation sequencing (NGS) data. One such approach uses feature frequency profiles (Sims et al., 2009) which we hypothesise will prove most useful in building a Tree of Life, due to their potential to capture the phylogenetic signal(s) across entire genomes. This particular feature frequency approach is simple to use but has shown some problems with efficiency and in taking biological features into account.

The aim of this project is to assess and compare phylogenetic methods such as multi-locus sequence, whole genome SNP, and NGS-based approaches using a large dataset of yeast genomes. To illustrate the different approaches, an evolutionary tree of Saccharomyces complex strains will be constructed alongside a key trait dataset. The success of the various approaches will be assessed by computational measures (e.g. Robinson Foulds distances, Mantel tests).

Sims GE, Jun S-R, Wu GA and Kim S-H (2009) Alignment-free genome comparison with feature frequency profiles (FFP) and optimal resolutions. *Proc Natl Acad Sci USA* 106: 2677-2682.

P61

Yeasts isolated from rumen fluid, a new option for cattle probiotic

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Yeasts have been used as probiotic in animal's diet to improve the rumen fermentation. However, the function of yeasts naturallypresent in the rumen is not well known. The aim of this study was isolate, identify and select yeast strains isolated from ruminal fluid which potential to be used as probiotic for cattle feeding. 77 strains isolated from ruminal fluid of 16 cattle, belonging to four herds and 3 commercial strains of *Saccharomyces cerevisiae* were used in this study. Initial screening was based on growth in sterile rumen liquid, growth at 39^{o} and 41°C, assimilation of lactic acid, and tolerance of acetic and propionic acids. The strains that showed best characteristics were used in a new screening based on *in vitro* fresh rumen fluid fermentation at three times (12, 24, and 48 hours). Yeast growth, neutral detergent fiber digestibility (NDF-D), pH, and acids accumulation were evaluated. Ten strains were selected at initial screening: LR78 and LR80 (*Candida pararugosa*), LR75 (*C. ethanolica*), LR41 (*C. rugosa*), LR50 (*Magnusiomyces capitatus*), LR 26 (*Meyerozyma caribbica*), LR20 and LR21 (*Pichia kudriavzevii*), LR42 (*Kluyveromyces marxianus*), and one of commercial strain COM83. There was a reduction in the yeast population during the fermentation time for all strains, however there was great variation among them on characteristics evaluated. The strains LR41, LR50, LR75, and LR78 showed greater ability to survive during the fermentation; LR26, LR41, LR42, and LR78 stimulate the production of acids and LR75 improve 25.9% the NDF-D. LR41, LR78 and LR75 are promising for use as probiotic.

P62

Species and genetic diversity of economically industrial potential of *Aureobasidium* strains isolated from Taiwan

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The objective of this study is to investigate genetic diversity and phylogeny of the economically industrial potential genus *Aureobasidium* isolated from Taiwan. Since 2005, totally 506 strains of the genus *Aureobasidium* isolated from samples collected in different niches from Taiwan. The strains were identified based on the D1/D2 domain of the large subunit (Domain of LSU) and Internal transcribed spacers (ITS) of rRNA gene and phylogenetic analysis showed 504 strains could be clustered into 15 clades. Furthermore, representative 204 strains of 504 strains were authenticated by sequencing the genes of EF1-*α* (Translation elongation factor-1*α*) and β-tubulin. Finally, 204 strains were classified into 11 clusters based on the four genes, showing the strains could be identified 11 distinct species, including four currently recognized species *A. thailandense, A. leucospermi, A. melanogenum, A.pullulans,* and seven tentative novel species distinctly different from currently recognized species.

P63

Genome mining for bioindustrial metabolic gene clusters in yeast

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The UK National Collection of Yeast Cultures (NCYC) has recently embarked on a large-scale genome sequencing project. The sequences of its ~4,000 diverse strains harbour a potential goldmine of novel compound diversity in the form of metabolic gene clusters. Although gene clusters have previously been found in yeasts (Wong and Wolfe, 2005) many are 'silent' - with no expression under standard culture conditions - and therefore represent an unknown and untapped resource for the production of chemicals of bioindustrial utility. This project aims to discover and analyse metabolic gene clusters in the NCYC genome data via the use of both existing and novel computational tools and methods.

One key metabolic gene cluster in yeast is the mannosyl-erythritol lipid (MEL) cluster first discovered in *Ustilago maydis*. MELs are a highly useful group of biosurfactants exhibiting a spectrum of physical properties and consequently have a wide range of industrial applications in addition to being more environmentally friendly and sustainable than synthetically derived alternatives. Here we present progress to date in mining the genomes of the now-obsolete genus *Pseudozyma* (Wang et al., 2015) for variants of the MEL cluster.

Wang Q. -M., Begerow D., Groenewald M.,Liu X. -Z., Theelen B., Bai F, -Y., and Boekhout T. (2015). Multigene phylogeny and taxonomic revision of yeasts and related fungi in the Ustilaginomycotina. Studies in Mycology 81: 55-83.

Wong S. & Wolfe K. H. (2005). Birth of a metabolic gene cluster in yeast by adaptive gene relocation. Nature Genetics 37, 777-82.

P64

Formulation of native citrus yeasts as biocontrol agents for phytopathogenic fungi

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Mexican and persian limes (*Citrus aurantifolia* and *C. latifolia*, respectively) are the main acid limes cultivated in Mexico, which is currently considered the main worldwide producer and exporter of lime fruits. However, diverse fungal diseases affect their production and packing processing, hence biocontrol alternatives are desired to diminish the use of chemical fungicides and to increase food safety. Some of the most important fungal diseases of *Citrus* sp. in Mexico are Fusarium dry rot (*Fusarium* sp.), brown rot (*Phytophthora* sp.), green/blue mold (*Penicillium* spp), Aspergillus rot, Sour rot (*Galactomyces citri-aurantii*), anthracnose (*Colletotrichum gloeosporioides*), and stem-/blossom-end rot (*Alternaria citri*, *Botryosphaeria* sp.) amongst others. Yeasts have biological and technological advantages to be used in biocontrol products, due to their high resistance to environmental stresses, easiness of cultivation, and as being unicellular fungi, they usually inconspicuously blend with the biocontrol formulations. However, specific yeast-filamentous fungi interaction has to be taken into account to assess the performance of a given strain, as well as formulation is a critical step to its industrial use, as it determines the economic feasibility of an otherwise very efficient BCA on the lab. In this work we isolated, tested and selected a final set of four citrus native yeasts (labelled LCBG-03, LCBG-23, LCBG-27 and LCBG-30) and developed a citrus medium to enhance their activity, and also various culture and processing parameters were assessed (pH, temperature, media, cell inoculum, sensitivity to centrifugation and final presentation) to select and formulate those with the highest biocontrol activity and longer shelf life.

P65

Lipid production by yeasts grown on crude glycerol from biodiesel industry

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The main carbon source used for growth by four yeast strains (Yarrowia lipolytica CCMA 0357, Y. lipolytica CCMA 0242, Wickerhamomyces anomalus CCMA 0358, and Cryptococcus humicola CCMA 0346) and their lipid production were evaluated, using different concentrations of crude and pure glycerol and glucose. Whereas crude glycerol (100 g/L) was the main carbon source used by Y. lipolytica CCMA 0357 (nearly 15 g/L consumed at 120 h) and W. anomalus CCMA 0358 (nearly 45.10 g/L consumed at 48 h), pure glycerol (150 g/L) was the main one used by C. humicola CCMA 0346 (nearly 130 g/L consumed). On the other hand, Y. lipolytica CCMA 0242 used glucose (100 g/L) as its main source of carbon (nearly 96.48 g/L consumed). Y. lipolytica CCMA 0357 demonstrated the highest lipid production (about 70% (wt/wt)), forming palmitic (45.73% of fatty acid composition), stearic (16.43%), palmitoleic (13.29%), linolenic (10.77%), heptadecanoic (4.07%), and linoleic (14.14%) acids. Linoleic acid, an essential fatty acid, was produced by all four yeast strains but in varying degrees, representing 70.42% of the fatty acid profile of lipids produced by C. humicola CCMA 0346.

P66

Identification of functional D-xylose isomerase genes that confer superior cellulosic bioethanol production to yeast strains

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Bioethanol has become the most important worldwide biofuel and lignocellulosic biomass is a great potential substrate for its production. *Saccharomyces cerevisiae* is still the dominant organism for industrial bioethanol production. Therefore, intense research has focused in the development of *S. cerevisiae* strains capable of fermenting hexose and pentose sugars present in lignocellulosic biomass. For that purpose, the identification of functional D-xylose isomerases is crucial.

The aim of this project is the *in vivo* characterization of D-xylose isomerase genes from different origins. The xylose-utilizing *S. cerevisiae* M315 was used as platform. This diploid strain has two copies of a cassette containing among others the gene coding for D-xylose isomerase from *Clostridium phytofermentas*.

We deleted the *C. phytofermentas* XI (*CpXylA*) genes in the M315 strain, so that it became unable to ferment xylose. The knockout strain M315-*CpXylAΔ/CpXylAΔ* was transformed with new XI genes expressed under a constitutive promoter and in a multi-copy plasmid. From 16 tested genes, two of them, respectively called *9XylA* and *12XylA,* were selected and genome integrated into M315-*CpXylAΔ/CpXylAΔ*. Fermentation experiments showed a significant improvement for xylose fermentation when compared to the original M315 strain. Also, in order to increase the expression levels of *CpXylA*, a mini-library of 47 codon-optimized *CpXylA* genes expressed in a multi-copy plasmid was tested. We selected one clone that showed a slight improvement of xylose fermentation in comparison to the original *CpXylA* gene.

P67

Developing methodologies to explore and exploit *Kluyveromyces marxianus* diversity

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Kluyveromyces marxianus is naturally found in dairy products (cheese, kefir, yoghurt). In addition, because of its rapid growth rate, thermotolerance and enzymes production it is widely adopted industrially. Although approximately 7 genome sequences are in the public domain, there is still little information on the level of diversity within the species. This limits our capacity to select strains for particular applications. To address this, a Multi-Locus Sequence Type (MLST) analysis was developed and used to analyse ~80 strains: 30 from culture collections and 50 from strains isolated from different dairy products. This revealed a high degree of diversity within the species. It is known that *K. marxianus* is able to produce a range of volatile molecules with applications as fragrances or flavors in food and beverages. For this reason, these strains were previously analyzed on the basis of their capacity to produce aroma compounds in whey. In addition, it was established that both carbon and nitrogen sources affected production of higher alcohols and acetate esters. Currently, we are investigating this in more detail and will report on how external parameters affect the expression of genes believed to be involved in production of these metabolites, for example via the Ehrlich amino acid degradation pathway. The ultimate aim is to understand how this yeast regulates flavour and aroma production with a view to using this information to select strains for specific applications or to control the pathways in pure or mixed cultures.

P68

Isolation, identification, and characterisation of naturally occurring yeasts that antagonise soilborne plant pathogens

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Fungi are the most important plant pathogens in soil, notoriously difficult to control, and a major constraint for crop production; often the only resort is crop rotation, fallow, or even an abandonment of the cropland. Yeast-like fungi have been identified as effective antagonists of fungal plant pathogens, but only few yeast species were developed as commercial plant protection products so far.

It is our aim to isolate and identify antagonistic yeasts, to characterise the modes of antagonism of the strongest antagonists against plant pathogens, and to assess their potential as plant protection agents in fruit and vegetable crops. In an initial screen of over 600 binary interactions (40 yeast isolates from agricultural samples against 16 filamentous fungi including plant pathogens, saprophytes and soil fungi), weakly and strongly antagonistic yeasts were identified. The growth characteristics, metabolic activities, rhizosphere competence, and antagonistic activities in soil and plants of the strongest antagonists have been studied and whole genome sequencing (of two isolates so far; *Metschnikowia pulcherrima*, *Candida subhashii*) has identified factors potentially involved in the mode of antagonism. In particular, we have identified potential antagonistic factors such as secreted enzymes and volatiles as mechanisms underlying the antagonistic activity of yeasts.

These results are the basis for assessing the biocontrol potential of antagonistic yeasts, required for a potential registration of such strains as plant protection agents, and the foundation for engineering and further improving the antagonistic activity of yeasts for the management of fungal plant pathogens.

P69

BASIDIOMYCETOUS YEASTS FOR PRODUCING LIPIDS FROM 2nd GENERATION CARBOHYDRATES

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Oleaginous microorganisms including yeasts can be used for obtaining oleochemical products, such as biofuels and building blocks for chemistry. In this framework, yeasts biodiversity can be considered a powerful source of novel strains whit high lipogenic aptitude.
Over 700 strains were screened for their lipid yields (Y_L) and the environmental basidiomycetous strains *Leucosporidium creatinivorum* DBVPG 4794, *Naganishia adeliensis* DBVPG 5195 and *Solicoccozyma terricola* DBVPG 5870, conserved in the DBVPG Collection (www.dbvpg.unipg.it) were tested for their lipogenic aptitude at 20 and 25°C on C3, C5, C6 and C12. Y_L from 4.3 ± 0.1 to 8.5 ± 1.0 g/L (42 ± 2.0 to 59 ± 2.1% cell dry weight) were found. Oleic acid was the dominant fatty acid, but a quite variable profile was observed considering both temperature and different carbon source.
The Y_L of the selected strains was evaluated on 2nd generation carbohydrates obtained from steam-exploded and enzymatically hydrolysed ligno-cellulosic biomasses, namely stranded driftwoods (SDW), cardoon stems (CA) and olive branches (OB). SDW and OB supported both grow and lipidogenesis of the three strains. The highest Y_L was achieved by *L. creatinivorum* on OB at 20°C and by *S. terricola* on CA at both 20 and 25°C (10.0 ± 0.1, 13.5 ± 0.2 and 10.0 ± 0.5 g/L, respectively). Interestingly, the last strain exhibited the highest lipid coefficient at 20°C on CA (Y_L/carbon source = 28.6 ± 0.4%), close to the maximum theoretical value.
This study highlighted the ability of basidiomycetous yeasts to grow and accumulate lipids from 2nd generation carbohydrates.

P70

Assessing biodiversity of *Saccharomyces sensu stricto* species at different altitudes using culturing method and metagenomic approach

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Natural species of the group *Saccharomyces sensu stricto* are commonly found inhabiting trees bark and surrounding soil, specifically oak trees. Previous attempts to study the diversity of these species in nature involved the use of enrichment culturing methods. In this lab, oak trees bark and soil were enriched using different sugars and incubated at different temperatures to isolate a variety of '*sensu stricto*' species. *Saccharomyces paradoxus* was isolated from soil and bark at 30 °C and 10 °C and *S. kudriavzevii* was isolated from bark samples enriched in melezitose incubate at 10 °C . To avoid culturing bias, mycobiome of soil surrounding oak, spruce and beech trees at altitudes 600m and 1400m were sequenced using Illumina Miseq. The ITS1-5.8s-ITS2 region is typically 850bp in '*sensu stricto*' species, to increase the selectivity this region was extracted and ITS1 was sequenced (total of 1 x 10^6 reads). Using this approach we were able to detect *S. mikatae*, which was never before isolated in Europe. The metagenomics approach also revealed distinct fungal populations for the different type of tree at the two altitudes. However, compared to other fungal species, a low number of *Saccharomyces sensu stricto* species were detected in general. Our results show coupling culturing method and high-through put sequencing improve the detection of *Saccharomyces* '*sensu stricto*' species in nature.

P71

Marine halotolerant yeasts for selective biotransformations of nitriles

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The biocatalytic ability of yeasts is a well-documented phenomenon and studies aimed at evaluating their potential as biocatalysts or sources of biocatalysts are still a very attractive field. In particular, the exploitation of extremophilic microorganisms from marine environment can represent a reliable alternative to mesophilic systems. Microorganisms from extreme environments are, in fact, thoroughly adapted for surviving and growing under severe conditions and, in particular, halotolerant microorganisms producing enzymes of potential industrial relevance are of great interest for developing biotransformations in high-salinity media and even in seawater. Moreover, it has been shown that there is a correlation between salts tolerance and solvents tolerance, making biocatalysts from halophilic microorganisms even more attractive.
A collection of marine yeasts isolated from deep-subseafloor sediments have been studied for their ability to grow in presence of high concentrations of NaCl (≤ 12%) and for some interesting biocatalytic traits.[1] In this work, the same collection was used for the individuation of nitrile hydrolyzing enzymes. Synthetic nitriles are widely employed in organic synthesis as precursors of various amides and acids and their conventional chemical hydrolysis requires harsh reaction conditions (acidic or alkaline pH and temperature above 100°C) and is plagued by the formation of by products and large amounts of waste. An alternative to this process is represented by nitrilases and/or nitrile hydratases, since they can directly convert a wide range of nitrile compounds into the corresponding carboxylic acids or amides under mild conditions and with high selectivity.

[1] Serra I et al. ChemCatChem 8, 3254-3260, 2016

P72

ASSIMILATION PROFILE OF TWO CANDIDA WILD-TYPE STRAINS TO FERMENT CARBOHYDRATES MIXTURES TO ALCOHOL

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The efficient fermentation of the carbohydrates present in the vegetable biomass to produce ethanol requires microorganisms capable of use hexoses and pentoses. The objective of this work was to determine the carbohydrate assimilation profile of two wild yeasts from a mixture of sugars in proportions similar to those found in citrus residues[1].The wild yeasts used were isolated from bovine ruminal fluid (LR2) and from termites stomach (T1). Genomic DNA was extracted from using the bromuro de hexadeciltrimetilamonio (CTAB) method, and was amplified by PCR using ITS4R and ITS5F primers. Clones were obtained from T1 and LR2 that were sequenced and identified as *Candida glabrata* with different phylogenetic distances. The Yeasts was inoculated in a medium based on nitrogen, mineral salts and a mixture of carbohydrates (51% glucose, 30% fructose, 8% galactose, 7% arabinose, 2% xylose and 2% sucrose). Both strains were able to assimilate all sugars present in the medium but exhibited statistically significant differences in the percentage consumed of each of them. T1 had a greater consumption of the sugars with exception of the arabinose which was consumed in greater quantity by LR2. The consumption of sugars from highest to lowest was: glucose, fructose, xylose, arabinose and galactose for T1, while for LR2 was glucose, fructose, arabinose, galactose and xylose. The alcohol production was slightly higher (24.28 g / l) with T1 with respect to LR2 (21.4 g / l). However, the maximum alcohol productivity was higher with LR2 (5.8 g/l.h) compared to the T1 (4.1 g/l.h).

P73

Extracellular production of novel β-fructofuranosidase enzymes from two mezcal- fermenting yeasts in *Pichia pastoris*

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In the process for making mezcal, the pines (stems) of the agave plants are cooked causing a non-complete hydrolysis of the stored fructans and the generation of Maillard compounds. In a second step, spontaneous fermentation is conducted by resident microorganisms, mainly yeasts. Depending on their action mode and substrate specificity, microbial fructan-degrading enzymes may be are useful for the production of either fructose syrups or prebiotic fructooligosaccharides. In this study, *Pichia pastoris* was engineered to constitutively express and secrete two novel β-fructofuranosidase enzymes from the mezcal-fermenting yeasts *Torulaspora delbrueckii* and *Candida apicola*. Genome sequencing and transcriptome analysis allowed us to identify putative β-fructofuranosidase genes in the two yeast strains. Sequence alignments revealed that the encoded enzymes of *T. delbrueckii* (65.28 kDa) and *C. apicola* (59.31 kDa) have 53 and 42 % of identity to *Saccharomyces cerevisiae* invertase, respectively, both containing the characteristic domains conserved at the active site of glycosyl-hydrolases family 32 (GH32). The presence of a typical N-terminal in the two enzymes suggests an extracellular location in the native hosts. The cloning of the entire gene sequence in the constitutive expression vector pGAPZB allowed protein secretion in *P. pastoris*. High activity yields of both *T. delbrueckii* and *C. apicola* fructofuranosidases were obtained in the *P. pastoris* culture supernatant reaching 154 and 156 U/ml respectively. Interestingly, only 66 and 1.64 U/ml were obtained in the supernatant of the wild strains. Experiments for the biochemical characterization of the two recombinant enzymes are in progress.

P74

Searching for new yeast strains efficiently by producing 2-phenylethanol in a whey-based medium

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Growing awareness among the general public increases the demand for healthy food and cosmetics free from artificial compounds, e.g. flavorings. Due to high costs, only a small portion of natural aromas are obtained by extraction from plants and, on an industrial scale, these substances are primarily produced by chemical synthesis. Therefore, there is a need to develop alternative methods for the production of natural flavorings, including biotechnological processes based on microbial activity.

2-Phenylethanol (2-PE), which is one of the most commonly used flavorings because of its rose scent, might be produced by yeast. However, the cost of biotechnological production of 2-PE is not attractive when compared with chemical synthesis [1]. One way of reducing costs might be the utilization of organic waste from the food industry, such as whey.

In the present study, we screened our yeast collection for strains able to grow and produce 2-PE in a whey-based medium. In effect, we selected eight yeast strains assigned to *Saccharomyces cerevisiae*, *Meyerozyma carribica*, *Pichia fermentas* and *Metschnikovia sp*., which seem to be promising 2-PE producers since they are able to achieve a 2-PE titer of 1.1 – 2.6 g/ L in simple and non-optimized batch cultures.

P75

Yeast Community Associated with Wild Tiger Lily (Lilium lancifolium Thunb.) and Characterization of Biosurfactant-Producing Yeast

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Identification of yeast species that have potential applications in biotechnology is greatly interesting. In this study, we investigated the yeast biota associated with wild tiger lily (Lilium lancifolium Thunb.). The first step in the identification of the yeast biota is the establishment of an effective isolation method. To this end, we compared the efficacy of different yeast media for the isolation of yeasts associated with wild tiger lily, and the media included antibiotics and fungistatic agents for the suppression of fungi. We isolated yeast species from flowers, leaves, and stems of the wild tiger lily because these niches have not yet been used for determining the yeast biodiversity. We were able to isolate 97, 94, and 82 yeast strains from the flowers, stems, and leaves, respectively. Yeast isolates were identified by phylogenetic analysis based on internal transcribed spacer region sequencing. Yeasts produce biosurfactants (BSs), which are important amphiphilic compounds that are used in the agricultural industry as well as cosmetic and pharmaceutical industries because of their low toxicity, biodegradability, and both commercial and academic interests. Using these isolated yeast strains, we developed rapid and simple screening methods for BS-producing yeast (BSPY) to design processes for the characterization of high-value yeast BSs and production of eco-friendly BSs. We screened many BSPYs from wild, and developed fermentation processes for BS production. Analysis of the chemical structure of these compounds by mass spectrometry and nuclear magnetic resonance revealed several potential novel BSs, including glycolipids.

P76

High-temperature ethanol production by *Kluyveromyces marxianus.*

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High-temperature fermentation can bring advantages to industrial fuel ethanol production in sugarcane mills, mainly due to the possibility of decreasing cooling costs, water usage and contamination levels. Based on that, thermotolerant yeasts have been studied, both at the phenotypic and at the genomic levels. Such studies could lead to the industrial use of a naturally thermotolerant yeast strain or to the metabolic engineering of non-thermotolerant yeasts, aiming at increasing their thermotolerance. *Kluyveromyces marxianus* encompasses several already described thermotolerant strains. This species has been described as a very rapid growing microorganism, displaying the capacity to assimilate various sugars, and respiratory-fermentative metabolism. We compared some previously selected *K. marxianus* strains with *Saccharomyces cerevisiae* for ethanol production at different temperatures. *K. marxianus* NCYC3396 and UFV-3 presented a similar ethanol yield on glucose at 48 °C, when compared to S. cerevisiae at 37 °C. The possibility of performing the industrial process at 48 °C is highly desired, since at this temperature the contaminants growth decreases sharply. We also performed genome comparisons among six different yeast species with the aim of identifying gene families potentially involved in the trait of thermotolerance. As a result, three genetic families were identified as being exclusively shared between *K. marxianus* and *Ogataea polymorpha*, which could be related to thermotolerance. This study reveals a non-*Saccharomyces* strain able to be potentially employed for high-temperature fuel ethanol production. Furthermore, genes potentially related to thermotolerance will be engineering into *S. cerevisiae*, in order to verify whether this could lead to increased its thermotolerance.

P77

Fermentative capacity of wild yeasts in *Opuntia ficus-indica* cladode flour medium like sole carbon source

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The genus Opuntia has the potential to be used as a raw material for alcohol production, their composition consists in polysaccharides such as cellulose, hemicellulose and mucilage[1]. The objective of this work was to compare four wild yeasts growth, and alcohol production, using Opuntia ficus-indica medium. The four yeasts were grown in medium with *Opuntia ficus-indica* cladodes flour (as the only source of carbon) at 10 and 20%. The yeasts previously isolated from ruminal fluid (LR2 and LR5) and termite stomach (T4 and T6) have been identified as *Candida glabrata* and *Kluyveromyces marxianus*, respectively. The maximum populations were obtained in mediums with 10% for all yeasts. The T4 had a maximum population at 24 hours, although it presented a lag phase of 8 hours. In contrast, the others did not present this lag phase but had a smaller growth. The population obtained in 20% of flour was about half to that obtained with 10% and was reached at 12 h for LR2 and LR5, and at 28 h for T4 and T6. In all cases the yeasts were able to produce alcohol, this production was higher in the medium with 20% of flour. The T4 was the major producer of alcohol in both percentages, followed by the T6 with the highest productivity of alcohol. The alcohol production was faster (4 - 8 h) with 10%; however this alcohol is later consumed by the yeasts. On the other hand, in the media at 20%, the alcohol produced remains in the medium.

P78

Investigation of technological, nutritional and sensorial characteristics of wheat bread influenced by different strains of *Saccharomyces cerevisiae*

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Bread is an important staple food in the human diet and besides brewing, yeast fermentation and therefore leavening of dough is one of the oldest biochemical processes in the world. Surprisingly, knowledge about dough fermentation in correlation to product quality parameters is scarce and still not completely understood. Only limited effort has focused on the investigation of the technological performance of different *Saccharomyces cerevisiae* strains in comparison to "Baker's yeast" in baked applications. While yeast selection is an established part of the production process in the beverage industry, less attention was drawn on dough fermentation. In this study, the suitability of various *S. cerevisiae* strains originating from the beverage industry were investigated to enhance bread quality. The results revealed that various yeast strains showed large differences in technological bread quality characteristics. Furthermore, glycaemic index (GI) of white wheat bread can be significantly decreased. PCA confirmed that the breads were different in terms of their technological properties, chemical composition and GI. In addition yeast strain dependent production of aroma compounds was shown, leading to a change in consumer acceptance. Due to their specific metabolite production, another important aspect are the process parameters (fermentation time and temperature). Therefore, response surface methodology (RSM) was used as a model design. A change in fermentation parameters showed further improvement of breads in terms of shelf life, GI and consumer acceptance. This study opens alternatives to better satisfy the high demand of consumers for an increasing variety of bread products by only changing the yeast culture.

P79

Deciphering the Genetic and Metabolic Bases of Yeast Aroma Properties

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During wine fermentation the yeast Saccharomyces cerevisiae plays a vital role in the conversion of grape derived non-aromatic precursors to aromas, and the formation of aroma compounds, like esters, higher alcohols and organic acids. To identify the genomic and metabolic bases for these processes, we performed a cross between two wine yeast strains, selected on the basis of their different need for nitrogen during fermentation. 130 F2-segregants were individually genotyped by whole genome sequencing and phenotyped during wine fermentation by measuring extracellular metabolites. Intracellular metabolic fluxes were estimated by constraint-based modeling. Quantitative trait locus (QTL)-mapping was used to identify allele variations influencing the aroma profile and the metabolic fluxes.
Most traits were transgressive and normal distributed among the population, indicating the interaction of several genes and alleles with opposite effects. Using linkage analysis we detected 97 QTLs on 46 loci explaining 55 quantitative traits. For some traits, for example propanol production, we furthermore detected interacting QTLs. By performing reciprocal hemizygosity analysis we could identify 13 allelic variants influencing the production of characteristic sensorial compounds, like amyl acetate, isoamyl alcohol and isoamyl actetate, as well as industrial relevant chemicals, like higher alcohols and fatty acids. In addition we identified and validated several alleles having a broad influence on intracellular metabolic fluxes, like metabolite transports, glycolytic fluxes and TCA pathways.
The identification of these genetic determinants increases knowledge of the links between genetic variation and industrial traits and provides a valuable foundation for the development of optimized strains by marker-assisted breeding strategies.

P80

"Improving a sustainable palm oil substitute from *Metschnikowia pulcherrima*"

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Palm oil it is a widespread product which demand keeps growing due to its unique characteristics and its low price. However its production it is already unsustainable and putting at risk several species. *Metschnikowia pulcherrima* has a high potential for industrial biotechnology due to its ability to produce multiple interesting metabolites such as lipids and its resistance to many stresses taken place in bioreactors. However as non-conventional yeast, there remains an information deficit. Our work will focus on shedding light on the regulation of the lipid production pathway which seems to be very dependent of nitrogen availability. The study of promoters with a role in these pathways will lead to develop genetic techniques which allow us to improve its lipid production. In order to do that bioinformatics approaches and genetic tools will be used. Bioinformatic goals include prediction of metabolism and generation of targets for manipulation – either genetic targets or environmental targets. Goals for development of genetic tools include control of expression and improved methods for modifying genes via editing or homologous recombination.

P81

Nixtamalization waste (nejayote) treatment using membrane technology and bioethanol production using its suspended solids

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Nixtamalization is the maize kernel lime-cooking process. In Mexico, Nixtamalization industry produced yearly between 16 and 22 million cubic meters of wastewater known as Nejayote[1].

Nejayote were hydrolyzed using amylase after that was added flocculants to remove some dissolved and not dissolved solids to obtain a paste after filtration. The supernatant recovery was filtered using a 5kDa MWCO ultrafiltration membrane to removed yeast inhibitors and the permeated was enriched for sugars. The paste was hydrolyzed using celluloses and hemicelluloses enzymes[2, 3]. The carbohydrates obtained after hydrolyzed were 77% retrieved in the retention fraction of the ultrafiltration. Reverse osmosis of permeate retrieved 16% of the initial Nejayote volume as water. The retention fraction and the hydrolyzed paste were mixed to obtain a fermenting must.

Moreover, was carried a screening of different yeast species in YPD to select inhibitor yeast resistant to ferulic and *p*-cumaric acid, thermotolerant and pentose assimilation were also evaluated to perform a simultaneous saccharification and fermentation process with the must produce. Five non-*Saccharomyces* yeast were selected for the fermentative ability, because the *Saccharomyces* yeast couldn´t grow in the inhibitors medium.

The selected strains of *K. marxianus* achieved a 0.154 g EtOH/g dry mass yield with a productivity of 0.854 g EtOH/Lh in the simultaneous process, similar to those reported in the literature using other lignocellulose residues also increased the productivity 3 times for the saccharification-fermentation simultaneous.

It is possible produced bioethanol from nejayote waste after filtration and using non-*Sacchoaromyces* yeast increasing the yield and productivity.

P82

A genetic breeding concept for exploration of the biodiversity of non-laboratory yeast strains, applied to heterologous protein production

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Saccharomyces cerevisiae is a widely used host for heterologous protein production. Here, we present a concept for introduction of several genetic modifications during breeding of new S.cerevisiae strains, enabling high-throughput screening for novel strains with improved protein production traits.
A number of genetic modifications, required or beneficial for heterologous protein expression, were introduced into a MATa laboratory strain using dominant selection markers. The genes encoding two important proteases (PEP4 and YPS1), TPI1 (which is used as plasmid selection marker), and the CAN1 ORF were replaced with dominant markers, flanked by loxP sites. In addition, the strain harboured a multi-copy vector for expression of the heterologous protein. This genetic trait donor strain was then crossed to a number of haploid S. cerevisiae strains of opposite mating type and with alternative genetic backgrounds to obtain heterozygous diploid strains. Following sporulation, haploid offspring containing all the desired deletions and the expression plasmid, could be isolated by selection on medium containing all relevant antibiotics. The dominant markers were afterwards removed by expression of a Cre recombinase, resulting in marker-free strains.
Screening for protein production by the haploid strains obtained in this way resulted in a surprisingly large diversity of target of interest product yields, with yields varying by 10-fold between strains and allowing for isolation of new strains with approximately 2-fold higher product yields than any of the parental haploid strains. In another case, we increased the expression yield of a difficult-to-express model protein by 5-fold using this approach.

P83

IDENTIFICATION OF WILD YEASTS IN ECUADORIAN CHICHAS

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Ecuadorian chichas are a variety of homemade beverages with a low alcoholic content, derived from the spontaneous fermentation of cereals (mainly maize) and/or fruits. These substrates are important habitats for bacteria and wild yeast populations that have not been fully characterized. Some of them may be related with specific organoleptic characteristics of the final product.
The aim of this study was to identify different species of yeast with fermentative potential, present in the natural microbiota of four Ecuadorian chichas of rice, oats, grape and yamor (traditional indigenous beverage which is brewed using seven varieties of corn).
Samples of the four types of Ecuadorian chichas, prepared under controlled conditions, were taken at three time-points of fermentation: initial (day 3), tumultuous (day 7) and final (day 14). A total of 254 yeast isolates were identified by conventional microbiological analysis and by polymerase chain reaction - restriction fragment length polymorfism (PCR - RFLP) of ITS1-5.8S rDNA - ITS2; results were confirmed by sequencing. Interdelta and mitochondrial DNA analysis for Saccharomyces cerevisiae strain characterization was also performed.
Twelve yeast genera were isolated from Ecuadorian chichas samples. The most representative yeast species were: *Hanseniaspora guillermondii, Hanseniaspora opuntiae, Saccharomyces cerevisiae, Torulaspora delbruecky, Pichia kluyveri, Candida* sp. and *Rhodotorula mucilaginosa*. *Hanseniaspora* species were dominant at the beginning of fermentation in all the analyzed beverages. *Saccharomyces cerevisiae* isolates were abundant in rice chicha and Torulaspora delbruecky isolates dominates in grape chicha. In further investigations we will study the potential industrial applications of these new yeast strains.

P84

Lactic acid stress imposes changes in Zygosaccharomyces parabailii transriptome

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Lactic acid represents great interest as a building block for bioplastic production. Nevertheless, weak acids are also well known to inhibit fermentation and induce cellular stress slowing down microbial growth and performance. Here we are interested in understanding how an interspecific hybrid strain of Zygosaccharomyces parabailii responds and tolerates this acid to eventually use it for lactic acid production. Most of the work on weak acid stress response has been studied in Saccharomyces cerevisiae so there is very little information on non-Saccharomyces yeast species. Zygosaccharomyces bailii, one of the parental species of our hybrid strain, is well known food spoilage yeast with remarkable ability to withstand low pH and high weak acid concentrations. For this reason, Z. bailii and are important yeast species to study lactic acid tolerance mechanism for biotechnological exploitation. Our group has recently revealed that lactic acid inhibits Z. parabailii fermentation inhibition without compromising cell viability. Moreover, we found macromolecular changes in the cells related to cell membrane and wall composition. In this study, we analyzed the transcriptional response of Z. parabailii cells upon lactic acid exposure using RNAseq and our recently obtained hybrid genome assembly and annotation. Differential expression analysis revealed Iron homeostasis significantly responsive to lactic acid stress. In addition, we found intriguing differential gene expression patterns of genes related to the cell membrane composition and major facilitator superfamily (MFS). RNAseq is a powerful tool for comprehensive analysis of important aspects of Z. parabailii robustness, which will be useful in biotechnology and food microbiology applications.

P85

Evaluation of antagonistic effect and mechanism of endophytic yeasts against plant pathogenic fungi

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In recent years, various researches have been conducted to find microorganisms for agriculture application. Among these, endophytic yeasts have been reported for their potential in controlling of various plant diseases caused by fungal pathogens. Therefore, this work aimed to investigate the antagonistic effect and possible antagonistic mechanism against plant pathogenic fungi of endophytic yeasts from leaf tissue. A total of 269 endophytic yeast strains, which consisted of 121 and 148 strains from rice and sugarcane leaves, respectively, were investigated for their antagonistic effect against plant pathogenic fungi by a dual culture method. The plant pathogenic fungi used in this work were five rice fungal pathogens (*Curvularia lunata*, *Fusarium moniliforme*, *Helminthosporium oryzae*, *Pyricularia grisea* and *Rhizoctonia solani*) and two sugarcane fungal pathogens (*Colletotrichum falcatum* and *F. moniliforme*). Only nine strains from rice leaves inhibited mycelial growth of fungal pathogens by 29.0-79.4%. Based on molecular identification, seven strains were identified as *Wickerhamomyces anomalus* and four strains were *Kodamaea ohmeri*. *W. anomalus* showed inhibition on mycelial growth of the three rice fungal pathogens (*C. lunata*, *F. moniliforme* and *R. solani*) and the two sugarcane fungal pathogens (*Co. falcatum* and *F. moniliforme*). All four strains of *K. ohmeri* could inhibit only *F. moniliforme* causing rice disease.

Further evaluation revealed their antagonistic effects were based on the production of antifungal volatile compounds, β -1, 3-glucanase, chitinase and siderophore. The nutrient concentrations had significant effect on fungal growth inhibition by these antagonistic yeast strains and their living cells were necessary for the antagonistic activity.

P86

Yeast diversity and susceptibility to organic acids during spontaneous fermentation of mawè, a cereal-based dough produced in West Africa

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Mawè is a West African spontaneously fermented cereal-based dough, which is dominated by lactic acid bacteria and yeasts. The succession and diversity of yeasts involved in mawè fermentation have, however, only been sparsely investigated. In four types of mawè from eight production sites in southern Benin, yeasts were identified and successions during the fermentations were determined. Furthermore, susceptibility of different identified yeasts to the organic acids found in mawè was studied. Isolated yeasts (334) were grouped by $(GTG)_5$-based repetitive PCR followed by 26S rRNA gene sequencing and ITS sequencing for Kluyveromyces marxianus. During the fermentation of all mawè types the average yeast counts increased from 4.8±0.75 to 7.4±0.41 $\log_{10}$ cfu/g from 0h to 36h, respectively, whereas the pH decreased from 5.4±0.52 at 0h to 4.1±0.30 at 36h. The predominant yeasts throughout all mawè fermentations were Pichia kudriavzevii, followed by K. marxianus and Saccharomyces cerevisiae. A minor part of the yeasts were Ogataea polymorpha, Candida glabrata and Wickerhamomyces anomalus. P. kudriavzevii and K. marxianus were present at all stages of fermentation with highest occurrence at the intermediate stage (6h, 12h and 24h), while S. cerevisiae occurred mostly at the end of the fermentation. When exposed to organic acids in concentrations found in mawè, S. cerevisiae, P. kudriavzevii and K. maxianus showed a lower tolerance, whereas C. glabrata had a slightly higher tolerance. This constitutes the first step for clarifying the role of yeast species during the fermentation and for ensuring growth of beneficial yeast species while preventing growth of opportunistic pathogens.

KEYWORDS: Mawè, spontaneous fermentation, yeasts, microbial succession

P87

Nondairy beverage produced by controlled fermentation withpotential probiotic starter cultures of lactic acid bacteria and yeast

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This work aimed to develop a nondairy fermented beverage from a blend of cassava and rice based on Brazilian indigenous beverage cauim using probiotic lactic acid bacteria (LAB) and yeast. The indigenous strains Lactobacillus plantarum CCMA 0743 (from cauim) and Torulaspora delbrueckii CCMA 0235 (from tarubá), and the commercial probiotic, L. acidophilus LAC-04, were used as starter cultures in single and cocultivations. The bacteria populations were around 8.0 log (CFU/mL) at the end of all fermentations as recommended for probiotic products.
Higher residual starch contents were noted in the single LAB cultures (10.6% [w/w]) than in co-cultures (< 6% [w/w]), showing that co-culture may help the digestibility. For all different assays (single and coculture), lactic acid was the main organic acid detected (> 1.6 g/L) and ethanol was lower than 0.5% (w/v) consisting in a non-alcoholic beverage.
The assays containing yeast showed the highest antioxidant activity (around 10% by DPPH and ABTS methods). Therefore, a nondairy fermented beverage was successfully obtained, and the co-culture of LAB and T. delbrueckii could increase the product's functional properties.

P88

Effect of pH and salinity on biofilm-like phenotypes of yeasts isolated from fermented olives

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The yeast biofilm-like phenotypes such as *media invasiveness* and *mat colony* are considered mechanisms to colonize and persist in environmental niches. In table olive fermentation, pH and salinity are two hurdles that yeasts have to overcome to succeed in developing a stable population. Eight yeast strains - among which *Candida*, *Pichia*, and *Wickerhamomyces* genera - isolated from table olive fermentations, and the two control strains of *Saccharomyces cerevisiae* ∑1278b and BY4742 were tested in YPD agar either carbon-rich (glucose 2%) or -deficient (glucose 0.1%) media also modified both for pH (4.3) and salinity (NaCl 5%) to simulate the brine. Conventional (2%) and low (0.3%) agar concentrations were used to study invasiveness and mat colony formation, respectively. The majority of the strains showed bigger mat colonies with 2% of glucose than 0.1%. Three strains exhibited an increase in area colonies growing in the modified media. The pH and salinity modifications determined invasive growth for six and two strains in the presence of either 2% or 0.1% of glucose, respectively. Two strains were unable to invade media. A wide diversity was observed among the strains and media; moreover, some of the strains displayed the two biofilm-like phenotypes in dissociated way. The observed phenotypic diversity could confer strain advantage during the olive fermentation process. Our results may be taking into account to select strains to drive fermentation process.

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P89

IMPAIRING GLYCEROL SYNTHESIS IN YEAST TO IMPROVE ETHANOL YIELD

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Ethanol production by fermentation of sugars using yeast is a known technology since thousands of years. Today the world is looking forward towards ethanol; as renewable fuel which can substitute gasoline. Industrial ethanol is produced in huge volumes. A desired aspect of an industrial ethanol production process is high yield. One way to improve ethanol yield is by reducing the formation of by-products like glycerol during fermentation. By reducing glycerol synthesis one can channelize the sugars towards ethanol. Here in this study two approaches were used to modify yeast in order to reduce glycerol synthesis. A set of knock-out strains was developed by deletion of genes involved in synthesis and regulation of glycerol. In the next approach the genetic diversity observed in yeast for quantitative traits like glycerol synthesis is exploited. Thus a set of knock-in strains has been developed by introducing SNPs at specific sites in within the targeted genes. The experiments were carried out in an industrial yeast strain of *Saccharomyces cerevisiae*. All strains were tested for glycerol and ethanol yields in an industrial process. It has been reported that impairing glycerol synthesis by deletion of genes like *GPD1* makes the yeast osmosensitive. Therefore the osmosensitivity of the modified strains was also tested. The modified strains had an improvement of ethanol yield with a reduction in the glycerol yield and had unaffected osmosensitivity compared to parent strain.

P90

Unravelling the melatonin biosynthetic pathway in *Saccharomyces cerevisiae*: pulses of different indolic compounds

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Melatonin (MEL) or N-acetyl-5-methoxytryptamine is a biosynthesized indolamine hormone from L-tryptophan in mammals, plants and fungi. This hormone has been recently found in beverages and food fermented products such as orange juice, bread and beer. It is considered a bioactive compound due to its antioxidant capability. In a previous study, MEL was found in wine but not in the grapes used for this vinification. This discovery involved wine yeasts as MEL producer in this kind of food during fermentation process. It is not well known about MEL production in yeast or its physiological role. In other organisms, the biosynthetic pathway is well defined from the L-Trp as a precursor. MEL biosynthesis requires four-step reactions. However, six genes have been implicated in the synthesis of melatonin in plants, suggesting the presence of multiple pathways.

We are trying to unveil the MEL biosynthesis in *Saccharomyces cerevisiae.* For this aim, we are carrying out several pulses with the intermediates of the pathway in cells with different growth stages (exponential and arrested cells in a SC and salt medium, respectively). The extra- and intracellular content of the cells was analyzed by HPLC-MS/MS. Our results showed that in yeast, the pathway is not lineal as it is in mammals. The results suggest that the pathway is integrated by different branches as we can infer from the conversion of non-conventional intermediates to MEL. This alternative pathway is more similar to the plants biosynthesis showing the complexity of this biochemical route and the need of further analysis.

P91

Biolipid production from lignocellulose and biodiesel-derived glycerol by oleaginous yeasts

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Oleaginous yeasts can accumulate lipids to more than 50% of their biomass, and they can produce biolipids for biodiesel-, food and animal feed production from lignocellulose hydrolysate or residual crude glycerol from biodiesel production without utilisation of arable land. Our research aims to produce biolipids from lignocellulose (especially hemicellulose) and crude glycerol.
We have tested a variety of ascomycetous and basidiomycetous oleaginous yeast strains for growth and lipid production on hemicellulose from wood [e.g. 1], or other resources, as well as from crude glycerol. Novel methods for in situ determination of lipid content and composition were established. Basidiomycetous yeasts (*Rhodotorul*a and *Rhodosporidium* spec.) usually produced lipids more rapidly and to higher concentrations from lignocellulose and crude glycerol than ascomycetes (*Lipomyces* spec.). Fermentation techniques were introduced to test lipid production under reproducible conditions [1]. When regarding a biorefinery approach, i.e. combining lipid production with biogas and electricity generation from fermentation residues, an energy balance similar to ethanol production was achieved [2]. Moreover, lipids from L. starkeyi could also be used as plant oil replacement in fish feeds.

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P92

Microsatellite genotyping reveals grouping of *Lachancea thermotolerans* isolates based on their geographic origin and isolation habitat

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Scientific interest in non-*Saccharomyces* yeasts is on the rise, as their uncommon physiological, metabolic and cellular functions warrant their further exploration and, ultimately, biotechnological application. One species with remarkable biotechnological potential about which little is known is *Lachancea thermotolerans* (formerly *Kluyveromyces thermotolerans*). This ubiquist occupies diverse ecological niches worldwide, including plant material, soil, insects, and horticultural crops, in particular grapes. In wine fermentations, *L. thermotolerans* in association with *S. cerevisiae* can lead to wine acidification, lower ethanol content and increased aromatic complexity. To gain an insight in the species' population diversity and structure, *L. thermotolerans* isolates were sourced from a range of natural and anthropic habitats, covering a wide geographic span. These were analysed using a set of 14 microsatellite markers, nine of which newly developed for *L. thermotolerans* genotyping. Of the 172 isolates, 136 distinct genotypes were observed, confirming the discriminatory power of the microsatellite analysis. Grouping of isolates could be observed based on both their continent/region of origin and isolation substrates. Analysis of molecular variance (AMOVA) confirmed that geographic origin and habitat significantly shaped *L. thermotolerans* diversity, accounting for 20.43% and 13.58% of variation, respectively (P values < 0.0001). Observed clustering was additionally supported by phenotypic tests conducted using different growth substrates and physicochemical conditions. Follow-up studies are providing in-depth phenotypical characterisation of strains, primarily in an oenological context, further testing the genotype-phenotype inter-relationships.

P93

Biodiversity bioprospecting for xylitol production: selection, physiological and genetic characterization of new yeast strains

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Growing demand for chemical products and environmental friendly processes has motivated the search for alternative solutions and renewable raw materials to replace some conventional processes. In this way, the lignocellulosic biomass has a great potential to be used for production of biofuels and value added chemicals. Xylitol is a five-carbon polyol with a wide-range of applications in odontologic pharmaceutical and food industries. At industrial scale, it is produced chemically by an expensive process of xylose hydrogenation at high temperatures and pressures, using nickel metal as catalyst. Since some yeast are able to convert xylose to xylitol, microbiological fermentation processes can be an interesting alternative to produce xylitol with lower cost. Considering this context, we prospected 400 yeast strains from Brazilian biodiversity for their ability to utilize xylose for growth. Following, the best performing strains were characterized physiologically. Xylose consumption profile and xylitol production by these cells were determined during cultivation in bioreactors using mineral medium and sugarcane bagasse hydrolysate under different aeration conditions. Sequencing of 26S r-DNA demonstrated that these strains are close related to the clade *Spathaspora* sp. and *Meyerozyma* sp. The new strains did not produce significant quantities of ethanol from xylose, but the xylitol production reached yields up to 0,70 $g.g^{-1}$, showing similar performance with strains commonly used for xylitol production. To better understand the genetic background of two yeasts, whole genome sequencing was performed and a comparative analysis was carried out. Data on genomes size, annotation and gene synteny will be presented and discussed.

P94

Metabolic engineering of *Saccharomyces cerevisiae* for the production of succinic acid using glycerol as a carbon source

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The advantage of using glycerol as carbon source in industrial biotechnology results from the fact that its conversion into the glycolytic intermediate pyruvate produces double the amount of reducing equivalents compared to glucose. This, in general, enables higher maximum theoretical yields of metabolic products whose production pathways require the respective reducing equivalents (e.g. NADH). One example for such a product of high commercial interest is succinic acid. Succinic acid, a 1,4-dicarboxylic acid, is regarded as one of the most important bio-based platform chemicals due to its numerous potential applications, such as its conversion to industrially valuable chemicals including adipic acid, tetrahydrofuran, gamma-butyrolactone, polyester polyols, and 1,4-butanediol. However, the industrial platform organism *Saccharomyces cerevisiae* naturally utilizes an FAD-dependent pathway for glycerol catabolism. This pathway transfers the electrons directly via $FADH_2$ to the mitochondrial respiratory chain with oxygen as the final electron acceptor. However, for the production of succinic acid, electrons derived from glycerol oxidation should be saved in the form of cytosolic NADH. Therefore, we used CRISPR-Cas9 to replace the native FAD-dependent pathway with an NAD^+-dependent route. Here, we present our current achievements towards the production of succinic acid from glycerol by engineering the reductive TCA pathway in the genetic background of a CEN.PK strain in which the glycerol catabolic pathway had been replaced as described above.

P95

DEVELOPMENT OF THE RECOMBINANT *SACCHAROMYCES CEREVISIAE* STRAIN FOR GLYCEROL PRODUCTION UNDER ANAEROBIC CONDITIONS

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Glycerol is widely used in cosmetic, paint, automotive, food, tobacco, pharmaceutical industries. Glycerol can be produced by microbial fermentation from sustainable carbohydrate feedstocks. Yeast *Saccharomyces cerevisiae* could be used for cost-effective glycerol production under anaerobic conditions. In *S. cerevisiae* glycerol synthesis occurs from dihydroxyacetone phosphate by subsequent action of glycerol-3-phosphate dehydrogenase (Gpd1) and glycerol-3-phosphate phosphatase (Gpp2). Synthesized glycerol is exported via channel formed by aquaglyceroporin Fps1. But, in physiological conditions the major part of intracellular dihydroxyacetone phosphate is isomerized to glyceraldehyde-3-phosphate by triose phosphate isomerase (Tpi1) and subsequently converted to pyruvate and eventually to ethanol. Enzyme acetolactate synthase (Ilv2) can convert pyruvate to acetolactate and CO2 thus decreasing amount of pyruvate available for alcohol dehydrogenase reaction which competes for NADH with Gpd-reaction.
The recombinant *S. cerevisiae* strain BY/tpi25/gpd1gpp2f/fps1m/ilv2 was constructed as follows: homologous recombination was used for partial substitution of *TPI1* gene promoter region with selective marker; multicopy integration module was used for expression of hybrid fused *GPD1-GPP2* ORF under the control of strong promoter; modified gene *FPS1* (with eliminated part encoding amino acid residues 76-230) and truncated gene *ILV2* were expressed under the control of *ADH1* promoter. This strain possessed reduced Tpi1 activity, increased Gpd1 and Gpp2 activities, constantly active form of Fps1 channel and cytosolic form of Ilv2. As a consequence, it accumulated up to 9 times more glycerol under micro-aerobic conditions and up to 4.7 times more glycerol under anaerobic conditions in comparison to the parental strain BY4742.

P96

Engineering of Cytosolic FeS Cluster Assembling for Increased Isobutanol Production in *Saccharomyces cerevisiae*

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Due to the limited nature of fossil fuel resources the demand for renewable bio-based chemicals for e.g. industrial applications and renewable energy will increase steadily. Here we will present the production of isobutanol, a platform compound from which multiple products with high market potential can be launched. Isobutanol can be converted synthetically to isobutylene, or can directly be used as solvent or drop-in fuel. Although high isobutanol yields were already achieved in bacteria, the production of isobutanol in yeast still lacks high yields. Nevertheless, *Saccharomyces cerevisiae* has certain advantages in industrial processes due to its tolerance to alcohols and to the harsh conditions present during the fermentation of lignocellulosic hydrolysates. In *Saccharomyces cerevisiae*, isobutanol is produced by a combination of the mitochondrial de-novo synthesis of valine and its cytosolic degradation. It has been shown that re-locating the anabolic enzymes acetolactate synthase (Ilv2), acetohydroxyacid reductoisomerase (Ilv5) and dihydroxyacid dehydratase (Ilv3) from the mitochondrial matrix into the cytosol increases the isobutanol production. However, our findings showed that competing pathways, co-factor imbalances, secretion of intermediates and cytosolic FeS cluster assembly on Ilv3 are still major issues that prevent high isobutanol yields in yeast.
Here, will mainly present strategies to enhance the cytosolic FeS cluster incorporation into Ilv3, in order to increase the conversion of 2,3-dihydroxyisovalerate (DIV) to α ketoisovaleric acid (KIV). Ilv3 seems to be the limiting step during isobutanol production, as DIV is secreted in high amounts into the fermentation medium.

P97

Engineering industrial *Saccharomyces cerevisiae* strains for ethanol production from starchy substrates and by-products

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The production of first generation bioethanol is currently obtained from various starchy substrates with a mature technology. Commercial biofuel established in the USA is currently produced from conventional corn dry-grind. This process requires exogenous enzymes, like alpha-amylases and glucoamylases, to breakdown the starch molecules into glucose, which is then fermented to ethanol by yeast *S. cerevisiae*. However, starch-to-ethanol processes are still expensive and the development of genetically engineering industrial yeast expressing glucoamylase enzymes could reduce commercial costs. This new class of engineered yeast allows an implementation of a consolidated bioprocessing strategy that significantly reduces enzyme cost during bioethanol production.

After an examination of typical fuel ethanol production processes, two fungal glucoamylases were identified for the complementarity of their enzymatic characteristics. The corresponding glucoamylase genes were cloned successively in multi-copy into the genome of a well-known industrial yeast strain, Ethanol Red. The recombinants obtained, displayed high amylolytic activities on liquefied corn and were able to ferment without exogenous cocktail in 50 hours. The best recombinant strain (or the recombinant strain selected) was effective for simultaneous saccharification fermentation (SSF) on different industrial mashes. Substitution of the conventional yeasts with AMG yeast reduced the amount of exogenous glucoamylase required for efficient fermentation by around 30-40%.

With these efforts, a new yeast product, ER-Xpress®, was launched by Lesaffre business Unit, LEAF and became available on the U.S. for bioethanol industry.

KEYWORDS: Industrial yeast, Glucoamylase, Biofuel, Starchy substrates

P98

Understanding the mechanisms of redox balancing in a synthetic *Saccharomyces cerevisiae* strain with an NADH-delivering glycerol catabolic pathway

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The main advantage of glycerol as carbon source for growing microorganisms in industrial bioprocesses is its higher degree of reduction in comparison to other generally used carbon sources, which is accompanied by higher theoretical yields of certain fermentation products. In this context, it has been a drawback of the yeast *S. cerevisiae* that many commonly used strains do not grow in synthetic glycerol medium. Although certain *S. cerevisiae* wild-type isolates are able to grow under these conditions, they use a glycerol catabolic pathway that is FAD-dependent and does not provide reducing equivalents for fermentative processes since the electrons are directly transferred via $FADH_2$ to the mitochondrial respiratory chain. Therefore, we recently replaced the native with an NADH-delivering pathway in several *S. cerevisiae* strains. Interestingly, these strains were able to cope with the surplus of cytosolic NADH.
The aim of the current study was to identify the mechanisms that are particularly responsible for maintaining the cytosolic redox balance in our engineered strains when glycerol serves as the sole carbon source. We tested the contribution of several potential mechanisms such as alcoholic fermentation, the activity of mitochondrial external NADH dehydrogenases, the L-G3P-shuttle and a futile cycle composed of glycerol utilization and production pathways. For the purpose of this study several strains were constructed carrying deletions in the genes encoding for the key enzymes, separately and in combinations. The resulting strains were thoroughly characterized with regard to growth on glycerol as well as product formation.

P99

Engineering *Saccharomyces cerevisiae* for production of ingenol precursors

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Ingenol-3-angelate is the active agent identified in the sap of *Euphorbia peplus* and has been recently FDA-approved for the topical treatment of actinic keratosis, a precancerous skin condition. As ingenol-3-angelate is isolated from *E. peplus* in yields of only 1.1 mg/kg, it is currently prepared semi-synthetically from the more abundant parent ingenol, obtained from dried seeds of *Euphorbia lathyris*. However, this process is still not optimal, and advances on the biosynthesis are prompted to render ingenol more readily accessible.
In plants the biosynthetic pathway of ingenol starts with the non-mevalonate pathway, after which prenyltransferases and a diterpene synthase produce casbene. The further biosynthetic steps are unknown, although it has been postulated that enzymes with oxygenation or oxidoreductase activity may catalyse the ring closures seen in ingenane diterpenes.
A *Saccharomyces cerevisiae* strain with an upregulated mevalonate pathway was constructed to optimize the flux to the production of the diterpene precursor geranylgeranyl diphosphate. Additional expression of plant-derived casbene synthases resulted in production of approximately 300mg/L of casbene, a 10-fold increase compared to the previously reported titres. Further biosynthetic enzymes involved in the formation of multicyclic diterpenoids were recently identified by integration of metabolomic and transcriptomic data generated from mature seeds of *E. lathyris*. Expression of the identified candidate enzymes in our yeast strain results in the production of oxidized casbanes, important intermediates within the ingenol biosynthetic pathway.
Overall these results represent a significant step towards the development of a microbial system for the production of ingenol and other bioactive multicyclic diterpenoids.

P100

Establishment of a vehicle-cargo system for production of hydroxylated fatty acids

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Industrial biotechnology uses nature's toolset for production of useful compounds and is therefore considered to be one of the key technological drivers for the growing bioeconomy. However, one particular genetic engineering target is frequently overlooked: the cellular export of the desired product.

Hence, our study focuses on the development of a widely applicable 'vehicle-cargo' cellular export system. The molecule of interest (cargo) will be covalently coupled to a vehicle molecule, the key to unlock the membrane barrier. It is recognized by a specific transporter which escorts the vehicle-cargo complex to the extracellular environment. Once outside, the cargo will be enzymatically released, after which the vehicle is re-shuttled inside the cell. Initially, the vehicle-cargo system will be developed starting from the yeast *Starmerella bombicola*. Its well-oiled machinery for sophorolipid synthesis will be diverted towards the production of hydroxylated fatty acids. The dual nature of the sophorose moiety makes it an ideal vehicle guiding the sophorolipids to the extracellular space; on the one hand the unusual β-1,2 linkage between the two glucose molecules ensures specificity towards the transporter and on the other hand, the glucose building blocks are readily taken up, allowing re-shuttling. Heterologous expression of enzymes selected from literature and own results in *S. bombicola* will accommodate vehicle uncoupling and simultaneously, the extracellular release of the hydroxy fatty acid cargo.

P101

Production of muconic acid from lignocellulose feedstock

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Development of sustainable processes to produce bio-based compounds is urgently required because of environmental problems caused by utilization of depleting fossil resources. Converting *Saccharomyces cerevisiae* into microbial cell factories by engineering new metabolic pathways facilitates the establishment of a renewable bio-based industry. Muconic acid serves as an interesting platform chemical for the production of several bio-polymers, such as polyurethane, nylon and polyethylene terephthalate. In the past, *Saccharomyces cerevisiae* has been tailored for the production of muconic acid by expression of a heterologous pathway consisting of three different genes. As such, dihydroshikimate (DHS), an intermediate of the shikimate pathway, is converted into muconic acid. We are applying a similar strategy using an industrial pentose-utilizing *S. cerevisiae* strain and achieved maximum titers of 15 mg/L muconic acid. However, as expected, the majority of the glycolytic flux was still directed towards ethanol, which confirms the need of disrupting pyruvate decarboxylase activity by obtaining the triple knock-out mutant for *PDC1, PDC5* and *PDC6*. The *pdc* negative strain cannot grow with glucose as sole carbon source. It requires a partial deletion in *MTH1* to ensure production of cytosolic Acetyl-CoA and, therefore, to restore growth on glucose. Initial results with this modified strain show higher amounts of muconic acid with lower carbon loss towards ethanol production.

P102

Co-localization of enzymes for improved production of flavonoids in Saccharomyces cerevisiae

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Saccharomyces cerevisiae has a long track of industrial use. Nevertheless, many bottlenecks still remain in constructing an industrial relevant strain. For example, the overall yield can be decreased by improper balancing of heterologous pathway genes and loss of intermediates due to side reactions by native enzymes, which can also lead to the accumulation of (toxic) intermediates. A solution to this problem is the co-localization of the heterologous pathway to bring the different enzymes in close proximity. This co-localization effect relies on the phenomenon of substrate channelling in which the substrates are passed from one enzyme to another, reducing the loss of intermediates to competing pathways and reducing the accumulation of intermediates by creating a local environment with more suitable enzyme conditions. Furthermore possible toxic intermediates are kept in a tight space inside the cell, limiting their negative effects. We therefor seek to find out how the co-localization of the flavonoid pathway in the yeast Saccharomyces cerevisiae can improve the overall yield. Flavonoids have valuable bioactive properties for human health. Currently they are obtained through plant extraction or chemical synthesis. However, these processes are time consuming, create a huge amount of waste and are dependent on external factors. Hence, microbial production might offer a promising alternative, allowing the production of these high-value products on an industrial scale.

P103

Engineering fatty acid composition in *Saccharomyces cerevisiae*

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Fatty acids serve as precursors for a number of industrially relevant molecule classes such as alkanes, alkenes, fatty alcohols and wax esters, which can be applied as biofuels, but also serve as ingredients in the food, cosmetic and pharmaceutical industry. Our aim is to develop sustainable production processes for these compounds through metabolic engineering of *Saccharomyces cerevisiae*.

The physical and chemical properties of the final products depend to a large extend on their chain length and degree of saturation, i.e. on the fatty acids used as substrates for the respective biochemical pathway. In many cases, the fatty acid precursors required for a specific product are not the ones that are most abundant in yeast. A major challenge is therefore to modulate the endogenous fatty acid metabolism in order to increase the synthesis of particular fatty acids. Approaches on how to specifically increase the production of medium chain, very long chain, saturated and unsaturated fatty acids, respectively, will be presented.

P104

The role of gallic acid decarboxylase in the tannic acid degradation pathway in *Arxula adeninivorans* - adaptation to harsh environments

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Tannins and hydroxylated aromatic acids, like gallic acid, are plant secondary metabolites protecting plants against herbivores and microorganism. Some microbes, such as the yeast *Arxula adeninivorans* are resistant against these antimicrobial properties and able to use tannins and gallic acid as carbon sources. In this study the *Arxula* gallate decarboxylase (Agdc1p) which degrades gallic acid to pyrogallol was characterized and its function in the tannin catabolism analyzed. The enzyme possesses the highest affinity to gallic acid (Km - 0.69 mM). Additionally protocatechuate, 3-hydroxybenzoic acid, 4-hydroxybenzoic acid, 2,3-dihydroxybenzoic acid, 2,4-dihydroxybenzoic acid and 2,5-dihydroxybenzoic acid are gallate decarboxylase substrates.
A. adeninivorans G1212/YRC102-AYNI1-AGDC1, which expresses the AGDC1 gene under the control of the strong nitrate inducible AYNI1 promoter achieved the maximum gallate decarboxylase activity of 1064.4 U L-1 and 97.5 U g-1 of dry cell weight in yeast minimal medium with nitrate as nitrogen and glucose as carbon sources. In the same medium no gallate decarboxylase activity was measured for control strain G1212/YRC102 with AGDC1 expression under the endogenous promoter. Gene expression analysis showed that AGDC1 is induced by gallic acid and protocatechuate.
In contrast to G1212/YRC102-AYNI1-AGDC1 and G1212/YRC102, *A. adeninivorans* G1234 [Δagdc1] is not able to grow on medium with gallic acid as carbon source but can grow in presence of protocatechuate. This confirms that Agdc1p plays an essential role in the tannic acid catabolism and could be useful in the production of catechol and cis,cis-mucoid acid. However, the protocatechuate catabolism via Agdc1p to catechol is not the only degradation pathway.

P105

Arxula adeninivorans – a suitable biocatalyst for new biotechnological products

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The yeast *Arxula adeninivorans* is a dimorphic, asexual hemiascomycete which is phylogenetically very distant from *Saccharomyces cerevisiae*. It has been shown to be most useful in a wide range of biotechnological applications: as gene donor in enzyme production, as host for heterologous gene expression, and as biological component in biosensors.

A. adeninivorans major advantage is its metabolic flexibility, which enables the utilization of a wide range of different carbon and nitrogen sources. For example, recent analyses of the genome and its transcriptome revealed a new pathway for the assimilation of n-butanol via butyraldehyde and butyric acid as well as new insights into the previously reported purine and the tannic acid catabolism. Additionally, the synthesis of several secretory enzymes with biotechnological potential, such as tannases and cutinases were identified. Due to these characteristics, *A. adeninivorans* can be exploited as a gene donor for the production of enzymes with attractive biotechnological applications.

Furthermore, its unusual thermo- and halotolerance as well as differential morphology-dependent glycosylation and the secretion characteristics render *Arxula* attractive as host for heterologous gene expression. Successful expression of bacterial *alcohol dehydrogenase* genes enables *A. adeninivorans* to be used as biocatalyst for the synthesis of chiral alcohols as building blocks for the chemical industry.

The combination of robustness with its great ability for heterologous gene expression makes *A. adeninivorans* a superior choice for the biological component in biosensor applications. Different *A. adeninivorans*-based biosensors detecting hormones as well as dioxins have been developed and consequently improved in the last decade.

P106

Effect of Aeration and Agitation Rates on the Microbial Lipid and Citric Acid Production of *Yarrowia lipolytica* K57 Strain

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Yarrowia lipolytica is an oleaginous yeast which can produce microbial lipid with more than 20% oil in its dry biomass. *Y. lipolytica* is also used for the production of citric acid. The aim of this study was to evaluate the *Y. lipolytica* K57 strain for microbial lipid and citric acid production.

Y. lipolytica K57 strain was used for microbial lipid and citric acid production by batch fermentation method which was carried out in two different agitations and aeration rates of 350-800 rpm, 1,0-0,5 vvm respectively at 28°C on bioreactor (Papanikalou et al, 2002).

The maximum citric acid production was obtained by agitation rates of 800 rpm and aeration of 0,5 vvm. The highest citric acid of 34 g/l and 0, 28 g/l of lipid were produced in these conditions. Yields of citric acid and lipid were found 0,66 g/g (g citric acid per g glucose consumed) and 0,064g/g (g lipid per g dry cell weight) respectively. 0,20 g/l/h of maximum production rate and 0,46 g/l/h of glucose consumption rate were estimated. 350 rpm and 1,0 vvm conditions of bioreactor was not suitable for high amount of citric acid and lipid productions.

Keywords: *Yarrowia lipolytica*, citric acid, microbial lipid

References: Papanikolaou S, Muniglia L, Chevalot I, Aggelis G, Marc I (2002) *J Appl Microbiol.* 92, 737-744.

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P107

ASTAXANTHIN PRODUCTION USING *PHAFFIA RHODOZYMA* (*XANTHOPHYLLOMYCES DENDRORHOUS*)

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Astaxanthin is one of the best-selling products in the carotenoid market with a market value of $369 million in 2014 [1]. Astaxanthin additions to animal diets predominantly serve as colorization aid, e.g. to give the desired pink colorization of the flesh of species being produced by aquaculture. Besides fish farming astaxanthin finds its application also in the pharmaceutical and food industries. Currently, the majority of astaxanthin used for the feed market is produced by chemical synthesis. However, the heterobasidiomycetous yeast *Phaffia rhodozyma* (*Xanthophyllomyces dendrorhous*) can be used as natural feed source of astaxanthin.

We investigated two different strategies to improve astaxanthin yields:

Combination of random mutagenesis of P. rhodozyma with metabolic engineering

Overexpression of late genes of the astaxanthin biosynthesis in the carotenogenic mutant, which had been derived from chemical mutagenesis, led to 9.7 mg/g cell dry weight in a 1.3 L bioreactor [2].

Fed-batch bioprocess optimization for non-GMO based astaxanthin production

Starting with an astaxanthin overproducing mutant of P. rhodozyma, optimization of the pH-regulation and adjustment of trace salt and vitamin concentration in the production medium led to around 7 mg/g cell dry weight and a final concentration of 0.7 g/kg of culture broth with a space-time-yield of 3.3 mg/(kg h) in a 20 L bioreactor [3].

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P108

Metabolic engineering of oleaginous yeast for production of isoprenoids

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The oleaginous yeast Yarrowia lipolytica is widely known for its efficient lipid biosynthesis. Its ability to accumulate a large fraction of its mass as lipids, added to the broad-range of substrates it can grow onto, including hydrophobic substrates, renders this non-conventional yeast as an attractive cell factory. Furthermore, it is generally regarded as safe (GRAS) and has been extensively used for the production of omega-3-fatty acids, lipases, citric acid and other products. We hypothesize that Y. lipolytica would be a suitable host for production of isoprenoids because it has a natural efficient production of isoprenoid basic precursor, acetyl-CoA, and co-factor, NADPH, in contrast to S. cerevisiae, which naturally makes very limited amounts of cytosolic acetyl-CoA and generates small amounts of reduced NADPH.
We explored the potential of oleaginous yeast Y. lipolytica as cell factory for the production of a wide range of isoprenoids. We developed a genetic toolbox, which enables fast, flexible and reliable strain engineering. This toolbox comprises of well-characterized integrative vectors targeting specific intergenic regions of Y. lipolytica chromosome. We demonstrated the utility of the toolbox by implementation of the biosynthetic pathways of the selected isoprenoids. We further optimized the mevalonate pathway to increase the flux towards isoprenoids. These platform strains show great promise for further development towards the biotechnological production of isoprenoid compounds.

P109

Extractive fermentation with rapeseed oil enhances 2-phenylethanol production by *Saccharomyces cerevisiae*

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The attention of the world in both the fragrance and flavors market, as well as the food industry, has mainly been directed at natural-based ingredients. 2-Phenylethanol (2-PE) is an aromatic alcohol with a pleasant rose odor. The natural compound can be obtained through extraction from the flowers of various plants, however, this method is expensive and product quality is raw material-dependent [1].
2-PE produced by yeast may be considered as a natural product and has a strong marketing advantage [2]. Because 2-PE inhibits yeast growth, simple batch cultivation is unprofitable and, therefore, ways to increase productivity are being sought. One such method is yeast strain modification, e.g. by hybridization [3], however, the use of in situ product removal (ISPR) might enhance the bioprocess itself [4].
Here, we present a new route for 2-PE production in a biphasic system. Using extractive fermentation with rapeseed oil, we were able to almost triple the final 2-PE concentration. Moreover, the developed method allows for the obtaining, via an environmentally friendly route, of two final products that can be used later as food or cosmetic additives - pure 2-PE and rapeseed oil with rose odor.

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P110

Production, characterization and practical application of fungal laccases expressed in *Saccharomyces cerevisiae*

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Laccases as "eco-friendly" oxidoreductases have a great potential for a wide range of industrial and biotechnological application. At present, the main technological applications of laccases are in textile industry in processes related to decolorization of dyes and wastewaters. The aim of this work is recombinant production of three laccases from different source of origin, characterization of their properties and application of these enzymes in biotechnology.
Laccase gene mutated by directed evolution from i) *Myceliophthora thermophila* (MtL), as well as two synthetic laccase genes with optimized codons for *S. cerevisiae* from ii) *Trametes versicolor* (TvL), and iii) *Trametes trogii* (TtL) were cloned into series of vectors by homologous recombination and successfully expressed in *Saccharomyces cerevisiae*. Cultivation conditions were optimized and secreted laccases were isolated from the medium by consecutive hydrophobic and gel chromatography. Laccase activity was measured spectrophotometrically using ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) as a substrate. Biochemical characterization of individual laccases showed differences in their specific activities, stabilities, and their abilities to decolorize dyes commonly used in textile industry and real wastewater.
Supported by NPU LO 1302 and TA CR grant TA0101 1461.

P111

Engineering yeast for the production of plant-derived color compounds

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Plant secondary metabolites are a valuable source of pharmaceuticals, nutraceuticals, and cosmetics. In most cases, only limited amounts of these compounds can be isolated from plants. In addition, the structures of these compounds are very complex, making chemical synthesis challenging and expensive. Therefore, to harness the potential of these natural products, we aim to express the plant biosynthetic pathway genes in the yeast Saccharomyces cerevisiae. Currently, our research group is focusing on the production of plant-derived color compounds, such as anthocyanins, carotenoids and other natural colors. In order to meet an increasing demand for natural food colors, it is desirable to develop novel production methods. Here, we present our results on engineering yeast as a cell factory for the production of anthocyanins and carotenoids.

P112

Towards sustainable production of lactic acid

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Lactic acid is an organic acid that is used in food, cosmetic and pharmaceutical industries and that serves as building block for polylactic acid (PLA), a biodegradable and biocompatible polymer. The global lactic acid market is estimated to increase from 714.2 kilo tons in 2013 to 1,960.1 kilo tons by 2020 with a revenue of USD 4.3 billion [1]. Currently, optically pure lactic acid is mainly produced by sugar fermentation of lactic acid bacteria. However, this production process has some drawbacks. Particularly, lactic acid bacteria are sensitive to low pH. Therefore, neutralizing agents have to be added to the production medium making the separation and purification of lactic acid very costly. In the presented project, we use *S. cerevisiae* as production organism for lactic acid. Budding yeast has many advantages over *Lactobacilli* such as the simple nutritional requirements or the tolerance to low pH, making it an excellent cell factory for producing organic acids. Since *S. cerevisiae* cells do not naturally produce lactic acid, metabolic engineering is required in the first place to develop a lactic acid producing strain. Initially, the genes coding for pyruvate decarboxylases involved in the reduction of pyruvate to ethanol were deleted. By overexpressing a heterologous lactate dehydrogenase, pyruvate can then be converted into lactic acid. Furthermore, a combinatorial approach of modifications of gene expression levels, cell sorting for high intracellular pH, and evolutionary engineering of the yeast production strain will improve the final lactic acid yield.

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P113

Transportome of the biosurfactant producer Starmerella bombicola

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The sophorolipid synthesizing yeast Starmerella bombicola is, from the industrial point of view, the most important microbial producer of biosurfactants. Due to its excellent production potential, it has also been transformed into an efficient platform for the synthesis of new to nature biosurfactants. However, for certain surface active compounds, the titers are significantly lower compared to the native ones. Commercially relevant production of these molecules cannot be obtained due to their hampered secretion and a lack of knowledge on the transport mechanisms involved. Indeed, cellular export is an important, but largely neglected theme in industrial biotechnology. This research, on the one hand, focuses on characterization of the S. bombicola ABC transporters in order to assign their function and role in sophorolipid biosynthesis, drug resistance and general metabolism. On the other hand, the study aims to test a number of strategies (e.g. heterologous expression of efflux pumps or protein engineering) that can lead to improved or advanced export. Particular attention is given to the sophorolipid MDR transporter (SL MDR), which was found in the middle of the sophorolipid biosynthetic gene cluster of S. bombicola [1]. In order to investigate the transport mechanism and substrate affinity, the SL MDR is expressed in Saccharomyces cerevisiae and evaluated by in vitro transport assays. Furthermore, using transporter databases and BLAST searches other S. bombicola endogenous efflux pumps were identified. While the created knockout strains will be used for the functional characterization of these transporters, overexpression as fluorescent- tag fusion proteins will allow their subcellular localization.

P114

DESIGN OF *S. CEREVISIAE* STRAINS TO UNDERSTAND ACETIC ACID PRODUCTION AND IMPROVE WINE YEAST PROPERTIES

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The increment of alcoholic degree of wine is one of the main challenges, related to global warming, that wine industry is facing nowadays. Our previous studies showed that, despite the Crabtree effect, which favors fermentation over respiration in S. cerevisiae, under suitable aeration conditions, this species is able to drive a sensitive reduction of alcoholic degree by respiration. The main drawback is a considerable increase in volatile acidity. We have also seen that the deletion of some genes in a homozygous industrial S. cerevisiae strain led to a significant reduction in volatile acidity, suggesting that some carbon catabolite repression genes could affect the yields of acetic.
The objective of this work is investigating the mechanisms involved in acetate production in S. cerevisiae under aerobiosis.
We used systems biology approaches (constraint based flux analysis) to predict and investigate the deletion of different genes, possibly related with acetic acid production, in the presence of oxygen. In parallel we also analyzed the behavior of a selection of strains deleted for genes related with CCR.
The results show that the impact of these deletions on the production of ethanol is actually small, although the effect of oxygenation on ethanol yields is confirmed. We are currently developing work in this line of research and will present results that will serve as a guide in the design of strategies to improve industrial strains to reduce the production of acetic and ethanol yields in wine making conditions.
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P115

Combined effects of environmental factors on phenotypic traits of *S. cerevisiae* X *S. kudriavzevii* hybrids isolated from spontaneous wine fermentation

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Although *Saccharomyces cerevisiae* plays a predominant role in wine fermentation, many natural hybrids between *Saccharomyces* species have been recently identified in the wine environment. For example, the Eg8 wine yeast strain, which was isolated in Alsace (France) in 1979 from a grape must spontaneously fermenting at low temperature, was recently described as a *S. cerevisiae* X *S. kudriavzevii* hybrid by Erny et al. (2012). Variants of this strain were commercialized because of their abilities to ferment at low temperature and to liberate high amounts of varietal aromas. However, they occasionally produced excessive amounts of acetate which was detrimental for wine quality. The aim of this study was to understand the environmental and genetic causes of this high acetate production. We first compared the technological properties of Eg8, industrial variants and related hybrids isolated from different areas. Even if the strains were described as close, they produced different amounts of acetate: from 0.45g/L to 1.1g/L. We then used a Box Behnken design to evaluate the combined effects of lipid amount, sugar concentration and temperature on the level of acetate produced by the hybrids during wine fermentation. We found that lipid and sugar concentrations are the main factors affecting acetate production. Moreover, we found a linear relation between lipid concentration and the liberation of 3MH, a varietal aroma which brings citrus flavour to the wine. We also sequenced the genomes of these strains (Illumina) in order to study their genetic relationships and to potentially associate phenotypic differences with major genetic variations.

P116

Rational development of a mixed starter-culture of *Hanseniaspora guilliermondii* UTAD222 and *Saccharomyces cerevisiae* UCD522 for industrial application

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The *Hanseniaspora guilliermondii* UTAD222 strain, previously isolated in our laboratory from a fermenting grape-juice from Douro Region has been selected to be used as an adjunct of *Saccharomyces cerevisiae* in wine fermentations. In previous experiments conducted in a natural grape-juice it was found that, although the co-inoculation with *H. guilliermondii* negatively affected *S. cerevisiae* UCD522 growth and fermentation rate, its presence significantly alter the levels of aroma compounds found at the end of the fermentation. In this study we aimed to increase knowledge on this yeast consortium towards increasing the efficiency, predictability and quality of wine production using this yeast consortium. For this purpose, Response Surface Methodology (RSM) based on central composite (CCD) was employed to statistically evaluate the combined effect of different conditions, on yeast growth and fermentative activity and on the production of sensory relevant wine compounds. Four independent variables, fermentation temperature, initial nitrogen and sugars concentration and inoculum levels of *H. guilliermondii* were evaluated. The analysis of the experimental data showed that overall, nitrogen was the main factor impacting fermentation kinetics and metabolites produced, followed by sugars levels and fermentation temperature. The effects of the co-inoculation with *H. guilliermondii* were highly sensitive to extremes of temperature and nitrogen levels. The inoculum level displayed positive linear effects on the synthesis of some higher alcohols and esters, in particular ethyl acetate. The results demonstrate that the approach used could be useful for the rational development of industrially appropriate mixed-cultures as a function of grape-juice composition and fermentation conditions.

P117

Biodiversity of maltose and maltotriose fermentation in Saccharomyces pastorianus lager brewing yeasts

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Lager brewing yeast Saccharomyces pastorianus, interspecific hybrid of S. eubayanus and S. cerevisiae, is one of the most important industrially used microorganisms. In lager beer brewing processes, brewing yeast ferments wort sugars (mainly maltose, maltotriose, and glucose) to ethanol and carbon dioxide at cold temperatures. Kinetics of sugar metabolism represent a major factor in determining the rate and extent of wort fermentation. In this context, uptake of maltose and maltotriose by plasma-membrane proton symporters is a highly relevant process. A variety of maltose/ maltotriose transporter genes have been identified in lager brewing yeasts, including MTT1/ MTY1, AGT1, and different versions of MALx1. Although several previous studies have sought to define the number of relevant transporters and to characterize them, results on substrate specificity of individual transporters are far from complete and sometimes contradictory. This study explores the biodiversity of maltose/maltotriose transporters in Saccharomyces yeast and characterizes individual transporters from in order to gain a better understanding of the individual contribution of each transporter to the fermentation performance of S. pastorianus and to gain a better understanding about the evolution of maltose/ maltotriose transport proteins from S. pastorianus and its parent strains S. eubayanus and S. cerevisiae.

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P118

IDENTIFICATION OF ANTIMICROBIAL PEPTIDES SECRETED BY Candida intermedia WITH ANTIFUNGAL PROPERTIES AGAINST Brettanomyces bruxellensis, A WINE SPOILAGE YEAST

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Brettanomyces bruxellensis has been described as a principal contaminant of the wine industry. To avoid its growth, the must is commonly supplemented with SO_2, one of the most used preservatives in the food industry. However, the use of this compound has been questioned for the potential health problems that entails its consumption. With the aim of exploring other alternatives, our laboratory has previously described a strain Candida intermedia LAMAP1790 as a productor of antimicrobial compounds. Thus, the objective of this work was to determine the antifungal activity of the compound produced by C. intermedia against B. bruxellensis and elucidate its chemical nature. Using joint seeding tests and viability after direct exposure to sterile culture medium of C. intermedia LAMAP1790, the secreted compound was found to have fungicidal activity on four strains of B. bruxellensis, without affecting the growth of Saccharomyces cerevisiae. Posteriorly, in order to determine the chemical nature of the compound, the proteins in the supernatant were fractionated and concentrated 250X, which revealed that the antifungal activity is related to the presence of two peptides with a molecular mass less than 5 kDa. Finally, to determine if these peptides are responsible for the antifungal activity, a proteolytic treatment was performed on the fractions 250X of C. intermedia. In these tests, it was observed that the antifungal compound has a proteinaceous nature and a molecular mass close to 3 kDa. These results allow pressuming that the secreted peptide could have similarity to antimicrobial peptides (AMPs) as the ones described in filamentous fungi, with possible biotechnological applications in the control of spoilage microorganisms in the wine industry.

Keywords: Antimicrobial peptides, Biocontrol, C. intermedia, B. bruxellensis.

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P119

Physiological and molecular characterization of Brettanomyces bruxellensis in the presence of p-coumaric acid

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Brettanomyces bruxellensis has been described as the main yeast contaminant in wine, because it is able to convert naturally occurring hydroxycinnamic acids in grape must into phenolic derivatives. These compounds are weak acids, have a lipophilic character and have been reported to exhibit antimicrobial activity by inhibiting the growth of many microorganisms. To evaluate the effect of these acids on the growth of B. bruxellensis, strains LAMAP1359 and LAMAP2480 were cultured in YNB medium in the absence and presence of p-coumaric acid (pCA). The presence of 100 ppm of pCA in the culture medium caused an increase in lag phase duration in both strains, in addition to a reduction in the specific growth rate for strain L2480. However, for strain L1359 grown in the presence of pCA, specific growth rate increases with respect to the control condition. The percentage of inhibition of growth was calculated, being for the LAMAP2480 strain of 9.8%, and for the strain LAMAP1359 of -27%, which means that in the presence of pCA, this last strain improves the growth kinetics increasing the specific growth rate. On the other hand, a comparative analysis of the transcriptome of both strains was carried out, which has allowed us to propose a model in which the entry of pCA into the cell generates a generalized stress in which the expression of proton and efflux of toxic compounds. These latter could be involved in the outflow of nitrogen sources such as amino acids or allantoin, which by decreasing in concentration would trigger gene expression of nitrogen metabolism. This knowledge will allow the design of new tools in the control of this microorganism causing millions of economic losses in the wine industry.

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P120

Amino acids and ammonium utilization by non-*Saccharomyces* yeasts from grape juice in fermentation

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Non-*Saccharomyces* wine yeasts, considered in the past as undesired or spoilage yeasts, can enhance the analytical composition, and aroma profile of the wine. The use of mixed starters of selected non-*Saccharomyces* yeasts with strains of *Saccharomyces cerevisiae* represents an alternative to both spontaneous and inoculated wine fermentations, taking advantage of the potential positive role that non-*Saccharomyces* wine yeast species play in the organoleptic characteristics of wine. In this context mixed starters can meet the growing demand for new and improved wine yeast strains adapted to different types and styles of wine. But their use is still poorly mastered, by lack of knowledge about their needs and behaviours in co-fermentation, including their consumption of amino acids and ammonium. To better control use of non-*Saccharomyces* in winemaking, the utilization of amino acids and ammonium by three strains of non-*Saccharomyces* was studied in grape juice. Both quantitative and qualitative differences, during the catabolism of 200 g/L of glucose, in the utilization of assimilable nitrogen were observed in various culture conditions. First with pure culture under aerobic condition and then with limited aeration, condition found in winemaking. The second culture condition is a sequential co-culture with *Saccharomyces cerevisiae* under limited aeration to understand the impact of the non-*Saccharomyces* yeasts on the availability of assimilable nitrogen to *Saccharomyces cerevisiae*. Our results demonstrate that non-*Saccharomyces* yeast possess specific amino acid profile consumption never reported before. Our results also underline that nitrogen consumption by yeast might be a source of competition that could explain some observed interactions.

P121

Exploring an autochthonous *Metschnikowia pulcherrima* strain in the production of Douro wines

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In the last two decades, several research groups have deeply examined the potential of non-*Saccharomyces* yeasts as adjuncts to *S. cerevisiae* to exploit their flavour complexing properties. In particular, the use of *Metschnikowia pulcherrima* strains leads to interesting technological outcomes, improving aroma and flavour and in reducing ethanol levels in wines. In this study, a sub-set of M. *pulcherrima* indigenous isolates from grapes, must or wines of Douro Demarcated Region was used. PCR-fingerprinting showed no correlation between the genotype patterns and the geographical origin of the strains, indicating that grape-juice or wines from a particular grape variety or winery are not preferentially colonized by a specific group. To study their potentially relevant features, all strains were surveyed for their ability to produce enzymes that can positive or negatively affect the wine quality and their ability to adjust to winemaking conditions. Genotypic and phenotypic variability was found within strains isolated from the same sample at different stages of fermentation. Mixed-culture fermentations using grape-juice of local varieties with *Saccharomyces cerevisiae* and a selected strain of *M. pulcherrima* were conducted. The formation of major volatile and non-volatile compounds was evaluated showing that the wines from mixed cultures presented differences in final composition. The results to be presented emphasize the potential of employing autochthonous non-*Saccharomyces* yeast strains as “regional starter-cultures” in the production of diversified high quality wines expressing the local *terroir*.

P122

Investigating the oenological potential of a extracellular aspartic protease from *Metschnikowia pulcherrima*

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A wide diversity of yeast species occur in grape juice. Amongst these, non-*Saccharomyces* are known to secrete extracellular enzymes but the actual activity of these enzymes remains mostly uncharacterised. *Metschnikowia pulcherrima* has recently been reported to secrete an aspartic protease that could eliminate/reduce protein haze and impact wine properties by breaking down grape and microbial proteins. Indeed, literature suggests that this protease is active under conditions resembling winemaking procedures.
In this study, the MpAPr1 aspartic protease of *Metschnikowia pulcherrima* was cloned and expressed in Komagataella pastoris. The enzyme was purified using cation exchange chromatography and its activity assessed in grape juice and at the end of alcoholic fermentation. Protein degradation was monitored through SDS-PAGE and HPLC. In order to assess the global impact of MpAPr1 activity and its suitability for winemaking, fermentation kinetics, yeast population dynamics and certain metabolic products of fermentation were also determined. The results showed that MpAPr1 only partially degraded grape proteins and no clear impact could be observed on protein haze formation. Nevertheless, this activity had a significant impact on the production of certain volatile aroma compounds by *Saccharomyces cerevisiae*, thereby confirming that MpAPr1 was active, albeit not optimally, under winemaking conditions. While further optimisation is required to improve MpAPr1 activity, this study not only confirms that proteases could be utilised in grape juice and during fermentation to improve wine properties, but also that the properties of non-*Saccharomyces* yeasts, in particular their ability to secrete enzymes, should be further explored and potentially exploited.

P123

Differences in nitrogen consumption abilities between *Saccharomyces cerevisiae* strains reflect diverse adaptation strategies to fermentations environments.

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Saccharomyces cerevisiae is the most commonly used microorganism in food. There is an important diversity between strains among this species largely due to domestication events that substantially modified their genomes. Recent work has demonstrated that this diversity has led to differences in the ability of strains to consume yeast assimilable nitrogen (YAN) (**1, 2, 3, 4**). As part of industrial process, nitrogen is a key nutrient affecting directly biomass production, fermentation progress and organoleptic quality. Understanding the bases underlying the differences YAN assimilation capacity would provide new insights for an improved management of nitrogen nutrition during fermentation processes.

Our aim was to further investigate the difference in YAN assimilation between strains from various lineages (Wine, Rum, North American, West African and Sake strains). We first observed that strains were clustered in two groups, high and low nitrogen consumers, could be suggesting an adaptation for their initial nitrogen matrix composition, resulting in a difference in their ability to consume differentially some specific N-compounds. Indeed, Wine and Rum strains presenting high nitrogen consumption capacity have preferred assimilation for ammonium, isoleucine, valine, phenylalanine, tyrosine and glutamine for which the percentage of assimilation between the two groups has a variation greater than 30%. Our data demonstrate that those differences in YAN consumption were correlated with differences in the uptake rate for these specific N-compounds. Variations in the nitrogen-sensing signalling pathway that modified the stress level of the cells, were also evidenced that likely reflected the constraints associated with the adaptation of fermentation environments.

P124

DEVELOPMENT OF A ROBOT-BASED METHOD TO MEASURE MULTIPLE ENZYME ACTIVITIES IN YEAST

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Many biotechnological processes rely on the metabolic activity of organisms of interest, particularly in the fields of metabolic engineering, food and agricultural biotechnology, environmental technology, etc. This is the case in oenology, where alcoholic fermentation is a key step of winemaking driven by wine yeast metabolic activity: sugar is metabolized in ethanol through glycolysis followed by ethanol production pathway. In oenology, like in other microbial processes, the improvement of the technological performance of the corresponding organisms requires the understanding of the metabolic pathways involved, including enzymes, the direct metabolic actors. In this work, a robot-based method was developed to measure multiple enzyme activities (MEA) in yeast focusing on the glycolysis and alcoholic fermentation pathway (13 enzymatic activities). This approach was then applied to the measurement of MEA in two wine yeast species, *Saccharomyces cerevisiae* and *S. uvarum*, grown in a synthetic grape juice. The main fermentation metabolites (acetic acid, malic acid, glycerol...) were also measured and the data integrated. The possible applications of such approaches will be discussed, for oenology but also more widely for systems biology studies.

P125

FERMENTATIVE BEHAVIOUR AND COMPETITION CAPACITY OF CRYOTOLERANT *Saccharomyces* SPECIES IN LOW NITROGEN WINEMAKING CONDITIONS

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Nitrogen metabolism of cryotolerant *Saccharomyces* species during winemaking is little known, although some works have demonstrated that *S. uvarum* shows lower nitrogen requirements than *S. cerevisiae.* We evaluated the nitrogen requirements of 32 strains belonging to *S. uvarum*, *S. eubayanus* and *S. kudriavzevii* species. The assays were performed in microplates containing synthetic must with different nitrogen concentrations (0, 40, 60, 80, 100, 120, 140, 160, 180, 200 and 300 mg/L), inoculated with 1 x 10^6 cells/mL and incubated at 25ºC. Yeast growth was followed by OD measuring and fitted to an exponential model. Kinetic parameters, obtained for all growth curves, evidenced that *S. eubayanus* strains showed higher μ_{max} than *S. uvarum* at all nitrogen concentrations, while *S. kudriavzevii* strains showed the lowest values. One *S. eubayanus* and one *S. uvarum* strains, showing low nitrogen requirements, were individually evaluated in competition assays against *S. cerevisiae* at different fermentation temperatures (12ºC, 20ºC and 28ºC) and nitrogen concentrations (60, 140 and 300 mg/L). Assays were performed in 80 mL synthetic must inoculated with 1 x 10^6 cells/mL of each species. *S. cerevisiae* dominated the fermentation at 28ºC while *S. uvarum* or *S. eubayanus* dominated at 12ºC, in all three nitrogen concentrations. At 20ºC, *S. cerevisiae* dominated (more than 90% implantation) the fermentation in musts containing 300 mg/L N while only 40% or 50% implantation was observed at low nitrogen concentrations (60 mg/L). Our results evidence the competition capacity of both *S. eubayanus* and *S. uvarum* in low nitrogen must fermented at intermediate temperature.

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DIFERENTIAL SULFITE RESISTANCE AS A DOMESTICATION SIGNATURE IN *Saccharomyces uvarum*

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A number of *S. uvarum* strains has been isolated from natural and anthropic habitats during the last years, but little information is still available on the distribution and possible domestication events in its cryotolerant species. Sulphite tolerance has been described as a domestication signature in *S. cerevisiae*; for that reason, we proposed the evaluation of the effect of this preservative in 61 *S. uvarum* strains obtained from diverse natural and anthropic habitats. The assays were performed in YPD-TA agar plates (pH 3.5 by tartaric acid addition). The plates contained 0, 1, 2, 3, 4, 5, 6, 7 and 8 mM sulphite and the inoculation was carried out by means of serial dilutions and dropping onto the agar plates. Strains isolated from natural habitats and apple chichas were only able to grow until 2mM sulphite concentrations. Contrarily, strains from cider or wine exhibited resistance to 4 or 5 mM sulphite. In particular, strains CECT12600 and BMV58 isolated from wine environments grew even at sulphite concentrations of 8 mM. These two resistant strains as well as two sensitive strains (NPCC 1314 from apple *chicha* and NPCC 1290 from natural habitat) were selected to perform microfermentations (100 mL) in synthetic must at 25ºC. At different fermentation times, samples were collected and the expression of *SSU1* gene quantified by quantitative RT-PCR. During the whole fermentation, wine strains overexpressed of *SSU1* significantly (10-25 fold higher expression than sensitive strains). Our results suggest that expression of *SSU1* gene is a domestication signature in *S. uvarum* evolution.

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Yeast-yeast interaction studies during alcoholic fermentation by flow cytometry

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Alcoholic fermentation (AF) is the main step for winemaking from grape must. It is mainly performed by the yeast Saccharomyces cerevisiae, even if other wine yeasts called non-Saccharomyces may be involved in the AF and may improve wine sensorial profile. Commercial mixed starters of non-Saccharomyces and S. cerevisiae strains are currently available, but there is a lack of knowledge about interaction mechanisms and a lack of tools to study the potential strain relationship. The recurrent problem with the use of these non-Saccharomyces yeasts is the lack of reproducible results year to year due to interactions between microorganisms. In order to study the interactions between yeasts, the first step was to discriminate the two species in mixed cultures during AF. This was possible thanks to the flow cytometry technique, which is able to discriminate S. cerevisiae and some non-Saccharomyces according to their size and granularity. Alternatively, a S. cerevisiae modified strain expressing a GFP can be used to separate the two population of yeasts. Monitoring of the yeast physiology during AF was performed in grape juice using flow cytometry to study different physiological parameters like the accumulation of Reactive Oxygen Species or lipids inside cells, intracellular pH and viability thanks to specific fluorochrome staining. These analyses can reveal the possible yeast-yeast interactions during AF by comparing pure cultures of each strain and the co-cultures of both strains.

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Investigation of the phenotypic variability and the norm of reaction in two distinct meiotic populations in an oenological context

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Complex traits are determined by numerous genes that interact with the environmental conditions. The norm of reaction, describes the pattern of phenotypic expression of a single genotype across a range of environments. This phenomenon is a critical point for wine industry that want to use robust strains to ensure successful fermentations in a wide range of grape musts. In this work, the phenotypic response of two populations of meiotic clones (~100 progenies) derived from two distinct *S. cerevisiae* crosses were measured in 3 environmental conditions. The impact of the grape variety and the micro-oxygenation was investigated for eleven quantitative traits including kinetics parameters and end-product compounds. By analyzing a data set of more 13000 data points (1218 fermentations x 11 traits) three main conclusions were done. First, environmental conditions strongly shaped trait variability confirming basic oenological knowledge. Second, a huge phenotypic variability was observed among progeny clones for both crosses confirming the strong genetic determinism of the traits investigated. This phenotypic variability was much higher than the one observed for a set of 31 wine starters suggesting that meiotic recombination emphases phenotypic novelty. Surprisingly, a similar phenotypic variability was observed in both crosses whatever the phenotypic and genetic distance measured within the parental pairs. Finally, we characterized for each progeny clone the norm of reaction by using self-Organizing Map (SOM). For several traits distinct patterns of phenotypic responses were discriminated. Altogether the results pave the way for exploiting and deciphering the genetic determinism of phenotypic robustness.

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The wine microbial ecosystem: Can we define the rules of engagement?

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The microbial population of natural grape juice constitutes a complex ecosystem that is usually dominated by yeast species originating from the grapes and the cellar equipment. Mostly the same non-*Saccharomyces* yeast species have been identified in all vine growing areas, but the specific species composition of individual grape juices differs significantly. In all cases, the indigenous yeast microbiota will start the fermentation process, and a succession of several yeast species will tend to dominate the early stages of fermentation and contribute considerably to the characteristics of a given wine. While each yeast species may add desirable or un-desirable features, the contribution by any individual species is a function of its numerical presence and metabolic activity, which in turn will depend on its relative fitness within the microbial ecosystem. To define and predict the oenological potential of a given wine microbiota, it is therefore essential to understand the rules and interactions governing this ecosystem. Here we describe an approach based on the use of a constructed microbial ecosystem consisting of eight yeast species that are most commonly found in South African grape musts. The data reveal antagonistic and synergistic interactions between these species, and suggest that such ecological interactions apply independently of the environmental and chemical parameters that characterise a specific grape must. The findings provide a sound base for characterising the broader biotechnological potential of a given yeast microbiota, and enhance the predictability of spontaneous fermentation. Such a predictive capability has the potential to revolutionise current wine making practices.

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Enological Properties of Some Wine Yeasts Isolated During The Fermentation of Cv. Narince Grapes in Capadocccia Region of Turkey

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Narince is a native white grape variety of *Vitis vinifera* L. grown in Capadoccia Region of Turkey and produces rich and balanced wines often with a greenish yellow tint and delicate fruity flavour.

In this study, enological properties of yeasts isolated from the spontaneous fermentation of cv. Narince were analysed. Yeasts were isolated and then identified by PCR-RFLP analysis of the 5.8 ITS rRNA region and sequence information for the D1/D2 domains of the 26S gene. Some yeast species identified were examined for the main enological properties such as fermantation rate, production of ethanol, volatile acidity, aroma compounds, foam and H_2S, tolerance to SO_2, temperature and pH, killer and enzymatic activities and floculation characteristic.

The species identified were *Saccharomyces cevresiaie*, *Candida zemplinia*, *Pichia kluyveri, Issatchenkia terricola, Issacthenkia orientalis, Whickerhamomyces anomalus, Lachancea thermotolerance* and *Hanseniaspora uvarum* and *Torulaspora delbruckii*. All yeast species completed the fermentations with the ethanol formation varying in the range of 9.7-12.2% v/v. Volatile acidity levels expressed as acetic acid were in the range of 0.5-0.9 g/L. They tolerated to SO_2, temperature and pH. Higher alcohols, esters, volatile acids, lactones and carbonyl compounds were analysed as aroma compounds in the range of 185.15-354.67 mg/L.

Key Words: Yeast, cv. Narince, *Saccharomyces cerevisiae*, Non-*Saccharomcyes* spp., Enological properties

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Transcriptomics analysis of different *Saccharomyces* species of interest for winemaking industry in synthetic must fermentation

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The use of commercial Saccharomyces cerevisiae is practically ubiquitous in industrial winemaking. However the sector is currently facing challenges such as the influence of climate change on must characteristics or the consumer new perspectives on alcohol consumption. One of the approaches followed in the last years to overcome those has been the use of non-conventional yeasts as starters in wine fermentation. Concretely, alternative species of the genus Saccharomyces show interesting characteristics such as rich aroma profiles, low ethanol and high glycerol yields, or adaptation to low temperature. Among them, S. uvarum and S. kudriavzevii have been suggested to have differences in the regulation of metabolism and physiology fostering these properties. In this work, we try to acquire a deepest knowledge of the molecular basis behind them by means of RNAseq analysis of these species in synthetic must fermentation, which can provide us with useful insight on wine starters' composition improvements, but also on evolutionary and adaptation aspects.

P132

Profiling the yeast microbiome in Pinot Noir vineyards and spontaneous fermentations at a British Columbian winery

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Wine is produced by one of two methods: inoculated fermentation, where a commercially-produced, single *Saccharomyces cerevisiae* yeast strain is used; or spontaneous fermentation, where yeast present on grape and/or winery surfaces carry out the fermentative process. Spontaneous fermentation is characterized by a diverse succession of yeast species, ending with one or multiple strains of *S. cerevisiae* dominating the fermentation. The resultant wines may be more organoleptically complex due to the participation of a wide range of yeast species. In the vineyard, yeast population composition is highly heterogeneous, differing between regions and even between adjacent vineyards. While the vineyard fungal microbiome has been profiled in wine regions worldwide, it has not been explored in Canada. Using amplicon sequencing of the intergenic transcribed spacer region, we characterize the yeast populations in three geographically separate British Columbian Pinot Noir vineyards (1km radius) farmed by the same winery. We also elucidate the impact of the winery environment on yeast population structure in spontaneous fermentations over two vintages by comparing yeast populations in aseptically fermented Pinot Noir grapes to populations in winery-conducted fermentations of grapes from the same vineyard. To examine yeast population dynamics during fermentation, we have profiled yeast populations at successive fermentation stages. Pinot Noir yeast species populations will be compared to previously described *S. cerevisiae* strain populations from the same fermentations. This study is the first to characterize vineyard-associated yeast populations in Canada and is the first step in a region-wide yeast population study aiming to identify species with novel oenological applications.

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EARLY TRANSCRIPTIONAL RESPONSE TO BIOTIC STRESS IN MIXED STARTER FERMENTATIONS INVOLVING *SACCHAROMYCES CEREVISIAE* AND *TORULASPORA DELBRUECKII*

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Advances in microbial wine biotechnology have led to the recent commercialization of several non-*Saccharomyces* starter cultures. These are intended to be used in either simultaneous or sequential inoculation with *Saccharomyces cerevisiae*. We analysed the transcriptional response to co-cultivation of *S. cerevisiae* and *Torulaspora delbrueckii*. The study is focused in the initial stages of wine fermentation with the goal of better understand the microbial interactions that can be stablished during wine fermentation with mixed-starters. Fermentations were carried out in bioreactors using synthetic grape must to mimic industrial conditions. Experiments were carried out in triplicate for fermentation kinetics, CO_2 performance and RNAseq analysis.

Both species showed a clear response to the presence of each other, even though the portion of the genome showing altered transcriptional levels was relatively small. Changes in the transcription pattern suggested a stimulation of metabolic activity and growth, as a consequence of the presence of competitors in the same medium. The response of *S. cerevisiae* seems to take place earlier, as compared to *T. delbrueckii*. Enhanced glycolytic activity of the mixed culture was confirmed by the CO_2 production profile during these early stages of fermentation. Interestingly, *HSP12* expression appeared induced by co-cultivation for both of *S. cerevisiae* and *Torulaspora delbrueckii* in the two time points studied. This might be related with a recently described role of Hsp12 in intercellular communication in yeast. Expression of *S. cerevisiae* PAU genes was also stimulated in mixed cultures.

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Study of interactions between *Saccharomyces cerevisiae* and *Torulaspora delbrueckii* in winemaking using a proteomic approach

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During the early stages of winemaking, non-*Saccharomyces* yeasts are usually predominant. However, due to their low ethanol tolerance and microbial competition, *Saccharomyces cerevisiae* develops rapidly and becomes the main agent of alcoholic fermentation. Among non-*Saccharomyces* yeasts, *Torulaspora delbrueckii* is proposed by several companies to be used in association with *Saccharomyces cerevisiae*. Nevertheless, interactions mechanisms between these two species are still unclear and consequences on wine remains uncertain.

In a previous work, a bioreactor with double compartment was used to study the behaviour of *S. cerevisiae* and *T. delbrueckii* in pure and mixed culture, with and without physical contact, during alcoholic fermentation of Sauvignon must. Our results shown that in mixed cultures with physical contact, *T. delbrueckii* population dropped of earlier, suggesting the existence of a cell-cell contact mechanism.

In this study, proteomic approach was used to identify proteins differentially affected by the two types of mixed culture (with and without physical contact) in comparison to pure cultures. Samples were taken at mid-stage of growth phase for each species. Then, after protein extraction, analysis was performed by liquid chromatography with tandem mass spectrometry. More than 740.000 spectra were detected and 32.889 peptides identified matching 3.635 proteins. Among them, when both species were cultivated with physical contact, proteins involved in oxidative stress response were higher for *T. delbrueckii*. Further experiments are in progress to determine if this mechanism can explain *T. delbrueckii* early growth in mixed culture with *S. cerevisiae*.

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Yeast based nutrients impact on *Saccharomyces cerevisiae* during grape must fermentation

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Saccharomyces cerevisiae is the main agent of alcoholic fermentation in wine. Since many years, most wine-makers inoculate grape must with industrial selected *S. cerevisiae* strains to ensure a reliable and predictable alcoholic fermentation. More recently, industrial yeast derivatives products (inactive yeast or autolysates) are available and intend to promote yeast implantation, particularly in case of assimilable nitrogen deficiency. Nevertheless, the exact composition of these products (lipids, vitamins, amino acids, micronutrients) and the effect of their different nutrients on *S. cerevisiae* are still unclear.

In this work, one industrial product was studied. Once its nutrient composition was specified, we sought nutrients impacting significantly *S. cerevisiae* growth and fermentation.In this perspective, several reconstituted products were developed, hence modifying the amount of each nutrient. A total of 40 compounds were studied. Experiments were carried out in Sauvignon Blanc grape must on one industrial strain of *S. cerevisiae,* using a Hadamard matrix. Our first results highlight 7 compounds, essentially amino acids, which impact positively *S. cerevisiae*, and 5 with a negative effect. Further experiments are in progress in different grape musts, more or less deficient in assimilable nitrogen. Moreover, the effects of nutrients on volatile thiols, aromatic compounds particularly important in white wines, will also be investigated.

With a better knowledge of these products composition and their effect on *S. cerevisiae*, this work should allow improving their uses, such as precising the time of supplementation, and eventually adapting formulations.

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Non-conventional yeasts in beer production

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Brewers are looking for beer with distinctive aromatic notes through the selection of hop varieties, malts and yeasts. The choice of the yeast to be used in the brewing process is though crucial to obtain a product that is valued by consumers and with distinctive features and flavors. Indeed, yeast transform the raw material flavor precursors into flavor-active compounds producing beer with distinctive aroma profile. In winemaking there is an increase interest on the use of mixed cultures with non-conventional yeast species to enhance some aromatic compounds In the brewing industry most beers are obtained with the use of a single *Saccharomyces* yeast strain. Recently, the attention has been focused on *Torulaspora delbrueckii,* a non-conventional yeast able to ferment maltose, and characterize the beer with a peculiar aromatic notes. With the present study we will evaluate the possible use of several non-conventional yeasts to use in brewing process. After a preliminary screening, strains belonging to the species *Lachacea thermotolerans*, *Wickerhamomyces anomalus, Zigotorulaspora florentina,* able to ferment the maltose, were selected and used in pure and mixed fermentation with *Saccharomyces cerevisiae* starter strain for beer production. The biomass evaluation, the fermentation kinetics, the main analytical and aromatic profile were evaluated. The results obtained showed that the use of non-conventional yeast in brewing process in mixed fermentation could be a suitable strategy to control flavor production during beer fermentation, and thus to obtain products with aromas and tastes that are different from those for beers brewed using pure *S. cerevisiae* starter strains.

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Contribution of Saccharomyces cerevisiae strains to health promoting compounds in wine

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Many studies have found that moderate consumption of wine is associated with health benefits, reducing the risk of cardiovascular and neurodegenerative diseases, and the onset of certain forms of cancers. These benefits are attributed to a series of bioactive compounds with antioxidant power, mainly polyphenols, capable of counteracting the negative action of free radicals. Phenolic compounds are naturally present in the grapes, but an additional amount originates during the vinification process. The aim of this work was to assess the ability of some wine yeasts to produce bioactive compounds during alcoholic fermentation. In particular, the content of higher alcohols (tyrosol, hydroxytyrosol and tryptophol), glutathione, melatonin, as well as the total antioxidant power were determined at the end of alcoholic fermentations carried out by four commercial and two indigenous Saccharomyces cerevisiae strains. In order to exclude the natural fraction of antioxidant compounds, a synthetic must was used. The results indicated that the production of these bioactive compounds is a strain-specific property and, therefore, the different yeast strains utilized during fermentation are able to modify the antioxidant activities in the final product.

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Differentiation of Kluyveromyces marxianus strains isolated from Mezcal fermentations by MALDI-TOF MS

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Since it development in the late 1980s, Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) has been applied for the determination of molecular masses of organic compounds. During the last decade, it has been shown that this technique possesses the capacity to identify microorganisms at the genus and specie levels directly from the biomass of isolated colonies generating a phenotypic profile or "molecular fingerprint". MALDI TOF MS is rapidly gaining popularity for this purpose in microbial ecology studies due to its time and cost effectiveness as compared to morpho-physiological or other molecular biology based methods.
In the present work, the capacity of MALDI-TOF MS for the differentiation of isolates of Kluyveromyces marxianus from different mezcal production regions was evaluated. In order to exam the validity of this approach the resulting dendogram was compared with that obtained by rep-PCR, a commonly applied gene-based fingerprinting technique.

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Impact of Saccharomyces cerevisiae ester genes deletion on metabolites production during wines alcoholic fermentation and sensorial characteristics.

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Many studies have highlighted the impact of yeast metabolism on foodstuff quality. The role of some esters on red wines aroma is a very good example of this general behaviour. Esters levels are highly influenced during fermentation by the yeast strain. *Saccharomyces cerevisiae* produce not only ethyl esters of short- to medium chain fatty acids (FAEE) but also acetate esters of some alcohols (AHA). Four genes playing a role in FAEE (*EEB1* and *EHT1*) and AHA (*ATF1* and *ATF2*) biosynthesis have been defined.

This study was designed to determine the impact of these 4 genes deletion on esters synthesis during alcoholic fermentation and organoleptic characteristics of the wines produced.

The mutant yeasts were done using the same genetic background than the strain FX10. First, single mutants were constructed by homologous recombination using deletion cassettes amplified by PCR. Then, quadruple (Fx10-ΔAE) deletion mutants were constructed crossing together single mutants and selecting appropriate meiotic clones from the resulting hybrid.

Fermentations of Fx10-ΔAE mutant yeast in enological medium were compared to the original FX10 using two different matrices. After alcoholic fermentation, esters were analyzed using GC-MS methods.

Statistical analyses showed a significant impact of the genes deletion on the ester contents in both matrices used. In this study yeast effect explains almost all the variation of ester levels between FX10 and FX10-ΔAE fermented wines. Gene deletion lead to a very weaker fruity character of the wines produced, fact which is totally in agreement with the strong decrease of the esters content.

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Higher antioxidant defences result in a better industrial performance in non-*Saccharomyces* wine yeasts

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In recent years there has been a growing interest in the usage of non-*Saccharomyces* yeasts as co-inoculums in the wine industry. There have been many reports in which different species have been used in mixed fermentations alongside *Saccharomyces cerevisiae*. Their ability to produce secondary metabolites, reduce alcohol content or their specific enzymatic activities affect the wine profile, resulting in wines with enhanced and more complex flavours and aromas, which makes them important biotechnological tools. Most of these non-*Saccharomyces* yeasts occur naturally in grapes and wine environments, and although there are already commercially available strains, most inoculums have not been produced at an industrial scale. We aim to characterize the behaviour and redox state of a set of non-*Saccharomyces* yeasts during yeast biomass propagation and dehydration, the two main industrial processes in active dry yeast production. We also intent on understanding the effects of the oxidative stress associated with these processes in their industrial performance. In this study we used a set of biochemical and physiological parameters that allowed us to determine the redox state of the studied yeasts, amongst them we analysed trehalose and gluthatione levels, gluthatione reductase and catalase activity, and lipid peroxidation. In order to analyse the industrial performance of our set of yeasts we measured the fermentative capacity and their viability after dehydration. Our results show high variability between the studied yeasts, those with increased antioxidant defences present a better tolerance and a reduction in fermentative capacity loss after dehydration, due to reduced oxidative damage.

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Functional analysis of *Pichia Kudriavzevii* isolated from traditional Korean fermentation starter Nuruk

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Saccharomyces cerevisiae SD1-2 and *Pichia kudriavzevii* N77-4 were isolated from traditional Korean alcohol drink and alcohol fermentation starter, respectively. Since both yeast strains were tolerant to high concentration of ethanol and also showed extracellular β-glucosidase and protease activities that contribute to increasing volatile components, they are suggested to play important roles in alcohol drink making. To analyze the basic characters of both strains and to enhance the utility of N77-4 strain in fermentation industries, functional analysis of SD1-2 and N77-4 strains were performed. Growth test showed that although SD1-2 strain was not able to grow well at 13°C and 41°C, N77-4 strain displayed robust growth at 13°C, 30°C and 41°C. Fermentation test revealed that SD1-2 and N77-4 strains produced 6.8 g/L and 7.4 g/L ethanol, respectively, in 20 g/L glucose medium. However, growth rate and ethanol production yield of N77-4 strain decreased in 250 g/L glucose medium. Spot test also showed that N77-4 strain showed sensitivity on media containing more than 200 g/L glucose. It is well known that high concentration of glucose induces several stress adaptation responses in *S. cerevisiae*. Elucidation of these adaptation responses in *P. kudriavzevii* is prerequisite for improving its resistance against high concentration of glucose toward enhancement of the ethanol production. Molecular analysis of these stress responses in N77-4 will be discussed.

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Genome sequence and annotation of the non-conventional yeast Hanseniaspora guilliermondii UTAD222

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Non-*Saccharomyces* yeasts have several interesting metabolic and enzymatic properties that are absent in *Saccharomyces cerevisiae* and that contribute to improve wine sensory profiles. Species of the *Hanseniaspora* genus are among those found to be more interesting for the production of wines with stylistic properties in co-culture with *S. cerevisiae*. In this work we have sequenced the genome of *H. guilliermondii* UTAD222, a strain that was isolated from a wine must and that was found to have multiple interesting oenological traits including high tolerance to ethanol, low production of H_2S and high proteolytic and β-glycosidase activities[1]. In this work the genome sequence of the UTAD222 strains is disclosed, as well as a corresponding manually curated annotation. Up to now this is the first genome sequence described for the *H. guilliermondii* species. The results of the analysis of the genome sequence of the UTAD222 strain will be discussed and compared with those of other species of the *Hanseniaspora* genus and with the genomes of *Saccharomyces cerevisae* wine strains, in a comparative genomics perspective. It is expected that the release of UTAD222 genome sequence could contribute to a better understanding of the role played by non-Saccharomyces species in wine fermentations. These result will also shed light into the poorly studied biology and physiology of the H. guilliermondii species, fostering its exploitation species as a cell factory since it has several interesting phenotypic traits that turn it attractive for industrial application.

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Involvement of FLO 5 in flocculation phenotype and adhesive properties of a Saccharomyces cerevisiae sparkling wine strain

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Yeast flocculation is an asexual calcium-dependent aggregation and, in Saccharomyces cerevisiae, it is regulated by the expression of FLO genes, which are hot spots of recombination. Despite there are several works focused on this topic, the specific contribution of FLO genes in flocculation ability is still unclear. The aim of this work was to study the distribution of FLO1 and FLO5 genes among 3 S. cerevisiae wine strains with different flocculation degree (F6789, F7101 and RT73). Subsequently, FLO1 and FLO5 genes were separately deleted in F6789 flocculent wine strain. After gene disruption, flocculation capacity, agar adhesion and Flo proteins distribution were evaluated. The inheritance of these two FLO genes was also followed. All strains presented different lengths for FLO1 and FLO5 genes. The deletion of FLO genes revealed that in F6789 FLO5 drove flocculation phenotype because its deletion eliminated most of the ability to flocculate. The invasive growth assay demonstrated that FLO5 gene influenced the cell-surface adhesion. In addition, the inheritance of FLO5 gene from F6789 was necessary and sufficient for flocculation development. Flo proteins distribution at the cell surface by an immunofluorescence staining contributed to demonstrate the key role of Flo5p in flocculation. In conclusion, different flocculins can have the same physiological function, because the members of FLO family have partial functional redundancy. The evolutionary pressure exercised by different environmental stimuli allows the predominance of one gene over the others.

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Rational selection of flocculent *Saccharomyces cerevisiae* strains for traditional sparkling wines production

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Traditional sparkling wines undergo two fermentation processes and yeasts involved in secondary fermentation should show specific characteristics, such as flocculation capacity and autolysis. This study aimed at the selection of flocculent strains to be used as starter culture for traditional sparkling wine production. Six flocculent *Saccharomyces cerevisiae* wine strains with different flocculation degree and autolytic activity and two commercial strains (FI and EC1118) were tested for the main oenological features (ethanol, reducing sugar, pH, volatile acidity, TTA, organic acids and glycerol) and aromatic profile during sparkling wine production in a winery. Moreover, the expression of genes (*FLO*1, *FLO*5, *FLO*8, *AMN*1 and *RGA*1) regulating flocculation was evaluated. Results demonstrated that FI strain showed the best fermentation kinetics, while even if flocculent yeasts started fermentation later reached the maximum pressure. However, flocculent strains produced wines more fruity than the controls after 6 months of aging, in fact esters and alcohols were the most produced groups. Gene expression analysis revealed that *FLO*5 was the most important gene for flocculation development in traditional sparkling wine, independently from flocculation degree. Probably, because in non flocculent strains pseudogenes were amplified. This study provides a further step to better understand the role of *FLO* genes in stress response during traditional sparkling wine production in cellar.

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Impact of nitrogen sources on the fermentative kinetic of non-*Saccharomyces* yeasts

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Alcoholic fermentation, an essential step of winemaking, has been mainly controlled over the past 50 yeasts through the use of selected starter cultures of *S. cerevisiae*. This is the safest way to ensure the completion of fermentation and to avoid undesirable off-compounds. The non-*Saccharomyces* yeasts, predominant in grape juice, are rapidly outcompeted by *S. cerevisiae* during fermentation because of their poor adaptation to increasing concentrations of ethanol and the lack of oxygen. Even though these species were used to be considered as spoilage microorganisms for a long time, their potential to improve the sensory quality of wines is now recognized. However, the metabolic specificities of non-*Saccharomyces* yeasts remain ill-characterised, in particular their ability to catabolize the nitrogen compounds. Therefore, it is critical to fill this shortcoming, nitrogen assimilation playing a key role in the fermentation process and the production of aroma compounds.

With this aim, fermentations were carried out in presence of an unique nitrogen source (27 experiments per strain). Monitoring the production of CO_2 thanks to a robot-assisted system allowed to compare the fermentation profiles of *S. cerevisiae* and non-*Saccharomyces* strains. We observed that non-*Saccharomyces* yeasts displayed a shorter lag phase than *S. cerevisiae*, likely reflecting a better adaptation of these species to wine environment. Different behaviours between strains were evidenced, depending on the provided nitrogen source, highlighting the preferred amino acids specific to each species.

This information will now allow to better manage the nitrogen nutrition during wine fermentations that use non-conventional yeasts sequentially inoculated with *S. cerevisiae*.

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Gene deletions helping reduce acetic acid production by *Saccharomyces cerevisiae* during aerated fermentation

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Our group is interested in reducing ethanol yield during wine fermentation, to counter the growing sugar content in grapes due to climate change, and avoid the negative impact of increasing ethanol content in the wine market. While respiratory metabolism appears as an attractive alternative to pure fermentation, to redirect carbon flux towards sensory neutral products (CO_2), both the Crabtree effect of *Saccharomyces cerevisiae* and the tendency to increased acetic acid production during aeration (as we have previously shown), constitute a hurdle to industrial application of respiration compatible fermentation conditions. However, mechanisms involved in acetic acid production by *S. cerevisiae* in the presence of oxygen are yet poorly understood. We addressed this topic by deletion of several genes, *HXK2*, *REG1*, *PDE2*, *PDC1* (homozygous) and *PYK1* (heterozygous) in the wine yeast strain FX0 (Laffort) background. Some of them had been previously related with the Crabtree effect. Our results show that oxygenation helps reducing ethanol content of wines. However, there is little impact of the deletions on this reduction. Interestingly, some of these deletions do results in a reduction in acetate yields during oxygenated fermentation of natural grape must.
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Improvement of maltose assimilation ability in Japanese *sake* yeast. -One of MALR genes is crucial for maltose assimilation ability.

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Maltose assimilation in the budding yeast *Saccharomyces cerevisiae* requires a series of MAL genes (*i.e.* maltose transporter (MALT), maltase (MALS), and a positive regulator of these genes (MALR)). Yeast cells lacking any one of them cannot assimilate maltose. As the maltose assimilation is crucial for beer brewing, comprehensive understanding of the MAL genes is important. In this study, we focused on the low maltose assimilation ability of Japanese *sake* yeast, and tried to improve it by genetic approach.

First, we obtained several mutants with a restored ability of maltose assimilation from a *sake* yeast strain by treatment of a *sake* yeast strain with ethyl methane sulfonate and the following selection on maltose medium containing antimycin A. Next, we examined which mutation conferred the maltose assimilation ability of the mutants. We found that the function of one of MALR genes is restored by regaining of one base at the site of deletion. The introduction of the restored MALR gene gave the original *sake* yeast an ability to grow well with maltose. Finally, we disrupted this MALR gene completely in the original *sake* yeast, and confirmed that the resulting disruptant exhibited little maltose assimilation ability compared to the weak but obvious assimilation of the original strain.

We concluded that this particular MALR gene, among other MALR-related sequences, was the causative gene of the low maltose assimilation ability in *sake* yeast.

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Differential nitrogen needs of *Saccharomyces cerevisiae* commercial wine strains: comprehensive phenotypic characterization

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Nitrogen is an essential nutrient for yeast during wine fermentation. The main objective of our study is to comprehensively determine the nitrogen demand of commercial *Saccharomyces cerevisiae* strains and explore the underlying genetic basis for different nitrogen requirements. Several approaches have been used to analyze the nitrogen requirements. In order to determine the influence of nitrogen concentrations on biomass formation, we monitored the growth profile in microtiter plates with synthetic must of 13 nitrogen concentrations. We also determined the biomass yield in chemostat during continuous culture with elevated nitrogen concentrations. Parallelly, in order to find out the influence of nitrogen concentration on fermentation speed, fermentations were carried out in synthetic must with nitrogen concentration 60, 140 and 300mg/L which represent nitrogen limited, standard and excess conditions. Competition fermentations have also been carried out between high and low nitrogen demanding strains. Moreover, the minimum nitrogen needed for keeping steady fermentation rate in chemostat cultures has been determined. Among all the industrial strains, one most representative strain of high and low nitrogen demand was selected respectively. We have concluded that under different nitrogen conditions, the growth and the fermentation profile is not always correlated. Analysis on molecular level will be done in the future work.

Furthermore, one of the most interested requests of wine industry is the timing and the composition of nitrogen supplementation for nitrogen-deficient fermentations. We are conducting experiments to introduce nitrogen at different fermentation stages and with various nitrogen sources to try to find out the best combination.

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