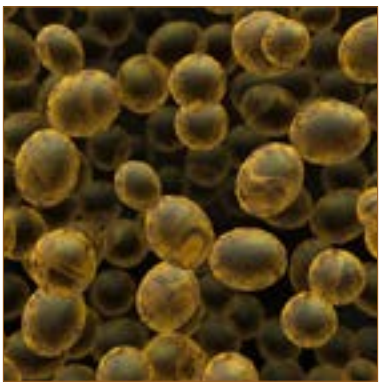
Poster Presentations

Yeast Congress 2018



World Yeast Congress

May 14-15, 2018 | Montreal, Canada

Evaluation of some probiotic properties of yeasts isolated from Turkish cheeses

Abudureyimu Maihebubai, Yavuz Beyatli, Zehranur Yüksekdağ and **Maimaiti Akepaer**
Gazi University Faculty of Science, Turkey

The possible beneficial properties of dairy foods and associated microorganisms for both human and animal health are increasingly investigated. In this study, *three Pichia kudriavzeii* (M16, M17 and M57) and one *Kluyveromyces marxianus* (M29) strains isolated from Turkish cheeses were evaluated for some functional properties relevant to their use as probiotic. All strains were able to grow under low acid condition (pH 3) and survived well (61.4-100%) in the presence of conjugated bile salts (0.3%) after 48 h of incubation, while producing exopolysaccharide (EPS) ranging between 55.3-130.7 mg/L. All yeast strains presented high auto-aggregation ability in the range of 70.6-88.7%. All strains also showed higher hydrophobic activities in acidic chloroform and toluene solvents compared with the neutral p-xylane solvent and basic ethyl acetate solvent. Only *P. kudriavzeii* M57 showed inhibitory activities on Bacillus cereus RSKK 863 (11.7 mm) and Pseudomonas aeruginosa ATCC 27853 (11.9 mm). In conclusion, the presented results indicate that both *Pichia kudriavzeii and Kluyveromyces marxianus strains* isolated from cheeses could be regarded as appropriate candidate for new probiotic yeast strains, they could be used as adjunct cultures for contributing to the quality and health related functional properties of dairy products.

Speaker Biography

Abudureyimu Maihebubai has recently completed her PhD from Gazi University and preparing for her Post-doctoral Program in Abroad. During her graduate studies, she has published 6 papers in reputed journals and has attended many international Congress.

e: mehbubem@gmail.com

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Hydroxyurea arrests *Saccharomyces cerevisiae* cells in G1/early S-phase of the cell cycle and limits rRNA synthesis

Alexia Muguet[1], Romain Charton[1], Joachim Griesenbeck[2], Michael J. Smerdon[3] and **Antonio Conconi[1]**
[1]Université de Sherbrooke, Canada
[2]Universität Regensburg, Germany
[3]Washington State University, United States

The chemotherapeutic agent Hydroxyurea (HU) inhibits the ribonucleotide reductase preventing the synthesis of dNTPs. Consequently, DNA replication is inhibited and cells arrest in G1/early S-phase of the cell cycle. Additionally, yeast exposed to the natural pheromone α-factor arrest cell division in G1. Cell growth hinges on the tightly regulated processes of ribosome biogenesis and rRNA synthesis. Thus, expression of rRNA genes and rRNA processing were analyzed in cell cycle arrested cells by both the chemotherapeutic agent HU and the pheromone α-factor. Chromatin endogenous cleavage, chromatin immuno-precipitation, chromatin spreading and Northern blotting were employed to investigate the effect of HU on the expression of rRNA genes and rRNA synthesis. The results indicate that in yeast arrested by HU the overall number of active promoters remains unchanged, and that rRNA genes chromatin stay poised for transcription. However, distribution of RNAPI on individual rRNA gene and rRNA processing are disturbed, lowering rRNA synthesis. Conversely, in α-factor arrested cells rRNA transcription was not affected. These results point out a hitherto unnoticed cellular response to HU that might participate in the inhibition of cell division. NSERC and Ministère des Relations Internationales du Québec (to AC), Bavarian State Chancellery (Bayerisch-Franzosisches Hochschulzentrum, to JG).

Speaker Biography

Alexia Muguet has a master in marine ecology from the Université Pierre et Marie Curie - Paris VI (France) (2014) and a master in microbiology from the Université de Bretagne Occidentale (France) (2015). She previously worked on microalgae ecophysiology before starting studying microorganisms at molecular level. During her master internship, she worked on the replication helicase MCM from *Pyrococcus abyssi*, an Euryarchaeota. As PhD student at Université de Sherbrooke (Québec), she is studying DNA repair mechanisms and chromatin on *Saccharomyces cerevisiae.* Her main work is analyzing the rRNA gene proteome linked to UV radiation and Nucleotide Excision Repair to highlight proteins involved in chromatin repair-dependent modifications. Alexia participated in two published papers and in in-redaction one.

e: Alexia.Muguet@USherbrooke.ca

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The effects of nanosecond pulsed electric fields on *Saccharomyces cerevisiae* cells

Povilas Šimonis
Center for Physical Sciences and Technology, Lithuania

Saccharomyces cerevisiae is one of the most well-studied and understood eukaryotic organisms. The studies of yeast cell allow reconstitution of possible molecular mechanisms of various abiotic effects. Pulsed electric field has been one of the most intensively investigated abiotic effects on biological tissues and cell suspensions for a past decade. It has been previously shown that a nanosecond pulsed electric field (nsPEF) permeabilize the plasma membrane, alter gene expression, cause phosphatidylserine translocation, affect the distribution of intracellular ions and even lead to the death of mammalian cells. There is still a lack of sufficient data related to the effects of nsPEF on yeast cells yet. In our study we analyzed the effects of square shaped electrical pulses of different duration (τ= 10-90 ns) and pulse number (pn= 1-5) with electric field strength (E) up to 220 kV/cm and showed that nanosecond pulses can induce the cell death, which in turn is dependent on the electric field pulse parameters and increase with the rise in E, τ and pulse number. Exposure of yeast cells to nsPEFs was accompanied by metacaspase activation, membrane permeability to propidium iodide and the externalization of phosphatidylserine. Furthermore, the investigation of yeast cells permeabilization to tetraphenylphosphonium ions (TPP+), which was induced by high power nanosecond duration electrical pulses, had demonstrated the following features: (i) The study of TPP+ ions absorption rate by yeast cells is an effective method for detection of short duration electric pulse influence on yeast cell wall properties; (ii) Shortening of the electric pulse duration makes it possible to achieve more homogeneous electrical treatment of yeast cell clusters and by this way to increase the effectiveness of single cell permeabilization; (iii) The significant acceleration of TPP+ ions absorption rate (up to 65 times) can be achieved without any influence on the vitality of the cells. We conclude that square shaped electric field pulses with nanosecond durations induce wide variety of effects including caspase-dependent apoptosis, oxidative stress, cell wall permeabilization, and that such abiotic treatment can be used in various applications starting from food safety ensurance and ending in medicine field.

Speaker Biography

Povilas Šimonis has finished master studies Biochemistry (Vilnius University) and started his Chemistry PhD (Center for Physical Sciences and Technology) in 2016. During his scientific career he participated in various schools related to application of pulsed electric fields including: EBTT – international scientific workshop and postgraduate course, school on applications of Pulsed Electric Fields for food processing. He is a member of ISEBTT (International Society of Electroporation – Based Technologies and Treatments). Presented his working results in more than 10 local and international conferences. Currently his scientific data is already published in Bioelectrochemistry Journal.

e: simonis.povilas@gmail.com

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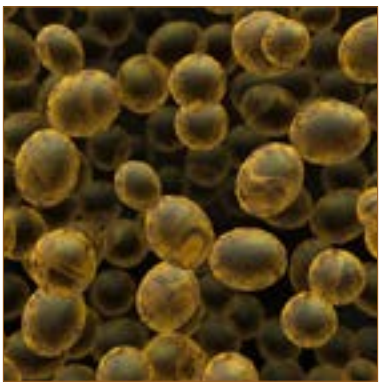

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Identification of some probiotic properties of yeast isolates from Turkish cheeses

Abudureyimu Maihebubai, Yavuz Beyatli and **Zehranur Yüksekdağ**
Gazi University Faculty of Science, Turkey

Yeasts not only play an important role in flavor and texture development during the production of cheese, also have shown probiotic effects on human health. In this study, four yeast isolates from Turkish cheeses were characterized to species level by phenotypic criteria using API ID 32 microbial identification kits and 18S rRNA sequence analysis. Three of them identified as *Pichia kudriavzevii* (M16, M17 and M57), while another one was *Kluyveromyces marxianus* (M29). Yeast strains were tested for their ability to survive in simulated gastric juice and intestinal environment. The survival of all tested yeasts was 88.9-145% after 4 hours of incubation in media with the addition of 1 g/L pancreatin and 46.3-80.4% after 3 hours of incubation in media with the addition of 3 g/L pepsin (pH 1.5). All yeast strains were able to assimilate cholesterol in the range of 9.3-28.8% over 48 hour's incubation. The DPPH radical scavenging activity of yeast strains was ranging between 75.1-80.5%. According to these results, the yeast strains could be considered as co-culture or probiotic in the preparation of fermented dairy products for contributing to the quality and health related functional properties of products.

e: mehbubem@gmail.com

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Intraspecific diversity of recombination in *S. cerevisiae*

Matthieu Falque
GQE– Le Moulon, INRA, Univ. Paris-Sud & Université Paris-Saclay, France

Allelic recombination due to meiotic crossovers is a major driver of genome evolution, as well as a key player for the selection of high-performing genotypes in economically important species. To get more insights into crossover regulation, we developed a high-throughput method to measure recombination rate and crossover interference in 26 *S. cerevisiae* strains representing a large part of the diversity of the species. 15 intervals were monitored, covering chromosomes VI and XI entirely, and part of chromosome I. Average recombination rates and recombination landscapes varied significantly across strains, and some regions showed up to 9.5-fold variation. We observed interference which varied across strains and was positively correlated with crossover number. Recombination rate was strongly and negatively correlated with whole-genome sequence divergence between homologs, but less so when using solely the sequences of the intervals probed for recombination and even less so when using the sequences in the DSB rich regions within these intervals, indicating that the negative correlations are not explained by cis-effects only. Finally, to investigate the genetic architecture of crossover rate, we built an incomplete diallel design from five parental strains and measured recombination in one region of chromosome XI for 10 different hybrids. The results suggest that recombination rate across hybrids may be mainly controlled by the level of sequence divergence between parental strains and by inbreeding effects, while additive effects of parental alleles were hardly significant. These results open the way to a better understanding of the genetic control of crossover formation, as well as building more efficient designs for yeast selection in industrial applications.

e: matthieu.falque@inra.fr

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CD^{+2} resistance mechanism in *Candida tropicalis* 3Aer isolated from industrial effluents

Abdul Rehman and **Zaman Khan**
University of the Punjab, Pakistan

Present investigation is elucidating the bioremediation potential and cadmium-induced cellular response with its molecular basis in *Candida tropicalis* 3Aer. Spectroscopic analysis clearly illustrated the involvement of yeast cell wall components in biosorption whereas bioaccumulation was confirmed by TEM, SEM and EDX scrutiny. TEM images divulged extracellular as well as cytoplasmic and vacuolar cadmium nanoparticle formation, further validated by presence of *ycf1* gene and increased biosynthesis of GSH under cadmium stress. Transcriptomic and proteomic approaches have rarely been applied to study change in cell architecture under environmental stress conditions, but this study is unveiling the altered expression of proteins and genes in *C. tropicalis* 3Aer under cadmium stress in concentration and time dependent manner, respectively. Fourteen proteins exhibited differential expression and found involve in cellular redox homeostasis, nitrogen metabolism, nucleotide biosynthesis and carbohydrate catabolism. Interestingly, C. *tropicalis* 3Aer is additionally equipped with nitrile hydratase enzyme, rarely been reported in yeast and thus have potential to remove nitriles (extremely toxic compounds) from environment. Cd^{+2} toxicity not only caused growth stasis but also upregulated the cysteine biosynthesis, protein folding and cytoplasmic detoxification response elements.

e: rehman_mmg@yahoo.com

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Evolutionary Diversification of Paralogous Genes in the yeast *Saccharomyces cerevisiae*: Its Physiological Role

Alicia González
Universidad Nacional Autónoma de México

For many years, it was accepted that the *Saccharomyces cerevisiae* (*S. cerevisiae*) lineage arose from a Whole Genome Duplication (WGD), making this yeast an interesting model to study diversification of paralogous genes. Recently, a phylogenetic study found compelling evidence indicating that *S. cerevisiae* lineage arose from an interspecies hybridization between one strain related to the Kluyveromyces, Lachancea and Eremothecium (KLE) clade and another one related to Z*ygosaccharomyces rouxii and Torulaspora delbrueckii* (ZT). Although whether the hybrid was the result of the fusion of two diploid cells or two haploid cells that underwent a WGD, is still an open question, both scenarios result in the formation of an allotetraploid with two copies of every gene. After the allotetraploid was formed, intragenic recombinations, full gene conversion, differential gene loss and selection pressures shaped *S. cerevisiae* genome to the one we observe today, harboring conserved blocks of duplicated genes. Retained duplicate genes (paralogs) can simply provide increased dosage of the same protein, or may go through a process of subfunctionalization or neofunctionalization, in which both copies of the gene lose a subset of their ancestral functions, while acquiring new properties. *S. cerevisiae* has been used as a model organism to analyze gene duplication dynamics and the functional fates of duplicated genes. In this conference I will present and discuss functional diversification pathways of three paralogous gene pairs, whose products are involved in amino acid metabolism and whose sub-subfunctionalization led to the separation and specialization of the ancestral function between the two duplicated genes. Examples of the subfunctionalization of paralogous pairs which was been achieved through: i) modifications of the coding sequence leading to paralogous proteins with particular kinetic properties *(GDH1/GDH3)*, ii) modifications of the regulatory region determining differential expression of each gene copy BAT1/BAT2 leading to the specialized functions of Bat1 and Bat2 encoded transaminases, and iii) selective organization of homo or hetero-oligomeric isozymes with peculiar biochemical properties (*LEU4/LEU9)*, will be presented and the functional repercussion of diversification will be amply discussed.

e: amanjarr@ifc.unam.mx

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Studies on biological denitrification using sequencing batch reactor

Jitendra Pal and **Anushya A**
National Institute of Technology Karnataka, INDIA

Nitrate pollution is caused through the introduction of excessive amount of nitrates in to surface and ground water mainly as a result of agricultural activities, human wastes or industrial pollution. Many industries such as nuclear industry, food processing, fertilizers, alcohol and metallurgy generates effluents with high concentration of nitrates. Nitrates are soluble in water and nitrate polluted water causes serious environmental and health issues. This study investigates the nitrate removal by heterotrophic denitrification process in Sequencing Batch Reactor (SBR). This process has been chosen because of its simplicity and higher efficiency for denitrification of wastewater. A facultative microorganism was isolated from NITK hostel septic tank waste water and screened under aerobic as well as anoxic conditions. To isolate a facultative bacterium from the sample, primary screening was done after enriching the culture. Further screening was done to identify a efficient denitrifying bacteria. The efficient bacterial strain (S26) was identified as Bacillus pumilus by 16S rRNA genome sequencing method. The effect of various parameters influencing denitrification by *Bacillus pumilus* was studied. As a result, denitrification could be obtained at PH 7-7.5, incubation temperature of 35- 40ºC with carbon source as acetate. Kinetics studies for denitrification were conducted and biomass yield at different nitrate concentrations were experimented and maximum specific growth rate of 0.074/h was observed during the 6^{th} hour of exponential phase. In future bionitrification experiments to be conducted in Sequencing Batch Reactor to study the effect of influent nitrate concentration and effect of carrier loading.

e: anushya.ashokan@gmail.com

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Benefits of probiotic yeasts in human and animal health

Ashima Vohra
University of Delhi, India

Saccharomyces boulardii is emerging as potential probiotic organism. This yeast has shown promising results in preventing enteral nutrition-related diarrhea, acute gastroenteritis, traveler's diarrhea and decreasing *Helicobacter pylori* treatment-related symptoms. The role of S. boulardii for both the prevention of Antibiotic-associated diarrhea (AAD) and treatment of irritable bowel syndrome and recurrent *C. difficile disease*, Crohn's disease, and giardiasis has been clearly demonstrated. Probiotic yeast has been considered superior over probiotic bacteria because of the natural resistance of yeast to antibacterial antibiotics. Probiotic yeasts have also shown a positive effect on poultry health and nutrition by reducing lactic acid acidosis, increasing fiber digestibility, secreting enzymes and improving animal performance by enhancing their growth rate and increasing milk, meat and eggs production. In the present study, *Saccharomyces cerevisiae* Id18 isolated from traditional Indian fermented food – Idli batter, exhibited probiotic attributes such as acid and bile salt tolerance, ability to grow at 37ºC, resistance to commonly used antibiotics, auto-aggregation ability and cell surface hydrophobicity. It showed antimicrobial action against enteric pathogens. It produced phytase, β-galactosidase, vitamin B12 and exopolysaccharides. It had the ability to assimilate cholesterol. This probiotic yeast, when used either alone or in combination with traditional dairy starter, significantly improved the nutritional properties and the shelf life of the fermented dairy product.

e: vohra_ashi@yahoo.co.in

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Yeast as a test-bed for drug discovery: From target engagement to drug resistance

Corey Nislow
University of British Columbia, Canada

Saccharomyces cerevisiae has served as genetic model organism for over a century, as a genomic powerhouse since it was the first eukaryote to have its genome sequenced in 1996, and more recently as a test-bed for the development and application of chemogenomic assays. Baker's yeast has also provided fundamental insights into evolutionary conserved biology as witnessed by three Nobel prizes attributable to yeast- in cell-cycle biology, secretion and autophagy. Its simplicity of cultivation, combined with its functional conservation allows for the discovery of novel chemical probes which can serve as tools to probe biological function and new leads for drug discovery. In this talk, I will describe how the HIHOP laboratory, established by Guri Giaever, has deployed yeast-based assays to discover novel-target-drug interactions, understand the mechanism by which drug resistance develops and map the chemical-genetic portrait of an organism.

e: corey.nislow@ubc.ca

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Bridging the gaps: An innovative and integrated undergraduate fermentation science course

Paul M Duffin
Transylvania University, USA

Undergraduate science is traditionally both taught and learned through disciplinary lenses. Often, students compartmentalize knowledge in courses and have difficulty making connections between disciplines. As brewing beer encompasses biology and chemistry, we (a physical chemist and a microbiologist) developed and team-taught an innovative and integrated undergraduate course on the science of fermentation. The course was taught during Transylvania University's May term where students take one intensive course for 5 weeks. The course explored the scientific principles of fermentation and was structured around students brewing standard 5-gallon batches of beers from malt extract. The course also covered the major characteristics of beer, the role of brewing ingredients/processes and how they affect the final product, and involved student measurements of various chemical and microbiological aspects of beer in the laboratory (e.g. microscopy, spectroscopy). Pre- and post-tests and attitudinal survey data from the students suggest that using this team-taught approach aided students to see the interconnectedness of biology and chemistry as they apply to brewing. At the end of the course students reported greater confidence in their ability to brew beer, increased understanding of beer and brewing in scientific terms, and the ability to identify beer styles bsed on taste, smell, and color. We can also report that the course affected two students deeply; one now works as a quality control chemist at a commercial brewery and one has become an avid home brewer

e: pduffin@transy.edu

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Screening and characterization of stress tolerant yeasts in Nipa Sap fermentation from Cagayan and Quezon Provinces, Philippines

James Paul T Madigal and **Shirley C Agrupis**
Mariano Marcos State University, philippines

In an effort to establish a holistic and S & T-based village-scale nipa bioethanol industry, an effort to identify super indigenous yeast species to improve ethanol fermentation and to identify microbial community structure residing in and responsible for natural fermentation of nipa sap. A total of 13 yeast isolates were obtained from the natural nipa sap fermentation in two selected sites of the Philippines, Brgy. Cabaggan, Pamplona Cagayan and Brgy. Tapucan, Mauban Quezon. They were purified and categorized into groups based on the combinations of cultural and molecular characteristics. Based on the banding patterns of isolates generated by microsatellite (GTG)5 fingerprinting, the thirteen isolates were clustered into four groups. Representatives of each group were sequenced using Internal Transcribed Spacer (ITS). After sequencing the ITS PCR genes and phylogenetic analysis using MEGA7, three isolates shared 99% identity of the ITS rDNA genes with *Saccharomyces cerevisiae* and the other one isolate showed 99% match with two P. *kudriavzevii* strains. These four representative yeast isolates were subjected to different stress tolerance tests. Among all yeasts, YC03 strain was the most highly acid tolerant (tolerated pH 2.0) and high temperature tolerant (tolerated 45°C). These interesting characteristics may find applications in further molecular biology researches such as the use of this strain as 'yeast cell factory'. The four profiled yeasts were evaluated for ethanol production efficiency using different ethanol production media and top ethanol producer was chosen. YMU1 strain gave the highest ethanol produced using different media with comparable ethanol yield (51.70 g L^{-1}) against reference strain *HBY3* (51.90 g L^{-1}) after 48 hour fermentation using synthetic media (YPD broth). However, using molasses-based medium and simulated nipa sap medium, ethanol produced by yeast strain YMU1 was observed to be significantly different from HBY3 after 48 hours of fermentation. Ethanol yield coefficient and specific growth rate of YMU1 were computed as 0.51 g g-1 and 0.0072 h-1, respectively. The application of selected isolates as fermenting organism in bioethanol production from various feedstock in addition to nipa sap could be investigated in further studies.

e: cigfah22@yahoo.com

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Yeast species as biological control agents of fungal plant pathogens

Katia Cristina Kupper
Centro de Citricultura "Sylvio Moreira", Brazil

The use of yeast species represents a promising strategy as biocontrol agents. Different yeast species are able to prevent infection, decrease host tissue colonization, and reduce plant pathogen survival and sporulation with varying degrees of efficiency. Despite the known biological roles of yeasts in the environment, however, much remains to be discovered regarding its modes of action in distinct environments and their antagonistic behavior toward other organisms. The purpose of this study was to isolate and select yeasts from citrus leaves, flowers and fruits as well as from citrus growing soils and determine the adequate strains for post-harvest diseases biocontrol both in vitro and in vivo. Additionally, to evaluate the modes of action of yeasts isolates that previously shown to be effective in controlling sour rot (*Geotrichum citri-aurantii*), green mold (*Penicillium digitatum*) and blue mold (*P. italicum*) in citrus fruits and, finally, to purify and characterize the killer toxin produced by yeast isolates and, verify their antagonistic activity on pathogens that occur in citrus postharvest. The results obtained in this study showed that the isolates ACBL-42 (*Sporobolomyces koalae*) and ACBL-77 (*Aureobasidium pullulans*), showed efficient control as a preventive and as well as curative measure for sour rot and were able to produce chitinase in the presence of the G. *citri-aurantii* cell wall. A. pullulans produced killer toxin against Geotrichum. *Saccharomyces cerevisiae* (ACB-K1) when applied as a preventive measure against P. *digitatum* promoted 73% healthy fruits. This yeast provided 100% disease control in 'Tahiti' acid lime fruits under refrigeration (10oC and 95%RH) when combined with a quarter dose of imazalil fungicide. Studies about modes of action of this yeast against *Geotrichum citri-aurantii* demonstrated that ACB-K1 produced hydrolytic enzymes (chitinases and β-1,3-glucanases), killer activity and inhibited conidial germination. The multiple modes of action (killer activity, production of chitinase and inhibition of conidial germination) presented by the Candida stellimalicola strains against P. *italicum* may explain why these yeasts provided control of blue mold in citrus fruits.

e: katia@ccsm.br

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A novel plasma membrane regulator of *Calcium homeostasis* in yeasts

Linghuo Jiang
Shandong University of Technology School of Agricultural Engineering and Food Science, China

Homologous to the vertebrate solute carrier SLC10A7, Rch1 is a novel regulator of *calcium homeostasis* in the plasma membrane of the budding yeast *Saccharomyces cerevisiae* and the human yeast pathogen Candida albicans. ScRCH1 is a functional homolog of CaRCH1. ScRch1 and CaRch1 negatively regulate the calcium uptake in response to high levels of extracellular calcium in *S. cerevisiae* and *C. albicans*, respectively. However, CaRch1 is constitutively expressed, while *ScRch1* is induced by a high level of calcium ions. Transcriptional expression of ScRCH1 is positively regulated by calcium/calcineurin signaling through the sole CDRE element in its promoter. Furthermore, distribution of ScRch1 proteins in the plasma membrane changes in a dynamic way, from multiple foci prior to cell division, accumulation at the bud neck during bud growth, and dispersion along the plasma membrane immediately prior to cytokinesis. Rch1 is a novel member regulating calcium homeostasis in yeasts.

e: linghuojiang@hotmail.com

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Real-time analysis of replicative senescence at single cell resolution

Maria Teresa Teixeira[1], Zhou Xu[1], Héloïse Coutelier[1], Erin Henninger[1], Pascale Jolivet[1], Stefano Mattarocci[1], Serge Pelet[2], Marie Doumic[3] and **Gilles Charvin[4]**

[1]Sorbonne Université, France

[2]University of Lausanne, Switzerland

[3]Université Pierre et Marie Curie, France

[4]Institut de Génétique et de Biologie Moléculaire et Cellulaire, France

Failure to maintain telomeres leads to their progressive erosion at each cell division and replicative senescence, a cell cycle arrest mediated by the DNA damage checkpoint signaling. To understand the flow of signaling events from telomeres to cell proliferation cessation, we set up a microfluidics-based live-cell imaging assay to investigate replicative senescence in individual *Saccharomyces cerevisiae* cell lineages following telomerase inactivation. Using this strategy, we found that most lineages experience an abrupt and irreversible transition consistent with a model where the first telomere reaching a critical short length triggers senescence onset. However, many lineages undergo frequent reversible DNA damage checkpoint cell-cycle arrests, beginning soon after telomerase inactivation (Xu et al, Nat Com, 2015). Here, we provide evidence that this novel phenotype stems from replicative stress at telomeres and gives rise to genomic instability, a hallmark of senescence escapers. First, we demonstrate that the DNA damage tolerance pathway is critical for viability immediately after telomerase inactivation. More specifically, Rad5 and Rad51 operate cooperatively and sequentially to bypass replication barriers at telomeres and the repair choice is modulated by Srs2 and orchestrated by PCNA modifications. Second, the long reversible arrests are suppressed in an adaptation defective mutant of the polo-like kinase Cdc5. This mutant strongly reduces the senescence-specific genome instability and alters the post-senescence survival patterns. Thus, replication stress at telomeres revealed by telomerase inactivation, initiates repair and adaptation pathways, leading to genomic instability and to potential post-senescence survival. Overall, our findings provide an essential mechanistic link between ageing and cancer emergence.

e: teresa.teixeira@ibpc.fr

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Bioengineering of yeast cell for biodiesel production

Priya Kumari and **Naseem A Gaur**
DBT-ICGEB Centre for Advanced Bioenergy Research, New Delhi

Due to increased oil demand, depleting fossil fuels and greenhouse gas emissions, biofuels production are getting much attention. The fatty acid based biofuels (fatty acids ethyl ester/biodiesel, fatty alcohol, etc.) produced from microbial cells have emerged as ideal alternatives to fossil oils, with significant pluses over plant, animal and algae oils. *Saccharomyces cerevisiae* is a most studied industrial model microorganism and also its fatty acid production ability has been increased by metabolic engineering approach. But still the cost of the process limits its industrial production therefore, more research is required. Here, we are addressing this issue by sequential metabolic engineering approach. In order to synthesize biodiesel in yeast cells, we integrated wax ester synthase (WS2) gene from *Marinobacter hydrocarbonoclasticus* into its genome. The genetic engineering approaches have focused on high-level biodiesel production by rewiring metabolism pathways to upsurge carbon flux towards fatty acid CoA synthesis, by increasing the cofactor supply, and disrupting the degradation pathway.

e: priya7654@gmail.com

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Naganishia qatarensis sp. nov. novel basidiomycetous yeast species from hypersaline marine environment

Rashmi Fotedar[1], Jack W. Fell[2], Anjana Anand[1], Ameena Al Malaki[1], Aisha Zeyara[1], Anna Kolecka[3] and **Teun Boekhout[3] Masoud Al Marri[1]**
[1]Department of Genetic Engineering, Biotechnology Centre, Ministry of Municipality and Environment, Qatar.

Two yeast strains isolated from the hypersaline marine environment from inland sea, Qatar, were classified as members of the genus *Naganishia* based on sequence analysis of the D1/D2 domains of the large subunit rRNA gene and the internal transcribed spacer (ITS) regions. The rRNA gene sequence analyses indicated that the two strains represented a novel species of the genus Naganishia, for which the name *Naganishia qatarensis* sp. nov. is proposed. They clustered in a strongly supported clade represented by *Naganishia albidus* in the *Tremellales* group in the phylogenetic tree drawn from ITS and D1/D2 sequence. The new species grows at 4°C and 35°C. The significance of findings will be discussed.

e: rfotedar@hotmail.com

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Improvement of chocolate flavor by yeast during cocoa fermentation

Rosane Freitas Schwan
Universidade Federal de Lavras, Brazil

Cocoa research performed during the last century has elucidated the basic physiology and ecology of cocoa fermentation and the biochemical changes that occur during cocoa fermentation, drying and roasting that lead to the development of the chocolate flavour. Biotechnological manipulation of the steps of microbial fermentation (microorganisms, amount of pulp, selected strains) can result in understandable and reasonably predictable effects on chocolate quality. Many different species of microorganisms have been isolated from cocoa fermentation and have been characterised and the microbial succession has been defined. Yeast are essential to the fermentation process and development of chocolate flavour. The concept of using starter cultures to conduct cocoa bean fermentations is not new. Initially, around 1960-1980, the aim was to induce a faster, more consistent fermentation, without adverse impact on chocolate quality. More specific investigations on the use of starter cultures have now been conducted where the main goals have been to develop a faster, more consistent fermentation process that yields cocoa beans with predicable qualities. The dynamic of *Saccharomyces cerevisiae,* P*ichia kluyveri and Hanseniaspora uvarum* during spontaneous and inoculated cocoa fermentations and their effect on sensory characteristics of chocolate were investigated. Yeast populations were assessed by qPCR. S. cerevisiae was predominant during spontaneous (average 5.4 log cell/g) and inoculated (average 7.2 log cell/g) fermentations. The *H. uvarum* seemed to be suppressed by the other two yeasts, as it showed similar population (approximately 4.0 log cell/g) even in the inoculated assay. Carbohydrates were consumed quickly at inoculated fermentation (68% and 42% were consumed in the inoculated and control assays respectively, at 24 h). Ethanol content was higher in the inoculated (8.3 g/kg at 48 h) than in the control (4.6 g/kg at 96 h) fermentation. Chocolate produced from the spontaneous fermentative process presented dominance of the bitter flavour, while obtained through inoculated fermentation process presented bitter, astringent, coffee and acid as dominant flavours. The inoculation accelerated the fermentative process in 48 h. The inoculation of yeast influenced the microbial profile, which affected the volatile compounds that affect sensory characteristics, resulting in chocolate with dominant bitter, cocoa, and fruity attributes.

e: rschwan@dbi.ufla.br

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Bioethanol production by thermotolerant yeast *Kluyveromyces marxianus* from sugarcane bagasse

Sachin Kumar, Meenu Hans and **Nilesh K Sharma**
Sardar Swaran Singh National Institute of Bio-Energy, India

Second generation (2G) bioethanol is a clean and renewable source of energy, which could be produced by lignocellulosic biomass (LCB) such as agricultural, forestry, municipal, and industrial wastes. LCB contains cellulosic and hemicellulosic fractions, which is yielded into pentose and hexose sugars using pretreatment and enzymatic saccharification. Most of the ethanol producing microorganisms are either hexose sugars utilizing or utilize pentose sugars inefficiently along with hexose sugars. However, the utilization of both pentose and hexose sugars is required for economical fuel ethanol production. An isolated thermotolerant yeast Kluyveromyces marxianus NIRE-K3 is able to utilize both pentose and hexose sugars. However, the utilization of pentose sugar (xylose) is very slow as compared to hexose sugar (glucose). The present study was carried out to develop *K. marxianus* NIRE-K3.2 for enhanced xylose utilization through two-phase evolutionary adaptation, and analyzed the bioethanol production potential of adapted *K. marxianus* NIRE-K3.2 from sugarcane bagasse (SCB) in comparison to native yeast. The two-phase evolutionary adaptation was carried out: first in YEPX medium (20gl^{-1} xylose) for 60 generations followed by second in minimal salt medium containing 20gl-^{1} xylose for 55 generations. Liquid ammonia pretreated SCB was enzymatically saccharified using Novozyme Cellic Ctec2. The maximum concentrations of glucose and xylose in hydrolysate were found to be 35.45gl^{-1}, and 14.92gl-^{1}, respectively. The fermentation of enzymatic hydrolysate was carried out using native NIRE-K3 and adapted NIRE-K3.2, separately at 45oC and pH5.5. NIRE-K3 showed utilization of 43.23% xylose, whereas, NIRE-K3.2 utilized 75.06% xylose present in hydrolysate. The ethanol yields obtained by NIRE-K3.2 was equivalent to 92.15% of the theoretical yield, whereas, 60.78% in case of NIRE-K3. The adapted strain NIRE-K3.2 showed 34% improved ethanol yield by utilizing xylose efficiently along with glucose as compared to that of native strain. The aforesaid results show the importance of evolutionary adaptation to develop enhanced xylose utilizing thermotolerant yeast *K. marxianus* NIRE-K3.2 for bioethanol production by utilizing both pentose and hexose sugars in SCB.

e: sachin.biotech@gmail.com

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Reducing ground water consumption in Pakistani distillery through very high gravity technology

Muhammad Arshad
University of Veterinary and Animal Sciences, Pakistan

Very high gravity technology (VHG) was implemented on industrial scale fermenters to study the reduction in fresh water consumption in the process as well as ethanol and by products formation during molasses fermentation. Different brixº 32, 36 and 40 with aeration rates 0.00, 0.20, 0.40, and 0.60 vvm has been applied. The maximum ethanol production was 12.2% (v/v) at 40º brix with 0.2 vvm aeration. Byproducts have the increasing trend with the brixº but aeration rate 0.2 vvm was found to be optimum for byproduct formation throughout the study. The high ethanol % attained had eased the distillation process and steam consumption reduced significantly. More over water consumption was reduced by 35% decreasing the stillage volume. Reduction in steam consumption decreases the overall water utilization by improving the economics of industrial ethanol production process significantly. Decrease in stillage volume is helpful in combating this environmental pollutant efficiently.

e: muhammad.arshad@uvas.edu.pk