A Newsletter for Persons Interested in Yeast

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Printed and Electronic Subscriptions

A reminder of some recent changes in the subscription rates and modalities. The printed version of the Yeast Newsletter will continue to be available to readers for USD$8.00 (Canada and U.S.A.) or USD$12.00 (all other countries). To facilitate accounting and administration, the subscription is due immediately upon receipt of the invoice that accompanies the December issue. Credit card payments can only be accepted for payments of USD$40.00 or more.

The electronic version is sent free of charge to readers whose accounts are in order. To be added to the electronic mailing list, please email me at lachance@uwo.ca.

Readers who have not renewed for 2005 were sent, in April, June, and October, reminder cards indicating that their subscriptions were due. Readers who have not replied were removed from the mailing list. Please encourage your colleagues who should be readers of the Yeast Newsletter to contact me for a subscription, as further reminders will not be sent.

Websites

Readers who have websites dealing with their activities with yeasts are invited to send the URLs so that they can be added as links to the YNL home page. URLs of other websites of potential interest to our readers are also welcome.

Please be sure to add a link to the YNL in your own web page.

http://publish.uwo.ca/~lachance/YeastNewsletter.html

Back Issues

We are still missing issues of the YNL published prior to November 1958 and would welcome these.

M. A. Lachance
Editor
European yeasts researchers interested to come to work at CBS for short time studies (up to three months) can do it in the framework of the SYNTHESYS program (www.synthesys.info).

Those interested should contact T. Boekhout <boekhout@cbs.knaw.nl> or V. Robert <robert@cbs.knaw.nl> in order to develop a proposal.

Recent publications

2004


2005


2006


Laboratory for Microbial and Biochemical Sciences, Georgia State University, Atlanta, GA 30303, U.S.A. Communicated by DG Ahearn, S Zhang, and C Mateus <zhangshangtong@yahoo.com>.

Recent publications.


Wild-type and efflux pump-deficient cells of Candida albicans adhering to silicone were compared with planktonic cells by flow cytometry for their relative resistance to fluconazole (FCZ). Flow cytometry data on cells carrying a fusion of green fluorescent protein to efflux pump promoters confirmed that enhanced tolerance of adhered cells to FCZ was due in part to increased expression of CaMDR1 and CDR1 promoters. Within 2 h of their attachment to silicone, the adherent cells demonstrated levels of FCZ tolerance shown by cells from 24-h biofilms. Following their mechanical detachment, this subset of cells retained a four- to eightfold increase in tolerance compared with the tolerance of planktonic cells for at least two generations. Enhanced efflux pump tolerance to FCZ appeared to be induced within the initial 15 min of attachment in a subset of cells that were firmly attached to the substrata.


Planktonic and attached cells of strains of Candida albicans, C. glabrata and C. krusei with varied susceptibilities to fluconazole (FCZ) were compared for their relative susceptibilities to Ag⁺ via cell recovery and flow cytometric analyses. All strains lost membrane permeability and were non-recoverable upon culture after one hour exposure in morpholino-ethanesulfonic acid (MES) buffer fortified with = 2.0 µg/ml Ag⁺. Cells attached to silicone over a 2-h period demonstrated enhanced tolerance to FCZ and to a lesser degree to Ag⁺. Minimal inhibitory concentrations of Ag⁺ in defined media increased in the order C. glabrata, C. krusei, C. albicans. Susceptibilities to Ag⁺ did not correlate with tolerance or resistance to FCZ.
Recent publications


When in vitro-cultivated potato (Solanum tuberosum) and tobacco (Nicotiana tabacum) are inoculated with antagonistic yeast Pseudozyma fusiformata, yeast cells become stable associated with plants. The colonized plants manifested improved growth characteristics and increased resistance to phytopathogenic fungus, Sclerotinia sclerotiorum, as compared with uncolonized plants. The combined technique of microbial colonization and plant micropropagation provides the basis for new technologies of plant cultivation, and plant-associated antagonistic yeasts are perspective for biocontrol of phytopathogens.

IV Department of Applied Microbiology, Lund University, PO Box 124, 221 00 Lund, Sweden. Communicated by MF Gorwa-Grauslund <marie-francoise.gorwa@tmb.lth.se>.

The department of Applied Microbiology pursues several axes of research on yeast.

**Yeast as biocatalyst for stereoselective reductions**

Saccharomyces cerevisiae is being genetically engineered to generate efficient biocatalysts for the reduction of dicarbonyl compounds of pharmaceutical or chemical interest. Work is focussed on (i) the isolation and expression of reductase genes from various sources and (ii) the engineering of pathways that provide NADPH that is the co-factor needed for the bioreduction.


**Design of pentose-utilising yeast**

Recombinant xylose- and arabinose- utilising Saccharomyces cerevisiae strains are designed for ethanol production from lignocellulosic hydrolysates using both metabolic engineering and inverse metabolic engineering strategies.


M. Jeppsson, B. Hahn-Hägerdal and M-F. Gorwa-Grauslund 2006 Reduced affinity of *Pichia stipitis* xylose reductase for NADPH increases ethanol production from xylose by recombinant *Saccharomyces cerevisiae*. Biotech Bioeng 93:665-673.


K. Öhgren, O. Bengtsson, M.F. Gorwa-Grauslund, M. Galbe, B. Hahn-Hägerdal and G. Zacchi 2006 Simultaneous saccharification and co-fermentation of glucose and xylose in steam-pretreated corn stover at high fiber content with *Saccharomyces cerevisiae* TMB3400. J Biotechnol - Accepted.

V Laboratorio de Microbiología Aplicada y Biotecnología (Applied Microbiology and Biotechnology Lab.), Centro Regional Universitario Bariloche, Universidad Nacional del Comahue. Quintral 1250, (8400), Bariloche, Argentina. Communicated by D Libkind <libkind@crub.uncoma.edu.ar>.

Recent publications.


Mycosporine-like amino-acids (MAAs) are found in aquatic bacteria, algae, and animals. A related compound, the mycosporine-glutaminol-glucoside (myc-glu-glu), has recently been reported in freshwater yeasts. Although animals depend on other organisms as their source of MAAs, they can efficiently accumulate them in their tissues. In this work we assessed the potential transfer of the yeast mycosporine myc-glu-glu from the diet into the copepod *Boeckella antiqua* and the ciliate *Paramecium bursaria*. For this purpose, we performed experiments to study the feeding of *B. antiqua* and *P. bursaria* on the yeast *Rhodotorula minuta* and their ability to bioaccumulate myc-glu-glu. Bioaccumulation of myc-glu-glu in *B. antiqua* was assessed through long-term factorial experiments manipulating the diet (Chlamydomonas reinhardii and *C. reinhardii* + yeasts) and radiation exposure (PAR and PAR + UVR). Shorter term experiments were designed in the case of *P. bursaria*. The composition and concentration of MAAs in the diet and in the consumers were determined by HPLC analyses. Our results showed that even though both consumers ingested yeast cells, they were unable to accumulate myc-glu-glu. Moreover, when exposed to conditions that stimulated the accumulation of photoprotective compounds (*i.e.* UVR exposure), an increase in MAAs concentration occurred in copepods fed *C. reinhardii* plus yeasts as well as in those fed only *C. reinhardii*. This suggests that the copepods were able to modify their tissue concentrations of MAAs in response to environmental clues but also that the contribution of yeast mycosporines to total MAAs concentration was negligible.

Publications in press.


Licenciate degree theses in Biological Sciences, Universidad Nacional del Comahue, Argentina.


Ultraviolet radiation (UVR) has great biological importance given it has the potential of inducing lethal or at least, deleterious effects in all organisms. Yeasts are unicellular fungi and several groups prevail in high UVR exposed environments. The production of secondary metabolites such as carotenoid pigments and mycosporines (MYC), could represent some of the photoprotection mechanisms of this microorganisms. However, the photoprotective role of carotenoid pigments is still controversial, and the experimental evidence accumulated up to now is not concluding. In this work, yeasts general response to UVR was studied using yeasts as model organisms. Photoprotective function of carotenoid pigments in yeasts is assessed by comparing pigmented strains and co-specific naturally occurring albino strains in laboratory experiments. All assays performed verified that UVR-B resistance in yeasts is frequently related to the ability of producing carotenoids, and that higher survival to UVR-B can be observed when carotenoids concentration increases. These results suggest that carotenoid pigments have an important photoprotective role in yeasts accounting for population success under high UVB conditions.


The glaciers of Nahuel Huapi National Park constitute unexplored environments concerning yeast biodiversity, and arise as possible reservoirs of cold-adapted microorganisms. Mount Tronador (3,554 m.a.s.l.) is located 71° 50’ W and 41° 10’ S in Chile – Argentina border and 4 of its 10 ice tongues are situated within Nahuel Huapi National Park (NHN). Occurrence of yeasts in rivers draining from three glaciers (Frias, Castaño Overo and Río Manso) has been studied for the first time and is reported here. One hundred and nine strains were isolated and identified up to the genus level by biochemical and molecular techniques. Basidiomycetous yeasts represented 90% of the isolates. Cryptococcus, Leucosporidiella, Dioszegia, Rhodotorula, Rhodosporidium, Mrakia, Sporobolomyces, Udeniomyces and Candida genera were found. Cryptococcus was the most frequently isolated genus in all samples (49% of the total strains), while Leucosporidiella accounted for 16%. From 21 identified species, Cryptococcus sp. and Leucosporidiella fragaria were the most representative ones. These results are in agreement with reports on yeasts diversity in Antarctic, Artic and Alps glaciers. The ability to synthesize mycosporines was detected in 33 of 78 strains isolated from these glacial environments all belonging to the Class Hymenomycetes (Orders Tremellales and Filobasidiales). Also seventy six cold-adapted strains were tested in their ability to synthesize cold-adapted enzymes. Most of the strains tested show lipolytic activity at 4 °C. The potential of these cold-adapted microorganisms as biotechnological sources of photo-protective compounds and cold adapted enzymes is an interesting and promising field of research.

Lic. Gabriel Russo 2006 Yeasts from an aquatic acid environment: Agrio River and Caviahue Lake (Caviahue-Copahue Provincial Park)

The Agrio River and the Caviahue Lake is located at 1,606 m a.s.l. in the Caviahue-Copahue Provincial Park at the Northwest of Neuquén Province (37°52’S 71°02’W). This environment is characterized by a pH gradient from 1.5 at the affluent of the Superior Agrio River up to pH 6.7, 15 km downstream Caviahue Lake. The acidity is due to the sulfuruous emanations from Copahue Volcano. The objective of the present work was to study the diversity of yeasts present in the acid aquatic environment of the Agrio River and the
Caviahue Lake, for which samples of water from seven places along the pH gradient were analyzed. Yeasts numbers were registered for each sample site by using three culture media at pH 5 and 3, and one which was formulated with the water of each sampling site. The isolated yeasts strains were identified by phenotypic and molecular techniques. The latter included the Micro/mini Satellite Primed Polymerase Chain Reaction (MSP-PCR) technique and the sequencing of the D1/D2 domain of the 26S ribosomal DNA. Highly variable yeast counts were observed showing an increment along the river and lower values in the Caviahue Lake. A total of 202 yeasts strains were isolated, which were classified, based on phenotypic characterization, in five groups corresponding to the genera Candida, Cryptococcus, Rhodotorula, Cystofilobasidium and Sporobolomyces. Posterior molecular studies allowed us to identify 23 species of which 9 represent novel species. Cryptococcus sp.1 and Rhodotorula mucilaginosa were the dominant species in number and distribution in the studied sites. Cryptococcus sp. 2 showed preference for acidified culture media. This species presented also its optimal growth pH at 3.5 which probably makes it the first acidophilic yeast isolated from Argentina. The comparison of yeasts obtained in the present study with works performed in acid aquatic environments of Spain and Portugal (Tinto River and Santo Domingo Mines respectively) allowed us to identify similarities in species composition and diversity of these extreme systems.

VI Laboratorio de Microbiologia, Instituto Superior de Agronomia (CBAA), 1349-017 Lisboa, Portugal. Communicated by M. Malfeito-Ferreira <mmalfeto@isa.utl.pt>.

Recent publications.


A total of 63 strains of Dekkera bruxellensis and 32 strains of Pichia guilliermondii isolated from wine related environments were identified by restriction analysis of the 5.8S-ITS region of the rDNA. These strains were subjected to intraspecific discrimination using mtDNA restriction and RAPD-PCR analysis. The isolates identified as D. bruxellensis yielded 3 different molecular patterns of mtDNA restriction using the endonuclease Hind I. The pattern A was the most frequent (58 strains) among strains from different sources, regions and countries. Pattern B (4 strains) and C (one strain) were determined in isolates from Portuguese wines. The discrimination among the pattern A strains was achieved by a RAPD-PCR assay with 3 primers (OPA-2, OPA-3 and OPA-9). A total of 12 haplotypes were obtained with the combination of the patterns provided by the 3 OPAs. The pattern 2 was the most frequent and extensively distributed being found in strains from different countries and from different sources like wine, barrique wood and insects. The strains of P. guilliermondii were characterized with restriction of mtDNA using the endonuclease Hind I yielding 7 different restriction patterns. These patterns were associated with different efficiencies of 4-ethylphenol production. Patterns A to D corresponded to 19 strains producing low levels of 4-ethylphenol (< 1 mg/l) while patterns F and G grouped 13 strains producing high levels of 4-ethylphenol (> 50 mg/l), when grown in synthetic media supplemented with 100 mg/l of p-coumaric acid. The high degree of polymorphism observed shows that intraspecific typing is essential for accurate yeast dissemination studies in wine related environments.


The behavior of Pichia guilliermondii strains producing high levels of 4-ethylphenol in synthetic media was studied in wines and grape juices. These strains lost their viability and did not produce 4-ethylphenol after 24 hr of inoculation in red wines with ethanol adjusted to 10 or 12 % (v/v) and pH 3.5, in the absence of free sulphite. Under the same conditions, at 12 % (v/v) ethanol, growth of Dekkera bruxellensis was observed. When grown in single culture in grape juices, selected strains of P. guilliermondii produced high levels of 4-ethylphenol. In mixed grape juice fermentations with Saccharomyces cerevisiae,
*P. guilliermondii* began to die after starter inoculation at 107 cfu/mL and did not produce 4-ethylphenol. Low starter inoculation rates (102 cfu/mL) added 72 hr after *P. guilliermondii* inoculation resulted in high production of 4-ethylphenol. In conditions mimicking cold pre-fermentative maceration processes, at 10ºC for 72 hr, *P. guilliermondii* did not grow, while at 25ºC growth attained a 104 fold increase. At this temperature, addition of 200 mg/L potassium metabisulfite after grape crushing did not eliminate *P. guilliermondii* inoculated at 104 cfu/mL in grape juice of pH 3.57. The possibility that high levels of 4-ethylphenol in wines are due to the activity of *P. guilliermondii* should be mostly related with uncontrolled growth in contaminated grape juices before starter inoculation. In wines, its ability to produce 4-ethylphenol seems to be much lower than that of *D. bruxellensis*.

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**Central Control and Testing Institute for Agriculture, Matúškova 21, 833 15 Bratislava, Slovakia. Communicated by E. Minárik.**

Summaries of recent publications.


   Wine yeast strains differ in their capacity to produce extracellular polysaccharides. Mannoproteins represent excellent means for intensifying malolactic fermentation. It is underlined that the amount of polysaccharides released by wine yeasts during alcoholic fermentation depends on the metabolic phase end state of yeasts, on the yeast strain and on the original amount of macromolecules in the wine. The ability of wine undergoing malolactic fermentation (MLF) is correlated with the macromolecule quantity released by wine yeast cell walls during alcoholic fermentation and by the contact time of the vine with yeasts.


   Magnesium is responsible for full utilization of wine yeasts in biochemical and biotechnological processes. Factors reducing Mg activities (e.g. calcium) show negative influences on yeast growth and metabolism. Calcium also displays negative properties on yeast cell physiology of full Mg uptake. Magnesium thus represents an exceptional cation in physiological metabolic processes retaining wine yeast viability and vitality. Mg-Ca antagonism in alcoholic fermentation of grape must is considered.


   Light smack may have different origins. UV-light evokes several modifications in sparkling wine composition by ester decomposition. Riboflavin is able to evoke the same decomposition type of ethylhexanate. Research results of photochemical changes in grape vine flavour are briefly discussed.


   Fructophilic yeasts *Candida stellata* and *C. bacillaris* display priority in fermenting fructose compared with glucose fermentation. These yeasts might be suited for residual saccharide fermentation of sweet wines. Morphological and physiological properties as well as possible technological utilization of *Candida stellata* are discussed.
The following are publications for 2005, 2006, or those in press.


   Using RFLP-analysis of PCR-amplified rDNA fragment, spanning the 5.8S rRNA gene and internal transcribed spacers ITS1 and ITS2, we found significant heterogeneity of the species *Zygowilliopsis californica*. Phylogenetic analysis of nucleotide ITS1 and ITS2 rDNA sequences differentiated three varieties: *Z. californica* var. *californica*, *Z. californica* var. *dimennae* and *Z. californica* var. *fukushimae*. More variable is the ITS2 region: 7-26 nucleotide substitutions. The varieties formed semi-sterile hybrids with meiotic segregation of control markers. Limits of the phylogenetic species concept are discussed.


   In consequence of the year-round investigation the number of ascosporogenous yeast *Saccharomyces* was found to be increased sufficiently on living and decaying leaves of plants in separate short periods. Owing to this the massive isolation of *Saccharomyces* strains was conducted, while formerly this yeast was known to be common only in substrates with high sugar content. All strains were identified as *Saccharomyces paradoxus* on the base of physiological features and the lengths of restriction fragment of 5,8S-ITS rDNA. The possible reasons of the short-time enlargements of *Saccharomyces* number in phyllosphere are discussed.


   Using restriction analysis of non-coding rDNA regions, multiplex PCR and molecular karyotyping we examined *Saccharomyces* strains isolated from red berry wines in Russia, Belarus and Ukraine. According to molecular analysis, all strains belong to *S. cerevisiae*. There is a correlation between microsatellite fingerprints of strains and the source of their isolation. Strains isolated from juices and from surfaces of different berries showed distinct PCR profiles. Genome composition of interspecific *Saccharomyces* hybrids of natural and laboratory origins was studied.


   Using molecular and genetic analyses we characterized 28 *Arthroascus* strains isolated from widely different geographic localities: Europe, North America, Far East Asia and Hawaii. Most of the strains have been assigned to the species *A. schoenii*. PCR-RAPD revealed two Japanese *Arthroascus* strains to have peculiar patterns. Comparative rDNA (D1/D2 26S, ITS1 and ITS2) sequence analysis showed that the two strains represent a new species and a new variety, respectively. Based on the results of sequence analysis, genetic hybridization and DNA-DNA reassociation we formally describe two new members of the genus *Arthroascus*: *A. babjevae* sp. nov. and *A. fermentans* var. *arxii* var. nov. Our results show that *A. schoenii* has a
world-wide distribution, while the species *A. javanensis* is only represented by the type culture CBS 2555 isolated in Indonesia. Cluster analysis revealed a correlation between PCR-RAPD fingerprints and geographic origin of the *A. schoenii* strains. Despite this molecular differentiation, *A. schoenii* strains collected in different regions of the world formed preponderantly fertile hybrids with normal recombination of control markers.

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**IX** Department of Microbiology and Molecular Genetics, Oklahoma State University, 422 Life Sciences East, Stillwater OK 74078, USA. Communicated by H Vishniac

<helen.s.vishniac@okstate.edu>.

Recent publication.


Yeast isolates from soil samples collected from a latitudinal gradient ( >77°S to >64°N) were subjected to multivariate analysis to produce a statistical foundation for observed relationships between habitat characteristics and the distribution of yeast taxa (at various systematic levels) in soil microbial communities. Combinations of temperature, rainfall (highly correlated with Net Primary Productivity), and electrical conductivity could explain up to ca. 44% of the distribution of the predominant yeast species, rainfall and pH ca. 32% of the distribution of clades in the most common orders (Filobasidiales and Tremellales), while vegetation type (trees, forbs, grass) played the same role for orders. *Cryptococcus* species with appropriate maximum temperatures for growth predominated in most soils. *Cryptococcus* species in the Albidus clade of the Filobasidiales predominated in desert soils; *Cryptococcus* species of other clades in the Filobasidiales and Tremellales in wetter and more-vegetated soils, with Tremellalean species favored in soils of lower pH or higher EC. The predominance of *Cryptococcus* species in soils has been attributed to their polysaccharide capsules, particularly important when competing with bacteria in arid soils.

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I have recently published a short summary of the current state of research on *Kluyveromyces lactis* as a model organism.


**XI** Institute of Fermentation Technology and Microbiology, Technical University of Lodz, 90-924 Lodz, Wolczanska 171/173, Poland. Communicated by D. Kregiel <dkregiel@p.lodz.pl> and W.Ambroziak <ambrowoj@p.lodz.pl>.

The following article is in press.


2 Lectures presented at the XIth School of Fermentation “Trends in Technology and Marketing of Beer”, 2006, April 29 – May 1, Lodz, Poland.

During the annual workshops initiated by Program Tempus S-JEP-09770/95, a series of lectures are conducted. The Schools of Fermentation are dedicated to technical staff of breweries as a cooperation on the line university – industry. At the XIth School of Fermentation, the lectures presented by scientists from Institute of Fermentation Technology and Microbiology covered a wide spectrum of problems from yeasts in brewery to quality of final product – beer.

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The following are abstracts of our recent work.


The diversity of yeasts in soil and litter samples taken from three Austrian natural forest reserves (Mülleröden, Saubrunn, Rotwald) was investigated. In total 82 yeast strains were isolated and identified using molecular methods. Partial sequencing of the 26S rDNA gene resulted in 25 different sequences belonging to eleven genera. For one sequence it was not possible to determine the genus membership. Eight species were identified via PCR-fingerprinting. The alluvial forest at Müllerboden showed the highest yeast diversity. The vast majority of the isolated strains belong to the ascomycetes yeasts, more than the half were members of the genus Cryptococcus (56 isolates belonging to seven species).


A new yeast, Cryptococcus zeae (type strain HB 1207T) is described. Six strains were isolated from corn and pests of corn in Austria. Microsatellite-primed polymerase chain reaction (MSP-PCR) fingerprints showed that the strains are members of the same species. Phylogenetical analyses of domains D1/D2 26S rDNA and ITS 1 - 5,8S - ITS 2 sequences showed Cryptococcus zeae to have the closest relationship to Cryptococcus luteolus. The D1/D2 sequences of C. zeae are 100 % fit to three Korean Cryptococcus sp. strains (AF459690, AF459691, AF459692). The new species is separable from the closest relative C. luteolus using only two physiological tests.

The department of Bioprocesses and Microbial Systems of the Chemical Engineering Lab. (CNRS UMR 5503) carries out different studies dealing with the industrial use of micro-organisms and specially yeast cells. Recent studies include the following: - Analysis of the interactions between Saccharomyces and non-Saccharomyces and also between Saccharomyces and lactic acid bacteria in wine making. A part of this work is done in cooperation with the Faculte d’Oenologie de Bordeaux (Pr. A. Lonvaud). - Studies on nutritional requirements of different strains of Saccharomyces in order to ensure a complete and fast fermentation of musts. - Studies on the yeast Brettanomyces. - Use of entrapped cells of yeast (Saccharomyces and non Saccharomyces) in wine making process. These studies are realized in cooperation with Proenol Lda in Portugal. At this time, the lab studies are achieved and many trials at industrial level are running. - Study on the effect of different pesticides on the yeast cell: the possible effects of different pesticides are analyzed on the kinetics of growth and production but also on the possible alterations of DNA (DNA adducts formation).
The main results are presented in the following publications.

Books

Papers

The following are abstracts of articles that were published recently and are in press.

The production of individual from of extracellular polygalacturonase by Aureobasidium pullulans from forest soil was found to depend on the pH of cultivation medium as well as on the nitrogen source in the precultivation or cultivation medium. Polygalacturonases were purified and characterized. The pH optima of polygalacturonases produced in the first phases of cultivation (24 or 48 h) and after 10 days as well as their molecular masses, isoelectric points, action pattern and ability to cleave polymeric and oligomeric substrates were different. Generally, polygalacturonases with random action pattern (EC 3.2.1.15) were produced only in the first phases of cultivation in acidic medium. The function of these enzymes for A. pullulans in the colonization of plant material rather than in the destruction of plant was hypothesized in physiological conditions. Exopolygalacturonases (EC 3.2.1.67) with terminal action pattern were produced in later phases of growth. Oligogalacturonase as well as strongly basic polygalacturonase with unusual action pattern on substrates were found.
Nowadays naturally occurring compounds with the potential antimitogenic and anticarcinogenic effects are of great importance for their prospective use in cancer chemoprevention and treatment. The new water soluble derivative of microbial polysaccharide β-D-glucan – carboxymethyl glucan (CMG) belongs to such a category of natural substances. CMG isolated from the cell wall of baker’s yeast Saccharomyces cerevisiae is included into the class of biopolymers known as biological response modifiers (BRMs) with a broad range of activities, above all ones interfering with cancer therapy. It was demonstrated on four experimental model systems that biological and consequential medicinal importance of CMG is based on the combined application with another active compound. In the Saccharomyces cerevisiae antimutagenicity assay CMG significantly reduced ofloxacin-induced mutagenicity in the yeast strain D7. CMG exerted bioprotective (anti-toxic and antimitogenic) effect after its simultaneous application with methyl methanesulphonate on the repair-deficient strain uvs10 of the unicellular green alga Chlamydomonas reinhardtii. In the Vicia sativa simultaneous phytotoxicity and anti clastogenicity assay CMG exerted statistically significant anticlastogenic effect against maleic hydrazide-induced clastogenicity in Vicia sativa L. Only in the Salmonella/microsome assay CMG did not exert statistically significant antigenotoxic effect, despite of the fact that it reduced 9-aminoacridine-induced mutagenicity in S. typhimurium TA97, but his revertants decreasing was statistically significant only at the highest CMG concentration used. The data presented unambiguously documented that even biopolysaccharides (e.g., derivatives of -glucan) belonging to the most abundant class of natural biopolymers may contribute to cancer prevention and therapy.

Radical-scavenging activity of the water-soluble derivative obtained from cell wall of the baker’s yeast Saccharomyces cerevisiae was investigated using the technique of electron paramagnetic resonance spectroscopy and its activity in the adjuvant arthritis Carbohydrate Polymers 61, 18–28, 2005.

Changes in the activity of cysteine (cathepsins B and L) and aspartyl (cathepsin D) proteases were investigated at the development of susceptible and resistant variants of murine lymphosarcoma (LS). It has been demonstrated that the variant resistant to the cyclophosphamide treatment is characterized by a lower activity of all three cathepsins in the tumor tissue. Application of a higher dose of cyclophosphamide led to a more pronounced increase of the studied enzymatic activity in mice with a resistant variant of LS, than in those with a susceptible one. Administration of a yeast polysaccharide derivative - sulfoethyl glucan - enhanced therapeutic effect of cyclophosphamide in mice with both variants of LS, while the most efficientdose was found to be that of 10 mg/kg body mass. In the intact mice, usage of both cyclophosphamide and sulfoethyl glucan led to a similar increase of the cathepsins activity in liver and spleen.

The extracellular polygalacturonases produced by *Aureobasidium pullulans* isolated from waters of the Danube river were partially purified and characterized. The pH optima of polygalacturonases produced in the first phases of cultivation (48 h) and after 10 d as well as their optima of temperature, thermal stabilities, molecular masses, isoelectric points, action pattern and ability to cleave polymeric and oligomeric substrates were compared. Polygalacturonases with a random action pattern (random cleavage of pectate forming a mixture of galactosiduronides with lower degree of polymerization) [EC 3.2.1.15] were produced only in the first phases of growth, while exopolygalacturonases [EC 3.2.1.67] with a terminal action pattern (cleavage of pectate from the nonreducing end forming d-galactopyranuronic acid as a product) were found during the whole growth. The main enzyme form with a random action pattern was glycosylated and its active site had the arrangement described previously for the active site of polygalacturonase of phytopathogenic fungi.


Since the content of hyaluronan (HA)-degrading enzymes in synovial fluid (SF), if any, is extremely low the high rate of HA turnover in SF is to result from a cause different from enzymatic catabolism. An alternative and plausible mechanism is that of oxidative-reductive degradation of HA chains by a combined action of oxygen and transition metal cations maintained in a reduced oxidation state by ascorbate.


The tolerance of seventy yeast strains belonging to 15 species, isolated from water and soil environments as well as from tree leaves, to four heavy metals – copper, zinc, nickel and cadmium were studied. We have found that the interspecific and intraspecific variations in metal tolerance among studied strains were considerable. The highest interspecific variations were observed toward copper and cadmium. The strains of the species *Sporobolomyces salmonicolor*, *Cryptococcus albidus*, *Cystofilobasidium capitatum*, *Saccharomyces cerevisiae*, and *Candida maltosa* belonged to the most sensitive ones. In general ascomycetous yeasts were more tolerant to heavy metals than basidiomycetous ones. The differences among strains that came from various natural sources were also found. The most sensitive yeast population originated from untilled soil whereas the most tolerant population was isolated from tree leaves.


Carboxymethylated derivatives were prepared from the (153)-_d-glucan isolated from the cell wall of baker’s yeast *Saccharomyces cerevisiae* and from the chitin-glucan complex of the mycelium of the industrial filamentous fungus *Aspergillus niger*. The polysaccharides were applied to peritoneal mouse macrophages and after a 2-h incubation the release of TNF-α by the stimulated macrophages was measured using an enzyme-linked immuno-sorbent assay. As the third polysaccharide stimulant, a water-soluble derivative of chitin was assayed and the observed cytokine release was compared with the control experiment. In three concentrations of the polysaccharides applied, carboxymethyl glucan revealed a dramatic increase in the TNF-α release, while addition of carboxymethyl chitin-glucan resulted only in a moderate enhancement, and carboxymethyl chitin was inactive. The results indicate that fungal polysaccharides, especially (153)-_d-glucan, are potent macrophage stimulators and activators of TNF-α release, which implies their potential application in antitumor therapy.


**Papers.**


3. David, M., Gabriel M., Kopecká M. Cytoskeletal structures and ultrastructural characteristics of the basidiomycetous yeast *Cryptococcus laurentii*. Before sending to press.

**Lectures.**


**Abstracts from conferences.**


**Recent papers.**


I shall present the following talk in August.

7. Lachance MA, Lawrie, D Dobson, J 2006 Sex, endemism, and gene flow in natural yeast populations. 8th International Mycological Congress, Cairns, Australia.
The following are summaries of our current projects.


   *Laboratory of Molecular Biology, Department of Molecular and Cell Biology, Division of Life Science, Graduate School of Agricultural Science, Tohoku University, Tsutsumidori-Amamiyamachi 1-1, Aoba-ku, Sendai 981-8555, Japan.

   Although chromatin structure was studied during the last 40 years, what is known now is mostly centered on the nucleosome structure and more or less on the 30-nm chromatin fiber. Chromatin structures higher than the 30 nm fiber are predominantly hypothetical. In order to shed some light on higher–order chromatin structures in the eukaryotic nucleus we combined a method for detection of DNA damages – the Comet assay with the well-known nucleases (MNase and DNase I). The result is a method with high sensitivity which effectively “senses” the differences in loop organization of nuclear chromatin. The budding yeast Saccharomyces cerevisiae serves as an indispensable tool in evaluation of eukaryotic chromatin structure. However, the chromatin of this yeast is distinctive. Linker histone Hho1p deficient strains are viable and show no distinguished phenotypic changes. In previous experiments we have demonstrated substantial differences in the susceptibility of chromatin to nucleases in such strains. These differences, according to our observations, in the higher order chromatin structure, could be observed by a very sensitive method developed by us - Chromatin Yeast Comet Assay (ChYCA). Act3p/Arp4 is an essential actin-related protein in Saccharomyces cerevisiae, involved in transcriptional regulation. By applying the method of ChYCA on Act3p/Arp4 mutant cells we demonstrate higher openness of chromatin to nucleases, when compared to wild type S. cerevisiae cells, presuming profound influence of Act3p/Arp4 on chromatin structure maintenance. Our work now advances toward revealing the characteristics of Arp4/Hho1p double mutants.

2. Peycheva E, Pevala V,* Kolarov J* and Miloshev G. DNA fragmentation during apoptosis in yeast Schizosaccharomyces pombe.

   *Department of Biochemistry, Comenius University, Mlinska dolina CH-1 str., 842 15 Bratislava, Slovakia

   Apoptosis is a highly regulated cellular process. Recently apoptosis-like processes have been also found in yeasts Saccharomyces cerevisiae and Schizosaccharomyces pombe. Therefore, they are successfully used as model organisms for investigating the regulation of apoptosis. The Bcl2 family of proteins known to be major regulators of apoptosis is present in higher eukaryotes but not in yeasts. The Yeast Comet Assay (YCA) facilitates detection of single and double-stranded DNA breaks as well as alkali-labile sites in its molecule. As the genomic DNA fragmentation is one of the hallmarks of the process the YCA method could enable perceptive investigation of DNA fragmentation during apoptosis. The kinetics of DNA fragmentation investigated by the method of YCA in S. pombe cells, expressing Bcl-XL or Bax proteins will be assessed.


   *Department of Developmental and Cell Biology, University of Rome ‘La Sapienza’, 5 Aldo Moro, 00185 Rome, Italy.

   The yeast Kluyveromyces lactis possesses several important characteristics which make them especially attractive and suitable for biotechnology purposes. After sequencing of K. lactis genome the efforts are now concentrated on characterization of their genes and gene products. At present our research is focused on characterization of the three K. lactis genes encoding proteins which displayed homology to S. cerevisiae carboxypeptidase Y greater than 50%. This includes in silico analysis of the coding sequences, and the upstream and downstream regulatory elements; comparison of the deduced amino acid sequences and search for common and specific conservative motifs in the predicted polypeptides. As a first step toward functional analysis, construction of strains with deletion of any of the three genes is in process. Phenotypic tests to study the impact of gene inactivation on cellular physiology will follow.
The KIPCL1 gene with an open reading frame of 1359 base pairs was isolated from K. lactis genomic library in a search for ScPRC1-related gene(s) in K. lactis. Sequencing and comparison of the KIPCL1 nucleotide sequence revealed identities with two S. cerevisiae genes, YBR139w and PRC1, and with three K. lactis ORFs. Our results showed that in K. lactis genome, KIPCL1 gene lies 1257 bp remote from the KlDUR3 gene. Alignment of the deduced KlPcl1p amino acid sequence disclosed strong similarities to carboxypeptidases from distantly related organisms, and KlPcl1p contains several highly-conserved regions characteristic of serine-type carboxypeptidase family. KlPcl1p mostly resembles the ScYbr139wp and KLLA0A09977g, whereas identities with ScPrc1p and KLLA0A09977g are slightly lower. However, in silico analyses revealed that KlPcl1p, just like ScPrc1p but in contrast to ScYbr139Wp, contains N-terminal signal sequence that could target the protein to the secretory pathway. We have also demonstrated that KIPCL1 is a non-essential gene for K. lactis. Inactivation of KIPCL1 neither impaired sporulation nor affected the growth ability of K. lactis cells under a variety of laboratory conditions. The nucleotide sequence of KIPCL1 was deposited in EMBL under accession no. AJ551275.

XVIII National Collection of Agricultural and Industrial Microorganisms, Corvinus University of Budapest, Faculty of Food Sciences, H-1118, Budapest, Somlói út 14-16, Hungary. Communicated by G. Péter <gabor.peter@uni-corvinus.hu>.

The following articles have been published since our last report.


Six strains of an unknown yeast species, phenotypically resembling Yarrowia lipolytica and isolated from chicken breast and chicken liver, were studied. The investigation of their small (18S) and large (26S) subunit rDNA revealed a robust genetic difference between these strains and the type strain of Y. lipolytica. A consistent difference in the physiological properties, suitable for separation of the two taxa, was also found. The description of the new anamorphic yeast species, Candida galli is given.


Two yeast strains, producing needle-shaped ascospores under suitable conditions, were isolated from grapes grown in Hungary. Based on these two strains, Metschnikowia viticola (type strain NCAIM Y.01705, CBS 9950, JCM 12561) is proposed as a new yeast species. Considering its phenotypic features, the restriction fragment patterns of 18S rDNA and the sequence of the D1/D2 domain of 26S rDNA, the proposed new species is closely related to Candida kofuensis.


Thirty-two strains, many of them isolated from wood-associated habitats, and designated as Kuraishia (Pichia) capsulata and Candida molischiana according to their phenotype, exhibited two types of HaeIII restriction fragment patterns of their small subunit rDNA with the neighboring ITS. One fragment pattern corresponded to that of the type strain of K. capsulata, whereas the other pattern was unique to the type strain of C. molischiana. Sequencing of the D1/D2 domain of the large subunit rDNA confirmed that the different HaeIII restriction fragment patterns of small subunit rDNA with the neighboring ITS reliably distinguished K. capsulata from C. molischiana. Ascospore formation was observed in several C. molischiana strains and K. molischiana (type strain: NCAIM Y.01725, CBS 9993) is proposed as the teleomorphic state of Candida molischiana.

The following papers have been recently published or are in press.


   The simple-septate basidiomycetes comprise more than 8000 species that show a high morphological and ecological heterogeneity. To gain insight in the phylogenetic relationships within this group we compared several ultrastructural features, such as septal pore apparatus, form and behaviour of the spindle pole bodies, types of host-parasite interaction, presence or absence of colacosomes, symplechosomes, atractosomes and cystosomes as well as nuclear rDNA sequences coding for small and large subunit rRNA. Based on our integrated analysis, we propose a new classification system for the simple-septate basidiomycetes with the subphylum Pucciniomycotina and the classes Agaricostilbomycetes, Atractiellomycetes, Classicolomycetes, Cryptomycocolacomyctes, Cystobasidiomycetes, Microbotryomycetes, Mixiomycetes and Pucciniomycetes. We also propose the pucciniomycotinous taxa Cystoasidiales, Erythroasidiales, Helicobasidiales, Mixiales, Naohideales, Pachnocyales, Spiculogloeales and Kondoaceae and the new subphyla Agaricomycotina (equivalent to the current Hymenomycetes) and Ustilaginomycotina (equivalent to the current Ustilaginomycetes).

Obituary

Joseph Owades Dies at 86; The Father of Light Beer
Adam Bernstein, Washington Post Staff Writer, Wednesday, December 21, 2005; B05
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Joseph L. Owades, 86, a biochemist credited with inventing, for better or worse, light beer but whose product lacked the macho marketing that later made Miller Lite a sensation, died of a heart ailment Dec. 16 at his home in Sonoma, Calif. Initially intrigued by the study of cholesterol, Dr. Owades entered the brewing trade through post-doctoral work in fermentation science. While working in Brooklyn, N.Y., at Rheingold Breweries, then an industry leader, he developed a process to remove the starch from beer. This reduced its carbohydrates and calories. "When I got into the beer business, I used to ask people why they did not drink beer," Dr. Owades once said. "The answer I got was twofold: One, 'I don't like the way beer tastes.' Two, 'I'm afraid it will make me fat.' "It was a common belief then that drinking beer made you fat," he said. "People weren't jogging, and everybody believed beer drinkers got a big, fat beer belly. Period. I couldn't do anything about the taste of beer, but I could do something about the calories." Introduced in 1967, his product was called Gablinger's Diet Beer. As Dr. Owades later said, the Gablinger's television advertisement showing a man with the girth of a sumo wrestler shoveling spaghetti into his mouth and downing a Gablinger's did little to help the cause. "Not only did no one want to try the beer," he said, "they couldn't even stand to look at this guy!" Plus, the name. Brooklyn Brewery President Steve Hindy once told the publication Modern Brewery Age that Gablinger's Diet Beer "doesn't exactly roll off the tongue." Moreover, Hindy said, Dr. Owades "didn't come up with 'tastes great, less filling.' And the beer ended up flopping."

With approval from his boss, Dr. Owades said, he shared his formula with a friend at Chicago's Meister Brau brewery, which soon came out with Meister Brau Lite. He routinely joked, "Being from Chicago, they couldn't spell 'light."' Miller Brewing acquired the light beer process when it bought assets of Meister Brau in the early 1970s. The "tastes great, less filling" marketing strategy, which used football players and other tough-knuckled types, helped Miller Lite flourish. Even if Gablinger's did not find eager takers, Dr. Owades was regarded as the father of light beer. He became an international consultant in beer, working through his Center for Brewing Studies. He moved to the Bay Area from Boston in the early 1980s. Although he lived near California's wine-growing region, he was never enthusiastic about aiding the wine business, because beer was simply more intriguing to him. "The making of wine does not require the skills of a biochemist," he told the San Francisco Chronicle. "The winemaker gets the liquid from which he makes wine prepackaged in little things called grapes. The brewer creates the liquid from which he makes beer."

Joseph Lawrence Owades was born July 9, 1919, in New York to parents from Ukraine. While growing up in the Bronx, he received a chemistry set from his mother, and his interest led him to study the science at City College of New York. He also received a master's and then a doctorate in biochemistry from Brooklyn Polytechnic Institute, now Polytechnic University. He briefly studied fermentation science at Fleischmann's Yeast before beginning a long career at Rheingold, where he rose to vice president and technical director. Soon after his work on Gablinger's, he held executive positions with Anheuser-Busch in St. Louis and Carling O'Keefe in Waltham, Mass. As a consultant since the mid-1970s, he helped craft formulas for Samuel Adams, New
Amsterdam Beer, Pete's Wicked Ale and Foggy Bottom Beer. When the long-defunct Rheingold name was revived in the late 1990s, Dr. Owades was hired to re-create his old recipe.

Some of his work was not terribly successful, including Yen Sum beer, a beverage he made with the herbal root ginseng. A clear malt drink, called Qruze and pronounced "cruise," was marketed at women. Owades said he wanted the aroma to have the allure of piña colada, but one beer scribe noted that it "smells a bit like suntan lotion." Dr. Owades held many patents and wrote about beer and brewing for technical journals. He held frequent seminars for beer enthusiasts, whether experts or novices, and could be cranky. "In this country, you can call anything an 'ale,' " he once said. He also described the odor of Corona as "skunky."

He was unpretentious as a teacher, refusing to use the periodic table as an educational tool. He preferred scribbling on a board: "The Stuff We Make Beer From." In 1969, he married Ruth Markowitz, who later sold a gardening catalogue to Williams-Sonoma, and then started the Calyx & Corolla flower catalogue business. Besides his wife, survivors include two sons and a brother.

Thanks to Wilfred Arnold for bringing this news item to our attention.

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**International Commission on Yeasts**

**Forty Years of the International Commission on Yeasts**

As indicated on the cover page, the Yeast Newsletter is the official publication of the International Commission for Yeasts (ICY). Many of the younger readers of the Newsletter may not know what ICY is and what is its history. ICY is an active international body, whose role is "to establish effective liaison between persons and organizations concerned in yeast investigations, and between them and the practical users of results of investigations, including yeast culture collections". This year we commemorate the 40th anniversary of the existence of the Commission. When and how did the story of ICY start? The creation of this body is linked to the second International Symposium on Yeasts which was held in July 1966, in Bratislava, Czechoslovakia, now the capital of Slovakia. The meeting was attended by 145 participants from 21 countries. During the Symposium the Czechoslovak representatives initiated the creation of an international organization which would stimulate scientific collaboration of people working with yeasts all over the world. A Council for International Collaboration in Yeast Science was founded. The late Dr. A. Kocková-Kratochvilová (deceased in 1992) was appointed Chair and Dr. Erich Minárik (82 this year), Secretary of the Council. Both were from Czechoslovakia.

The other endowing members of the Council were: K Beran (Czechoslovakia), A. Eddy (UK), P. Elinov (USSR), H. Klaushoffer (Austria), N.I. Kudrjavcev (USSR), U. Leopold (Switzerland), R. Muller (GDR), S. Nagai (Japan), O. Necas (Czechoslovakia), H.J. Phaff (USA), C.F. Robinow (Canada), H. Soumalainen (Finland), T. Tsuchiya (Japan), L.J. Wickerham (USA), T. Wikén (Holland) and S. Windisch (GFR), all well recognized yeast researchers at the time. During the meeting it was also agreed that the existing Yeast Newsletter, edited by H.J. Phaff at the University of California, would serve as the official publication of the Council. Thanks to
Prof. Phaff and later to Prof. M.A. Lachance, current editor, the Yeast Newsletter is still alive and bringing interesting and important information to our groups.

In early years after its foundation, the Council underwent changes in names and affiliations. In 1970, under the new name, International Commission on Yeasts and Yeast-like Microorganisms (ICY), it became a part of the Mycology Division of the International Union of Biological Sciences (IUBS). In 1981 ICY also joined the Mycology Division of the International Union of Microbiological Societies.

The main activity of ICY is the organization of International Symposia on Yeasts (ISY) at 3-5 years intervals, and more frequently, sometimes every year and in a different country, International Specialized Symposia on Yeasts (ISSY). The main organizer of each ISY becomes Chair of the ICY until the next ISY. Current the ICY Chair is Prof. Leda Mendonça-Hagler, who organized the successful 11th ICY, (11th International Congress on Yeasts), in Rio de Janeiro, Brazil, in August 2004. She will hold the Chair till the 12th ICY planned to be organized by Prof. A. Sibirny in the Ukraine in 2008.

On behalf of the readers and the editorial board of the Newsletter let us wish the International Commission on Yeast many successful activities and achievements in years to come.

Peter Biely, Associate Editor

**Forthcoming Meetings**

**FEMS 2006 - 2nd FEMS Congress of European Microbiologists**

*Madrid, Spain. July 4th-8th 2006*

On-line registration is still open at [www.fems2006.org](http://www.fems2006.org)

**19th International Conference on the Biology of Kluyveromyces**

*16th-17th September 2006, Parma Italy*

We are pleased to invite you to attend the 19th International Conference on the Biology of *Kluyveromyces* that will be held on 16th-17th September 2006 in Parma Italy. The scientific program will begin on Saturday 16th at 2 p.m. and will end on Sunday 17th at 12 a.m. As in previous years, the Meeting will be very informal with short talks of approximately 15-20 minutes each and we will try give each group the opportunity to present his work. The following projection facilities will be available: overhead projector and computer projection. The Registration Fee is 120,00 Euro and will include the abstract book, the get together dinner on September 16th the working lunch on September 17th and coffee breaks. The Registration Fee must be paid directly at the Meeting and receipts will be issued on payment. Registration of participants will take place at the Centro Santa Elisabetta Università degli Studi di Parma, Viale delle Scienze 43100 Parma - on Saturday September 16th, 2006 from 10 a.m.

The deadline for Registration and Hotel Reservation is June 24th 2006. For Abstract submission the deadline is August 4th. Please contact <k.lactis@unipr.it> to receive an electronic registration form. You can find some useful informations at [http://www.provincia.parma.it/](http://www.provincia.parma.it/)

Organizers: Paola Goffrini and Claudia Donnini.

All correspondence should be send to:

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**Brief News Item**

**New Affiliation: G. D. Clark-Walker**

On 10th March I retired from my position in the Research School of Biological Sciences. However I shall take up an appointment as an Adjunct Professor in the Research School of Chemistry to join a group working on DNA replication. We hope to look at protein-protein interactions using the yeast two-hybrid system. I can still be reached at the coordinates below.

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**Publication of Interest**

**Yeasts in Food and Beverages**

ISBN 3-540-28388-9

Yeasts play a key role in the production of many foods and beverages. This role now extends beyond their widely recognized contributions to the production of alcoholic beverages and bread to include the production of many food ingredients and additives, novel uses as probiotic and biocontrol agents, their significant role as spoilage organisms, and their potential impact on food safety. Drawing upon the expertise of leading yeast researchers, this book provides a comprehensive account of the ecology, physiology, biochemistry, molecular biology, and genomics of the diverse range of yeast species associated with the production of foods and beverages.

Contents

1  The Commercial and Community Significance of Yeasts in Food and Beverage Production.  
2  Taxonomic and Ecological Diversity of Food and Beverage Yeasts.  
3  Molecular Methods to Identify and Characterize Yeasts in Foods and Beverages.  
4  Yeast Ecological Interactions. Yeast-Yeast, Yeast-Bacteria, Yeast-Fungi Interactions and Yeasts as Biocontrol Agents.  
5  Physiological and Molecular Responses of Yeasts to the Environment.  
6  Molecular Mechanisms Involved in the Adaptive Evolution of Industrial Yeasts.  
7  Principles and Applications of Genomics and Proteomics in the Analysis of Industrial Yeast Strains.  
8  Carbohydrate Metabolism.  
9  Yeasts as Biocatalysts.  
10  Production of Antioxidants, Aromas, Colours, Flavours, and Vitamins by Yeasts.  
11  Food and Beverage Spoilage Yeasts.  
12  The Public Health and Probiotic Significance of Yeasts in Foods and Beverages.  
13  The Development of Superior Yeast Strains for the Food and Beverage Industries: Challenges, Opportunities and Potential Benefits.