

# Yeast

## A Newsletter for Persons Interested in Yeast

Official Publication of the International Commission on Yeasts  
of the International Union of Microbiological Societies (IUMS)

DECEMBER 2005

Volume LIV, Number II

Marc-André Lachance, Editor  
University of Western Ontario, London, Ontario, Canada N6A 5B7  
<lachance@uwo.ca>

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

### Associate Editors

Peter Biely  
Institute of Chemistry, Slovak Academy of Sciences  
Dúbravská cesta 9, 842 3  
8 Bratislava, Slovakia

Patrizia Romano  
Dipartimento di Biologia, Difesa  
e Biotecnologie Agro-Forestali  
Università della Basilicata,  
Via Nazario Sauro, 85,  
85100 Potenza, Italy

G.G. Stewart  
International Centre for Brewing and Distilling  
Department of Biological Sciences, Heriot-Watt University  
Riccarton, Edinburgh EH14 4AS, Scotland

P. Buzzini, Perugia, Italy	19	P. Strehaiano, Toulouse, France	33
D. Kręgiel, Lodz, Poland	20	H. Prillinger, Vienna, Austria	34
A. Caridi, Gallina, Italy	20	J. Londesborough, Espoo, Finland	35
I.Yu. Chernov, Moscow, Russia	21	C.A. Rosa, Belo Horizonte, Brazil	35
J.A. Barnett, Norwich, United Kingdom	23	H.M. Daniel, Louvain-la-Neuve, Belgium	37
W.I. Golubev, Puschino, Russia	23	M. Kopecka, Brno, Czech Republic	38
J. du Preez, Bloemfontein, South Africa	23	E. Minárik, Bratislava, Slovakia	39
E. Breierova, Bratislava, Slovakia	25	Á. Fonseca and J.P. Sampaio, Caparica, Portugal	40
J.W. Fell, Miami, Florida, USA	26	M.A. Lachance, London, Ontario, Canada	41
D. Libkind, Bariloche, Argentina	27	Network: Yeasts in Food and Beverages	42
L.C. Mendonça-Hagler, Rio de Janeiro, Brazil	28	International Commission on Yeasts	49
W. Middelhoven, Wageningen, The Netherlands	30	Recent meeting	50
G.I. Naumov and E.S. Naumova, Moscow, Russia	30	Forthcoming meetings	51
J.L. Ochoa, La Paz, BCS, México	32	Publication of interest	52

---

# Editorials

---

## 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](mailto: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 have been 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.

---

I wish all our readers a happy and scientifically rewarding new year!

M. A. Lachance  
Editor

**I. Industrial Yeasts Collection, Dipartimento di Biologia Vegetale, Università di Perugia, Borgo 20 Giugno 74, I-06121 Perugia, Italy. Communicated by P. Buzzini <pbuzzini@unipg.it>.**

Some of our yeast friends might be interested to know that Prof. Alessandro Martini will be retiring as of November 1<sup>st</sup> 2005. He generously made the decision of leaving 3 years early to allow the creation of a researcher position for a young biologist. All of us at DBVPG thank him for many years of patience and guidance; as well as for his innumerable uphill fights for the

DBVPG yeast collection which continues to struggle along against all odds. During retirement Prof. Martini's "life with yeast" will continue by way of his editorial activities in front of his home computer (Macintosh, naturally!). His DVD on yeast biology is now available from Insight Media, and he plans other contributions in the near future.

1. Turchetti, P. Buzzini 2003 Selection of own sources of microorganisms and planning of large-scale screening programs as the first steps for the discovery of novel antimycotic agents of microbial origin. *Chem. Today* 21:40-41.
2. Selvi, S., G. Cardinali and M. Ciani 2003 Variability of HXT2 at the protein and gene level among the *Saccharomyces sensu stricto* group. *FEMS Yeast Res* 4:247-52.
3. P. Buzzini, B. Turchetti, A. Martini 2004 Assessment of discriminatory power of three different methods based on killer toxin sensitivity for the differentiation of *Saccharomyces cerevisiae* strains. *J. Appl. Microbiol.* 96:1194-1201.
4. P. Buzzini, L. Corazzi, B. Turchetti, M. Buratta, A. Martini 2004 Characterization of the *in vitro* antimycotic activity of a novel killer protein from *Williopsis saturnus* DBVPG 4561 against emerging pathogenic yeasts. *FEMS Microbiol. Lett.* 238:359-365.
5. P. Buzzini, B. Turchetti, R. Facelli, R. Baudino, F. Cavarero, L. Mattalia, P. Mosso, A. Martini 2004 First large-scale isolation of *Prototheca zopfii* from milk produced by dairy herds in Italy. *Mycopathologia* 158:427-430.
6. Ganter, P. F., G. Cardinali, M. Giammaria and B. Quarles 2004 Correlations among measures of phenotypic and genetic variation within an oligotrophic asexual yeast, *Candida sonorensis*, collected from *Opuntia*. *FEMS Yeast Res* 4:527-540.
7. Wardrop F.R., Liti G., Cardinali G., Walker G.M. 2004 Physiological responses of Crabtree positive and Crabtree negative yeasts to glucose upshifts in a chemostat. *Annals Microbiol* 54:103-114.
8. A. Martini & A. Vaughan-Martini 2004 Biological Diversity of Yeasts DVD. Insight Media, <http://www.insight-media.com/>.
9. B. Turchetti, P. Pinelli, P. Buzzini, A. Romani, D. Heimler, F. Franconi 2005 *In vitro* antimycotic activity of some plant extracts towards yeast and yeast-like strains. *Phytother. Res.* 19:44-49.
10. P. Buzzini, S. Romano, B. Turchetti, A. Vaughan, U. M. Pagnoni, P. Davoli 2005 Production of volatile organic sulfur compounds (VOSCs) by basidiomycetous yeasts. *FEMS Yeast Res.* 5:379-385.
11. Romani, S. Menichetti, P. Arapitsas, C. Nativi, B. Turchetti, P. Buzzini 2005 *O*-methylglucogalloyl esters: synthesis and evaluation of their antimycotic activity. *Bioorg. Med. Chem. Lett.* 15:4000-4003.
12. P. Buzzini, A. Martini, M. Gaetani, B. Turchetti, U. M. Pagnoni, P. Davoli 2005 Optimization of carotenoid production by *Rhodotorula graminis* DBVPG 7021 as a function of trace element concentration by means of response surface analysis. *Enzyme Microb. Technol.* 36:687-692.
13. B. Turchetti, P. Pinelli, P. Buzzini, A. Romani, D. Heimler, F. Franconi 2005 *In vitro* antimycotic activity of some plant extracts toward yeast and yeast-like strains. *Phytotherapy Res* 19:44-49.
14. A. Vaughan-Martini, C. P. Kurtzman, S.A. Meyer & E. O'Neill. 2005 Two new species in the *Pichia guilliermondii* clade: *Pichia caribbica* sp. nov., the ascospore state of *Candida fermentati*, and *Candida carpophila* comb. nov.. *FEMS Yeast Res.* 5:463-469.
15. P. Buzzini & A. Vaughan-Martini. 2005 Yeast biodiversity and biotechnology. In: *Yeast Handbook on Biodiversity and Ecophysiology of Yeasts*. C. A. Rosa & G. Péter (eds.) Springer Verlag, Berlin. Chap. 22. 533-559.
16. Corte, L., Lattanzi, M., Buzzini, P., Bolano, A., Fatichenti, F. and Cardinali, G. (2005). Use of RAPD and killer toxin sensitivity in *Saccharomyces cerevisiae* strain typing. *J. Appl. Microbiol.* 99:609-617.

17. Pasticci, M. B., Baldelli, F., Camilli, R., Cardinali, G., Colozza, A., Marroni, M., Morosi, S., Pantosti, A., Pitzurra, L., Repetto, A., Bistoni, F. and Stagni, G. 2005 Pulsed field gel electrophoresis and random amplified polymorphic DNA molecular characterization of *Ralstonia pickettii* isolates from patients with nosocomial central venous catheter related bacteremia. *New Microbiol.* 28:145-149.

---

## II. Institute of Fermentation Technology and Microbiology, Technical University of Lodz, Wolczanska 171/173, 90-924 Lodz, Poland. Communicated by D. Kręgiel <dkregiel@p.lodz.pl>.

---

The following are summaries of some of your recent work.

1. Ambroziak W & Kręgiel D. Adhesion of industrial yeast strains on hydroxylapatite for fermentation processes.

There is a growing interest in using immobilized cell systems for different fermentation processes. This fact is explained by the many advantages of immobilized cell technology over the traditional batch free-cell systems. The most important one is the possibility of using immobilized cells systems in continuous operations which retain high cell densities per unit of bioreactor volume and very high fermentation rates. Important problem in immobilization techniques is the physiological state of immobilized cells. Our studies are focused on physiologic and metabolic responses of yeast cells to

immobilization, showing the activation of energetic metabolism upon this process. The main aims of this research are: to develop a suitable methods for adhesion of industrial yeasts on hydroxylapatite, measurement of stability of system cell-carrier, detection vitality and viability of immobilized yeast cells, including dye exclusion techniques (DAPI, primulin, acridine orange, trypan blue), ATP measurement and cellular enzyme activities assays. The studies are realized thanks to financial support of UE 6PR Grant NMP3-CT-2003-504937 PERCERAMICS.

2. Kręgiel D & W Ambroziak. A novel method of yeast immobilisation for fermentation processes.

Different methods, such as adhesion to a surface, matrix entrapment, flocculation and membrane techniques have been used for preparation of biocatalysts. The most widely applied method in continuous ethanol production is cells entrapment by using calcium alginate gels. Alginate beads obtained by classical dripping technique are highly inhomogeneous - cavities and fractures are present. In the immobilization technique, the combination of cell entrapment and nutrient limitation led to the formation of a peculiar microenvironment. This micro-environment surrounding the cells are widely used to explain physiological and morphological changes of immobilized yeasts. On the other hand, the immobilization has been attributed to protective effects of the support. The main idea of actually realized project is application of unique technique of formation

is multichamber cores eg. from alginate gels, with immobilized yeasts cells by using foaming solutions of basic components. Received cores will be enclosed with protective cover which will be increased their mechanical resistance. We are going to study ethanol production by growing cells of different yeast strains: conventional brewing and distillery yeast *Saccharomyces cerevisiae* and unconventional amylolytic yeast *Schwanniomyces (Debaryomyces) occidentalis*. Our preliminary studies on immobilized cells in different systems (mono- and mixed cultures) and their results show high activity of entrapped yeast cultures and efficiency of fermentation processes. The studies are realized thanks to financial support of KBN Grant 2PO6T08129.

---

## III. Dipartimento di Scienze e Tecnologie Agro-Forestali e Ambientali, Università di Reggio Calabria, Piazza San Francesco 7, I-89061 Gallina (RC), Italia. Communicated by A. Caridi <acaridi@unirc.it>.

---

Recent publication.

1. Caridi A 2005 A simple method for assaying the aptitude of yeast to adsorb phenolic compounds. International Workshop on Advances in Grapevine and Wine Research, p.41, Venosa (Italy), September 14-17, 2005.

There is a remarkable variability in the adsorption capacity of phenolic compounds by wine yeast strains. Adsorption of phenolic compounds released from grapes is significant, especially in red wine technology. The goal of this research was to develop a new, very simple method for the determination of this capability based on visual evaluation of the colour of yeast colonies. The assumption is that colony colour reflects phenol binding of the biomass. Two new chromogenic plating media were prepared: grape-skin agar (homogenised grape-skin 200 g/L, peptone from casein 7.5 g/L, yeast extract 4.5 g/L, agar 15 g/L) and grape-seed agar (homogenised grape-seed 100 g/L, peptone from casein 7.5 g/L, yeast extract 4.5 g/L, agar 15 g/L). The media were sterilised at 121 °C for 15 min, poured into Petri dishes, inoculated with a small quantity of yeast

biomass, and incubated at 25 °C for 7 days. The biomass colour - ranging from white to dark hazel - was correlated with the varying yeast aptitude to adsorb phenolic compounds: white biomass colour was explained as zero or low adsorption; dark hazel biomass colour as high adsorption. Evident colour differences among the strains were observed; these differences were confirmed by microvinification trials. The present research has shown that it is possible to quickly and simply select wine yeasts regarding their ability to interact with phenolic compounds. This indirect method has the potential to be applied mainly in industrial technology. It notably decreases times and costs of testing, allowing the opportunity to amplify the number of tested strains.

- Caridi A., Galvano F., Tafuri A., Ritieni A. - Yeast selection for ability to remove ochratoxin A during winemaking. International Workshop on Advances in Grapevine and Wine Research, P22 p.48, Venosa (Italy), September 14-17, 2005.

Increasing interest has been recently generated by the possibility of using microbiological-binding agents to remove mycotoxins. Since 1996, ochratoxin A (OTA) has been reported in grapes, grape juices and wines. In alcoholic beverages, OTA is formed prior to alcoholic fermentation, during which, however, it is partially removed or degraded. Interestingly, this decrease in OTA is dependent on the yeast strain used. Different decontamination procedures using *Saccharomyces* strains were recently proposed for OTA removal. The present work aims to investigate the ability of different wine yeasts to remove OTA during winemaking. At the end of the fermentation, wines and lees were analysed for toxin content. There were significant differences among the yeast strains for the residual OTA values

both in wines and in lees. The OTA-removal from grape must was probably carried out by the yeast cell wall, acting as a sponge; the concentration of the mycotoxin in the lees was up to 18 times greater than the residual content in the corresponding wines. It seems probable that parietal yeast mannoproteins, because of their ability to bind mycotoxins, are responsible for the OTA adsorption from contaminated grape musts. This binding ability has previously been shown using modified mannanoligosaccharide derived from the cell wall of *Saccharomyces*. In conclusion, it seems possible to reduce the OTA content of grape must up to 90% using expressly selected wine yeasts.

---

#### IV. Department of Soil Biology, Faculty of Soil Science, Moscow State University, Vorobyovy Hills, Moscow 119899, Russia. Communicated by I.Yu. Chernov. <yes@soil.msu.ru>.

---

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

- G.A. Lisichkina, I.P. Babjeva, D.Yu. Sorokin 2003 Alkalitolerant yeasts from natural biotopes. *Microbiology* 72(5):618-621.

Using a solid nutrient medium containing alkaline buffer (pH 10) and an antibiotic, alkalitolerant yeasts were isolated from samples of soda-rich saline soils (solonchaks) of Armenia (Arazdayan) and the Trans-Baikal region (the Kungur Steppe). The species diversity of the yeast communities of the tested soda-rich soils was relatively insignificant. They only contained alkalitolerant representatives of asporogenic capsulated yeasts

belonging to the species *Cryptococcus laurentii*, *Cr. albidus*, *Rhodotorula glutinis* and *Rh. mucilaginosa*. *Cr. laurentii* representatives clearly dominated the isolates obtained, their number exceeding that of the other species by two to three orders of magnitude. All of the isolates grew on acidic malt agar, suggesting that they did not include obligate alkaliphiles.

- A.M. Yurkov, I.A. Maximova, I.Yu. Chernov 2004 The comparative analysis of yeast communities in birch forests of the European part of Russia and Western Siberia. *Mikologia i Phytopatologia* 38(6):71-79 (in Russian).

Features of yeast communities distribution and its taxonomic structure in two birch forests on a podzolic soil in Moscow and Novosibirsk regions have been investigated. Yeast communities in these biogeocenoses were found to be very similar despite of significant geographical distance. Broadly distributed yeast species *Cryptococcus albidus*, *Cr. laurentii*, *Cr. terricola*, *Rhodotorula fujisanensis*, *Rhodotorula glutinis* dominated in both variants of birch forests. The character of their distribution on different substrates in both cases was approximately identical. At the same time, some features differed Siberian birch forest from the European ones. They are: less ratio

of yeast species typical for boreal litter, higher ratio of *Cryptococcus albidus* variants widespread in arid zones. Also another character of yeast distribution on spatial-succession series of plant substrates with maximum on dry aboveground plant parts (not on green parts as in Moscow region) has been observed. Yeast quantity in soil was significantly higher in European birch forest. All these features of the yeast communities taxonomic structure in Siberian forest should be caused by continental climate of Western Siberia. It showed also some similarity with the steppe and desert biogeocenoses.

- A.M. Yurkov 2005 First isolation of yeast *Saccharomyces paradoxus* in West Siberia. *Microbiology* 74(4):459-463.

Two ascomycetous yeast strains have been isolated near Novosibirsk from oak exudate. The strains have been identified as *Saccharomyces paradoxus* Bachinskaya based on the results of biochemical tests. The conspecificity of the isolates with

*S. paradoxus* was confirmed by electrophoretic karyotyping and restriction analysis of the ITS region of its rDNA. This first isolation of *S. paradoxus* in Siberia provides evidence for the continuity of its natural habitats.

- A.M. Yurkov, I.Yu. Chernov 2005 Geographic races in some species of ascomycetous yeasts in Moscow and Novosibirsk regions. *Microbiology* 74(5):597-602.

Strains of three ascomycetous species *Hanseniaspora guilliermondii*, *Torulaspora delbrueckii* and *Debaryomyces hansenii* isolated from aboveground parts of plants in similar ecosystems but distant geographic regions (Moscow and Novosibirsk regions) have been investigated. Strains in each species were indistinguishable by phenotypic features and general DNA characteristics based on restriction analysis. At the

same time comparison of strains using MSP-PCR techniques allows to reveal a sufficient intraspecific variability inside investigated species. On the basis of similarity of electrophoregrams strains in each species were found to be clustered according to region of isolation. This phenomenon could be expounded as the existence of geographic races inside major phenotypic species of yeasts.

5. I.Yu. Chernov 2005 Latitude-zonal and spatial-successional trends in distribution of yeasts. Zh. Obshch. Biol. 66(2):123-135 (in Russian).

The distribution of yeasts in natural habitats is analyzed in the different nature zones of the former USSR (from tundra to desert) using the results of long-term research. Yeast community structure is changing in parallel to different stages of plant debris decay as well as to latitude-zonal gradient. These changes are not fluctuating but trend ones. As mineralization of plant debris proceeds the availability of sugars decreases and habitat become more extreme for yeasts which are typical saccharolytics. It causes decrease in species abundance and species diversity. At

the same time in zonal gradient the most significant changes take place in the relative abundance of dominant species, genera or higher taxonomic groups. The thermotolerant species occurred more often in the southern regions while psychrophilic species dominate in the north. Soil yeast communities become more polytrophic in the north latitudes where mineralization of organic matter is rather low. Species inhabiting climatic pessimum areas usually form chlamydospores.

6. I.P. Babjeva, I.Yu. Chernov 2004 Biology of Yeasts. KMK Scientific Press 221 pp. (in Russian).

An illustrated textbook for students with general information about main divisions of zymology. The textbook includes the following chapters:

Origin and development of knowledge about yeasts	Yeasts in plants, plant debris and soils
Yeast cell. Cytology	Geographical distribution of yeasts
Components of yeast cell	Functions of yeasts in natural ecosystems
Ontogenetic changes of cell structures	Yeasts as causative agents of diseases
Peculiarities of cytology under different growth conditions	Candidosis
Morphology and asexual reproduction	Cryptococcosis
Micromorphology of yeasts	Malassezia
Cell circle	Industrial usage of yeasts
Sexual reproduction and life circles	Traditional processes
Ascomycetous yeasts	Yeasts in modern biotechnology
Basidiomycetous yeasts	Yeasts taxonomy
Peculiarity of metabolism	Species concept of yeasts
Alcoholic fermentation	Feature and criteria used in yeasts taxonomy
Respiration	Standard description of yeasts
Secondary products of metabolism	Systematic of anamorphic yeasts
Nitrogen sources	Classification of yeasts
Limitative factors	Identification of yeasts
Distribution of yeasts in nature	Yeasts collections
Specific sugary loci	

7. A.M. Glushakova, I.Yu. Chernov 2005 Yeast communities dynamic on leaves of annual plants in genus *Impatiens*. Mikologia i Phytopatologia (in press, in Russian).

Total number and species structure dynamics of epiphytic yeasts on annual plants was researched on leaves of genus *Impatiens* three species: *I. nolitangere*, *I. glandulifera* and *I. parviflora*. It was shown that in the contrast with all the year round green-leaf plants (*Oxalis acetosella* L.) the number of yeasts on annual plants sharply increases up to the end of vegetation when leaves fade. Species structure of epiphytic yeast society on leaves of *Impatiens* changes during the vegetation. At

that essential differences in dynamics of ascomycetous and basidiomycetous yeasts were discovered. Number of basidiomycetous yeasts is more stable. It reveals seasonal trends dependant on regular hydrotermical changes. In the contrast ascomycetous yeast dynamics is explosive. Apparently it isn't strongly connected with weather conditions but with endogen rhythms of plants, for example with changes in exudates composition during ontogenesis.

8. I.A. Maximova, I.Yu. Chernov. Spatial structure of yeasts communitien on fruits of *Sorbus aucuparia* L. Microbiology (in press, in Russian).

Epiphytic yeast communities on the surface of the *Sorbus aucuparia* fruits have been researched. The object was to make a quantitative assessment of the yeast communities' differentiation of the same but distant substrate. *Sorbus* fruits were sampled in nine geographical points in Russia and Moldova. In each point three trees were selected, then three corymbs with fruits were gathered from each tree and finally three single fruits were taken from each corymb. Factor variance analysis results demonstrated that total yeasts number variation, taxonomic diversity and relative abundance of the dominant yeast groups regularly increase with distance. Conversely average similarity between yeast groups of single fruits (Serencen index)

regularly decreased with distance. Yeast groups are most similar on the fruits of a single corymb (77%), a bit less - between different corymbs (60%), less on different trees in one point (55%) and the least similar in different geographical points (37%). The results got demonstrate that general number and taxonomic diversity of a single yeast group depend not only on ecological factors but on whereness and closeness of the yeast groups. Apparently it can be explained by the cross contamination of fruits by yeast cells. The aggregation alike in the distribution of the microorganisms' species (conditioned by the migration and clonal resettlement) should be taken into account when there diversity in natural habitats is estimated.

---

**V. School of Biological Sciences, University of East Anglia, Norwich NR4 7TJ, England, Communicated by J.A. Barnett <j.barnett@uea.ac.uk>.**

---

Current publications.

1. Barnett, J.A. 2005. Glucose catabolism in yeast and muscle. *Comprehensive Biochemistry* 44:1-132.
2. Barnett, J.A. & Entian, K.-D. 2005. A history of research on yeasts 9: regulation of sugar metabolism. *Yeast* 22:835-894.
3. Barnett JA. 2005. Some different kinds of yeast. In *Advances in Science and Industrial Production of Baker's Yeast*. Proceedings of the VH-Yeast Conference April 25-26 Berlin; 67-79.
4. Barnett, J.A. & Eddy A.A. 2006. A history of research on yeasts 10: metabolite transport. *Yeast in preparation*.

---

**VI. Russia Collection of Microorganisms, Institute for Biochemistry and Physiology of Microorganisms, Russian Academy of Sciences, Pushchino, 142290, Russia. Communicated by W.I. Golubev <wig@ibpm.pushchino.ru>.**

---

Current publications.

1. Golubev, W.I. 2005. Wine yeast races maintained in the Russia Collection of Microorganisms (VKM). *Prikl. Biochim. Mikrobiol.* 41(5):592-595 (English translation 521-523).

A list of pure cultures of over 70 races of wine yeasts maintained in the Russia Collection of Microorganisms is

published. Publications on the isolation and investigation of these strains are indicated.

2. Golubev, W.I., Sampaio J.P. Alves, L. and Golubeva, E.W. 2005. *Cryptococcus silvicola* nov. sp. from nature reserves of Russia and Portugal. *Ant. van Leeuwenh.* (in press).

Nitrate-positive strains of a filobasidiaceous anamorphic yeast related to *Cryptococcus cylindricus* were isolated from forest litter in a Russian nature reserve and from a lichen in Portuguese one. Mycocinotyping and rDNA sequence analyses

revealed that the strains represent a novel species, for which the name *Cryptococcus silvicola* (type strain VKM Y-2939 = CBS 10099) is proposed.

3. Golubev W.I., Golubeva E.W. 2005. Characterization of *Schizosaccharomyces hominis*. *J. Microbiol. Epidemiol. Immunobiol.* 20:74-75.

The strain VKM Y-650 of *Schizosaccharo-mycetes hominis* Benedek described as the causative agent of "schizosaccharomycosis" was examined for its cultural,

morphological, physiological and biochemical properties. This name is placed in synonymy with *Schizosaccharomyces pombe* Lindner.

4. Kulakovskaya T.V., Shashkov A.S., Kulakovskaya E.V., Golubev W.I. 2005. Ustilagic acid secretion by *Pseudozyma fusiformata* strains. *FEMS Yeast Res.* (in press).

Eight strains of *Pseudozyma fusiformata* were examined for antifungal activity. All of them had the same spectrum of action and were active against many species of yeasts, yeast-like and filamentous fungi. They secreted glycolipids, which were purified from the culture liquid by column and thin-layer

chromatography. According to nuclear magnetic resonance and mass-spectroscopy experiments all strains produced ustilagic acid, a cellobioside containing 2,15,16-trihydroxypalmitic acid as aglycon, 3-hydroxycaproic acid and acetic acid as O-acyclic substituents.

---

**VII. Department of Microbial, Biochemical & Food Biotechnology / UNESCO MIRCEN, University of the Free State, P.O. Box 339, 9300 Bloemfontein, South Africa. Communicated by James du Preez <dprezjc.sci@mail.uovs.ac.za> [www.uovs.ac.za/biotech](http://www.uovs.ac.za/biotech)**

---

The following articles from our department have recently appeared or are in press.

1. Baretseng, A.S., Kock, J.L.F., Pohl, C.H., Pretorius, E.E. and Van Wyk, P.W.J. 2005. Uncovering the first double brimmed hat-shaped ascospores in *Ambrosiozyma platypodis* Van der Walt. *Antonie van Leeuwenhoek* 87:169- 170.
2. Baretseng, A.S., Kock, J.L.F., Pohl, C.H., Pretorius, E.E., Botes, P.J., Van Wyk, P.W.J. and Nigam, S. 2005. The presence of 3-hydroxy oxylipins on surfaces of hat-shaped ascospores of *Ascoidea africana* Batra & Francke-Grosmann. *Can J Microbiol* 51(1):99-103.

3. Sebolai, O.M., Kock, J.L.F., Pohl, C.H., Botes, P.J., Strauss, C.J., Van Wyk, P.W.J. and Nigam, S. 2005. The presence of 3-hydroxy oxylipins on the ascospore surfaces of some species representing *Saccharomyces* Schionning. *Can J Microbiol* 51(7):605-612.
4. Ciccoli, R., Sahi, S., Singh, S., Prakash, H., Zafiriou, M-P., Ishdorj, G., Kock, J.L.F. and Nigam, S. 2005. Oxygenation by cyclooxygenase-2 (COX-2) of 3-Hydroxyeicosa-tetraenoic acid (3-HETE), a fungal mimetic of arachidonic acid, produces a cascade of novel bioactive 3-hydroxy-eicosanoids. *Biochem. J.* 390:737-747.

Cyclo-oxygenases-1/2 (COX-1/2) catalyse the oxygenation of AA (arachidonic acid) and related polyunsaturated fatty acids to endoperoxide precursors of prostanoids. COX-1 is referred to as a constitutive enzyme involved in haemostasis, whereas COX-2 is an inducible enzyme expressed in inflammatory diseases and cancer. The fungus *Dipodascus uniuucleata* has been shown by us to convert exogenous AA into 3(R)-HETE [3(R)-hydroxy-5Z,8Z,11Z,14Z-eicosatetraenoic acid]. 3R-HETE is stereo-chemically identical with AA, except that a hydroxy group is attached at its C-3 position. Molecular modeling studies with 3-HETE and COX-1/2 revealed a similar enzyme-substrate structure as reported for AA and COX-1/2. Here, we report that 3-HETE is an appropriate substrate for COX-1 and -2, albeit with a lower activity of oxygenation than AA. Oxygenation of 3-HETE by COX-2 produced a novel cascade of 3-hydroxyeicosanoids, as identified with EI (electron impact)-GC-MS, LC-MS-ES (electrospray) and LC-MS-API (atmospheric pressure ionization) methods.

Evidence for in vitro production of 3-hydroxy-PGE<sub>2</sub> (3-hydroxy-prostaglandin E<sub>2</sub>) was obtained upon infection of HeLa cells with *Candida albicans* at an MOI (multiplicity of infection) of 100. Analogous to interaction of AA and aspirin-treated COX-2, 3-HETE was transformed by acetylated COX-2 to 3,15-di-HETE (3,15-dihydroxy-HETE), whereby C-15 showed the (R)-stereochemistry. 3-Hydroxy-PGs are potent biologically active compounds. Thus 3-hydroxy-PGE<sub>2</sub> induced interleukin-6 gene expression via the EP<sub>3</sub> receptor (PGE<sub>2</sub> receptor 3) in A549 cells, and raised cAMP levels via the EP<sub>4</sub> receptor in Jurkat cells. Moreover, 3R,15S-di-HETE triggered the opening of the K<sup>+</sup> channel in HTM (human trabecular meshwork) cells, as measured by the patch-clamp technique. Since many fatty acid disorders are associated with an 'escape' of 3-hydroxy fatty acids from the β-oxidation cycle, the production of 3-hydroxy-eicosanoids may be critical in modulation of effects of endogenously produced eicosanoids.

5. Van Heerden, A., Kock, J.L.F., Botes, P.J., Pohl, C.H., Strauss, C.J., Van Wyk, P.W.J. and Nigam, S. 2005. Ascospore release from bottle-shaped asci in *Dipodascus albidus*. *FEMS Yeast Res* (In press).

Some yeasts utilize different mechanisms to release ascospores of different lengths from bottle-shaped asci. Using electron microscopy, confocal laser scanning microscopy, gas chromatography-mass spectrometry and digital live imaging, the individual release of many oval ascospores from tight-fitting narrow bottle-necks, is reported in the yeast *Dipodascus albidus*. These ascospores are surrounded by compressible, oxylipin coated sheaths enabling ascospores to slide past each other when forced by turgor pressure and by possible sheath contractions

towards the narrowing ascus-neck. In this paper, the release mechanisms of ascospores of various lengths from bottle-shaped asci and produced by different yeasts are compared. We suggest that different release mechanisms, utilizing compressible sheaths or hooked-alignment have possibly evolved to compensate for variation in ascospore length. Alternatively, perhaps sheaths and hooks were two evolutionary solutions to the same biomechanical problem i.e. to release ascospores irrespective of length from bottle-shaped asci.

6. Leeuw, N.J., Kock, J.L.F., Pohl, C.H., Bareetseng, A.S., Sebolai, O.M., Joseph, M., Strauss, C.J., Botes, P.J., Van Wyk, P.W.J. and Nigam, S. 2005. Oxylipin covered ascospores of *Eremothecium coryli*. Antonie van Leeuwenhoek (In press).

*Eremothecium coryli* is known to produce intriguing spindle-shaped ascospores with long and thin whip-like appendages. Here, ultra structural studies using scanning electron microscopy, indicate that these appendages serve to coil around themselves and around ascospores causing spore aggregation. Furthermore, using immunofluorescence confocal laser scanning microscopy it was found that hydrophobic 3-hydroxy oxylipins

cover the surfaces of these ascospores. Using gas chromatography - mass spectrometry, only the oxylipin 3-hydroxy 9:1 (a monounsaturated fatty acid consisting of a hydroxyl group on carbon 3) could be identified. Sequential digital imaging suggests that oxylipin-coated spindle-shaped ascospores are released from enclosed asci by probably protruding through an already disintegrating ascus wall.

7. Strauss, C.J., Kock, J.L.F., Van Wyk, P.W.J., Lodolo, E.J., Pohl, C.H. and Botes, P.J. 2005. Bioactive oxylipins in *Saccharomyces cerevisiae*. *J Inst of Brew* (In press).

We found that some strains of *Saccharomyces cerevisiae* (include strains used in fermentation processes) produce short chain (mainly 8 carbon) oxylipins and not potent inflammatory long chain (20 carbon) oxylipins such as prostaglandins. When acetylsalicylic acid (aspirin) was added to cultures of *Sacch. cerevisiae* UOFS Y-2330, flocculation was significantly inhibited as well as the production of 3-hydroxy 8:0 thereby linking flocculation and this oxylipin. Furthermore, no traces of 3-hydroxy 8:0 could be detected at the start of flocculation in this

yeast. This research is based on (i) reports that yeasts in general can produce bioactive prostaglandins, (ii) findings suggesting a link between aspirin-sensitive prostaglandins and biofilm formation by *Candida albicans*, (iii) the discovery that the addition of low concentrations of aspirin abolish yeast biofilm formation and sexual cell aggregation and (iv) the recent discovery of a novel potent aspirin-sensitive pro-inflammatory 3-hydroxy prostaglandin E<sub>2</sub> synthesized by *Candida albicans* in conjunction with mammalian cells probably during candidiasis.

**VIII. Culture Collection of Yeasts, Institute of Chemistry, Dúbravská cesta 9, 845 38 Bratislava, Slovakia. Communicated by Emilia Breierová <chememi@savba.sk> [www.chem.sk/yeast](http://www.chem.sk/yeast)**

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

1. Sláviková E. and Vadkertiová R. 2003 Effects of pesticides on yeasts isolated from agricultural soil. *Z. Naturforsch.* 58c:855-859.

The effect of six various pesticides on the growth of yeasts isolated from agricultural soil was investigated. Two herbicides (with the effective substances lactofen and metazachlor), two fungicides (with the effective substances fluquinconazole and prochloraz), and two insecticides (with the effective substances cypermethrin + chlorpyrifos and triazamate) were tested. It is evident that there are considerable differences in inhibition effects of studied pesticides. The fungicide with the

effective substance prochloraz inhibited the growth of majority of yeast strains. Insecticide triazamate at concentration 0.6 mM restricted or inhibited growth of all tested strains. The strains of the genus *Cryptococcus* were the most sensitive to pesticides, while the strains of the species *Cystofilobasidium capitatum*, *Debaryomyces occidentalis* var. *occidentalis*, and *Trichosporon cutaneum* were the most resistant.

2. Márová I., Breierová E., Kočí R., Friedl Z., Slovak B., Pokorná J. 2004 Influence of exogenous stress factors on production of carotenoids by some strains of carotenogenic yeasts. *Ann. Microbiol.* 54:73-85.

The aim of this study was to compare composition and content of carotenoids produced by some yeasts strains in optimal growth conditions and in the presence of exogenous stress factors. Nine strains of carotenogenic yeasts were grown aerobically on glucose medium. As the stress factors 10 mmol/l H<sub>2</sub>O<sub>2</sub> and 5-10% NaCl were used, which were added into media i) at the beginning of growth and ii) to the exponentially growing cells. Changes of growth parameters as well as carotenoid production (lycopene,  $\alpha$ -carotene and  $\beta$ -carotene) were followed. Ergosterol production was followed as additional parameter of biomass quality. Analyzed strains partially differed in the

spectrum of produced carotenoids; the highest content of  $\beta$ -carotene was detected in *S. salmonicolor* CCY 19-4-10. Stress factors added to yeast cultures resulted in different responses. As good producers of enriched biomass could serve above all strains *R. glutinis* and *S. salmonicolor* grown under salt stress. Carotenoids act as lipid-soluble membrane antioxidants whose production is considered as an adaptive mechanism against adverse stress effects. Ability of red yeasts to adapt by means of overproduction of industrially significant metabolites could be of increasing interest for potential biotechnological applications.

3. Stratilova E., Dzurova M., Breierova E., Omelkova J. 2005 Purification and biochemical characterization polygalacturonases produced by *Aureobasidium pullulans*. *Z. Naturforsch.* 60c:91-96.

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 a 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.

4. Breierova E., Gregor T., Jursikova P., Stratilova E., Fiserova M. 2004 The role of pullulan and pectin in the uptake of Cd<sup>2+</sup> and Ni<sup>2+</sup> ions by *Aureobasidium pullulans*. *Ann. Microbiol.* 54:247-255.

Three yeast-like strains of *Aureobasidium pullulans* that efficiently remove heavy metal ions from aqueous solution were studied. The production of the pullulan played an important role in the heavy metal accumulation. For better protection of cells against metals, this polysaccharide was added (0.3% w/v) into the

cultivation medium and the result was compared the effect of pectin (0.3% w/v). Pectin due to its acidic character bound the heavy metals more effectively, while pullulan was better as a protective substance inhibiting penetration of heavy metals into the cells.

5. Raptá P., Polovka M., Zalibera M., Breierova E., Zitmanova I., Marova I., Èertik M. 2005. Scavenging and antioxidant properties of compounds synthesized by carotenogenic yeasts stressed by heavy metals - EPR spin trapping study. *Biophysical Chem.* 116:1-9.

Free radical scavenging and antioxidant activities of metabolites produced by carotenogenic yeasts of *Rhodotorula* sp. and *Sporobolomyces* sp. grown under heavy metal presence were studied using various EPR experiments. The thermally initiated decomposition of K<sub>2</sub>S<sub>2</sub>O<sub>8</sub> coupled with EPR spin trapping was shown to be the best choice to characterize antioxidant properties of yeast's samples. EPR spectroscopy revealed that yeast walls

showed higher ability to scavenge free radicals than those from inside the cells. Since carotenogenic yeast differ to each other in resistance against the heavy metals due to their individual protective system, quenching properties and antioxidant activities of carotenogenic yeasts were modulated by Ni<sup>2+</sup> or Zn<sup>2+</sup> ions variously.

6. Košíková B. and Sláviková E. 2004 Biotransformation of lignin polymers derived from beech wood pulping by *Sporobolomyces roseus* isolated from leafy material. *Biotechnol Lett* 26:517-519.

The ability of the yeast, *Sporobolomyces roseus*, isolated from leafy material, to modify lignin derived from beech wood pulping was examined by FTIR and <sup>13</sup>C NMR spectroscopy, which revealed oxidative cleavage of the C<sub>α</sub>-C<sub>β</sub> linkages between lignin units. Using veratryl alcohol as a model

substrate confirmed that *Sp. Roseus* could oxidize veratryl alcohol into veratric acid. This yeast might be suitable for the pretreatment of lignocellulosic materials and/or biotransformation of technical lignins.

7. Miadoková E., Svidová S., Vlèková V., Kogan G., Rauko P. 2004. The role of microbial polysaccharides in cancer prevention and therapy. *J. Cancer Integrative Med.* 2:173-178.

With increasing integration of native and conventional therapy in today's healthcare arena, naturally occurring compounds are of great importance for their prospective use in cancer chemoprevention and treatment. The new water-soluble

derivative of polysaccharide β-D-glucan, or sulfoethyl glucan (SEG), belongs to this category of natural substances. SEG is included in the class of biopolymers known as biological response modifiers with a broad range of activities.

8. Vlèková V., Dúhová V., Svidová S., Farkašová A., Kamasová S., Vlèk D., Kogan G., Rauko P., Miadoková E. 2004 Antigenotoxic potential of glucomannan in four model test systems *Cell Biol. Toxicol.* 6:325-332.

Antimutagenic, anticlastogenic, and bioprotective effect of polysaccharide glucomannan (GM) isolated from *Candida utilis* was evaluated in four model test systems. The antimutagenic effect of GM against 9-aminoacridine (9-A-A)- and sodium azide (NaN<sub>3</sub>)-induced mutagenicity was revealed in the *Salmonella typhimurium* strains TA97 and TA100, respectively. GM showed anticlastogenic effect against N-nitroso-N'-methylurea (NMU) induced chromosome aberrations in the *Vicia sativa* assay. The bioprotective effect of GM co-treated with methyl-methane-sulphonate (MMS) was also established in

*Chlamydomonas reinhardtii* repair deficient strains *uvs10* and *uvs14*. The statistically significant antimutagenicity potential of GM was not proved against 4-nitroquinoline-1-oxide (4-NQO)-induced mutagenicity in *Saccharomyces cerevisiae* D7 assay. It may be due to bioprotectivity of α-mannan and β-glucan, which are integral part of *S. cerevisiae* cell walls. Due to the good water solubility, low molecular weight (30 kDa), antimutagenic/ anticlastogenic, and bioprotective activity against chemical compounds differing in mode of action, GM appears to be a promising natural protective (antimutagenic) agent.

9. Èertik M., Breierova E., Juršikova P. 2005 Effect of cadmium on lipid composition of *Aureobasidium pullulans* grown under addition of extracellular polysaccharides. *Int. Biodeter. Biodegrad.* 55:195-202.

Effect of cadmium on the growth and lipid composition in three species of *Aureobasidium pullulans* grown at presence or absence of extracellular polysaccharides was studied. Maximally tolerated Cd<sup>2+</sup> concentration for all strains was found up to 1.0 mM. However, addition of either pectin or pullulan to the medium caused an increase of tolerance against Cd<sup>2+</sup> up to 2 mM. Yeasts enhanced lipid accumulation in cells in occurrence of cadmium. Cadmium and extracellular polysaccharides evoked changes in fatty acid profile of yeasts lipids. Index of fatty acid unsaturation of total lipids (TL), neutral lipids (NL), phosphatidylcholine (PC), and phosphatidylethanolamine (PE)

was always higher when cadmium was employed to medium. It was due to increase of oleic and linoleic acid levels in all isolated lipid fractions. Moreover, addition of protective polysaccharides further induced biotransformation of oleate to linoleate. PC displayed high amounts of C18 unsaturated fatty acids, while PE exhibited elevated levels of myristic and palmitic acids. Thus, biosynthesis of C18 unsaturated fatty acids in *A. pullulans* is probably associated with PC. Involvement of fatty acid desaturases in adaptable mechanisms of yeasts surrounded by cadmium and polysaccharides is discussed.

**IX. Rosenstiel School of Marine and Atmospheric Science, Division of Marine Biology and Fisheries, University of Miami, Key Biscayne, Florida, USA 33149. Communicated by J.W. Fell <jfell@rsmas.miami.edu>.**

Dr. Mara R. Diaz was the first recipient of the MMSA-Pfizer Medical Mycology Scholar award. She presented the award address on June 7, 2005 at the Annual General Meeting of the American Society for Microbiology, Atlanta, Georgia as part of

a new Symposium jointly sponsored by the ASM and the Medical Mycological Society of the Americas. Dr. Diaz's recent research has focussed on the high-throughput detection of pathogenic yeasts of the genus *Trichosporon*.

**Recent publications.**

1. Middelhoven WJ, Scorzetti G & Fell JW. 2004. Systematics of the anamorphic basidiomycetous yeast genus *Trichosporon vadense* Behrend with the description of five novel species, viz. *T. smithiae*, *T. dehoogii*, *T. scarabaeorum* and *T. gamsii*. *Int J Syst Evol Microbiol* 54:975-986.
2. Sampio JP, Golubev WJ, Fell JW, Gadanho M & Golubev NW. 2004 *Curvibasidium cygneicollum* gen. nov., sp. nov. and *Curvibasidium pallidicorallinum* sp. nov., novel taxa in the Microbotryomycetidae (Urediniomycetes), and their relationship with *Rhodotorula fujisanensis* and *R. nothofagi*. *Int J Syst Evol Microbiol.* 54:1405-1411.

3. Sampaio JP, Inacia J, Fonseca A, Gadanho M, Fell JW & Scorzetti 2004 *Auriculibuller fuscus* gen. nov., sp. nov. and *Bullera japonica* sp. nov., novel taxa in the Tremellales. *Int J Syst Evol Microbiol* 54:987-993.
4. Lopandic K, Sugita T, Middelhoven WJ, Herzberg M, Fell JW, Zelger S & Prillinger H. 2004. *Trichosporon caseorum* sp. nov. and *Trichosporon lactis* sp. nov., two basidiomycetous yeasts isolated from cheeses. *Frontiers in Basidiomycota Mycology* 99-116.
5. Fell JW & Scorzetti G 2004 Reassignment of the basidiomycetous yeasts *Trichosporon pullulans* to *Guehomyces pullulans* gen. nov, comb., nov. and *Hyalodendron lignicola* to *Trichosporon lignicola* comb. nov. *Int J Syst Evol Microbiol* 54:995-998.
6. Thanh VN, Smit MS, Moleleki N & Fell JW 2004 *Rhodotorula cycloclastica* sp. nov., *Rhodotorula retinophila* sp. nov., and *Rhodotorulal terpenoidalis* sp. nov., three limonene-utilizing yeasts isolated from soil. *FEMS Yeast Research* 4:857-863.
7. Diaz M & Fell JW 2004 High throughput detection of pathogenic yeasts in the genus *Trichosporon*. *J Clin Microbiol* 42:3696-3706.
8. Fell, JW, Statzell-Tallman A & Kurtzman CP. 2004 *Lachancea meyersii* sp. nov., an ascosporegenous yeast from mangrove regions in the Bahama Islands. *Studies in Mycology* 50:359-363.
9. Kurtzman CP, Statzell-Tallman A & Fell JW 2004 *Tetrapisispora fleetii* sp. nov., a new member of the Saccharomycetaceae. *Studies in Mycology* 50:397-400.
10. Summerbell RC, Levesque CA, Siefert KA, Bovers M, Fell JW, Diaz MR, Boekhout T, de Hoog GS, Stalpers J, Crous P 2005. Microcoding: the second step in DNA barcoding. *Phil Trans R Soc B* 1721-1729.
11. Diaz MR & Fell JW. 2005 Rapid identification of the varieties and genotypes of *Cryptococcus neoformans* species complex using a high-throughput suspension array. *J. Clinical Micro* 43:3662-2672.
12. Diaz MR, Boekhout T, Kiesling T & Fell JW 2005 Comparative analysis of the intergenic spacer regions and population structure of the species complex of the pathogenic yeast *Cryptococcus neoformans*. *FEMS Yeast Res* (in press).
13. Kurtzman CP & Fell JW 2004 Yeasts. In: *Biodiversity of Fungi Inventory and Monitoring Methods* Eds Mueller G, Bills G and Foster M. pp 337-343. Elsevier.
14. Starmer WT, Fell JW, Catranis, CM, Aberdeen V, Ma LJ, Zhou S & Rogers SO, 2005. Yeasts in the Genus *Rhodotorula* Recovered from the Greenland Ice Sheet. In: *Life in Ancient Ice*. Eds Castello JD & Rogers SO Princeton Univ Press.

The following publication details a method that is equally useful for detection of yeast species.

15. LaGier MJ, Scholin CA, Fell JW & Goodwin KD. An electrochemical RNA hybridization assay for detection of the fecal indicator bacterium *Escherichia coli*. *Mar Pollution Bull* (in press).

---

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

---

Recent Publications.

1. Libkind, D., Sommaruga, R., Zagarese, H., van Broock, MR. 2005. Mycosporines in carotenogenic yeasts. *Syst Appl Microbiol* 28:749-754.

Publications in press.

2. Pérez, P., Libkind, D., Diéguez, M.C., Summerer, M., Sonntag, B., Sommaruga, R., van Broock, M and H. E. Zagarese. Mycosporines from freshwater yeasts: a trophic cul-de-sac? *Photochem Photobiol Sci*

Mycosporine-like aminoacids (MAAs) are natural sunscreens that passively filter out the most damaging UV wavelengths of solar radiation. MAAs are present in aquatic bacteria, algae, and animals, and 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 within their tissues. In this work we analyse the

transference of the yeast mycosporine myc-glu-glu through the diet in the copepod *Boeckella antiqua* and the ciliate *Paramecium bursaria*. For this purpose, experiments were performed 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 reinhardtii* and *C. reinhardtii* + yeasts) and radiation treatments (PAR and PAR+UVR), while shorter feeding trials were set up 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 the copepod was exposed to UV radiation, favoring the accumulation of photoprotective compounds, they were unable to accumulate yeast-derived mycosporines as no signal of the yeast compound myc-glu-glu was observed in the chromatographs performed on their tissues. An increase in MAAs concentration occurred both in copepods fed *C. reinhardtii* plus yeasts, as well as in those fed only *C. reinhardtii*, suggesting that the contribution of yeast mycosporines to the total MAAs concentration observed was negligible. The lack of assimilation of myc-glu-glu was also confirmed by the results obtained from *P. bursaria* that ingested and completely digested yeast cells.

3. Libkind, D. & van Broock, M.R. Biomass and carotenoid pigments production by Patagonian native yeasts. *World J Microbiol Biotechnol*.

New yeast isolates from unexplored Patagonian habitats were studied for the production of biomass and carotenoids as the first step towards the selection of hyper-producing strains and the design of a process optimisation approach. Patagonian yeast isolates considered as potential biomass and carotenoid sources were studied using ammonium sulphate and urea as nitrogen

sources in semi-synthetic medium (MMS), and agro-industrial by-products (cane molasses, corn syrup, raw malt extract) as carbon sources. Maximum pigment production (300 µg g<sup>-1</sup>) was achieved by *Rhodotorula mucilaginosa* CRUB 0195 and by novel species *Cryptococcus* sp. CRUB 1046. β-carotene, torulene and torularhodin were major the carotenoids found.

Publications submitted.

4. Libkind, D., Diéguez, M., Moliné, M., Pérez, P., Zagarese, H., Sommaruga, R. & van Broock, M. Occurrence of photoprotective compounds in yeasts from freshwater ecosystems of northwestern Patagonia. *Photochem Photobiol*.

In this paper we review the research on the occurrence, induction and role of photoprotective compounds (PPCs) present in native aquatic yeasts from freshwater Patagonian ecosystems. We focus on the effect of ultraviolet radiation (UVR) as a factor controlling the level of photoprotection of yeasts, and explore its potential significance for affecting yeast distributional patterns. The evidence presented here arises from three years of collaboration between the laboratories of Microbiology and Photobiology (CRUB, Comahue University, Argentina) within the framework of the IAI CRN26 project "Enhanced ultraviolet-B radiation in natural ecosystems as an added perturbation due to ozone depletion". The research presented here combines field surveys and laboratory work, including the isolation and culture

of native yeasts strains, as well as laboratory assays under different radiation exposure conditions. Our survey indicates that yeasts are common dwellers of oligotrophic Patagonian waterbodies, and provides the first evidence of the distribution of PPC (carotenoid and mycosporine) producing yeast in temperate freshwaters. Our survey evidenced the widespread occurrence of the UV-absorbing compound mycosporine-glutaminol-glucoside, whose presence in freshwater yeasts has only recently been reported. Collectively, our work suggests a relationship between the ability to produce PPCs, the tolerance to UV exposure and the ability to dwell in highly exposed habitats. In addition, we have shown that the ability to synthesise myc-glu-glu may be useful as a chemotaxonomic marker in yeast systematics.

5. Moliné, M., Libkind, D., Diéguez, M.C., van Broock, M. Photo-protective role of carotenoid pigments in yeasts: experimental study contrasting naturally occurring pigmented and albino strains. *Photochem Photobiol Sci*.

Organisms naturally exposed to high irradiance levels frequently have high levels of photoprotective compounds that filter out or help preventing photo-oxidation. Carotenoid pigments have been recognised to provide protection from ROS (reactive oxygen species) quenching. The synthesis and accumulation of high amounts of carotenoid pigments is frequent in several yeasts species. In this work we analyse through laboratory experiments the photoprotective function of carotenoid

pigments by contrasting responses of naturally occurring albino and pigmented strains of the yeasts *Sporobolomyces ruberrimus* and *Cystofilobasidium capitatum*. Strains cospecificity was confirmed by PCR fingerprinting and rDNA sequencing. In all experiments and for both species, pigmented strains presented higher resistance to UVR than albino strains. Our results indicate that carotenoids pigments afford UVR protection in yeasts and that carotenoid concentration is related to UV-B sensitivity.

---

**XI. Instituto de Microbiologia Prof. Paulo de Góes, Laboratório de Ecologia Microbiana e Taxonomia e Coleção de Culturas de Leveduras. Univ. Federal do Rio de Janeiro (UFRJ). CCS-CP 68028 - CEP 21944-590, Brasil. Communicated by Leda C. Mendonça-Hagler <leda@mls.com.br>.**

---

**A brief report on ICY 2004**

The Eleventh International Congress on Yeasts, Yeasts in Science and Technology: the Quest for Sustainable Development (ICY 2004), was held at Hotel Glória, Rio de Janeiro, Brazil, 15-20<sup>th</sup> of August under the coordination of Leda C. Mendonça-Hagler. The congress was attended by over 240 delegates, representing 34 countries. ICY 2004, as expected for a general meeting, covered a broad spectrum of topics, including

the following: Genomics, Proteomics, Taxonomy and Evolution, Metabolism, Cell Morphology, Ecology and Biodiversity, Responses to Stress, Biotechnology, Food and Beverages, Industrial Fermentations, Pathogenic Yeasts and Novel Methodologies. The current status of ethanol production and other biotechnological yeast processes received a special focus. These themes are relevant to Brazil, the major world producer of ethanol from fermented sugar cane and traditionally a leading

country on the use of sustainable technologies to protect its environment and rich biodiversity.

The ICY 2004 program included 8 plenary lectures, 8 symposia, 6 oral presentation sessions and 6 poster sessions. Ninety speakers delivered lectures and oral presentations on new developments in yeast research and technology. More than one hundred posters were presented. A workshop on yeast in green chemistry and a course on yeast taxonomy took place as Pre-ICY 2004 events. A meeting of the International Commission on Yeasts was held during ICY 2004 (August 17<sup>th</sup>) with the presence of 36 commissioners. The congress social activities included a welcome cocktail, a typical Brazilian dinner and a day of city tour.

On behalf of the Local Organizing Committee we express our appreciation and gratitude to ICY Commissioners for their support and to all delegates for their participation, with a special mention of the large number of young scientists. It was a pleasure for the Brazilian yeast group to host ICY 2004, held the first time in South America. A book of abstracts of presentations was edited (Mendonça-Hagler L.C. & O.V. de Sousa, 2004 *Eleventh Congress on Yeasts – Yeasts in Science and Biotechnology: The Quest for Sustainable Development. Book of Abstracts*. U. Fed. Rio de Janeiro, 232pp). A comprehensive conference report was published in FEMS Yeast Research (T. Deák, 2005. 5:485-489).

Recent publications and theses.

1. Gomes, N.C. O. Fagbola, R. Costa, N.G. Rumjanek, A. Buchner, L.C. Mendonça-Hagler, K. Smalla. 2003. Dynamics of fungal communities in bulk and maize rhizosphere soil in the tropics. *Appl. Environm. Microbiol.* 69:3758-3766.
2. Costa A.M.M, A.C.S. Santos, A.S. Cardoso, M.B. Portela, C.M. Abreu, C.S. Alviano, A.N. Hagler, R.M.A. Soares. 2003 Heterogeneity of metallo and serine extracellular proteinases in oral clinical isolates of *Candida albicans* on HIV positive and health children from Rio de Janeiro, Brazil. *FEMS Immunol. Medical Microbiol.* 38:173-180.
3. Portela M.B. I.P.R. Souza, A.M.M.Costa, A.N. Hagler, R.M.A. Soares, A.C.S. Santos. 2004. Differential recovery of *Candida species* from subgingival sites in HIV-positive and health children from Rio de Janeiro, Brazil. *J. Clinical Microbiol.* 42:5925-5927.
4. Maciel-Souza M.C.; A. Macrae; A.G.T. Volpón; P. S. Ferreira; L.C. Mendonça-Hagler. 2005. Chemical evaluation and microbial response from an oil-spill contaminated mangrove, Brazilian. *J. Microbiol.*, 2005. In press.
5. Oda R., L. C. Mendonça-Hagler. 2004. A Biotecnologia e o Desenvolvimento Sustentável da Biodiversidade. In: *Biossegurança em Biotecnologia*. P. Binsfeld (ed). Interciência SP. P 209-228.
6. Hagler A. N. 2006. Yeasts as indicators of quality. In Rosa C.A. and Peter, G. Biodiversity and Ecophysiology of Yeasts. 2006. P. 519-536. In press.
7. Mendonça-Hagler, L. C. S., I.S. Melo, M.C. Valadares-Ingliš; B. M. Aniano, J.O. Siqueira, P.V. Toan, R. Wheatley. 2005. Non-target and biodiversity impacts in soil. In: *Environmental Risk Assessment of Genetically Modified Organisms: Vol II. Methodologies for assessing Bt Cotton in Brazil*. (Eds. A. Hilbeck, D. Andow, E. Fontes). CABI Pub. In press.
8. Mendonça-Hagler L.C., M.J.S. Vital, R. Costa, A. Macrae, A.N. Hagler 2003. Yeast diversity from Amazon and Atlantic rainforest soils. 23th ISSY. Budapest, Hungary Book of Abstracts
9. Cabral A., P. Carvalho, T. Pinotti, P. I. Hargreaves, A. N. Hagler, L. C. Mendonça-Hagler and A. Macrae. 2004. Intra and interspecific mycocinogenic activity from yeasts isolated from Brazilian soils and fruits. In: *Yeasts in Science and Technology: The Quest for Sustainable Development*. 11<sup>th</sup> Intern. Congress on Yeasts. Rio de Janeiro, Brazil. Book of Abstracts. p. 197.
10. Hagler, A. N. & L.C. Mendonça-Hagler 2004. 30 years isolating yeasts from diverse habitats in Brazil. In: *Yeasts in Science and Technology: The Quest for Sustainable Development*. 11<sup>th</sup> Intern. Congress on Yeasts. Rio de Janeiro, Brazil. Book of Abstracts. P. 29.
11. Costa R., N.C.M. Gomes, K. Smalla, A.N. Hagler, L. Mendonça-Hagler. 2004. Phenotypic and genotypic characterization of ascomycetous yeasts isolated from maize rhizosphere in Brazil. In: *Yeasts in Science and Technology: The Quest for Sustainable Development*. 11<sup>th</sup> Intern. Congress on Yeasts. Rio de Janeiro, Brazil Book of Abstracts. p. 134.
12. Carvalho P., T. Pinotti, A. Cabral, K. Garcia, A. Fernandes, A. Huzar, L. Mendonça-Hagler, A. Macrae, A. Hagler. 2004. Bioprospecting strategy to isolate more species from yeast rich habitats. In: *Yeasts in Science and Technology: The Quest for Sustainable Development*. 11<sup>th</sup> Intern. Congress on Yeasts, Book of Abstracts. Rio de Janeiro, Brazil. P.189.

13. Cabral A., R. Costa, P.M.B. Carvalho, T. Pinotti, A. Macrae, A.N. Hagler, L.C. Mendonça-Hagler. 2004. Bioprospecting for killer yeasts from Brazil for the biocontrol of plant pathogenic fungi. 10th Intern. Symposium on Microbial Ecology. Cancun, Mexico. Book of Abstracts.
14. Mendonça-Hagler L.C., M.J. S. Vital, R. Costa, A. Cabral, A. Macrae, A.N. Hagler, 2004. Yeasts associated with tropical rainforests, their antagonistic activities and their post-harvest biocontrol potential. ASM General Meeting. N. Orleans. Book of Abstracts
15. Cabral A, P. Carvalho, T. Pinotti, A. N.Hagler, L. Mendonça-Hagler, A. Macrae. 2005. Killer Yeast Control the Growth of the Phytopathogen *Crinipellis perniciosa* (causal agent of Witches Broom). XI International Congress of Mycology, IUMS 2005: Book of Abstracts: Microbes in a Changing world. San Francisco, USA.
16. Anderson de Souza Cabral. Leveduras killer e seu potencial no controle de fungos fitopatogênicos. 2004. M. Sc. Dissertation (Plant Biotechnology) – UFRJ.
17. Maria do Carmo Maciel Souza. Comunidades microbianas associadas a manguezais impactados por petróleo na Baía de Guanabara, RJ. 2005. Ph. D. Thesis (Biological Sciences). - UFRJ.

---

**XII. Laboratorium voor Microbiologie, Wageningen University, Hesselink van Suchtelenweg 4, 6703 CT Wageningen, The Netherlands. Communicated by W.J. Middelhoven <Wout.Middelhoven@wur.nl>.**

---

Papers describing *Trichosporon wieringae* sp.nov. and *Cryptococcus allantoinivorans* sp.nov. appeared in printed version as Antonie van Leeuwenhoek 86:329-337, 2004 and

87:101-108, 2005, respectively, thus rendering the species descriptions the valid status. Abstracts of these papers already appeared in YNL of December 2004.

Submitted for publication.

1. Polysaccharides and phenolic compounds as substrate for yeasts isolated from rotten wood and description of *Cryptococcus fagi* Middelhoven et Scorzetti sp.nov.

Pieces of rotten wood collected in the forest near Wageningen appeared to be inhabited by several yeast species. In total 14 ascomycetous and 6 anamorphic basidiomycetous yeast species could be identified, most ones represented by only one strain but *Candida bertae* by 2 and *Trichosporon porosum* by 6 strains, all from different samples. Earlier research of Grinsberg, Ramírez and González in Chile, two decades ago, revealed a similar biodiversity in wood that had undergone whiterot. Some of the species described by them were also detected in the present study that in addition to known species yielded three novel species, viz. *Cryptococcus fagi* and two *Candida* spp. to be described elsewhere. Decaying plant material is rich in polysaccharides and phenolic compounds. These were assimilated as sole carbon source by the basidiomycetes but

generally not by the ascomycous true yeasts. The polysaccharides used included starch and inulin, but also compounds rarely studied by yeast physiologists such as pullulan, dextran, xylan, polygalacturonate, galactomannan and tannic acid. Carboxymethylcellulose, colloidal chitin, arabinogalactan and gum xanthan were also supplied but none of the yeasts could grow on them. The ascomycetes are notable for rapid growth on n-hexadecane, a few strains excepted. Far-reaching physiological similarity of *Leucosporidiella (Rhodotorula) creatinivora* and *Leucosporidium scottii* was observed, of own isolates as well as of the type strains. It is suggested that *L. creatinivora* is an anamorph of *L. scottii* rather than a separate species. This is confirmed by great similarity of rDNA sequences.

---

**XIII. State Scientific-Research Institute for Genetics and Selection of Industrial Microorganisms (GosNIIGenetika), I-Dorozhnyi 1, Moscow 117545, Russia. Communicated by G.I. Naumov and E.S. Naumova <gnaumov@yahoo.com>.**

---

Many thanks to I. Masneuf-Pomarede (Bordeaux), M.Th. Smith (Utrecht), S. Casaregola (Paris-Grignon), M. Aigle (Bordeaux) for fruitful collaboration in 2005 and A. M. ten Berge for kind attention during our stay in Delft. Both of us received the EGIDE grants (France) to work in Paris-Grignon on molecular genetics of the yeast *Kluyveromyces* (October-November 2005). G.I.N. is

grateful to the Organising Committee of the ESF-EMBO Symposium 2005 on Comparative Genomics of Eukaryotic Microorganisms (Sant Feliu de Guixols, Spain) for the invitation to give a lecture and for financial support to participate in the symposium.

The following are publications for 2005 or in press.

1. Naumov G.I. 2005 Why does the yeast *Kluyveromyces wickerhamii* assimilate but not ferment lactose? Dokl. Biol. Sci. 403:310-312.
2. Korshunova I.V. Naumova E.S., Naumov G.I. 2005 Comparative molecular-genetic analysis of the beta-fructosidases of yeasts *Saccharomyces*. Molecular Biology (Moscow) 39:366–371.

3. Naumova E.S., Sukhotina N.N., Naumov G.I. 2005 Molecular markers for differentiation between the closely related dairy yeast *Kluyveromyces lactis* var. *lactis* and wild *Kluyveromyces lactis* strains from the European “krassilnikovii” population. *Microbiology (Moscow)* 74:329–335.

A comparative molecular genetic study of 37 *Kluyveromyces* strains of different origin has made it possible to find molecular markers that can differentiate between the dairy yeast *Kluyveromyces lactis* var. *lactis* and the genetically close wild *Kl. lactis* strains from the European “krassilnikovii” population, which are unable to ferment lactose. A restriction fragment length polymorphism analysis of the IGS2 rDNA region

reveals two different *AluI* profiles, one of which corresponds to *Kl. lactis* var. *lactis* while the other corresponds to yeasts from the “krassilnikovii” population. The *AluI* restriction profile of the IGS2 region of the rDNA also makes it possible to differentiate between the physiologically similar species *Kl. marxianus* and *Kl. lactis*. The origin of clinical *Kl. lactis* var. *lactis* isolates is discussed.

4. Naumova E.S., Naumov G.I., Masneuf-Pomarede I., Aigle M., Dubourdiou D. 2005 Molecular genetic study of introgression between *Saccharomyces bayanus* and *S. cerevisiae*. *Yeast* 22 (in press).

The genomic constitution of different *S. bayanus* strains and natural interspecific *Saccharomyces* hybrids has been studied by genetic and molecular methods. Unlike *S. bayanus* var. *uvarum*, some *S. bayanus* var. *bayanus* strains (the type culture CBS 380, CBS 378, CBS 425, CBS 1548) harbour a number of *S. cerevisiae* subtelomeric sequences: Y', pEL50, *SUC*, *RTM* and *MAL*. The two varieties, having 86–100% nDNA–nDNA reassociation, are partly genetically isolated from one another but completely isolated from *S. cerevisiae*. Genetic and molecular data support the maintaining of var. *bayanus* and var. *uvarum* strains in the species *S. bayanus*. Using Southern hybridization

with species-specific molecular markers, RFLP of the *MET2* gene and flow cytometry analysis, we showed that the non-*S. cerevisiae* parents are different in lager brewing yeasts and in wine hybrid strains. Our results suggest that *S. pastorianus* is a hybrid between *S. cerevisiae* and *S. bayanus* var. *bayanus*, while *S. bayanus* var. *uvarum* contributed to the formation of the wine hybrids S6U and CID1. According to the partial sequence of *ACT1* gene and flow cytometry analysis, strain CID1 is a triple hybrid between *S. cerevisiae*, *S. kudriavzevii* and *S. bayanus* var. *uvarum*.

5. Naumov G.I., Naumova E.S., Barrio E., Querol A. 2005 Genetic and molecular study of inability of the yeast *Kluyveromyces lactis* var. *drosophilum* to ferment lactose. *Microbiology (Moscow)* (in press).

Lactose fermentation (Lac<sup>+</sup>) in dairy yeast *Kluyveromyces lactis* var. *lactis* is controlled by *LAC4* (β-galactosidase) and *LAC12* (lactose permease) genes. Twelve *Kl. lactis* var. *drosophilum* natural homothallic Lac<sup>-</sup> strains of different origin were analyzed by complementation analysis with genetic heterothallic lines of *Kl. lactis* var. *lactis* having genotypes *lac4LAC12* and *LAC4lac12*. We showed that natural Lac<sup>-</sup> strains do not possess the gene clusters *LAC4* and *LAC12*.

Both Southern hybridization of chromosomal DNAs with *LAC4* and *LAC12* cloned genes and recombination analysis strongly suggest that *Kl. lactis* var. *drosophilum* strains do not have even silent copies of the genes. In contrast, natural Lac<sup>-</sup> strains of *Kl. marxianus* are mutants on lactose permease (analog of *lac12* gene), but have active β-galactosidase gene (analog of *LAC4* gene). The origin of the cluster *LAC4LAC12* of dairy *Kl. lactis* yeasts is discussed.

6. Naumov G.I., Naumova E.S. 2005. Genetics of non-fermentation of lactose in some wild *Kluyveromyces* yeasts. XVIII<sup>th</sup> Meeting on the Biology of *Kluyveromyces*. Bratislava (Slovakia), August 6, 2005, 7.

Genetics of lactose fermentation is studied closely in the dairy yeast *Kl. lactis* var. *lactis* (Dickson and Riley, 1989). The *LAC4* and *LAC12* genes encoding β-galactosidase and lactose/galactose permease have been identified. Besides, galactose-lactose regulatory *LAC9* and *LAC10* genes were revealed. We have conducted a molecular-genetic study of inability to ferment lactose by non-dairy yeast *Kl. lactis* var. *drosophilum*, *Kl. wickerhamii* and some *Kl. marxianus* strains. The *LAC* genotypes of these homothallic yeasts have been determined by complementation analysis on the basis of hybridization with heterothallic testers of *Kl. lactis* var. *lactis* having *lac4* or *lac12* mutations. Hybrids between auxotrophic strains were obtained on selective medium and then transferred to Durham tubes to check lactose fermentation. According to the complementation analysis, 12 natural Lac<sup>-</sup> strains of *Kl. lactis* var. *drosophilum* from different geographic populations (‘drosophilum’, ‘phaseolosporus’, ‘krassilnikovii’, ‘vanudenii’, ‘aquatic’ and ‘oriental’) are not able to ferment lactose due to the

absence of active *LAC4* and *LAC12* genes. Southern hybridization of chromosomal DNA with the *LAC4* and *LAC12* probes revealed no hybridization signals and, therefore, absence of even silent corresponding sequences in these yeasts. On the other hand, two *Kl. marxianus* strains have mutant permease gene (analogue of *lac12*) and active β-galactosidase gene – analogue of *LAC4*. We addressed the question why the yeast *Kl. wickerhamii* can assimilate lactose but is not able to ferment it (Kluyver effect). While crossed with *Kl. lactis* var. *lactis* testers having genotype *LAC12 lac4* (active lactose permease and no β-galactosidase), *Kl. wickerhamii* strains restored the ability to ferment lactose. On the contrary, hybrids with *Kl. lactis* var. *lactis* testers having genotype *LAC4 lac12* (active β-galactosidase and no lactose permease) cannot ferment lactose. The complementation analysis indicates that the yeast *Kl. wickerhamii* harbors normal β-galactosidase gene, but they do not have their own lactose permease gene. Thus, the Kluyver effect is connected with absence of active lactose permease.

7. Naumov G.I. 2005. Factors of yeast speciation. ESF-EMBO Symposium on Comparative Genomics of Eukaryotic Microorganisms; Sant Feliu de Guixols (Spain), November 12-17, 2005.

Studying natural genetic diversity of different yeasts, we have found that the genetic hybridization analysis can be successfully used not only for identification of single genes or their complex systems, but also for identification of such taxonomic units as genera, species and varieties. Based on the

results of genetic study of the yeast genera *Saccharomyces*, *Kluyveromyces*, *Arthroascus*, *Williopsis*, *Zygowilliopsis*, *Galactomyces* and literature data on *Metchnikowia* sensu stricto and *Metchnikowia* sensu lato, we proposed the concept of a genetic genus in yeasts, which suggests that member species

possess the compatible mating type system responsible for their crossing. The genetic genera are well-separated clusters of the phylogenetic trees based on sequence data of rRNA genes. Biological species of the genetically defined genus can be crossed in any combination, however the resulting hybrids remain sterile having non-viable ascospores. Crossing of biological species is a potential factor for the combinative variability. Compatible mating type system and the sterility of interspecific hybrids allow the determination of both generic and species boundaries in yeasts. We have determined several biological species in the genera *Saccharomyces*, *Arthroascus*, and *Williopsis*, some of which are new for science. Moreover, genetic approaches in combination with molecular methods gave us an opportunity to reveal the complex composition of *Saccharomyces*

*paradoxus*, *Kluyveromyces lactis* and *Arthroascus fermentans*, which are represented by geographic or/and ecological populations – the species *in statu nascendi*. They can be classified as taxonomic varieties having partial genetic isolation and regular recombination of control markers. Double (*S. cerevisiae* x *S. bayanus*, *S. cerevisiae* x *S. kudriavzevii*) and triple hybrids (*S. cerevisiae* x *S. bayanus* x *S. kudriavzevii*) occur at low frequency among natural *Saccharomyces* isolates. One *S. cerevisiae* x *S. kudriavzevii* hybrid was found to be fertile in any generation due to tetraploidization of diploid spores under germination and can be classified as a hybrid species. Therefore, the ecological and geographic isolations, along with interspecific hybridization, are important factors of the yeast speciation.

8. Naumova E.S., Ivannikova Yu.V., Martynenko N.N., Naumov G.I. Comparative analysis of genomes of cultured *Saccharomyces* yeasts. (XXIIInd Int. Conf. on Yeast Genetics and Molecular Biology, 7-12 August 2005, Bratislava). *Yeast*, 22 (S1):S33.
9. Naumov G.I., Sukhotina N.N., Naumova E.S. Differentiation of dairy yeast *Kluyveromyces lactis* var. *lactis* and its closest wild relatives from European population 'krassilnikovii'. (XXIIInd Int. Conf. on Yeast Genetics and Molecular Biology, 7-12 August 2005, Bratislava). *Yeast*, 22 (S1):S43.
10. Gazdiev D.O. 2005. Taxonomic study of *Williopsis*, *Zygowilliopsis* and *Saccharomyces* yeasts isolated from natural sources in Far East. Ph.D. Thesis, Moscow State University, Moscow.
11. Korshunova I.V. 2005. Evolutionary genetics of beta-fructosidases and alpha-galactosidases of the *Saccharomyces* yeasts. Ph.D. Thesis, GosNIIgenetika, Moscow.

---

**XIX. Centro de Investigaciones Biológicas del Noroeste (CIBNOR), La Paz, Baja California Sur México. Box 128, La Paz, BCS, 23000, México. Communicated by J.L. Ochoa <jlochoa@cibnor.mx>.**

---

Publications.

1. Ochoa J.L., Ramírez-Orozco M. & Márquez F. 2003 Glycerol and glucose dissimilation by *Debaryomyces hansenii*: Substrate influence on yeasts' growth and biomass yield. In: Non-conventional Yeast Handbook. (Wolf K., Breunig K., Barth G. eds). Springer-Verlag Berlin. Heidelberg, Germany. pp. 51-57.  

An experimental exercise to compare the effect of different carbon sources in yeast biomass production. The biomass yield and growth rate of *Debaryomyces hansenii* in D-glucose- or D-glycerol-containing medium were compared. It was shown that both, specific growth rate and biomass yield, are higher in D-glycerol than in D-glucose in spite of each having a similar specific consumption rate. With this experiment it is possible to calculate the bioenergetic yield to confirm the higher efficiency of D-glycerol metabolism with respect that of D-glucose in *D. hansenii*.
2. Ramírez-Orozco M., & Ochoa J.L. 2003 Growth of *Debaryomyces hansenii* in seawater culture medium. In: Non-conventional Yeast Handbook. (Wolf K., Breunig K., Barth G. eds). Springer-Verlag Berlin. Heidelberg, Germany. pp. 47-49.  

Sea water can be used as solvent for the preparation of media to grow marine yeasts. Advantages can be lower risks of contamination in large scale production.
3. Ochoa J.L. y Vázquez-Juárez R. 2004 Las levaduras marinas como herramienta científica y biotecnológica. Universidad & Ciencia. Número Especial 1. Biodiversidad en el Trópico Húmedo. pp. 39-50.  

This paper is intended to promote interest in marine yeasts among Spanish speaking scientists. Little information on yeasts is available in that language.
4. Ochoa J.L. y Latisnere-Barragan H. (2005). Colección de Levaduras Marinas de México. Ciencia. AMC. (In Press).
5. Latisnere H., Virgen M., Martínez J., Ochoa J.L. (2005) Levaduras Marinas. Biodiversitas. (In Press)

Mexican patent.

- Ochoa J.L., Ramírez-Orozco M, & Hernández-Saavedra NY. 2005 Procedimiento para obtener superóxido dismutasa tipo cobre-zinc de levaduras marinas. (Procedure for obtaining Cu,Zn superoxide dismutase from marine yeasts). Pat No. MX 225540 (Jan 11, 2005).

Research Projects.

- Marine Yeast Collection of México. Ochoa J.L., Virgen M.

We are happy to report the creation of a Marine Yeast Collection of México. For a start, we have isolated various specimens from the west coast of the Baja California Peninsula within 24-27° N, and 111-115° W from water surface down to 100 m depth. So far, the collection comprises 64 exemplars belonging to the *Candida*, *Debaryomyces*, *Rhodotorula*, and

*Yarrowia* genera. The traditional biochemical assays reveal differences in assimilation of various carbon sources between strains of the same genus. Our goal is to generate a distinctive Marine Yeast Collection that can be used as source of research material. This work has been supported by CONABIO (National Commission on Biodiversity).

- Fungi and yeasts from Mexican lime (*Citrus aurantifolia*). Hernández-Montiel L., Latisnere-Barragan H, and Ochoa J.L.

A research project is in progress aimed to isolate and identify the main post-harvest pathogens of the mexican lime (*Citrus aurantifolia*). Throughout this work, we have been able to isolate 16 cultivars which have been identified as members of

*Aspergillus*, *Geotrichum* and *Penicillium* genera. Also, one *Candida* and two *Debaryomyces* yeast strains were isolated in clean waxed fruits.

- Chlorine dioxide susceptibility of Fungi and yeasts isolated from Mexican lime (*Citrus aurantifolia*). Hernández-Montiel L., Farias S., Latisnere-Barragán H, and Ochoa J.L.

Aimed at introducing more efficient methods for citrus fruit disinfection, we have been analyzing the susceptibility of citrus pathogens towards chlorine dioxide. Preliminary results indicate that it is feasible to control fungus growth in vitro using

low levels of chlorine dioxide (about 1.0 mg/L), ten times lower than the amount of the fungicide Benomyl required for the same purpose.

---

## **XV. Département Bioprocédés et Systèmes Microbiens, UMR-CNRS 5503, 5 rue Paulin Talabot, 31106 Toulouse cedex, France. Communicated by Pierre Strehaiano.**

---

Among the research projects developed in the Department of Bioprocesses and Microbial Systems of the Chemical Engineering Laboratory, some dealing with the use of yeast cells in industry. Recent studies include the following. (1) Analysis of the parameters for the production of a selected hybrid yeast strain. (2) Analysis of interactions between *Saccharomyces* and non-*Saccharomyces* yeasts and also between *Saccharomyces* and lactic acid bacteria in winemaking. A part of this study is done in cooperation with the Faculty of Enology of Bordeaux (Pr. A. Lonvaud). (3) The use of immobilized yeast cells in

alginate. In cooperation with Proenol Lda (Portugal) the use of cells entrapped in double layered alginate beads was developed for sparkling wine making (cells of *S. cerevisiae*), for must or wine deacidification (cells of *Schizosaccharomyces pombe*), and for the treatment of stuck or sluggish fermentations (specially prepared cells of *S. cerevisiae*). (4) A study of the contamination by *Brettanomyces* in winemaking. (5) The effect of some pesticides on the behaviour of yeast during the fermentation process.

The following papers have resulted from these projects.

- Ramon Portugal F., Pingaud H., Strehaiano P. 2004 Metabolic transition step from ethanol consumption to sugar/ethanol consumption by *Saccharomyces cerevisiae*. *Biotechnol Lett* 26:1671-1674.
- Pommier S., Strehaiano P., Delia M.L. 2004 Modelling the growth dynamics of interacting mixed cultures: a case of amensalism. *Int J Food Microbiol - Special Issue Quimper On ligne*, December 8 2004.
- Aranda J. S., Strehaiano P., Taillandier P. 2004 Trehalose accumulation in *Saccharomyces cerevisiae* cells: experimental data and structured modelling approach. *Bioprocess Engin J* 17:129-140.
- Divol B., Strehaiano P., Lonvaud Funel A. 2005 Effectiveness of dimethyldicarbonate to stop alcoholic fermentation in wine. *Food Microbiol* 22:169-178.
- Serra A., Strehaiano P., Taillandier P. 2005 Influence of temperature and pH on *Saccharomyces bayanus* var. *uvarum* growth; impact of a wine yeast interspecific hybridization on these parameters. *Int J Food Microbiol* 104:257-265.
- Strehaiano, P, Ramon-Portugal F., Taillandier, P. In Press. Chapter 9. Yeasts as biocatalysts. In *Yeast handbook* (Ed: G. Fleet, A. Querol).

The following are abstracts of our recent work.

1. M. Wuczkowski, C. Bond and H. Prillinger. In press. *Geotrichum vulgare*, a novel asexual arthroconidial yeast. *Int J Syst Evol Microbiol*

Two strains of a new yeast species were isolated from different habitats, one from soil in an alluvial zone national park in Austria and one from a drain in a Turkish soft drinks factory. Analysis of the sequences of the D1/D2 region of their large subunit ribosomal DNAs and PCR fingerprints show that they belong to the same species, described as *Geotrichum vulgare*.

Analysis of the sequence showed that this species is related to the ascogenous genus *Galactomyces*, the closest relative is *Geotrichum silvicola* CBS 9194<sup>T</sup>, a recently described species. The type culture of *Geotrichum vulgare* is HA1379<sup>T</sup> in the ACBR culture collection (CBS10073<sup>T</sup>, NRRL Y-27915<sup>T</sup>).

2. K. Lopandic, S. Zelger, L.K. Bánszky, F. Eliskases-Lechner, H. Prillinger. In press. Identification of yeasts associated with milk products using traditional and molecular techniques. *Food Microbiol* (in press).

An integrated approach including phenotypic (morphological, biochemical and physiological characterisation) and genotypic (RAPD-PCR, sequencing of D1/D2 domain of 26S rRNA encoding gene) methods was used for the identification of yeasts isolated from different milk products. There were 513 isolates in all, 460 ascomycetous and 53 basidiomycetous yeasts. The yeast isolates were characterised on the basis of their biochemical and physiological properties, and the D1/D2 domain of 26S rDNA was sequenced in selected strains. Relying on the obtained results from the both data sets, corresponding type strains were selected and compared with the respective yeast isolates from milk products by RAPD fingerprinting. The strains showing a degree of similarity >80% were considered conspecific. By means of the applied techniques it was possible to identify 92% yeast isolates at species level. *Debaryomyces*

*hansenii*, *Geotrichum candidum*, *Kluyveromyces marxianus*, *Yarrowia lipolytica* and *Candida zeylanoides* are the most frequently isolated species. The majority of the yeasts were isolated from fresh and sour curd cheese. A comparison of the results obtained by phenotypic and genotypic investigation revealed that the identification based on testing of biochemical and physiological properties was supported by genotypic characterisation in only 54% of examined isolates. The results described in this work show that the applied molecular identification is a reliable approach to the identification of yeasts associated with milk products in contrast to the time consuming biochemical and physiological tests. The identification of new yeast species requires additional genetic markers such as sequencing of different genes or DNA:DNA hybridisation.

3. K. Lopandic, O. Molnár, M. Suzuki, W. Pinsker, and H. Prillinger. 2005. Estimation of phylogenetic relationships within the Ascomycota on the basis of 18S rDNA sequences and chemotaxonomy. *Mycol. Progress* 4:205-214.

Small subunit rRNA gene sequences (18S rDNA), cell wall carbohydrate composition, urease activity and ubiquinone components were analysed within a larger number of ascomycetous yeasts and dimorphic fungi to evaluate their congruence in predicting phylogenetic relationships. The glucose-mannose pattern distinguishes the Hemiascomycetes from the Euscomycetes and the Protomycetes which are characterised with the glucose-mannose-galactose-rhamnose-(fucose) profile. The glucose-mannose-galactose pattern was found in the cell walls of all the three classes. Different coenzyme Q component (CoQ5 to CoQ10) were found within the representatives of the Hemiascomycetes. Whereas CoQ9, CoQ10 and CoQ10H2 predominate within the Euscomycetes, CoQ9 and

CoQ10 characterise the Protomycetes. Chemotaxonomic studies coupled with additional molecular and co-evolution studies support the idea that the Hemiascomycetes occupy a basal position in the phylogeny of Ascomycota. These results are not in line with the phylogenetic studies based on the sequences of 18S rRNA encoding gene. The maximum parsimony analysis indicated that Hemiascomycetes and Protomycetes might represent sister groups, opposing to the earlier reported results, where the Archiascomycetes (Protomycetes) or the Hemiascomycetes had been considered to be the most primitive ascomycetous fungi. Instead of the class Archiascomycetes, the term Protomycetes was introduced reflecting much better the properties of the whole class.

4. O. Molnár and H. Prillinger 2005 Analysis of yeast isolates related to *Metschnikowia pulcherrima* using the partial sequences of the large subunit rDNA and the actin gene; description of *Metschnikowia andauensis* sp. nov. *System. Appl. Microbiol.* 28:717-726.

Thirty two yeast isolates were cultured from guts or excrements of 3 different pests of corn or from the stem of healthy corn. The strains were analyzed using MSP-PCR (micro/minisatellite-primed polymerase chain reaction), sequences of the D1/D2 region of the large subunit rDNA and a 979 bp long part of the actin gene (act-1). They seem to belong

to three groups that are all sister groups of *Metschnikowia pulcherrima*, *M. fructicola* and *M. chrysoerlae*. A new species, *Metschnikowia andauensis* (HA 1657<sup>T</sup>) is described. In contrary to *M. pulcherrima* and *M. fructicola*, *M. andauensis* is well separated in the act-1 phylogenetic tree too.

---

**XVII. VTT Biotechnology, P.O.Box 1500, FIN-02044 VTT, Finland. Communicated by John Londesborough <john.londesborough@vtt.fi>.**

---

Publications since our last communication include.

1. Dietvorst, J., Londesborough, J. and Steensma, H.Y. 2005 Maltose utilization in lager yeast strains: *MTT1* encodes a maltotriose transporter. *Yeast* 22:775-788.
2. Huuskonen, A. and Londesborough, J. (2005) Selection of brewer's yeast mutants suitable for VHG fermentations. Proceedings of the 30th EBC Congr, Prague, 14-19 May 2005, CD-ROM, 35.
3. Richard, P., Verho, R., Londesborough, J. and Penttilä, M. 2005 Feedstocks for the future: chemicals and materials from renewable resources *in* Genetic engineering of *S. cerevisiae* for pentose utilisation. ACS Symposium Series 921 (in press).
4. Ruohonen, L., Aristidou, A., Frey, A.D., Penttilä, M., and Kallio, P.T. 2005 Expression of *Vitreoscilla* hemoglobin improves the metabolism of xylose in recombinant yeast *Saccharomyces cerevisiae* under low oxygen conditions. *Enzyme Microbiol Technol* (in press).
5. Salusjärvi, L., Pitkänen, J.P., Aristidou, A., Ruohonen, L., and Penttilä, M. 2005 Gene expression analysis of recombinant xylose-fermenting *Saccharomyces cerevisiae* reveals novel responses to xylose as a carbon source. *Appl Biochem Biotechnol* (in press).
6. Vidgren, V., Ruohonen, L. and Londesborough, J. 2005 Characterisation and functional analysis of the *MAL* and *MPH* loci for maltose utilization in some ale and lager yeast strains. *Appl. Environ. Microbiol.* (in press).
7. Walsh, M., Mulder, L., Geurts, W., Huuskonen, A., Vidgren, V. and Londesborough, J. 2005 Pilot scale propagation and fermentation of three high gravity resistant mutant lager yeast strains in 18 °P all malt wort. 30<sup>th</sup> Int. EBC Congr. Prague, 14-19 May 2005, CD-ROM, 44.

The following PhD thesis was to be defended on 18th November 2005.

8. Juha-Pekka Pitkänen 2005 Impact of xylose and mannose on central metabolism of yeasts. Department of Chemical Technology, Helsinki University of Technology, Espoo, Finland.

---

**XVIII. Departamento de Microbiologia, ICB, C.P. 486, Universidade Federal de Minas Gerais, Belo Horizonte, MG, 31270-901, Brazil. Communicated by C. A. Rosa <carlrosa@icb.ufmg.br>.**

---

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

1. Rosa C.A. & Lachance M.A. 2005. *Zygosaccharomyces machadoi* sp. n., a yeast species isolated from a nest of the stingless bee *Tetragonisca angustula*. *Lundiana Int. J. Biodivers.* 6:27-29.

A new yeast species, *Zygosaccharomyces machadoi*, was discovered in garbage pellets of the stingless bee *Tetragonisca angustula* in Brazil. Analysis of the sequence of the D1/D2 domains of the large-subunit rDNA showed that the new species is related to *Zygosaccharomyces rouxii*. *Z. machadoi* probably

causes food spoilage in hives of stingless bees. It assimilates only a few carbon sources such that it is difficult to distinguish it from other *Zygosaccharomyces* species based on conventional physiological tests. The type strain of *Z. machadoi* is UFMG-J01-63.2<sup>1</sup> (= CBS 10264<sup>1</sup>).

2. Lacerda I.C.A., Miranda R.L., Borelli B.M., Nunes A.C., Nardi R.M., Lachance M.A. & Rosa C.A. 2005. Lactic acid bacteria and yeasts associated with spontaneous fermentations during the production of sour cassava starch in Brazil. *Int. J. Food Microbiol.* 105:213-219.

Sour cassava starch is a traditional fermented food used in the preparation of fried foods and baked goods such as traditional cheese breads in Brazil. Thirty samples of sour cassava starch were collected from two factories in the state of Minas Gerais. The samples were examined for the presence of lactic acid bacteria, yeasts, mesophilic microorganisms, *Bacillus cereus* and faecal coliforms. Lactic acid bacteria and yeasts isolates were identified by biochemical tests, and the identities were confirmed by molecular methods. *Lactobacillus plantarum* and *Lactobacillus fermentum* were the prevalent lactic acid bacteria in product from both factories, at numbers between 6.0

and 9.0 log cfu.g<sup>-1</sup>. *Lactobacillus perolans* and *Lactobacillus brevis* were minor fractions of the population. *Galactomyces geothricum* and *Issatchenkia* sp. were the prevalent yeasts at numbers of 5.0 log cfu.g<sup>-1</sup>. A species similar to *Candida ethanolica* was frequently isolated from one factory. Mesophilic bacteria and amylolytic microorganisms were recovered in high numbers at all stages of the fermentation. *B. cereus* was found at low numbers in product at both factories. The spontaneous fermentations associated with the production of sour cassava starch involve a few species of lactic acid bacteria at high numbers and a variety of yeasts at relatively low numbers.

3. Moraes M.E., Rosa C.A. & Sene F.M. 2005. Preliminary notes on yeasts associated with necrotic cactus stems from different localities in Brazil. *Braz. J. Biol.* 65:299-304.

The yeast species found in necrotic stems of three columnar cacti (*Pilosocereus machrisii*, *Pilosocereus vilaboensis*, and *Praecereus euchlorus*) at eight localities in Brazil were described and a similarity analysis using Sorensen distances was used to compare the composition of yeast species at these localities. Of 56 necrotic cactus stems sampled, 32 produced yeast colonies. Ten species of yeast or yeast-like microorganisms were identified from 53 isolates, with *Pichia*

*cactophila*, *Candida sonorensis*, *Geotrichum* sp., and *Sporopachydermia cereana* being the most common. The remaining species occurred in low proportions in the cacti surveyed. The similarity analysis provided a dendrogram (UPGMA) that clustered the yeast communities from different cactus species and indicated that host cactus species was unimportant in this clustering.

4. Morais P.B., Lachance M.A. & Rosa C.A. 2005. *Saturnispora hagleri* sp. nov., a yeast species isolated from *Drosophila* flies in Atlantic rainforest in Brazil. *Int. J. Syst. Evol. Microbiol.* 55:1725-1727.

Six strains representing a novel yeast species belonging to the genus *Saturnispora* were isolated from two species of the *Drosophila fasciola* subgroup (*Drosophila repleta* group) in an Atlantic rainforest site in Rio de Janeiro State, Brazil. Four strains were isolated from crops and one from external parts of *Drosophila cardinae*. The other strain was isolated from external

parts of *Drosophila fascioloides*. Analysis of the D1/D2 large-subunit rDNA sequences indicated that the novel species is closely related to *Saturnispora dispersa*. The name *Saturnispora hagleri* sp. nov. is proposed to accommodate these strains. The type strain is UFMG-55<sup>T</sup> (=CBS 10007<sup>T</sup>=NRRL Y-27828<sup>T</sup>).

5. Martins F.S., Nardi R.M., Arantes R.M., Rosa C.A., Neves M.J. & Nicoli J.R. 2005. Screening of yeasts as probiotic based on capacities to colonize the gastrointestinal tract and to protect against enteropathogen challenge in mice. *J. Gen. Appl. Microbiol.* 51:83-92.

Probiotics are defined as viable microorganisms that exhibit a beneficial effect on the host's health when they are ingested. Two important criteria are used for selection of probiotic microorganisms: they must be able to survive in the gastrointestinal environment and to present at least one beneficial function (colonization resistance, immunomodulation or nutritional contribution). Generally, in vitro assays demonstrating these properties were used to select probiotics but it is unclear if the data can be extrapolated to in vivo conditions. In the present work, twelve *Saccharomyces cerevisiae* strains isolated from different environments (insect association, tropical fruit, cheese and "aguardente" production) and pre-selected for in vitro resistance to simulated gastrointestinal conditions were inoculated in germ-free mice to evaluate their real capacity to colonize the mammal digestive tract. Using these data, one of the yeasts (*S. cerevisiae* 905) was selected and tested in gnotobiotic (GN) and conventional (CV) mice for its capacity to protect

against oral challenge with two enteropathogenic bacteria (*Salmonella Typhimurium* and *Clostridium difficile*). The yeast reached populational levels potentially functional in the gastrointestinal portions where the enteropathogens tested act. No antagonism against either pathogenic bacterium by the yeast was observed in the digestive tract of GN mice but, after challenge with *S. Typhimurium*, mortality was lower and liver tissue was better preserved in CV animals treated with the yeast when compared with a control group (p<0.05). Histopathological results of intestines showed that the yeast also presented a good protective effect against oral challenge with *C. difficile* in GN mice (p<0.05). In conclusion, among the 12 *S. cerevisiae* tested, strain 905 showed the best characteristics to be used as a probiotic as demonstrated by survival capacity in the gastrointestinal tract and protective effect of animals during experimental infections.

6. Ramos J.P., Rosa C.A., Carvalho E.M.M., Leoncini O. & Valente P. 2005. Heteroduplex mobility assay of the D1/D2 region of the 26S rDNA for differentiation of clinically relevant *Candida* species. *Antonie van Leeuwenhoek* (in press).

The Heteroduplex Mobility Assay (HMA) method using the PCR amplified D1/D2 26S rDNA was tested for the differentiation of pathogenic *Candida* species. Strains belonging to the same species are not expected to form heteroduplexes in this assay when their PCR products are mixed. D1/D2 HMA experiments between all *Candida* type strains tested showed heteroduplex formation, including *C. albicans* and *C. dubliniensis*. There was no heteroduplex formation when most

of the clinical and non-type strains were tested against the type strain of their presumptive species, except when *C. albicans* WVE and *C. dubliniensis* TAI were analysed. Additional HMA experiments, phenotypic characterisation, and D1/D2 sequencing identified these isolates as *C. tropicalis* and *C. parapsilosis*, respectively. HMA provides a rapid and relatively simple molecular tool for the confirmation of potentially pathogenic *Candida* species.

7. Oliveira E.S., Rosa C.A., Morgano M.A. & Serra G. E. 2005. The production of volatile compounds by yeasts isolated from small Brazilian cachaça distilleries. *World J. Microbiol. Biotechnol.* (in press).

The production of volatile compounds by 24 strains of *Saccharomyces cerevisiae* and one strain each of *Candida apicola*, *C. famata*, *C. guilliermondii*, *Hanseniaspora occidentalis*, *Pichia subpelliculosa* and *Schizosaccharomyces pombe* was evaluated in connection with the production of cachaça. They were isolated from small cachaça distilleries (27), industrial cachaça distilleries (2) and one sugarcane alcohol distillery, and tested in synthetic medium for the production of acetaldehyde, ethyl acetate, propanol, isobutanol, isoamyl alcohol, acetic acid and glycerol. The *Saccharomyces* strains showed a narrow range of variation in the production of such compounds, near 50% of the average of each volatile compound

concentration. Principal component analysis showed the separation of the strains into six groups, and acetic acid production was the variable of greatest impact in the differentiation of the strains. The strains of *S. pombe* formed a distinct group (Group 2), and the strains of *C. apicola* and *H. occidentalis* formed a joint group (Group 6) as did Sc13 and Sc4 (Group 4). Group 1 was formed exclusively of *S. cerevisiae*. The closest non-*Saccharomyces* strains were *C. apicola* and *H. occidentalis*, with a similarity index of about 0.95. The strain *P. subpelliculosa* showed general characteristics more similar to those of the *S. cerevisiae* strains than to the non-*Saccharomyces* strains.

---

**XIX. Mycothèque de l'Université Catholique de Louvain, Croix du Sud 3, B-1348 Louvain-la-Neuve, Belgium. Communicated by H.M. Daniel <daniel@mbla.ucl.ac.be>.**

---

The following posters were presented recently.

1. Pini F, Evrard P, Daniel HM, Natali C, Michaud L, Bruni V, and Fani R 2005 Molecular and genetic characterisation of cold-adapted yeasts isolated from Terranova Bay (Antarctica). Poster presented at the Third International Conference on the Oceanography of the Ross Sea, 10-14 October 2005, Venice, Italy.

Three basidiomycetous species of cold-adapted yeasts were isolated from sediments at two sites near the Italian base in Terranova Bay (Antarctica). The viable cell counts were approximately  $2 \times 10^3$ /ml for each strain. The three strains were identified by sequence analysis of the D1/D2 region of the LSU (26 rDNA) and the ITS1/5.8/ITS2 region as *Leucosporidiella yacutica*, *Cryptococcus carnescens* and an undescribed species of *Mrakia*. They were deposited at the Mycothèque de l'Université catholique de Louvain under the numbers MUCL 46211, MUCL 46209 and MUCL 46210. Phylogenetic analyses

of LSU and ITS sequences were performed to investigate the evolutionary relationships of the newly isolated strains with other strains and species. The strains were further characterised by the determination of their growth rates on solid medium and their physiology in a microplate system, both at temperatures of 4, 14 and 27 °C. We also used a rRNA-targeted oligonucleotide probe to detect yeast cells in antarctic water samples by FISH. A gene library of *Leucosporidiella yacutica* was constructed to detect and clone genes involved in histidine biosynthesis or adaptation to low temperature.

2. A. F. Jiménez AF<sup>1</sup>, Evrard P, Decock C and Daniel HM 2005 Ecological survey of yeasts in Cuba. Poster presented at the 5th Latin American Mycological Congress, August 1-5, 2005, Brasília, Brasil.

<sup>1</sup>Instituto de Ecología y Sistemática, Carretera de Varona, km 3 ½ Capdevila, Boyeros, A.P.8029, C.P. 10800 Boyeros, Ciudad de la Habana, Cuba.

Cuba is the only island of the Caribbean that still harbours relatively undisturbed forested areas. Approximately 6000 vascular plant species are detected of which 50 percent are endemic, identifying the island as a biodiversity hotspot [1]. A review of fungal collections in the Caribbean resulted in about 75000 records of mostly filamentous and lichen-forming fungi, most of which were associated with plants [2]. The yeast communities that can be detected on plants are determined by insect vectors and have been recognised as specific and stable for a variety of habitats, chemical nature of substrates, presence of killer yeasts, climate and geography [3, 4]. Ecological studies, seeking conclusions regarding the global distribution, the mechanisms of dispersal and the role of yeasts within the plant-insect ecosystem, have not included the Caribbean or Cuba. Systematic surveys are needed to establish a baseline of the yeast populations and their ecological roles.

We report on yeasts collected from flowers of approx. 90 plant species. The sampling sites included forests, parks and gardens in 6 of the 14 Cuban provinces and sampling was performed between 2001-2004. In this initial study, 208 yeast strains were isolated representing 67 taxa of which 8 are potentially new species and an additional 9 are currently undescribed species that are known from other locations. The yeast isolates consisted of 194 strains from 60 species of ascomycetous yeasts and 14 strains from 7 species of basidiomycetous yeasts. Approximately 50% of the ascomycetous yeast isolates were assigned to the *Debaryomyces/Lodderomyces* clade and approx. 20% to the *Stephanoascus/Metschnikowia* clade [8]. These two clades contained also all the detected undescribed and new species with the exception of one undescribed basidiomycete. The yeast species with the highest numbers of isolates coincide with species that have been

recognised to be heterogenic by molecular typing methods (eg. *Pichia guilliermondii*, *Candida parapsilosis* and *C. intermedia/C. pseudointermedia*).

The identification was performed with the miniaturised and automated ALLEV system [5] that includes 96 morphological and physiological criteria based on the methods of Kreger-van Rij [6] and Van der Walt & Yarrow [7]. Strains that could not be assigned to a species with confidence by this system were identified by sequencing of the D1/D2 region of the ribosomal DNA (rDNA) large subunit (LSU) in 46 cases and the ITS1-5.8S-ITS2 region for 4 basidiomycetous strains. Sequence analysis has mainly identified strains of recently described yeast species, of potential new species and isolates that fall into species complexes.

- 1 CABS webpage: Center for applied biodiversity science <http://www.biodiversityhotspots.org/xp/Hotspots/caribbean/>.
- 2 Minter DW, Rodriguez Hernández M, Mena Portales J (2001) 946 pp. UK, Middlesex, Isleworth; PDMS Publishing.
- 3 Starmer WT, Schmedicke RA, Lachance MA (2003) FEMS Yeast Research 3:441-448.
- 4 Lachance MA, Bowles JM, Starmer WT (2003) FEMS Yeast Research 4:105-111.
- 5 Robert V, Evrard P, Hennebert GL. (1997) Mycotaxon 64:455-463
- 6 Kreger-van Rij NJW (1987) In AH Rose & JS Harrison (Ed), The yeasts vol. 1, 2nd ed, Academic Press, London, p. 5-61
- 7 Van der Walt JP, Yarrow D (1984) In NJW Kreger-van Rij (Ed) The yeasts, a taxonomic study, Elsevier, London, p. 45-104.
- 8 Kurtzman CP, Robnett CJ (1998). Antonie Leeuwenhoek 73:331-371.

The following paper has appeared recently.

3. Himmelreich U, Somorjai RL, Dolenko B, Daniel HM and Sorrell TC 2005 A rapid screening test to distinguish between *Candida albicans* and *Candida dubliniensis* using NMR spectroscopy FEMS Microbiology Letters 251:327-332

Nuclear magnetic resonance (NMR) spectroscopy combined with a statistical classification strategy (SCS) successfully distinguished between *Candida albicans* and *Candida dubliniensis*. 96% of the isolates from an independent test set were identified correctly. This proves that this rapid approach is

a valuable method for the identification and chemotaxonomic characterisation of closely related taxa. Most discriminatory characters were correlated with metabolite profiles, indicating biochemical differences between the two species.

---

**XX. Department of Biology, Faculty of Medicine, Masaryk University, Tomesova 112 62500 Brno, Czech Republic. Communicated by Marie Kopecka <mkopeccka@med.muni.cz>.**

---

The following are papers and lectures resulting from recent work in the department.

1. Kopecká M, Gabriel M., Takeo K, Yamaguchi M, Svoboda A., and Hata K 2003 Analysis of microtubules and F-actin structures in hyphae and conidia development of the opportunistic human pathogenic black yeast *Aureobasidium pullulans*. *Microbiology (UK)* 149:865-876.
2. Svoboda A 2004 Cell wall-cytoplasm signalling. *J. Appl. Biomed.* 2:81-85.
3. Gabriel M, Kopecká M, Yamaguchi M, Svoboda A, Takeo K, Yoshida S, Ohkusu M, Sugita T, Nakase T In press Cytoskeleton in the unique cell reproduction by conidiogenesis of the long neck yeast *Fellomyces (Sterigmatomyces) fuzhouensis*. *Protoplasma*.
4. David M, Gabriel M, Kopecká M Microtubule cytoskeleton and ultrastructural characteristics of *Malassezia pachydermatis*. In preparation for *Cell Biol. Int.*
5. Kopecká M, Gabriel M, Svoboda A, Takeo K, Yamaguchi M 2002 Microtubules and actin cytoskeleton in human pathogens *Cryptococcus neoformans* and *Aureobasidium pullulans* and the effect of cytoskeletal inhibitors. Poster. EMBO Workshop "Genetics after the Genome" Brno Czech Republic 16. - 19. 5. 2002.
6. Kopecká M, Gabriel M, Svoboda A, Takeo K, Yamaguchi M, Ohkusu M, Hata K 2002 Microtubules and actin cytoskeleton in growth and conidiogenesis in *Aureobasidium pullulans*. Plenary Lecture. XXXth Annual Conference on Yeasts, Smolenice (Slovak Republic) May 29. – 31, 2002.
7. Gabriel M, Kopecká M, Svoboda A, Takeo K, Yamaguchi M, Ohkusu M, Hata K 2002 Cytoskeleton in human fungal pathogens *Cryptococcus neoformans* and *Aureobasidium pullulans* and the effect of cytoskeletal inhibitors. Poster. XXXth Annual Conference on Yeasts Smolenice (Slovak Republic) May 29. – 31, 2002.
8. David M, Gabriel M, Kopecká M 2002 The study of potentially pathogenic lipophilic yeast *Malassezia pachydermatis*. Poster. XXXth Annual Conference on Yeasts Smolenice (Slovak Republic) May 29. – 31, 2002.
9. Gabriel M, Kopecká M, Takeo K, Yamaguchi M, Svoboda A, Nakase T, Sugita T 2003 The cytoskeleton during the conidiogenesis. In: XI. Cytoskeletální klub, Vranovská Ves 23.-25. 4. 2003, 18.
10. David M, Gabriel M, Kopecká M 2003. The first findings on the microtubule cytoskeleton in *Malassezia pachydermatis*. In: XI. Cytoskeletální klub, Vranovská Ves 23.-25. 4. 2003, p. 21.
11. David M, Gabriel M, Kopecká M 2004 Microtubules and actin structures in the basidiomycetous yeast, *Cryptococcus laurentii*. XXXII. Annual conference on Yeasts, Smolenice, May 2004 (poster).
12. Kopecká M, Gabriel M, Yamaguchi M, Takeo K and Svoboda A 2004 Conidiogenesis in pathogenic fungi: cytoskeleton as target for antifungals (invited lecture). 8<sup>th</sup> Asia-Pacific Conference on Electron Microscopy (8APEM) in conjunction with 60<sup>th</sup> Annual Meeting of the Japanese Society of Microscopy "Microscopy for Human Life", Kanazawa, Japan 7.-11.6.2004. Program B12: Mycology and Parasitology p.28.
13. Kopecká M, Gabriel M, Yamaguchi M, Svoboda A and Takeo K 2004 Cytoskeleton in human pathogenic yeasts (invited lecture). Japan Women University Mejirodai, Bunkyo-ku, Tokyo, Japan, 14. 6. 2004.
14. Gabriel M, Kopecká M, Takeo K, Yamaguchi M, Svoboda A, Nakase T, Sugita T 2003 The Cytoskeleton during the Conidiogenesis. In: XI. Cytoskeletální klub, Vranovská Ves 23.-25. 4. 2003, p.18.
15. David M, Gabriel M, Kopecká M 2003 The first findings on the microtubule cytoskeleton in *Malassezia pachydermatis*. In: XI. Cytoskeletální klub, Vranovská Ves 23.-25. 4. 2003, p. 21.
16. David M, Gabriel M, Kopecká M 2004 Microtubules and actin structures in the basidiomycetous yeast *Cryptococcus laurentii*. 32<sup>th</sup> Annual Conference on Yeasts Smolenice, Slovakia, May 12.-14. 2004. Chemical Institute of Slovak Academy of Sciences, Bratislava (2004), p.39. ISSN 1336-4839.

17. Kopecká M, Gabriel M, Yamaguchi M, Takeo K, and Svoboda A 2004 Conidiogenesis in pathogenic fungi: cytoskeleton as target for antifungals. In: Proceedings 8<sup>th</sup> Asia-Pacific Conference on Electron Microscopy (8APEM) in conjunction with 60<sup>th</sup> Annual Meeting of the Japanese Society of Microscopy "Microscopy for Human Life". June 7 to 11, 2004, Kanazawa, Japan, p.1043-1044. Japanese Society of Microscopy, Japan. ISBN 4-9902 106-0-3.

---

**XXI. Research Institute for Viticulture and Biology, Matúškova 25, 831 01 Bratislava, Slovakia. Communicated by E. Minárik.**

---

Summaries of recent publications.

1. Minárik E 2005 Inhibition of malolactic bacteria by vine yeasts *Vinařský obzor* 98:10 (in Slovak).

Cryotolerant vine yeast strains are more tolerant to malolactic fermentation (MLF) than mesophilic yeasts. Cryotolerant yeasts produce  $\beta$ -phenylethanol and succinic acid. Antibacterial metabolites activate towards lactic acid bacteria (LAB) and insufficient nutrients were found. Substances

released by *Saccharomyces cerevisiae* often show inhibition by LAB. The nature of the inhibitory substances and other factors promoting their production in vine are elucidated. It is nevertheless not clear what is the reason for the lack of specific nutrients that is inevitable for LAB.

2. Minárik E 2005 Interactions affecting malolactic bacteria and wine yeasts during malolactic fermentation. *Vinařský obzor* 98:6 (in Slovak).

The interaction of lactic acid bacteria (LAB) and wine yeasts (*Saccharomyces cerevisiae*) during malolactic fermentation (MLF) in wine is of complex nature. 1. Some wine yeast strains may show stimulatory or inhibitory influence. 2.

The influence of wine yeasts also depends on grape must composition and winery practice. 3. Some LAB strains display inhibitory activity to wine yeasts (*Saccharomyces cerevisiae*).

3. Minárik E 2005 Inhibition of wine yeasts by lactic acid bacteria. *Vinařský obzor* 98:7-8 (in Slovak).

Inhibition of wine yeasts (*S. cerevisiae*) in grape must by lactic acid bacteria (LAB) does not often occur. Nevertheless one has to keep in mind this possibility in enological practice. The character of such interactions may be different especially

when growing factors, e.g., yeast nutrients, are utilized by LAB. Direct inoculation of *Oenococcus oeni* in young vine on lees (method "sur lies") often confirmed this phenomenon.

---

**XXII. CREM – Centro de Recursos Microbiológicos, Secção Autónoma de Biotecnologia, Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, 2829-516 Caparica, Portugal. Communicated by Á. Fonseca <amrf@fct.unl.pt> and J.P. Sampaio <jss@fct.unl.pt>.**

---

Book chapter.

1. Fonseca, Á. and Inácio, J. Phylloplane yeasts (Chapter 13), in: *Yeast Handbook, Vol. 1: 'Biodiversity and Ecophysiology of Yeasts'*, G. Peter & C.A. Rosa (editors), Springer-Verlag (in press; scheduled for publication in 2005).

Contents: The phylloplane as a microbial habitat; Methods for detection, enumeration, and identification of epiphytic microorganisms; Plant surfaces as yeast habitats (epiphytic yeasts); Diversity of phylloplane yeasts; Population

dynamics on the phylloplane: variation in space and time; What are the makings of a 'phylloplane yeast'?; Future directions in ecological studies of epiphytic yeasts; References.

The following papers have been recently published.

2. Gadanho, M. and Sampaio, J.P. 2005. Occurrence and diversity of yeasts in the Mid-Atlantic Ridge hydrothermal fields near the Azores archipelago. *Microb Ecol* (DOI: 10.1007/s00248-005-0195-y).

The yeast community associated with deep-sea hydrothermal systems of the Mid-Atlantic Rift was surveyed for the first time. This study relied on a culture-based approach using two different growth media: a conventional culture medium for yeasts supplemented with sea salts (MYPss) and the same medium additionally supplemented with sulphur (MYPssS). For the evaluation of species diversity, a molecular approach involving MSP-PCR strain typing and sequence analysis of the D1/D2 domains of the 26S rDNA was followed. In the seven water samples that were studied the number of cfu/l ranged from 0 to 5940. The non-pigmented yeasts were much more abundant than the pink-pigmented ones. This disproportion was not observed in studies of other marine systems and may be due to the unique conditions of hydrothermal vents, characterized by a

rich animal and microbial diversity and therefore by the availability of organic compounds utilizable by yeasts. Higher counts of non-pigmented yeast were obtained using MYPss, whereas for pink yeasts higher counts were obtained using MYPssS. Moreover, among pink yeasts some of the MSP-PCR classes obtained were composed of isolates obtained only on MYPssS, which might be an indication that these isolates are adapted to the ecosystems of the hydrothermal vents. Twelve phylotypes belonged to the Ascomycota and seven phylotypes belonged to the Basidiomycota. The non-pigmented yeasts were identified as *Candida atlantica*, *C. atmosphaerica*, *C. lodderae*, *C. parapsilosis*, *Exophiala dermatitidis*, *Pichia guilliermondii* and *Trichosporon dermatis*, whereas the pigmented yeasts were identified as *Rhodospodium diobovatum*, *R. sphaerocarpum*,

*R. toruloides* and *Rhodotorula mucilaginosa*. Some of the yeasts that were found belong to phylogenetic groups that include species reported from other marine environments and eight

phylotypes represent undescribed species. The new phylotypes found at MAR hydrothermal fields represent 33% of the total number of yeast taxa that were found.

- Inácio, J., Portugal, L., Spencer-Martins, I. and Fonseca, Á. 2005. Phylloplane yeasts from Portugal: Seven novel anamorphic species in the Tremellales lineage of the Hymenomycetes (Basidiomycota) producing orange-coloured colonies. *FEMS Yeast Res* 5:1167-1183.

A survey of epiphytic yeasts on leaves of selected Mediterranean plant species collected at the 'Arrábida Natural Park' (Portugal) yielded about 850 isolates, mostly of basidiomycetous affinity. Amongst the basidiomycetes, 35 strains showed the following characteristics: production of orange-coloured colonies, ability to produce starch-like compounds, assimilation of D-glucuronic acid and/or inositol, inability to utilize nitrate, and formation of ballistoconidia by many of the isolates. This group of yeasts was assigned to the Tremellales lineage of the Hymenomycetes and was further characterised using a combination of conventional phenotypic identification tests with molecular methods, namely PCR fingerprinting and rDNA sequencing. Eight additional strains presumptively

identified as *Bullera armeniaca*, *B. crocea* or *Cryptococcus hungaricus* were also studied. Twenty-eight strains could be assigned to or were phylogenetically related to recognised species of *Dioszegia* in the 'Luteolus clade', but the 15 remaining strains belonged to other clades within the Tremellales. Ten phylloplane isolates were identified as *Dioszegia hungarica*, one as *D. aurantiaca*, another as *D. crocea* and three others were ascribed to the recently described species *D. zsoldii*. Seven novel species, viz. *Cryptococcus amylolyticus*, *C. armeniacus*, *C. cistialbidi*, *Dioszegia buhagiarrii*, *D. catarinonii*, *D. fristingensis* and *D. takashimae*, are proposed for the remaining strains that did not correspond to any of the hitherto recognised species.

- Almeida, J.M.G.C.F. 2005. Yeast community survey in Tagus estuary. *FEMS Microb Ecol* 53:295-303 (abstract was included in the last issue).

The following papers have been accepted for publication.

- Gadanhó, M., Libkind, D. and Sampaio, J.P. Yeast diversity in the extreme acidic environments of the Iberian Pyrite Belt. *Microbial Ecology*.

In the Iberian Pyrite Belt (IPB) acid rock drainage gives rise to aquatic habitats with low pH and high concentrations of heavy metals, a situation that causes important environmental problems. We investigated the occurrence and diversity of yeasts in two localities of the IPB: São Domingos (Portugal) and Rio Tinto (Spain). Yeast isolation was performed on conventional culture media (MYP), acidified (pH 3) media (MYP3) and on media prepared with water from the study sites (MYPw). The main goal of the study was to determine the structure of the yeast community and a combination of molecular methods was employed for accurate species identifications. Our results showed that the largest fraction of the yeast community was recovered on MYPw rather than on MYP and MYP3. Twenty seven yeast species were detected, 48% of which might represent undescribed taxa. Among these an undescribed species of the

genus *Cryptococcus* required low pH for growth, a property that has not been observed before in yeasts. The communities of S. Domingos and R. Tinto showed a considerable resemblance and eight yeast species were simultaneously found in both localities. Taking into consideration the physicochemical parameters studied, we propose a hierarchic organization of the yeast community in terms of high, intermediate or low stress conditions of the environment. According to this ranking the acidophile yeast *Cryptococcus* sp. 5 is considered the most tolerant species, followed by *Cryptococcus* sp. 3 and *Lecytophora* sp. Species occurring in situations of intermediate environmental stress were *Candida fluvialilis*, *Rhodospodium toruloides*, *Williopsis californica* and three unidentified yeasts belonging to *Rhodotorula* and *Cryptococcus*.

- Gadanhó, M. and Sampaio, J.P. Microeukaryotic diversity in the extreme environments of the Iberian Pyrite Belt: a comparison between universal and fungi-specific primer sets, temperature gradient gel electrophoresis and cloning. *FEMS Microbiology Ecology*.

The Iberian Pyrite Belt (IPB) is rich in complex polymetallic sulphides and one of the most important pyrite regions in the world. The IPB extends from Portugal to Spain and in several areas its aquatic reservoirs display extreme conditions characterized by low pH and high concentrations of several heavy metals. In this study the diversity of microeukaryotes was analysed at the abandoned mines of São Domingos (Portugal) and at Rio Tinto (Spain). We employed a molecular approach including direct DNA extraction from water samples followed by amplification of a fragment of small subunit rDNA. We used a set of eukaryotic universal primers and analysed the amplicons by molecular cloning and temperature gradient gel electrophoresis (TGGE). In addition, a fungi-specific primer set was also used in TGGE experiments. The fungi-specific primers

contributed to a substantial increase of the number of fungal taxa found in this study. This situation is probably a consequence of the low density of fungal structures as compared to the number of cells of other microeukaryotes. Several microorganisms, belonging (or closely related) to the ascomycetous yeast *Pichia acaciae*, the basidiomycetous yeasts *Cryptococcus humicola* and *Cystofilobasidium bisporidii*, the green algae *Chlamydomonas noctigama* and *Chlorella protothecoides* var. *acidicola* and some uncultured microeukaryotes were present at both localities, which suggest that specific microbes are adapted to the peculiar conditions of the IPB extreme environments. However, in spite of the similarities, a higher algal richness was observed at S. Domingos, whereas for R. Tinto the richness of fungi was more prominent.

I am currently working on the biodiversity of yeasts of peninsular Malaysia, with special attention to the yeast community of fermenting bertam palm buds (as well as morning glories), in collaboration with Dr. Frank Wiens, an ecologist interested in the palms and the mammals that feed on the fermenting nectar of palm. Current manuscripts in preparation or submitted deal with two new subclades of *Metschnikowia* species, one from Africa and another from Malaysia. We are developing a means of

characterizing population structure using polymorphic DNA markers that can be detected by single-strand conformation polymorphism (SSCP) electrophoresis. We have successfully applied the approach to the biogeography of *Metschnikowia lochheadii* and will be extending it to other interesting species in the future. Preliminary results will be presented at the Cairns IMC meeting this summer.

The following is the abstract of a poster presented at the IUMS Congress in San Francisco last summer. I hope eventually to publish an extensive review paper on the matter of endemism and microbial ubiquity.

1. Lachance MA 2005 Endemism in yeasts. IUMS Congress, San Francisco (Poster).

**Background:** One currently advocated interpretation of Beijerinck's principle, "everything is everywhere", is that the microbial world consists of a relatively small number of very widely distributed species and consequently that the study of microbial biogeography is a vain pursuit (Fenchel & Finlay 2004 Bioscience 54:777-784). The large number of species descriptions based on single strains of dubious origins is a testimony to the neglect endured by yeast ecology due in part to the ubiquity paradigm. Here, I explore the question of ubiquity in two groups of related yeasts that were sampled extensively across the globe.

**Methods:** Collections were obtained through several studies by the author and various colleagues (Starmer, Phaff, Bowles, Rosa, Ganter, and others). Phylogenetic reconstructions were derived from rDNA sequences using commonly accepted methods.

**Results:** Numerous strains assigned to the *Sporopachydermia* clade each can be assigned to one of 21 phylotypes, 17 of which probably represent separate species. *S. lactativora* stands out as the only one that might be treated as cosmopolitan; the

three known isolates came from Antarctic seawater, industrial waste in the U.S., and a clinical specimen in Finland, respectively. In every other case the phylotypes have distributions that can be attributed to vicariance or dispersal, and most appear to be endemic. A large collection of heterothallic *Metschnikowia* and related species from nitidulid beetles and other floricolous insects can be assigned clearly to 22 biological species. Again, the distributions follow patterns that are best explained in terms of biogeographic history, and again, endemism is the rule rather than the exception. Of special interest is the recent discovery that endemic beetles that live on endemic plants in Hawai'i harbour at least six endemic sister species of *Metschnikowia*.

**Conclusions:** Large collections of related yeasts isolated from natural habitats in globally diverse localities are rare. When the members of such collections are identified correctly, their global distributions are best explained in terms of speciation history. The possibility that some yeast species might be ubiquitous cannot be rejected outright, but at this time evidence in support of such a phenomenon is lacking.

The following book chapter is in press.

2. Lachance MA 2006. Yeast biodiversity: how many and how much? pp. 1-10. In: Rosa CA and Péter G (Eds.) Biodiversity and Ecophysiology of Yeasts, Series: The Yeast Handbook 580 pp.

---

**Network: Yeasts in Food and Beverages  
Publications on the Biodiversity of Wine Yeasts  
Communicated by P. Romano <pot2930@iperbole.bologna.it>**

---

**FRANCE: UMR Sciences pour l'Oenologie, INRA - 2, place Viala, 34060, Montpellier, France.  
Communicated by Sylvie Dequin <dequin@ensam.inra.fr>**

---

1. Valero E., Schuller D., Cambon B., Casal M., Dequin S. 2005. Dissemination and survival of commercial wine yeast in the vineyard: a large scale, three years study. FEMS Yeast Research 5(10):959-969.

The use of commercial wine yeast strains as starters has been extensively generalised over the past two decades. In this study, a large-scale sampling plan was devised over a period of three years in six different vineyards to evaluate the dynamics and survival of industrial yeast strains in the vineyard. A total of 198 grape samples were collected at various distances from the wineries, before and after harvest, and yeast strains isolated after spontaneous fermentation were subsequently identified by molecular methods. Among 3780 yeast strains identified, 296 isolates had a genetic profile identical to that of commercial yeast strains. For a large majority (94%), these strains were recovered

at very close proximity to the winery (10-200m). Commercial strains were mostly found in the post harvest samples, reflecting immediate dissemination. Analysis of population variations from year to year indicated that permanent implantation of commercial strains in the vineyard did not occur, but instead that these strains were subject to natural fluctuations of periodical appearance/disappearance like autochthonous strains. Our data show that dissemination of commercial yeast in the vineyard is restricted to short distances and limited periods of times and is largely favoured by the presence of water runoff.

- Schuller D., Valero E., Dequin, S., Casal M. 2004. Survey of molecular methods for the typing of industrial yeast strains. *FEMS Microbiology Letters* 231(1):19-26.

A survey of the genetic polymorphisms produced by distinct methods was performed in 23 commercial winery yeast strains. Microsatellite typing, using six different loci, an optimized interdelta sequence analysis and restriction fragment length polymorphism of mitochondrial DNA generated by the enzyme *HinfI* had the same discriminatory power: among the 23

commercial yeast strains, 21 distinct patterns were obtained. Karyotype analysis gave 22 patterns, thereby allowing the discrimination of one of the three strains that were not distinguished by the other methods. Due to the equivalence of the results obtained in this survey, any of the methods can be applied at the industrial scale.

- Schuller D, Alves H, Dequin S., Casal M. 2005. Ecological survey of *Saccharomyces cerevisiae* strains from vineyards in the vinho verde region of Portugal. *FEMS Microbiology Ecology* 51:167-177.

One thousand six hundred and twenty isolates of *Saccharomyces cerevisiae* were obtained from 54 spontaneous fermentations performed with grapes collected in 18 sampling sites of 3 vineyards (Vinho Verde Wine Region, Northeast of Portugal) during the 2001-2003 harvest seasons. All isolates were analyzed by their mtDNA RFLP (*HinfI*) and a pattern profile was verified for each isolate, resulting in a total of 297 different profiles, all revealed to belong to the species *S. cerevisiae*. The strains corresponding to sixteen of these profiles showed a wider temporal and geographical distribution, being characterized by a generalized pattern of sporadic presence, absence and reappearance. One strain (ACP10) showed a more regional distribution with a perennial behavior. In different fermentations

ACP10 was dominant or not, showing that the final outcome of fermentation was dependent on the specific composition of the yeast community in the must. Only 24% of grape samples collected before harvest initiated a spontaneous fermentation, compared to 71% for grapes collected after harvest, in a time frame of about 2 weeks. The associated strains were also much more diversified (267 patterns among 1260 isolates compared to 30 patterns among 360 isolates in the post- and pre- harvest samples respectively). These studies are indispensable for the development of strategies aiming at the preservation of biodiversity and genetic resources as a basis for further strain development.

---

**SOUTH AFRICA: Department of Microbial, Biochemical and Food Biotechnology, University of the Free State Bloemfontein, South Africa. Communicated by J.L.F. Kock <kockjl.sci@mail.uovs.ac.za>.**

---

- Strauss C .J., Kock J.L.F., Viljoen B.C., Botes P.J., Hulse G., Lodolo E. 2004. Lipid turnover during inverse flocculation in *Saccharomyces cerevisiae* UOFS Y-2330. *J. Inst. Brew.* 110(3):207-212.

In this study we uncovered that *Saccharomyces cerevisiae* UOFS Y-2330 does not only demonstrate inverse flocculation, but is also characterised by two different lipid turnover patterns. During Flo1 phenotype flocculation, this yeast showed two neutral lipid accumulating stages (i.e. at 8 h and from 12 h). This is probably triggered by flocculation, which can be regarded as a survival mechanism where cells accumulate especially neutral lipids as reserve energy source - a similar mechanism is probably

operative when cells enter stationary growth. Contrary to Flo1 behaviour, this strain in NewFlo phenotype mode demonstrates only a single lipid accumulation phase i.e. when cells enter stationary growth, which coincides with increase in flocculation. In addition, an increase in phospholipids was experienced during active growth in both flocculation behaviours i.e. Flo1 and NewFlo probably as a result of active membrane production.

- Strauss C.J., Kock J.L.F., Van Wyk P.W.J., Lodolo E.J., Pohl C.H., Botes P.J. 2005. Bioactive oxylipins in *Saccharomyces cerevisiae*. *J. Inst. Brew.* (In press).

We found that some strains of *Saccharomyces cerevisiae* (include strains used in fermentation processes) produce short chain (mainly 8 carbon) oxylipins and not potent inflammatory long chain (20 carbon) oxylipins such as prostaglandins. When acetylsalicylic acid (aspirin) was added to cultures of *Sacch. cerevisiae* UOFS Y-2330, flocculation was significantly inhibited as well as the production of 3-hydroxy 8:0 thereby linking flocculation and this oxylipin. Furthermore, no traces of 3-hydroxy 8:0 could be detected at the start of flocculation in this

yeast. This research is based on (i) reports that yeasts in general can produce bioactive prostaglandins, (ii) findings suggesting a link between aspirin-sensitive prostaglandins and biofilm formation by *Candida albicans*, (iii) the discovery that the addition of low concentrations of aspirin abolish yeast biofilm formation and sexual cell aggregation and (iv) the recent discovery of a novel potent aspirin-sensitive pro-inflammatory 3-hydroxy prostaglandin E<sub>2</sub> synthesized by *Candida albicans* in conjunction with mammalian cells probably during candidiasis.

- I. Cocolin, L., Rantsiou, K., Iacumin, L., Zironi, R., Comi, G. 2004. Molecular detection and identification of *Brettanomyces/Dekkera bruxellensis* and *Brettanomyces/Dekkera anomalus* in spoiled wines. *Appl. Environ. Microbiol.* 70:1347-1355.

In this paper we describe the development of a PCR protocol to specifically detect *Brettanomyces bruxellensis* and *B. anomalus*. Primers DB90F and DB394R, targeting the D1-D2 loop of the 26S rRNA gene, were able to produce amplicons only when the DNA from these two species were used. No amplification product was obtained when DNA from other *Brettanomyces* spp. or wine yeasts were used as the templates. The 305-bp product was subjected to restriction enzyme analysis with *DdeI* to differentiate between *B. bruxellensis* and *B. anomalus*, and each species could be identified on the basis of the different restriction profiles. After optimization of the method

by using strains from international collections, wine isolates were tested with the method proposed. Total agreement between traditional identification and molecular identification was observed. The protocol developed was also used for direct detection of *B. bruxellensis* and *B. anomalus* in wines suspected to be spoiled by *Brettanomyces* spp. Application of culture-based and molecular methods led us to the conclusion that 8 of 12 samples were spoiled by *B. bruxellensis*. Results based on the application of molecular methods suggested that two of the eight positive samples had been infected more recently, since specific signals were obtained at both the DNA and RNA levels.

1. Manzano M., Cocolin L., Longo B., Comi G. 2004. PCR-DGGE differentiation of strains of *Saccharomyces sensu stricto*. *Antonie van Leeuwenhoek* 85:23-27.

A quick molecular biology method based on the polymerase chain reaction (PCR) and Denaturing Gradient Gel Electrophoresis (DGGE) was developed for distinguishing strains belonging to the *Saccharomyces sensu stricto* group. Differentiation was obtained between *S. cerevisiae*, *S. paradoxus* and *S. bayanus/S. pastorianus* although no distinction was possible between *S. bayanus* and *S. pastorianus* using the

amplification of the ITS regions. The ability to distinguish different strains of *Saccharomyces sensu stricto* group could allow for a better understanding the ecology of these species on grapes as well as in musts and wines and the method developed can be useful for the quick identification of *Saccharomyces sensu stricto* strains from numerous isolates.

2. Manzano M., Cocolin L., Iacumin L., Cantoni C., Comi G. 2005. A PCR-TGGE (Temperature Gradient Gel Electrophoresis) technique to assess differentiation among enological *Saccharomyces cerevisiae* strains. *International Journal of Food Microbiology* 101:333-339.

In this paper new primers, annealing to the ITS2 region, were used to obtain a PCR product that was subsequently subjected to Temperature Gradient Gel Electrophoresis (TGGE) analysis. The PCR-TGGE method performed was able to

distinguish *S. cerevisiae* and *S. paradoxus* and to distinguish between strains of *S. cerevisiae*. Moreover direct analysis of *S. cerevisiae* and *S. paradoxus* ecology in musts were also performed.

3. Manzano M., Medrala D., Giusto C., Bartolomeoli Urso R., Comi G. 2005. Classical and molecular analyses to characterize commercial dry yeasts used in wine fermentations. *J. Appl. Microbiol.* (in press).

The aim of the work was to apply PCR-TGGE and RE assays to identify commercially available starters of *S. cerevisiae sensu stricto* complex. To characterize an analyzed pool of 62 active dry yeasts of different brands used in wine fermentation practices, classical microbiological tests were also performed as well as evaluation of contamination with lactic acid bacteria and non-*Saccharomyces* yeasts. PCR-TGGE and RE were used in order to provide fast and reliable methods to identify and differentiate enological yeasts. Proposed molecular methods

enabled to identify particular strains within 36 hours after colony isolation and directly from dry yeast suspension. The methods are highly recommended to obtain reliable results on yeast strain differentiation in a significantly shorter time if compared to classical fermentation tests. The obtaining of yeast strain differentiation in a short time and without plating is a good tool for a rapid discrimination among enological strains used as starters in enological practices.

1. Giovani G., Nannelli F., Rosi I. 2004. Influence of cold pre-fermentative maceration on the microbiological profile of Sangiovese must (biotype prugnolo gentile). *Proceedings 2<sup>nd</sup> International Symposium "Il Sangiovese identità e peculiarità: vitigno tipico e internazionale"*. Firenze (Italy), 17-19 November 2004 (in press).

Cold pre-fermentative maceration or cryomaceration is a must treatment that consists of retarding the beginning of alcoholic fermentation by cooling the crushed grapes. While some knowledge has been acquired regarding the effect of this type of treatment on the increasing of colour stability and varietal aroma of wines from Sangiovese grapes, there is scarce information available about its effect on the microbial ecology of musts obtained from these grapes. This study was conducted to evaluate the effect of different cooling techniques (method and time of application) on the microbiological profile of musts before and during alcoholic fermentation. The results showed that the wild yeast population underwent a reduction as a function of the type of cooling applied to the must. During alcoholic fermentation, the non *Saccharomyces* yeast population

in general, and the apiculate yeasts in particular, remained higher in the trials with longer cooling times (e.g. 72 h vs 36 h). However, within the same must pre-treatment period, the yeast population decreased more rapidly in the trial where the must was cooled with liquid N<sub>2</sub>. Similarly, the *Saccharomyces cerevisiae* population showed greater viability, both at the beginning and end of alcoholic fermentation, in the trials where the musts were cryomacerated for 36 h, independent of the type of cooling applied. At the end of alcoholic fermentation in all treatments, the non *Saccharomyces* population fell to zero, and the analysis of dominance showed that the *Saccharomyces cerevisiae* strain inoculated was able to dominate the environment.

2. Giovani G., Puccioni S., Millarini V., Rosi I. 2004. Effect of different ways of inoculation of a *Saccharomyces cerevisiae* strain on the microbiological and chemical profile of Sangiovese wine. Proceedings 2<sup>nd</sup> International Symposium “Il Sangiovese identità e peculiarità: vitigno tipico e internazionale”. Firenze (Italy), 17-19 November 2004 (in press).

The aim of this work was to evaluate the effect of different inoculation doses at different times on the microbiological and chemical profile of a Sangiovese wine. Fermentations were set up with three inoculation doses (10, 20 and 30 g/hl active dried yeast) carried out 0, 12, 36 h after obtaining the must. The fermentation kinetics, evolution of yeast populations and analytical profile of the wines were assayed for all samples. The results showed that the musts inoculated after 12 h, and especially those after 36 h, underwent modifications in composition due to the spontaneous microflora, which in turn

lead to a different trend of *Saccharomyces cerevisiae* and non *Saccharomyces* yeast population evolution. Moreover, with regard to the samples inoculated after 36 h, the analysis of dominance carried out at the end of fermentation revealed that when the lowest inoculation doses (10-20 g/hl) were applied, the strain was not able to completely dominate the environment. The analytical profile of the wines was also found to be different as a function of the time and dose of active dry yeast added to the must.

3. Fia G., Millarini V., Bertuccioli M., Sieczkowsky N., Rosi, I. 2004. Influence of the *Saccharomyces cerevisiae* strain on the chemical properties and sensory identity of Sangiovese wine. Proceedings 2<sup>nd</sup> International Symposium on Sangiovese. Proceedings 2<sup>nd</sup> International Symposium “Il Sangiovese identità e peculiarità: vitigno tipico e internazionale”. Firenze (Italy), 17-19 November 2004 (in press).

Many studies have shown that the quality of wine is influenced by the *Saccharomyces cerevisiae* strain that dominates fermentation. This study was carried out to investigate the possibility to emphasise the typical sensory properties of a wine by using autochthonous yeast strains, rather than strains isolated in other zones. Ten fermentations were conducted in duplicate with two autochthonous and three different commercial strains. Sangiovese grapes, obtained from two different Chianti Classico areas, were vinified following the technological scheme of the this area. Six months after harvesting, wines were analysed by chemical and descriptive sensory methods, according to a complete randomised block design. A panel of 10 trained judges recorded the intensity of four attributes: overall aroma, red fruit (blackberry) aroma, artificial fruit (prune) aroma, and wildflower aroma. The obtained profiles were compared with

reference profile for Sangiovese wine. The data show that the wines of two commercial strains attained higher scores for overall and artificial fruit (prune) aroma, which do not confer typicality characteristics of wine obtained from Sangiovese grapes. On the contrary, wines obtained from two autochthonous strains and one commercial strain had higher scores for red fruit (blackberry) and wildflower aroma, typical sensory properties of Sangiovese wine. Chemical data have shown that the grape origin determined the differences between the wines. Only for the wines obtained with an autochthonous strain the effect of the strain reduces the differences due to the grape origin. In conclusion, this study suggests that some strains can influence the sensory identity of a wine and that the origin of the yeast strain is not correlated to these traits.

4. Cratini F., Millarini V., Rosi I. 2004 Fermentation of Sangiovese wines in difficult environmental conditions. Proceedings 2<sup>nd</sup> International Symposium “Il Sangiovese identità e peculiarità: vitigno tipico e internazionale”, Firenze (Italy), 17-19 November 2004 (in press).

In oenology, it is not unusual to experience very slow and incomplete fermentations, and numerous studies have been dedicated to solutions for this problem. The present investigation attempts to highlight the effect of the concentration of some microelements (Cu, Zn, Mg, Ca) and various nutrients on the viability and fermentative activity of yeast. This effect has been studied under difficult environmental conditions, such as high sugar concentration in the must and sudden rise in temperature during the initial phases of fermentation. The results obtained point out that a brusque rise in temperature leads to a reduction

of viability and a slowing down of the fermentative activity of the yeast. This effect was particularly evident in the samples containing high concentrations of copper and zinc (40 mg/l and 5 mg/l, respectively) in the initial must. Addition of various nutrients, after thermal shock, was not effective in stimulating the fermentative activity of the yeast. In order to avoid slow or incomplete fermentation, it is thus important to maintain strict control of the fermentation temperature and the content of copper and zinc in the starting musts.

5. Sebastiani F., Pinzauti F., Cavalieri D., Casalone E., Rosi I., Fia G., Polsinelli M., Barberio C. 2004. Study of biodiversity of *Saccharomyces cerevisiae* strains isolated from Sangiovese grapes of Chianti area. *Ann. Microbiol.* 54 (4):415-426.

Biodiversity of 175 *Saccharomyces cerevisiae* strains isolated from Sangiovese grapes of Chianti area was determined by genetic and molecular approaches. Genetic analysis was carried out on 97 strains examining markers like the ability to ferment five sugars, copper resistance, H<sub>2</sub>S production, homothallism, sporification and spore viability, growth rate. Molecular analysis was performed: a) by RAPD with three primers on 135 strains, 57 of which examined also by genetic analysis, b) by hybridisation with a polymorphic probe on fifteen strains barely discriminated by RAPD, and c) by specific PCR amplification with two primers (DC4FA, DC4RA) designed on

the 5'- and 3'- DNA sequences of the polymorphic probe. A remarkable biodiversity was detected by all the techniques. Genetic analysis and RFLP with a polymorphic probe were the most powerful methods, permitting to distinguish, as single strains or groups, 80% and 73% of the strains, respectively. Specific PCR amplification with primers DC4FA and DC4RA showed also to be a highly discriminative method. The overall results enabled us to distinguish 80 single strains out of the 175 examined. This biodiversity can be employed to select new wine starter strains of *S. cerevisiae*.

6. Fia G., Giovani G., Rosi I. 2005. Study of  $\beta$ -glucosidase production by wine-related yeasts during alcoholic fermentation. A new rapid fluorimetric method to determine enzymatic activity. *J. Appl. Microbiol.* 99:509-517.

The  $\beta$ -glucosidase activity is involved in the hydrolysis of several important compounds for the development of varietal wine flavour. The aim of the present study was to investigate the production of  $\beta$ -glucosidase in a number of wine-related yeast strains and to measure and identify this activity over the course of grape juice fermentation.  $\beta$ -glucosidase activity was measured as the amount of 4-MU released from 4-MUG substrate. Intact cells of some grape and wine-spoilage yeasts showed  $\beta$ -glucosidase activity much higher than those observed in wine yeasts "sensu stricto". During fermentation, three *Saccharomyces cerevisiae* strains, one *Hanseniaspora valbyensis* strain, and one

*Brettanomyces anomalus* strain showed  $\beta$ -glucosidase activity both intra- and extra-cellularly. In the studied strains,  $\beta$ -glucosidase activity was at its maximum when the cells were in the active growth phase. However, a lowering of medium pH to values around 3 during fermentation led to total loss of activity. During the course of this study, a new, rapid and reproducible method to assay  $\beta$ -glucosidase activity was developed. The fact that *Saccharomyces* and non-*Saccharomyces* yeast strains are able to express  $\beta$ -glucosidase activity during the alcoholic fermentation sheds new light on the contribution of these yeasts in the aroma expression of wines.

7. Giovani G., Rosi I. 2005. Release of parietal polysaccharides from *Saccharomyces cerevisiae* autolytic mutants during alcoholic fermentation. Paper presented as poster at XXIV ISSY, Oropesa del Mar, Castellon-Spain- September 28<sup>th</sup>-October 2<sup>nd</sup>, 2005.

*Saccharomyces cerevisiae* can release parietal polysaccharides-particularly mannoproteins- during alcoholic fermentation of grape juice. It has previously been reported that the amount of parietal polysaccharides released is highly dependent on yeast strain, on metabolic phase of cells, as well as on fermentation conditions. Various positive effects on wine quality of these yeast-produced macromolecules have been proposed: increase of colour and decrease in astringency of red wines, regulation of volatility of the substances responsible for odour, protective effect of the tartaric and protein precipitation of wine, stimulation of malolactic fermentation. In order to increase the release of parietal polysaccharides into the fermentation medium, a wine strain of *S. cerevisiae* was subjected to UV mutagenesis to obtain thermosensitive autolytic mutants affected in cell wall integrity. The screening was based on the release of active alkaline phosphatase to the medium and growth to restrictive temperatures (37 °C). Fourteen thermosensitive mutants were obtained and 5 of them were

utilized in fermentation trials. As substrate of fermentation was used a polysaccharide-free synthetic medium. For each mutant and for the parental strain duplicate fermentations were carried at 28, 32 and 34 °C. The evolution of fermentation was followed by CO<sub>2</sub> loss. At the end of fermentation cell viability, ethanol and total polysaccharide concentration were determined. Results showed that the thermosensitive mutants released a quantity of polysaccharides into the fermentation medium that was nearly twice as much as compared to the parietal strain. This release was revealed to not be dependent on the temperature of fermentation. On the contrary, viability and fermentative performance of mutants and parental strain decreased at 34 °C. As a correlation between the loss of cell viability and the quantity of polysaccharides released by the yeast strain was not found under any conditions, it can be assumed that the mutation led to a phenotype with less stable cell walls and thus an easier release of macromolecules into the medium.

---

**ITALY: Dipartimento di Biologia, Difesa, Biotecnologie Agro-orestali, Via Ateneo Lucano 10, 5100-Potenza, Italy. Communicated by Patrizia Romano <pot2930@iperbole.bologna.it>.**

---

1. Romano P., Paraggio M., Capece A. 2004. Wine *Saccharomyces cerevisiae* improved by using traditional approaches. *Bulletin de l'O.I.V.* 77(883-884):631-641.

Numerous studies in these last years have demonstrated the existence of a considerable variability in the expression of technological traits among wine strains of the species *Saccharomyces cerevisiae*. By using these differences as source of genetic variability, strain improvement can be achieved by breeding program. This technique doesn't modify the natural genetic complement, but it facilitates natural breeding by

crossing strains selected and chosen from the environment. By applying this method we obtained wine strains possessing specific and stable technological characteristics, suitable for the fermentation of Aglianico of Vulture wine. These recombinant strains don't represent a hazard for the human health because they are not genetically modified, but are the product of a programmed combination of selected traits of the parental strains.

2. Sipiczki M., Romano P., Capece A., Paraggio M. 2004. Genetic segregation of natural *Saccharomyces cerevisiae* strains derived from spontaneous fermentation of Aglianico wine. *J. Appl. Microbiol.* 96:1169-1175.

Investigation of the meiotic segregation of karyotypes and physiological traits in indigenous *Saccharomyces* strains isolated from Aglianico (South Italy) red wine. Segregation was studied in F1 and F2 descendants. Tetrads were isolated from sporulating cultures by micromanipulation. The spore clones were subjected to karyotype analysis by pulse-field gel electrophoresis (Bio-Rad model CHEF-DR II) and to various physiological tests. Certain chromosomes of the isolates showed 2:2 segregation patterns in F1 but proved to be stable in F2. Resistance to CuSO<sub>4</sub>, SO<sub>2</sub>

tolerance, the fermentative power and the production of certain metabolites segregated in both F1 and F2 generations and showed patterns indicating the involvement of polygenic regulation. The analysis revealed a high degree of genetic instability and demonstrated that meiosis can improve chromosomal and genetic stability. Winemaking is critically dependent on the physiological properties and genetic stability of the fermenting *Saccharomyces* yeasts. Selection of clones from F2 or later generations can be a method of reduction of genetic instability.

3. Paraggio M., Fiore C. 2004. Screening of *Saccharomyces cerevisiae* wine strains for the production of acetic acid. *World J. Microbiol. Biotechnol.* 20:743-747.

In this study eighty wine strains of *Saccharomyces cerevisiae* were characterized for the production of acetic acid. A significant variability in the production levels was determined among the strains, which produced from a few mg/l to more than 1 g/l. Fifteen strains, differing in acetic acid production, were tested in fermentation of grape musts of different varieties (Aglianico, Sangiovese, Cannonau, Bombino nero, Nero d'Avola, Vermentino, Fiano). The results emphasized a great strain

variability in function of the grape must composition. The cluster analysis, performed on these data, subdivided the strains in tree groups, characterized by a similar pattern in acetic acid production. This study confirming the high/low production of acetic acid as a strain characteristic, emphasized also that the strain behaviour depends on the grape must composition and therefore to the vine variety.

4. Capece A., Fiore C., Maraz A., Romano P. 2005. Molecular and technological approaches to evaluate strain biodiversity in *Hanseniaspora uvarum* of wine origin. *J. Appl. Microbiol.* 98:136-144.

This study regards the characterization by molecular and physiological methods of wild apiculate strains, isolated from Aglianico del Vulture grape must. The restriction analysis of 18S rDNA allowed the identification of strains at the species level, which were predominantly *Hanseniaspora uvarum*. The RAPD analysis and the valuation of technological traits, such as the metabolic and enzymatic activities, were useful to evaluate the polymorphism of this species. The RAPD analysis clustered the wild *H. uvarum* strains in four main genetic groups and a very high phenotypic variability confirmed this genetic polymorphism. The technological variables, which determined the strain

biodiversity differed significantly, demonstrating that these technological traits are strain dependent. A certain correlation was found between the strain behaviour and its isolation zone, indicating the influence of the environment on the genetic patrimony of the population. The genetic and technological biodiversity recorded among *H. uvarum* wild strains represents the basis for organizing a collection of apiculate strains exhibiting oenological characteristics at different levels, such as high/low production of secondary compounds, and, therefore, potentially useful for a selection programme.

5. Fiore C., Arrizon J., Gschaedler A., Flores J., Romano P. 2005. Comparison between grape and agave must yeasts for traits of technological interest. *World J. Microbiol. Biotechnol.* 21:1141-1147.

In Mexico there are different alcoholic beverages obtained from agave juice, which is cooked, fermented and distilled. For tequila production only *Agave tequilana* Weber blue variety is allowed. In this study we compared yeast strains of different species (*Saccharomyces cerevisiae*, *Kloeckera africana* and *K. apiculata*, *Candida magnolia* and *C. krusei*) and of different origin (agave and grape juice) for parameters of technological interest, such as SO<sub>2</sub> and copper resistance, ethanol tolerance and

enzymatic activities. All agave strains resulted more resistant to SO<sub>2</sub> and agave non-*Saccharomyces* yeasts were more tolerant to ethanol, whereas grape strains exhibited positive results for  $\beta$ -glucosidase and  $\beta$ -xylosidase activities. As regards fermentations of *Agave tequilana* juice added with ethanol at different concentrations, only *Saccharomyces* agave strains were more tolerant to ethanol than grape strains.

6. Capece A., Sciancalepore A., Sunseri F., Romano P. 2005. Molecular tools for assessing genetic diversity in *Saccharomyces cerevisiae* and in the grapevine cultivar Aglianico del Vulture typical from South Italy. *J Wine Res* 15(3):179-188.

In grapevine (*Vitis vinifera* L.), cultivar identification problems have frequently been solved using ampelographic and chemical analysis. However, these methods resulted in several ambiguous attributions, particularly when different clones of the same cultivar have to be identified. The availability of reliable and reproducible tools to identify genetic differences at clonal level would facilitate the classification of clones and cultivar. At the same time, molecular tools are also well developed in order to classify the autochthonous yeast strains (*Saccharomyces cerevisiae*) isolated in the area of Aglianico del Vulture cultivation. In this work, 6 vineyards of the ancient cultivar

Aglianico del Vulture and 60 *Saccharomyces sensu stricto* strains were characterized. Molecular tools, such as RAPD-PCR, microsatellite, ARDRA and AFLP were applied in order to study the genetic variability among the vineyards of Aglianico del Vulture and among the *S. cerevisiae* isolates. The molecular markers revealed different fingerprinting patterns either on grapevine or *S. cerevisiae* strains. The genetic differences detected in yeast and plant would represent the genetic variability usable for a selection of the best plant-yeast combination in order to preserve the typical Aglianico del Vulture wine features.

- Flores Berrios E.P., Alba González J.F., Arrizon J., Capece A., Gschaedler Mathis A. 2005. The use of AFLP for detecting DNA polymorphism, genotype identification and genetic diversity between yeasts isolated from Mexican agave distilled beverages and from grape musts. *Lett Appl Microbiol* 41:147–152.

The objectives were to determine the variability and to compare the genetic diversity obtained using amplified fragment length polymorphism (AFLP) markers in analyses of wine, tequila, mezcal, stool and raicilla yeasts. A molecular characterization of yeasts isolated from Mexican agave musts, has been performed by AFLP marker analysis, using reference wine strains from Italian and South African regions. A direct correlation between genetic profile, origin and fermentation

process of strains was found especially in strains isolated from agave must. In addition, unique molecular markers were obtained for all the strains using six combination primers, confirming the discriminatory power of AFLP markers. Significance and impact of the study: This is the first report of molecular characterization between yeasts isolated from different Mexican traditional agave-distilled beverages, which shows high genetic differences with respect to wine strains.

- Arrizon J., Fiore C., Acosta G., Romano P., Gschaedler A. 2005. Fermentation behaviour and volatile production by agave and grape must yeasts in high sugar Agave tequilana and grape must fermentations. Antonie van Leeuwenhoek, in press.

Few studies have been performed on the characterization of yeasts involved in the production of agave distilled beverages and their individual fermentation properties. In this study, a comparison and evaluation of yeasts of different origins in tequila and wine industry was carried out for technological traits. Fermentations were carried out in high (300 g l<sup>-1</sup>) and low (30 g l<sup>-1</sup>) sugar concentrations of *Agave tequilana* juice, in musts

obtained from Fiano (white) and Aglianico (red) grapes, and in YPD medium (added with 270 g l<sup>-1</sup> of glucose) as a control. Grape yeasts exhibited a reduced performance in high-sugar agave fermentation, while both agave and grape yeasts showed similar fermentation behaviours in grape musts. Production levels of volatile compounds by grape and agave yeasts differed in both fermentations.

- Romano P., Capece A., Fiore C. 2005. Yeast/Vine Interaction as Selection Tool to Optimize Wine Typicality. International Workshop on “Advances in Grape and Wine Research”, Venosa (Potenza), Italy, September 15-17 (in press in *Acta Horticulture*).

The conversion of grape sugars to alcohol and other end-products by specific yeast populations may yield wines with distinct organoleptic quality. In order to reduce the risk of undesirable changes of wine flavour, nowadays commercial starter cultures are widespread used in winemaking. In addition to their principal role of transforming grape sugars into alcohol without off-flavours development, starter cultures have to possess technological properties related to the winemaking process, such as useful enzymatic activities and production of secondary compounds related both to wine organoleptic quality and human health. The actual trend is the selection of starter cultures able to complement and optimize grape quality in order to obtain a wine, which could be the result of the optimal interaction yeast/vine. The selection of starter cultures is mainly addressed to the

principal actor in wine fermentation, *Saccharomyces cerevisiae*, characterized by high ethanol and sulphur dioxide tolerance and high fermentation power, which allows to dominate and complete grape must fermentation. Among strains of this species it's demonstrated the existence of a strong polymorphism and it is widely reported that each fermentation seems to have its own population of different *S. cerevisiae* strains, which contribute to the wine chemical composition and produce wines differing in the expression of technological traits. This presentation deals with results of studies performed on numerous wild *S. cerevisiae* strains, isolated from grapes of different varieties, in order to emphasize the significant biodiversity of this species and the need of a strong selection procedure for the individuation of suitable starter cultures in function of grape must to ferment.

- Capece A., Fiore C., Romano P. 2005. Molecular and technological biodiversity in apiculate yeasts of wine origin. International Workshop on Advances in Grape and Wine Research, Venosa (Potenza), Italy, September 15-17 (in press on *Acta Horticulture*).

Among the non-*Saccharomyces* yeasts which dominate the early fermentation stages, *Hanseniaspora uvarum* represents the prevalent species, due to its wide diffusion on the grapes and in grape must just pressed. Numerous studies in the last decade have demonstrated that these yeasts survive at significant levels for longer periods during fermentation than previously thought and their growth is not suppressed in inoculated fermentations with selected cultures of *S. cerevisiae*. Until a few years ago these yeasts were considered as spoilage species exhibiting undesirable oenological traits, while recently numerous studies have demonstrated the existence of a significant biodiversity also in *H. uvarum* population for technological traits. During the last ten years numerous *H. uvarum* wild strains, isolated and identified at the species level by molecular techniques, have been included in the collection of Wine Microbiology Laboratory of Basilicata University. They have been analyzed to evaluate their genetic and technological variability. For the genetic characterization, the strains were submitted to RAPD-PCR

analysis by using the primer P80 (5'CGCGTGCCCA3') and M13 and the results obtained emphasized the existence of a significant genetic polymorphism among the strains. The *H. uvarum* strains were characterized for parameters of technological interest in oenology, such as the evaluation of enzymatic activities influencing wine quality ( $\beta$ -glucosidase and  $\beta$ -xylosidase activities), as well as for the capacity to form by-products, such as higher alcohols, acetic acid, acetaldehyde, acetoin, during grape must fermentation. The evaluation of technological parameters revealed the existence of a wide phenotypic biodiversity, correspondent to the genetic polymorphism. The wide strain biodiversity found in *H. uvarum* represents a source of natural different biotypes/phenotypes, useful to an appropriate selection program addressed to the choice of *H. uvarum* strains possessing positive oenological traits, for the potential application as starter of the early fermentation phase in mixed or sequential fermentation with *Saccharomyces cerevisiae*.

11. Fiore C., Romano P., Serafino E. 2005. *Saccharomyces cerevisiae* wine strains differ in production of extracellular enzymes. Poster presented at XXIV ISSY, Oropesa del Mar, Castellon (Spain), September 28<sup>th</sup>-October 2<sup>nd</sup>, 2005.

The aromatic fraction of wines is composed by a wide variety of compounds with different aromatic properties. Some of these compounds are already present in the must, others are modified during the vinification process, and others are produced during the fermentative process by yeast activity. Wine yeasts generate many secondary products and produce extracellular enzymes, that are key determinants of wine quality. Yeast enzymatic activities, such as proteases, xylosidases and glucosidases are some of the enzymes secreted by yeasts, that can considerably affect aroma formation. This work has been focused on the characterization of natural strains of *Saccharomyces cerevisiae* with the aim to determine the ability of these yeasts to produce extracellular enzymes. Therefore, a considerable number of *S. cerevisiae* strains, isolated from grapes of different varieties, have been tested for the production of extracellular enzymes (glucanase, cellulase, xylanase, amylase,  $\beta$ -xylosidase,  $\beta$ -rhamnase,  $\beta$ -arabinase and proteolytic activity), with the aim

to individuate some strains with characteristics of interest in winemaking. Only a few strains possessed  $\beta$ -D-xylosidase, and only four strains exhibited the highest level of this enzymatic activity (2  $\mu$ mol/p-NP/h/ml), and proteolytic activity, by hydrolyzing all the proteins at an acceptable level (1  $\mu$ mol tyrosine/ml). Fifty selected strains were tested on plate media for the evaluation of additional enzymatic activities, such as cellulase,  $\alpha$ -amylase,  $\beta$ -glucanase,  $\beta$ -arabinase,  $\beta$ -rhamnase,  $\beta$ -xylanase and  $\beta$ -glycosidase. All the strains of *S. cerevisiae* exhibited  $\beta$ -glucanase activity and a high percentage also cellulase activity, a few strains exhibited a significant activity for all the enzymes tested with the exception of  $\beta$ -glucanase. These findings underline the importance to ascertain the potentiality of wild wine yeasts for the production of oenologically significant enzymes and it would be therefore advisable for any selective program to insert the test for enzymatic activities of interest in winemaking.

---

**ITALY: Dipartimento di Scienze Agrarie - Università degli Studi di Modena e Reggio Emilia - Reggio Emilia - Italy. Communicated by P. Giudici <giudici.paolo@unimore.it> and A. Pulvirenti <pulvirenti.andrea@unimore.it>**

---

1. Pulvirenti, A., Castellari, L., De Paola, M., Giudici, P. 2004. Study of the prevalence of yeasts selected during cellar fermentation. Bulletin De L'OIV 77:662-675.

During alcoholic fermentation, prevalence of the yeast stock inoculate on indigenous yeasts is considered to occur when the product obtained has the characteristics of a must fermented with the pure stock, i.e. without other yeasts present. In the first part of our work, we wanted to show that the establishment of the inoculated stock could be assessed on the basis of the correlation

between the expected part, we evaluated the characteristics of the product obtained. In the second part, we evaluated the numerical relationship between the yeasts corresponding to the inoculated stock and the sum of indigenous yeasts, a ratio that led us to assert the prevalence of the former yeasts stock.

2. Pulvirenti, A., Solieri, L., De Vero, L., Giudici, P. 2005 Limitations on the use of PCR/RFLP of the r-DNA-NTS2 region for the taxonomic classification of the species *Saccharomyces cerevisiae*. Can J Microbiol 51(9):759-764.

Different molecular techniques were used in order to test to most effective for the identification of *Saccharomyces cerevisiae* strains. In particular, PCR-RFLP of the Internal Transcribed Spacer (ITS) regions; PCR-RFLP of the Non Transcribed Spacer 2 (NTS2) region; sequencing of the D1/D2 domain and electrophoretic karyotyping were applied to 123 yeast strains isolated from different sourdoughs and tentatively attributed to the species *Saccharomyces cerevisiae*. All the strains tested showed an identical PCR-RFLP pattern of the ITS regions, an identical nucleotide sequence of the D1/D2 domain and the typical electrophoretic karyotype of the *S. cerevisiae* species. On the contrary, 14 out of the 123 strains tested showed some

polymorphism with the BanI restriction analysis of the NTS2 region. Our results indicate that while the sequencing of the D1/D2 domain, the PCR-RFLP of the ITS regions and the electrophoretic karyotype can be employed successfully to identify *S. cerevisiae* strains the PCR-RFLP analysis of the NTS2 region does not allow a consistent and accurate grouping for *S. cerevisiae* strains. The fact that the NTS2 region of a small number of strains (8,78% of the total strains tested) is different from the one of the other *S. cerevisiae* strains, confirms that molecular methods should always be tested on a great number of strains.

3. Giudici, P., Solieri, L., Pulvirenti, A., Cassanelli, S. 2005. Strategies and perspectives for genetic improvement of wine yeasts. Applied Microbiology Biotechnology 66:607-613.

Recent developments in expression profile and proteomic techniques cleared up that the main oenological traits are complex and influenced by several genes, each of them identified as absolutely essential. Only for monogenic properties the genetic improvement programs of wine yeasts can be performed by alteration of individual genes. Ideally the most productive way of

improving the whole cell biocatalysts is by evolution of the cell entire genome. In this article we briefly review the main genetic improvement techniques applied in new and optimized wine strains construction, paying particular attention to blind and whole genome strategies, such as the sexual recombination and genome shuffling.

---

# International Commission on Yeasts (ICY)

## A Commission of the Mycology Division of IUMS

### Meeting of Commissioners, October 1 2005

#### XXIV ISSY- Hotel Marina d'Or - Oropesa del Mar- Spain

---

#### Minutes of Meeting

**Present:** Leda C. Mendonça-Hagler (Chair), Lex Sheffers (Vice-Chair), Lucia de Figueroa, Lodewyk Kock, Matti Korhola, Anna Maraz, Tokichi Miyakawa, José M. Peinado, Patrizia Romano, Rafael Sentandreu, Johan Thevelein, Graeme Walker.  
**Apologies:** Charoen Charoenchai, Graham Fleet, Merja Pentilla, Doris Rauhut, Isabel Spencer-Martins.

**Report from the Chair:** Leda C. Mendonça-Hagler welcomed the delegates to the meeting and gave apologies for those who could not attend. She expressed her appreciation to Prof. Rafael Sentandreu and its group for the excellent organization of XXIV ISSY and their support to the Commissioners meeting. She presented the agenda and requested the inclusion of any additional item.

**Minutes of the previous meeting:** Leda C. Mendonça-Hagler reported on the ICY meeting which took place on August 17<sup>th</sup>, 2005, at Hotel Glória, Rio de Janeiro, Brasil, during the Eleventh International Congress on Yeasts. The Minutes for this last meeting were published in the December 2004 issue of the Yeast Newsletter. ICY meeting in Rio de Janeiro was attended by 34 Commissioners. It was agreed by the Commissioners that dormant members should be automatically removed from ICY (if they had not attended any of the previous four ICY/ISSY symposia) and new delegates would be recruited to represent their country. The Commissioners list was updated by Lex Scheffers. During the meeting, L. C. Mendonça-Hagler and Lex Scheffers were elected respectively, the Chair and Vice-Chair of the International Commission on Yeasts, in accordance with ICY statutes.

#### Reports on meetings:

**ICY 2004: Rio de Janeiro, Brazil, 15/08-20/08: Yeasts in Science and Biotechnology. The quest for Sustainable Development.** L. Mendonça-Hagler, the organizer of ICY 2004, gave a brief report on the general meeting. ICY2004 was attended by over 240 delegates from 34 countries. A full report on ICY 2004 was published in FEMS Yeast Research, (2005) 5: 485-489 (by T. Deák).

**ISSY 24 (2005) - Oropesa del Mar, Spain, 28/09-02/10:** Prof. R. Sentandreu reported on the ongoing XXIV ISSY, which was attended by some 180 delegates and had a central theme: Cell Surface: Genomics, Proteomics and Functional Analysis: A tribute to the scientific career of José Ruiz-Herrera. The Commissioners commented on the program's high scientific level and the attractive beach resort, Oropesa del Mar. On behalf of ICY, L. Mendonça-Hagler expressed her gratitude to the Spanish group for their dedication and proposed a toast to Prof. Sentandreu and the organizing committee, for the excellent work done to achieve the success of XXIV ISSY.

**ISSY 25 (2006) June 18-21, Hanasaari, Espoo, Finland: Systems Biology of Yeasts** <http://www.issy25.vtt.fi>  
Prof. M. Pentilä is organizing this symposium. In her absence, Matti Korhola reported on the preparations for the meeting, fostering information on the venue and presenting a preliminary program. He announced that he would make a presentation, with pertinent information on the symposium, which was delivered during the XXIV ISSY closing ceremony.

**ISSY 26 (2007) Italy:** Prof. Patrizia Romano is organizing this Symposium. She reported on the preparations for the meeting, announcing Ravello (Italy) as the venue. It is planned to be held in June. This Symposium will address yeasts in food and beverages as the main theme.

**ICY 2008 - 12th International Congress on Yeasts - Ukraine:** During the ICY meeting in Budapest (2003) the Commissioners agreed to accept Prof. A. Sibirny's proposal to be the organizer of ICY 2008, probably in Kiev. A progress report was made by A. Sibirny in Rio de Janeiro (2004). (No further report was presented).

**ISSY 27 (2009) - France:** Prof. Monique Bolotin-Fukuhara proposed to organize ISSY27 in France. The proposal was welcomed by the Commissioners during ICY 2004 in Rio de Janeiro. (No further report was presented).

**ISSY 28 (2010) - Thailand -** Leda C Mendonça-Hagler reported on a letter of intention received from Dr. Charoen Charoenchai with a proposal to organize a Symposium in Thailand. The proposal was accepted by the Commissioners with great interest and welcomed the opportunity to have a meeting in Asia.

**Report on IUMS 2005:** 11<sup>th</sup> International Congress of Mycology, San Francisco, July 24-29. ICY was represented at IUMS 2005 meeting by L. Mendonça-Hagler, who reported on current and future activities. ICY was recognized by IUMS as a very successful COMCOF. The congress was hosted by the American Society for Microbiology, which appointed a national (US) organizing committee, chaired by John Taylor. Graham Fleet, Vice-Chair of the Mycology Division of IUMS and International Chair of the Organizing Committee for the Mycology Congress, devoted considerable effort to achieve a good balance between topics on yeasts and filamentous fungi, as well as international representation of speakers. While there was no ICY sponsored/organized session, there was an excellent representation of yeast topics throughout the program. One dozen of ICY Commissioners attended IUMS 2005. ICY Commissioners expressed their appreciation to Graham Fleet for his representation, at IUMS, on behalf of the yeast scientific community.

**Report on YNL -** Prof. Marc-André Lachance updated Leda Mendonça-Hagler on the activities related to the Yeast Newsletter, during IUMS 2005, in San Francisco. The Commissioners recognized Prof. Lachance's large contribution in managing the **Yeast Newsletter**.

#### Other business and activities

The Commissioners discussed the possibility of having more than one International Specialized Symposium a year. After this discussion, the majority of the Commissioners were in favor to continue with one meeting a year.

#### Meeting Close

Leda Mendonça-Hagler closed the meeting. On behalf of ICY, she expressed her gratitude to the organizers for the success of XXIV ISSY, held in the pleasant surroundings and with the warm hospitality shown at Oropesa del Mar, Spain, and also to Prof. Rafael Sentandreu and his group for arranging a lavish lunch for the ICY meeting.

Leda C. Mendonça-Hagler

---

---

# Recent Meeting

---

## 33rd Annual Conference on Yeasts of the Czech and Slovak Commission for Yeasts, Smolenice, Slovakia, May 11-15, 2005

---

The 33rd Annual Conference on Yeasts, organized regularly by the Czech and Slovak Commission for Yeasts and the Institute of Chemistry, Slovak Academy of Sciences, took place in the Smolenice Castle, the Congress Center of the Slovak Academy of Sciences, during May 11-15, 2005. The Conference was attended by 26 participants from the Czech Republic, 40 participants from Slovakia, and 1 participant from Hungary and Poland. The program consisted of three sessions dedicated to Yeasts in Modern Biotechnologies, Molecular Biology and Genetics and Yeast in Human and Veterinary Medicine. The lectures were complemented by 42 posters. The titles of lectures and posters are listed below.

### Lectures in the session Yeast in Modern Biotechnologies

- Sulo P. The true prehistory of biotechnology. From pot to jackpot.
- Kočí R., Márová I., Drábková M., Hulínová T., Ondruška V. Production of industrial metabolites by red yeast *Sporidiobolus salmonicolor*.
- Čertík M., Masrnová S., Sitkey V., Minárik M., Breierová E. Physiological regulation of microbial production of astaxanthin.
- Rapta P., Čertík M., Breierová E., Márová I. Radical scavenging and total antioxidant capacity of yeast extracts.
- Čebík M. BioTech – distributor of New Brunswick Scientific in Czech Republic and Slovakia.
- Vajcziková I., Breierová E., Antalová Z., Sláviková E. Yeast infection of the wine squash and the natural microflora of this squash.
- Rebroš M., Rosenberg M. Ethanol production by entrapped microorganisms: yeasts or bacteria?
- Selectký R., Šmogračková D. Production low-alcoholic and special beers using mutant brewer's yeast.
- Vajcziková I. Sensorial evaluation of soft drink, wine and brandy.

### Lectures in the session Molecular Biology and Genetics

- Farkaš V. 55 years from the discovery of sugar nucleotides.
- Maceková D., Farkaš V. On the nature of binding of capsular poly-saccharides to cell surface in *Cryptococcus neoformans*.
- Nosek, J., Kosa, P., and Tomáška, L. Living in the wild. Homothalism and sporulation - tools for genome renewal.
- Laurenčík M., Seman M., Sulo P. Yeast microflora in Bryndza cheese.
- Fekete V., Poláková S., Čierna M., Lacková M., Sulo P. Petite positive islands in petite negative yeast sea.
- Nosek, J., Kosa, P., and Tomáška, L. Organelle genomics: What can we learn from mitochondrial genomes of yeasts?

### Lectures in the session Yeasts in Human and Veterinary Medicine

- Hamal P., Raclavský V. Typing of pathogenic yeasts by molecular genetic techniques.
- Bergendiová K., Skutilová E. Recurrent onychomycosis and immunity-Case report.
- Hrubíško M., Paulovičová E., Vargová H. Chronic colpitis: its immunological profile and treatment.
- Kliment M., Červenková D., Kertys P. Comparison between two therapeutic schemas effects in the therapy of recurrent vulvovaginal candidiasis.
- Raclavský V., Trtková J., Sehnalíková P., Kvasničková E., Buchta V., Hamal P. Detection and identification of pathogenic yeasts by molecular genetic techniques.

- Kogan G., Miadoková E., Vlčková V., Rauko P., Slameňová D., Machová E. Yeast cell wall polysaccharides as alternative anticancer agents.
- Đurana R., Lacík I., Paulovičová E., Bystrický S. Preparation of precursors for the synthesis of glycoconjugate vaccines against pathogenic yeasts.
- Polčic P., Kolarov J. Yeast as a model to study mammalian apoptosis.
- Dawson K.A., Andrieu S., Bobček R. Impact of monensin and *Saccharomyces cerevisiae* on ruminal functions and improvement of the performance in dairy cattle.

### List of posters

1. Letavayová L., Vlčková V., Vlasaková D., Marková E., Brozomanová J. DNA damage induced by sodium by sodium selenite in *Saccharomyces cerevisiae*.
2. Maláč J., Urbánková E., Sigler K., Gášková D. Composition of the growth medium affects the MDR pump activity in *S. cerevisiae*: diS-C3(3) fluorescence assay.
3. Poláková S., Sulo P. Shuffling of mitochondrial genomes during the evolution via interspecific hybrid speciation.
4. Sidorová M., Suvaková E., Kozovská Z., Hikkel I., Šubík J. Drug-sensitizing effect of some loss-of-function *pdr3* mutations in *S. cerevisiae*.
5. Černická J., Šubík J. Multiple drug resistance mechanisms resulting in decreased susceptibilities to antimycotics in *C. albicans* clinical isolates.
6. Holíč R., Griac P. Study of Sfh1p, homolog of phosphatidylinositol /phosphatidylcholine transfer protein (Sec14p) in yeast.
7. Urbánková E., Marešová L., Gášková D., Sychrová H. Plasma membrane potential of *S. cerevisiae* cells and potassium transport systems.
8. Vránová D., Vadkertiová R. Method for rapid identification of *Saccharomyces* species by PCR-RFLP.
9. Džugasová V., Šubík J. Anionic phospholipids are essential for growth of the *Saccharomyces cerevisiae* *op1/aac2* mutant on minimal medium.
10. Fekete V., Sulo P. Acid rain in lab hell (Effect of low pH on chronological ageing).
11. Gášková D., Chládková-Moquin K., Hendrych T., Sigler K. Dependence of chemical stress-induced damage to yeast cells on the status of MDR pumps.
12. Poláková S., Slamka T., Minárik G., Sulo P. Complete nucleotide sequence of the mitochondrial DNA from *Brettanomyces custersianus*.
13. Ondrovičová G., Liu L., Singh K., Gakh G., Perečko D., Janata J., Parkhomenko N., Granot Z., Orly J., Suzuki C. K., Kutejová E. Mitochondrial lon proteases employ mechanism of recognition and degradation of endogenous substrates unlike the other ATP-dependent proteases.
14. Imrichová D., Černická J., Šarinová M., Gbelská Y., Šubík J. Regulation of *KNQ1* gene expression in *Kluyveromyces lactis*.
15. Takács K., Pesti M. Gene expressions in *Schizosaccharomyces pombe* Dpap1 signal transduction mutant exposed to cadmium.
16. Vlčková V., Naďová S., Dúhová V., Svidová S., Kogan G., Miadoková E. Carboxymethyl glucan-yeast polysaccharide with antimutagenic and bioprotective effects.
17. Dudíková J., Mislovičová D., Kolarova N. Extracellular polysaccharide components from acapsular strain *Cryptococcus laurentii* CCY 17-3-6.

18. Kucejová B., Kucej M., Petrežsélyová S., Abelovská L., Fričová D., Ryčovská A., Nosek J., Tomáška L. MDM31 and MDM32 proteins – novel players in mitochondrial magnesium homeostasis and organelle volume control.
19. Zemanová J., Nosek J., Tomáška L. Development of tools for the functional analysis of *Candida parapsilosis* genome.
20. Kosa P., Tomáška L., Nosek J. Comparative analysis of yeast mitochondrial genomes.
21. Kubešová J., Mikulcová A., Ptáček P., Márová I. Use of *Saccharomyces cerevisiae* D7 strain for study of antimutagenicity/ genotoxicity of plant food.
22. Rapta P., Brezová V., Zalibera M., Čertík M. Comparison of total antioxidant capacity of pigments produced by stressed yeasts evaluated by different spectroscopic techniques (FRAP, ABTS and EPR spin trapping assays).
23. Czabany T., Špaňová M., Mrózová Z., Hapala I., Čertík M. Effect of cerulenin and exogenous fatty acids on lipid metabolism in yeasts.
24. Sláviková E., Košíková B., Sasinková V. The use of various yeast strains for removal of pine wood extractives.
25. Šajbidor J., Breierová E., Garajová S., Čertík M. Dual effect of ethanol and starvation on lipid composition of *Saccharomyces cerevisiae*.
26. Stratilová E., Džúrová M., Breierová E., Omelková, J. Purification of individual forms of pectate hydrolases produced by *Aureobasidium pullulans* from forest soil.
27. Breierová E., Oláhová M., Čertík M., Omelková J. Kinetic and morphologic analysis of yeast growth during utilization of heavy metals.
28. Márová I., Kočí R., Hrdličková J., Drábková M. Production of carotenoids by red yeasts and transgenic bacteria: a comparative study.
29. Omelková J., Breierová E., Stratilová E. Influence of protective substances on maintenance of *Sporobolomyces salmonicolor*.
30. Šmogrovičová D., Selecký R. Bioluminometric determination of active yeast biomass.
31. Blaskó A., Belágyi J., Dergez T., Deli J., Vágvölgyi C., Pesti M. Effect of altered carotenoid composition of *Phaffia rhodozyma* and *Xanthophyllomyces dendrorhous* on the plasma membrane order parameter.
32. Vadkertiová R., Sláviková E. Metal tolerance of yeasts isolated from water, soil and plant environments.
33. Rosenberg M., Rebroš M., Sláviková L., Krištofiková L. Semicontinuous production of ethanol by *Saccharomyces cerevisiae* entrapped in PVA gel.
34. Trtková J., Smilková L., Plachý R., Hamal P., Raclavský V. Identification of pathogenic yeast species based on melting curve analysis of RAPD-products (McRAPD).
35. Tomšíková A. Employment of acquired humoral and cell-mediated immunity in the prevention and therapy of fungal infections.
36. Paulovičová E., Hrubisko M., Machová E. Cross-reactive mannan antigens of pathogenic *Candida* spp.
37. Ližičárová I., Matulová M., Machová E., Capek P. Isolation and structural characterization of a mannan from the yeast *Candida dubliniensis*.
38. Bystrický S., Paulovičová E., Machová E., Ližičárová I. Preparation and immunogenicity of *Candida dubliniensis* cell wall mannan-conjugate.
39. Siegfried L., Hrabovský V., Sabol M., Tóthová K. Susceptibility to antifungal agents of *Candida* strains isolated from patients with cancer.
40. Hamal P., Ohshima T., Maeda N., Makimura K., Yamaguchi H., Abe S. Comparative analysis of *Candida dubliniensis* karyotypes.
41. Baculíková M., Mentel M., Gavurníková G., Kolarov J. Effect of growth conditions on respiration and survival of the dimorphic yeast *Yarrowia lipolytica*.
42. Drobčová B., Zeman I., Kolarov J. Influence of the specific mitochondrial defects on the function of Bcl-2 family proteins and on the yeast aging.

The conference language was this year, with a few exceptions in Slovak or Czech, English. The meeting thus became a good opportunity for young scientists to test their abilities to present their lectures and posters in the language of current science. The Organizing Committee awarded for first time prizes to young scientists for the best oral presentation and for the best poster.

At the meeting of the Committee of the Czech and Slovak Commission for Yeasts held during the Conference it was decided that the 34th Annual Conference on Yeasts will be organized again in Smolenice Castle in May or around May 2006. It is planned to open future conferences more to foreign scientists, and to attract mainly yeast researchers from the other two V4 countries, which are Hungary and Poland.

Communicated by Peter Biely

---

## Forthcoming Meeting

**ISSY 2006**

**Systems Biology and Metabolic Engineering of Yeasts  
June 18-22 2006, Hanasaari, Finland**

---

The ISSY 2006 meeting on "Systems Biology of Yeasts - from models to applications" will be held 18-21 June 2006 at Hanasaari, an island on the outskirts of Helsinki.

Further information may be obtained from [anita.tienhaara@vtt.fi](mailto:anita.tienhaara@vtt.fi) (please enter "ISSY25" in the subject line) or from the web site <http://issy25.vtt.fi/>

---



---

## Publication of Interest

---

Carlos A Rosa and Gábor Péter (Eds) 2006 Biodiversity and Ecophysiology of Yeasts. Series: The Yeast Handbook, 580 pp., 40 illustrations - ISBN: 3-540-26100-1.

### Table of contents

1. Yeast Biodiversity: How Many and How Much? - Marc-André Lachance
2. Yeast Systematics and Phylogeny – Implications of Molecular Identification Methods for Studies in Ecology - Cletus P. Kurtzman and Jack W. Fell
3. Yeast Biodiversity and Culture Collections - Vincent Robert, Joost Stalpers, Teun Boekhout and Shu-hui Tan
4. Genomics and Biodiversity in Yeasts - M. Bolotin-Fukuhara
5. Methods for Investigating Yeast Biodiversity - K. Boundy-Mills
6. Sugar Metabolism in Yeasts: an Overview of Aerobic and Anaerobic Glucose Catabolism - Fernando Rodrigues, Paula Ludovico and Cecília Leão
7. Diversity of Nitrogen Metabolism Among Yeast Species: Regulatory and Evolutionary Aspects - Francine Messenguy, Bruno André and Evelyne Dubois
8. Environmental Factors Influencing Yeasts - Tibor Deak
9. Yeast Responses to Stresses - An Tanghe, Bernard Prior and Johan M. Thevelein
10. Antagonistic Interactions Among Yeasts - W.I. Golubev
11. Yeasts in Soil - Alfred Botha
12. Yeast Biodiversity in Freshwater, Marine and Deep-Sea Environments - Takahiko Nagahama
13. Phylloplane Yeasts - Á. Fonseca and J. Inácio
14. Yeast and Invertebrate Associations - Philip F. Ganter
15. Yeasts in Extreme Environments - Peter Raspor and Jure Zupan
16. Yeast Biodiversity in the Antarctic - Helen S. Vishniac
17. Yeast Biodiversity in Tropical Forests of Asia - Takashi Nakase, Sasitorn Jindamorakot, Somjit Am-in, Wanchern Potacharoen and Morakot Tanticharoen
18. Yeast Communities in Tropical Rain Forests in Brazil and other South American Ecosystems - Paula B. Morais, Fernando C. Pagnocca and Carlos A. Rosa
19. The Biogeographic Diversity of Cactophilic Yeasts - William T. Starmer, Virginia Aberdeen and Marc-André Lachance
20. Black Yeasts and Meristematic Fungi: Ecology, Diversity and Identification - Katja Sterflinger
21. Yeasts as Indicators of Environmental Quality - Allen N. Hagler
22. Yeast Biodiversity and Biotechnology - Pietro Buzzini and Ann Vaughan-Martini

