

Yeast

A Newsletter for Persons Interested in Yeast

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Editorials

In Memoriam - Robert R. Davenport (1933-2001)

I learned recently of the passing of Professor Davenport while reviewing a manuscript describing the new species *Candida davenportii* named in his honour. The senior author, Malcolm Stratford, was kind enough to accept my invitation to write an obituary. Dr. Davenport made important contributions to the theory and practice of yeast ecology, in particular the need to develop efficient approaches to processing large numbers of isolates if meaningful inferences are to be made. In 1977, as I was planning my post-doctoral future, I wrote to Dr. Davenport to enquire on the possibility to hosting me in his laboratory. His response was forthcoming and encouraging. Fate later decided differently. Reading Malcolm's tribute, I regret that I did not get to know Dr. Davenport better.

Nobel Prize Goes to Yeast Researchers

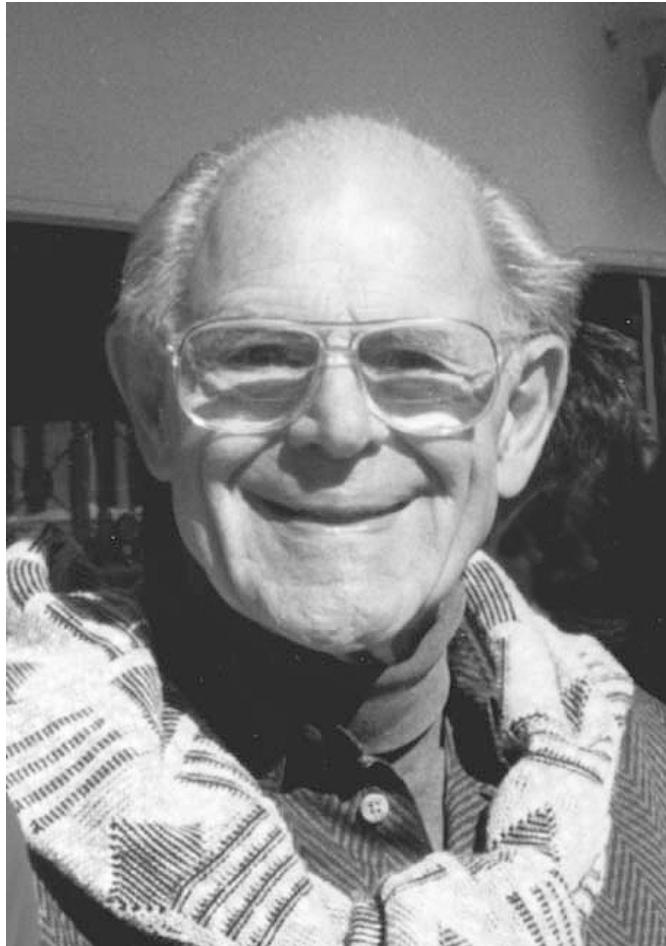
The Nobel Prize for Physiology or Medicine was awarded this year to Lee Hartwell, Tim Hunt, and Paul Nurse, for their outstanding achievements in understanding the cell division cycle and its regulation. All three did much of their work on either *Saccharomyces cerevisiae* or *Schizosaccharomyces pombe*. I am sure our readers will join me in congratulating the laureates for this well-deserved award.

Important Information for Credit Card Users

Our first experience with credit cards as a means of payment has been mostly positive, as it is now possible for readers in some parts of the world to avoid paying very high bank charges for the issuance of cheques. However, the management of credit cards involves in some cases a considerable amount of extra work. In addition, claims made to VISA with expired cards or inaccurate information are subject to a substantial penalty. I must therefore ask our readers who must pay by credit card (1) to make their payment as soon as possible after receipt of the annual invoice, (2) to insure that their card expiry date is not within the next four months, (3) to double-check the accuracy of the information provided, and (4) to renew their subscription for two or more years. Readers who have both cards are urged to use MASTERCARD and not VISA.

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

M.A. Lachance
Editor



Herman Jan Phaff (1913-2001)

“Dedicated, modest, encyclopedic, a role model, a man who touched so many lives.”

These words are from Kyria Boundy-Mills, Curator of the Phaff Culture Collection, and the last of a long suite of individuals to be introduced to yeasts by one whom many regard as the very personification of the concept of yeasts. Herman Phaff died in August from the complication of a fall, while vacationing in the Caribbean with his wife Diane. Most of our readers have known Herman in one capacity of another. In the last issue, celebrating the 50th anniversary of the Yeast Newsletter, it was noted that Herman Phaff served as its editor for 34 years. I dedicate this issue to him.

The Phaff Culture Collection website <http://www.Phaffcollection.org> lists several testimonials from family, friends, and colleagues, including a touching statement by Arnold Demain that was read on the occasion of the memorial service held in October. The service took place on the University of California Davis campus. It celebrated Herman’s love of music with stunning cello performances on his own instrument. His contributions as a caring educator were remembered through testimonials by his former students.

Herman’s scientific life has been recapitulated on several occasions in the literature [v.g., Ann. Rev. Microbiol. (1986) **40**:1-28; J. Indust. Microbiol. (1995) **14**: 429-431 and 432-435]. An obituary by Sally Meyer will appear in the first 2002 issue of the International Journal of Systematic and Evolutionary Microbiology, for which Herman served as Associate Editor for many years. The following special section is a modest attempt to recognize what Herman Phaff has meant to many.

Those of us who walk in Herman Phaff’s footsteps do so with love, pride, admiration, and gratitude.

M. A. Lachance

Obituary published in the *Davis Enterprise*, Davis, California

Herman Jan Phaff, Professor emeritus of Food Science and Technology at the University of California, Davis, died suddenly on August 24th while on vacation in the Caribbean. Dedicated, modest, possessed of an encyclopedic mind, he was a role model, and the loss to his family and friends, to his University, and to the worldwide scientific community is immeasurable. His was a truly rich, lustrous life, and he touched many with his quiet warmth and boundless intellectual curiosity.

Born in Winschoten, the Netherlands in 1913, Herman Jan Phaff spent his childhood in the environment of his family's winery, which stimulated his interest in microorganisms and consequently had a strong impact on his later life. He attended the Technical University in Delft, where he earned a B.S. degree in chemical engineering with a thesis on "The elaboration of extracellular pectin-hydrolyzing enzymes by fungi," a subject of importance to the wine industry.

In 1939, at the recommendation of renowned microbiologist A.J. Kluyver, Mr. Phaff came to California to pursue graduate study at UC Berkeley in the Division of Fruit Products, later known as the Department of Food Technology. His initial research was done with Emil Mrak (later chancellor at UC Davis), and in his Ph.D. studies he worked under the direction of Maynard Joslyn and H.A. Barker. His research focussed on yeast taxonomy, ecology and physiology. Mr. Phaff was studying in the United States during WWII; his brother, who fought in the Dutch resistance, was lost to the Nazi forces.

In 1943 Mr. Phaff accepted a faculty position at UC Berkeley, and he moved to UC Davis in 1954 when the Food Science department was transferred. In all, he was an active member of the University of California faculty for 58 years. During that time, he published two books and hundreds of research papers and was the recipient of numerous awards, including being named as University of California at Davis Faculty Research Lecturer in 1969. In 1985, Professor Phaff co-authored a definitive bibliography of viticulture and enology publications with the distinguished professor Maynard Amerine.

Although he officially retired from full-time research in 1983, Professor Phaff maintained a very active research laboratory. He continued daily study and work on a collection of over 6000 yeast strains, representing over 400 of the 700 known yeast species - many of which are available only from his collection. The collection contains the strains Professor Phaff collected on global expeditions throughout his research career and also includes several hundred yeast strains collected in the 1930s by Professors Mrak and Cruess. Few microbial culture collections of this size and diversity have been collected and maintained by any institution, let alone by a single researcher. This collection is utilized by academic and industrial researchers throughout the world.

On October 4, 1996, the UC Davis Department of Food Science and Technology, the California Institute of Food and Agricultural Research, and the College of Agricultural and Environmental Sciences held a symposium presented in Professor Phaff's honor, at which The Herman J. Phaff Culture Collection: Yeasts and Yeast-Like Microorganisms as an official Biological Collection of the University of California was formally dedicated.

Professor Phaff was also noted for his professional skill as a cellist and his passion for the arts and culture. He was a founding member of the Davis Comic Opera Company and the UCD symphony orchestra - playing it its inaugural concert in 1959 - and a dedicated player in chamber music groups in the Bay area and Davis for five decades. He was chairman of the Committee for Arts and Lectures at UC Davis and in 1960 was made an honorary member of the Music Department.

Professor Phaff and his wife, Diane, shared a love of music and the arts, and the couple sponsored visiting musicians on campus, hosted performances in their home, and were long-time supporters of UC Davis Presents and other community cultural groups. Professor Phaff's late wife, Marinka, who died in 1985, was also a strong supporter of the Davis community cultural scene and is well remembered for her involvement with the arts.

As a fitting bookend to his illustrious life, Professor Phaff was awarded the College of Agricultural and Environmental Sciences Award of Distinction for 2001. Sadly, notification of the award arrived only on Monday, August 27th. His wife will accept the award in his honor.

Herman Jan Phaff never stopped learning and seeking to enrich the scientific community, and the Phaff Culture Collection is the culmination of his life's work as a scientist. The family asks that gifts in his honor be made out to "UC Regents" and directed to the Herman J. Phaff Culture Collection, UC Davis Food Science Department, Cruess Hall, Davis, California 95616.

Professor Phaff is survived by his wife, Diane; his sister, Re Phaff, of London, England; his nephew Japp Phaff, wife Madelon, and their three children, of Breda, Netherlands; his niece Helma Phaff and her son, of Heemstede, Netherlands; his nephew Frans Frederik Wethmar of Amsterdam, Netherlands; his step-daughters, Kim Rowland and Correy Rowland Cavanaugh; his son-in-law, Paul Cavanaugh; and his granddaughters, Maggie and Julia Horowitz. He is also survived by his cat and two beloved terriers.

A memorial service for Professor Phaff will be held on October 29th at the Buehler Alumni Center on the UC Davis campus. His family invites all his students, colleagues and associates to attend and celebrate the life of a great scholar, teacher, musician, family man, and friend.

**Address presented by Sally A. Meyer on the occasion of the
memorial service held in Davis, California, in October 29, 2001**

HERMAN J. PHAFF; my professor, my mentor, my friend, my colleague. He was all of these and more to me and to many of you. He was special! He was a man for all seasons; a man will all reason. He was our leader; he was an outstanding scientist; a fine musician; a great outdoorsman. He was serious. He was gentle. A man who lived life to its fullest, a giant in his field of yeast biology. His interests were broad and his accomplishments many fold. He was a constant student of many things.... or maybe just everything. He taught us many things; how to work and how to celebrate the accomplishments of our work. He enjoyed it all.

Several months ago I was asked to contribute a letter of recommendation for the nomination of Herman for the UC Davis College of Agricultural & Environmental Sciences Award of Distinction. It is not often a student is asked to write a letter of recommendation for a professor. It is usually the student asking the professor for the recommendation. Of course, I was honored and delighted to support the nomination of Herman for this award. I would like to read a portion of my letter:

"I enthusiastically support the nomination of Herman J. Phaff for the College of Agricultural and Environmental Sciences Award of Distinction. There is no one more deserving than Herman for this award. Herman has been a teacher, a mentor and a friend to many of us who have passed through his laboratory over the years. His dedication to his field of yeast biology is tremendous and his contributions too numerous to count. Over the years he has been a constant student of the yeasts and has integrated the newer technologies with former knowledge to keep at the forefront of yeast biology. He has been a leader in his field for many years (more than half a century!) and attracted many students, post-doctoral fellows and visiting scientists from around the world to Davis whose sole desire was to study under Herman's guidance and direction. Many of these individuals in universities and in industry have gone on to make noteworthy contributions in the yeast field."

Herman has been the 'centerpoint' of a unique family of yeast scientists whose contributions have been paramount in yeast research. He is our master, our guru who we turn to for advice and suggestions as needed. And he, in turn, is delighted and proud of our successes and contributions. I know of no other group of scientists who are better friends and colleagues and as a scientific body has done more in a particular field than those that make up the 'Phaff group'.

Besides being an outstanding scientist and teacher, Herman has dedicated much of his time (even after retirement) to the editorship of scientific journals and has consistently assisted non-English speaking scientists with the language so their work would be accepted and published in recognized journals.

Herman Phaff is synonymous with 'yeasts'. His world renown reputation established Davis as a center of yeast research. His accomplishments for the yeast world are far greater than any other

individual and he has helped so many students/colleagues along the way. His life is exemplary. Herman is truly an outstanding individual who deserves this Award of Distinction."

My professor and I shared something in common. He came from a wine-making family and I from a beer-making family. Together we shared our interest in yeasts, as well as the products they made. We learned more about yeasts than our families ever thought existed.

As students, visitors in the lab, friends and colleagues, we all have enjoyed the hospitality he extended to us, the wonderful dinner parties and gatherings at his home, the celebrations when someone completed a thesis or passed the oral exam. We all have stories to tell and memories to share about our professor, or friend or colleague. We worried about him as he drove to the Bay Area on Wednesday afternoons to meet his musician friends because he studied Russian while he was driving! I recall the day he told a group of us he wouldn't be around the lab in the afternoon because he was going to the "library". We burst out laughing. He didn't know WHY, but we explained. "Library" was the code word we used to mean 'skiing'. In our jargon, he was going skiing! Or the day, the snails he collected for me escaped their containers and were hiding in the strangest places all around the lab. And I recall the day my professor signed my thesis, I sighed with relief.....thinking "I DID IT! I AM FINISHED!!" and he turned to me and said, "Now, Sally, you can call me 'Herman'." And I said, "Thank you, Doctor Phaff."

I have.... in my mind.... thanked him many, many times over the years for allowing me to be part of his life, for giving me guidance and direction. I treasure the memories I have of my professor, my mentor, my friend and my colleague. He was special!

Message from Lex Scheffers, Chair, International Commission on Yeasts

With sadness I heard that **Dr. Herman J. Phaff** died suddenly on August 24th, 2001, while on vacation in the Caribbean. At the age of 87, he still was active in yeast science. In August 2000 he indeed participated in the 10th International Symposium on Yeasts in The Netherlands and at that occasion he once more attended a meeting of the International Commission on Yeasts, of which he has been an illustrious member for so many years. His death means an immeasurable loss to the worldwide community of yeast research.

Current Status of the Herman J. Phaff Yeast Culture Collection

Kyria Boundy-Mills, Curator

Collection Holdings. The Herman J. Phaff Yeast Culture Collection at the University of California, Davis, contains over 6400 strains, including primarily ascomycetes and basidiomycetes. A number of euascomycetes, yeast-like algae, and filamentous fungi are also included. Over 400 of the 700 currently known yeast species, as well as at least 100 new, unpublished yeast species, are represented in the collection. The size, diversity, and reputation of this collection place it in the top 10 yeast culture collections in the world.

The majority of yeast strains in the collection were isolated by Dr. Phaff over the last 58 years on his collecting expeditions, which took him, along with students and collaborators, to North and South America, Asia, Hawaii, the Caribbean, Australia, and New Zealand. Strains were isolated from a great variety of natural habitats, including insects, soils, flowers, plant exudates, necrotic or infested plant tissues, marine algae, crustaceans, and fresh water streams and lakes, wine, beer, and other fermented beverages, fruit, shellfish, meat and dairy products, honey, and many other foods. In addition to strains isolated by Dr. Phaff, a number of strains were deposited into the collection by retiring UC professors (including Drs. Cruess, Mrak, York and Lewis), other culture collections, and various food and clinical sources.

Strains are stored at -80°C, as lyophilized pellets, and/or as oil slants. A complete inventory of all strains in the collection was performed in 2000. Methods of strain storage, strain distribution and data management are being updated.

Collection database. All strains in the collection are currently catalogued in an inventory database, in FileMaker Pro format. This database includes information on the ID number, species, and origin of strains. A searchable version of this database is currently available on our web site, <http://www.Phaffcollection.org>. We are currently compiling a new

database that will also include the vast amounts of physiological data gathered for species identification. This state-of-the-art database utilizes BioloMICS, a database application developed for the Centraalbureau voor Schimmelcultures (CBS) in the Netherlands. This will be made available to the public online in the next few months.

Collection facilities. The collection is administered by the California Institute of Food and Agricultural Research, and housed in the Department of Food Science and Technology, Cruess Hall, University of California Davis. The main lab is located in room 138 Cruess Hall, and is approximately 416 square feet. A temperature-controlled culture room of approximately 170 square feet is located across the hall. We are very excited about the recent announcement of a gift of \$25 million from Robert and Margrit Mondavi to be used toward new building that will house the Department of Food Science and Technology and the Department of Viticulture and Enology.

We have recently purchased new equipment, including a PCR thermocycler, microcentrifuge, refrigerator, and many small items.

Current uses of yeast strains from the collection. Current research projects being performed at the Phaff Yeast Culture Collection include identification and publication of new yeast species, analysis of various yeast species for expression of degradative enzymes, and detection of biofilm formation by fermentative and spoilage yeasts.

Strains from the Phaff collection are also currently being used by academic and industrial researchers across the globe, in applications that include conversion of waste biomass to value-added products, nutraceuticals, pesticides, antioxidants, starter cultures for wine and bread, production of degradative enzymes, and food-grade pigments. Strains can be selected and ordered online through our web site.

Current funding Although designated as an official biological collection of the University of California in 1996, the Phaff Yeast Culture Collection does not have a permanent source of funding for collection maintenance. A grant through the University of California BioSTAR program is currently supporting the half-time curator and assistant curator salaries, equipment and supplies. This grant will expire in August 2002.

A few thousand dollars per year is obtained through institutional grants and strain distribution fees. These funds are used for undergraduate assistance and supplies.

We have established an endowment fund to support maintenance of this highly treasured but under-funded collection, and we are embarking on a fund raising campaign. The endowment will be used to fund a curator, and eventually an endowed chair in yeast taxonomy and microbial ecology. Your assistance is requested in identifying potential donors, including industries and individuals. Please contact Curator Kyria Boundy-Mills for details. Phone: (530) 754-5575. Email: klbmills@ucdavis.edu

I. Laboratorium voor Microbiologie, Wageningen University, Hesselink van Suchtelenweg 4, 6703 CT Wageningen, The Netherlands. Communicated by W.J. Middelhoven <WoutMiddelhoven@algemeen.micr.wau.nl>

Essay: Assimilation of n-hexadecane

Growth on hydrocarbons is shown by several yeast species. Of these, *Candida guillermondii*, the anamorph of *Pichia guillermondii*, is grown in Russia on kerosene for large-scale production of single cell protein. The yeast assimilates the straight-chain hydrocarbons, leaving the branched-chain fraction unattacked that still can be used for combustion. Assimilation of n-hexadecane is an indicator of unusual biochemical capacities. In my experience over the last 18 years, ascomycetous yeasts able to assimilate uric acid, amines and phenol or other benzene compounds as sole carbon source all are hexadecane utilizers. However, in the basidiomycete realm such a correlation is absent.

Growth tests on n-hexadecane have not gained much

popularity among yeast taxonomists as many strains grow very slowly. In the 1998 edition of The Yeasts, a Taxonomic Study (Eds. Kurtzman & Fell) some of the larger genera were screened for hexadecane assimilation. Sally Meyer applied the test on *Candida* species, Clete Kurtzman did so with *Pichia* and demonstrated that it distinguishes species, Jack Fell and Adele Statzell-Tallman studied *Cryptococcus* but detected no growth. In the other yeast monograph, i.e. that of Barnett, Payne and Yarrow (2000), no attention is paid to hexadecane assimilation, but the first edition of 1983 contained a list of species responding positively and an identification key to these species was provided. David Yarrow told me that this list was based on literature data, e.g. those of Markovetz and Kallio (1964) and

Scheda and Bos (1966), not on own observations.

It came to my attention that many species in the list of 1983 according to the 1998 edition of The Yeasts failed to grow on hexadecane. I wondered what could be the cause of this discrepancy. Probably it was caused by different techniques used for the growth tests. Unfortunately, no standard protocols exist and many workers use their own methods, with variable results. Hydrocarbons are organic solvents, insoluble in water, but highly lipophilic. Close contact with yeast cells may cause membrane damage and impairment of mitochondria and other cell compartments rich in lipids. It is plausible that yeast species may differ in sensitivity.

To check this, I tested some yeast strains, own isolates as well as type strains, on hexadecane, applying different growth conditions. In the first paper dealing with hydrocarbon assimilation from a taxonomic viewpoint (Markovetz and Kallio, 1964) two methods were used and were claimed to give identical

results. In the first method the yeast was inoculated into 100 ml basal growth medium in a shaken conical flask to which 1 ml of pure n-hexadecane was supplied. In the other method slants of Yeast Nitrogen Base Agar were lightly inoculated and 0.5 ml of the liquid substrate was added along the glass wall avoiding contact with the agar slant as much as possible. Some yeast species will show growth at the bottom, in close contact with the substrate, but other species show growth on the whole slant, suggesting that the substrate had reached the cells via the vapour phase. I applied this "slant culture method" successfully, not only for hexadecane assimilation but also for demonstrating growth on phenol, cresoles and other toxic benzene compounds. Experimental details were given by Middelhoven et al. (2001). I was unaware that most other yeast workers used a third method, colloquially named Kurtzman's method: a loopfull of n-hexadecane is added to a culture tube with a few ml of YNB, lightly inoculated with a preculture and vigorously shaken to ensure contact between the cells and the substrate.

Species	Strains tested	BPY1983	The Yeasts 1998	Slant culture	Liquid YNB
<i>Arxula adeninivorans</i>	1	?	+	4d	DW
<i>Candida catenulata</i>	1	+	-	+	?
<i>Candida chiropterorum</i>	1	+	-	+	?
<i>Candida magnoliae</i>		+	-	?	?
<i>Candida parapsilosis</i>	2	+	+/-	4d	-
<i>Candida zeylanoides</i>		+	-	?	?
<i>Cerinosterus cyanescens</i>	1	?	?	8d	10d
<i>Cryptococcus curvatus</i>		+	-	?	?
<i>Cryptococcus humicola</i>		+	-	?	?
<i>Pichia castillae</i>		+	+	?	?
<i>Pichia farinosa</i>		+	+	?	?
<i>Pichia guillermondii</i>	2	+	+	8d	4d
<i>Pichia mexicana</i>		+	+	?	?
<i>Trichosporon asahii</i>	1			20d	DW
<i>Trichosporon aquatile</i>	1	+	?	W	-
<i>Trichosporon veenhuisia</i>	1	?	?	8d	-
<i>Yarrowia lipolytica</i>	2	+	+	+	-

In the table assimilation of n-hexadecane is given for 17 yeast species, as recorded in the list of 1983 (BPY1983), in The Yeasts of 1998, or observed in the present study; ? means not done. In some cases the number of days required for clearly discernible growth is given.

It is evident that Kurtzman's method (liquid YNB) is perfect in the case of several *Pichia* species; for *P. guillermondii* it appears to be superior to the slant culture method. It also works well with *Cerinosterus cyanescens*, a basidiomycetous yeastlike fungus. However, false negative results were obtained with *Trichosporon* and some *Candida* and *Cryptococcus* species. *T. veenhuisia* had recently been introduced as a hexadecane utilizer (Middelhoven et al., 2000), but it does so only in the slant culture, not in liquid YNB. *Arxula adeninivorans* grows very well in the slant culture, but gave only a weak response in liquid YNB.

These literature data and own observations make it clear that for demonstration of growth on n-hexadecane the slant culture method gives the most reliable results. It should be recommended for further use.

Thanks are due to David Yarrow, Clete Kurtzman and Adele Statzell-Tallman for historical notes.

References:

- A.J. Markovetz and R.E. Kallio. Assimilation of alkanes and alkenes by yeasts. J.Bacteriol. 87(1964)968
- W.J. Middelhoven, G. Scorzetti and J.W. Fell. *Trichosporon veenhuisia* sp.nov., an alkane-assimilating anamorphic basidiomycetous yeast. Int. J. Syst. Evol. Microbiol. 50(2000)381-387
- W.J. Middelhoven, G. Scorzetti and J.W. Fell. *Trichosporon porosum* comb.nov., an anamorphic basidiomycetous yeast inhabiting soil, related to the *loubieri/laibachii* group of species that assimilate hemicelluloses and phenolic compounds. FEMS Yeast Research 1(2001)15-22
- R. Scheda and P. Bos. Hydrocarbons as substrates for yeasts. Nature 211(1966)660

II. Russian Collection of Microorganisms, Institute for Biochemistry and Physiology of Microorganisms, Russian Academy of Sciences, Pushchino 142292, Russia. Communicated by W.I. Golubev <WIG@ibpm.serpukhov.su>.

Recent publications.

1. Puchkov E.V., Wiese A., Seydel U., Kulakovskaya T.V., 2001. Cytoplasmic membrane of a sensitive yeast is a primary target for *Cryptococcus humicola* mycocidal compounds (microcin). *Biochim. Biophys. Acta* **1512**:239-250.

A basidiomycetous yeast strain, *Cryptococcus humicola* 9-6, secretes a mycocidal compound (microcin) which is lethal for many yeasts. In this study a new protocol for microcin purification has been developed, and TLC-purity product was obtained. Using fluorescein as a pH-sensitive probe it was found that microcin treatment of *Cryptococcus terreus*, a model microcin-sensitive yeast, immediately caused transient alkalization followed by acidification of cells' cytoplasm. Upon completion of this process, endogenous respiration as well as activity of unspecific esterases were inhibited, and alterations in cell wall and/or capsule started. Microcin was shown to make the cell leaky for intracellular ATP. The mycocidal effect of

microcin did not depend on the cell cycle phase of *Cr. terreus*. Based on these observations and on electrical measurements on planar phospholipid bilayers, which indicated a microcin-induced membrane permeabilization, it is suggested that the cytoplasmic membrane of the sensitive yeast is a primary target of microcin action. The conjectured mode of microcin action involves gradual increase of the cytoplasmic membrane's unspecific permeability. Intracellular ion homeostasis changes induced by microcon are considered to be the main cause of enzyme inhibition, alterations in the outer layers of the cell envelope and, finally, division arrest.

2. Golubev W.I., Kulakovskaya T.V., Golubeva E.W., 2001. *Pseudozyma fusiformata* VKM Y-2821 is a producer of antifungal glycolipid. *Mikrobiologiya* **70** (in press).

The yeast *Pseudozyma fusiformata* (Ustilaginales) secretes fungicidal agent that was of low Mr, resistant to proteolysis and thermostable. Among 280 species of yeasts and yeast-like fungi examined more than 80% of them were sensitive

to it under acid conditions. The toxin was extracted with methanol and purified by column and thin-layer chromatography. It consists of glucose and saturated fatty acids.

III. State Scientific-Research Institute for Genetics and Selection of Industrial Microorganisms, I-Dorozhnyi 1, Moscow 113545, Russia. Communicated by G.I. Naumov and E.S. Naumova <gnaumov@yahoo.com>.

We thank M. Jakobsen (Royal Veterinary and Agricultural University, Copenhagen) and M. Sipiczki (University of Debrecen, Hungary) for fruitful collaboration in yeast research

in 2001. We are grateful to the Organising Committee of the ISSY2001 (Lviv) for the invitation to give a lecture. The following are publications for 2001 or in press.

1. Naumov G.I., Naumova E.S., Schnürer J. 2001. Genetic characterization of the nonconventional yeast *Hansenula anomala*. *Res. Microbiol.* **152**: 551-562.
2. Kondratieva V.I., Naumov G.I. 2001. The phenomenon of spore killing in *Schizosaccharomyces pombe* hybrids. *Dokl. Biol. Sciences* **379**(4): 570-573.
3. Tokareva N.G., Naumova E.S., Bab'eva I.P., Naumov G.I. 2001. Identification of strains of *Zygowilliopsis californica* having different origin by polymerase chain reaction with universal primers. *Microbiology (Engl. Transl.)* **70**(5): 576-582.
4. Naumov G.I., Naumova E.S., Korshunova I.V. 2001. Genetic identification of cultured *Saccharomyces* yeasts from Asia. *J. Gen. Appl. Microbiol.* (in press).
5. Naumov G.I., Naumova E.S. 2001. Molecular and genetic approaches in classification and identification of Saturn-spored yeasts *Williopsis*, *Zygowilliopsis* and *Komagataea*. 21st Int. Spec. Symp. on yeasts (ISSY 2001), 21-25 August 2001, Lviv, Ukraine, p. 47.

6. Naumova E.S., Naumov G.I., Naumoff D.G., Korshunova I.V., Jakobsen M. 2001. Introgression between non-conventional *Saccharomyces paradoxus* species and *S. cerevisiae*: α -galactosidase genes *MEL12-MEL15*. 21st Int. Spec. Symp. on yeasts (ISSY 2001), 21-25 August 2001, Lviv, Ukraine, p. 57.
7. Naumov G.I., Naumova E.S., Schnürer J. 2001. Genetic characterization of the nonconventional yeast *Hansenula anomala*. 21st Int. Spec. Symp. on yeasts (ISSY 2001), 21-25 August 2001, Lviv, Ukraine, p. 58.
8. Naumov G.I., Naumova E.S., Korshunova I.V., Jakobsen M. 2001. Superfamily of alpha-galactosidase genes of *Saccharomyces cerevisiae*: the new divergent African genes *MEL12-MEL15*. (XXth Int. Conf. on Yeast Genetics and Molecular Biology, 26-31 August 2001, Prague). Yeast, 18 (S1): S320.
9. Špirek M., Groth C., Petersen R.F., Langklær, Naumova E.S., Naumov G.I., Piškur J. 2001. Diversity in the genome organisation of *Saccharomyces* sensu lato yeasts. (XXth Int. Conf. on Yeast Genetics and Molecular Biology, 26-31 August 2001, Prague). Yeast, 18 (S1): S321.
10. Tokareva N.G. 2001. Molecular and genetic approaches in classification of yeasts with Saturn-shaped spores. Ph.D. Thesis, Moscow State University, Moscow.
11. Naumoff D.G. 2001. Molecular and genetic analysis of some microbial glycosyl hydrolase families. Ph.D. Thesis, State Scientific-Research Institute for Genetics and Selection of Industrial Microorganisms, Moscow.

IV. Department of Environmental Biology, University of Guelph, Guelph, Ontario, Canada N1G 2W1. Communicated by H. Lee.

The following is the abstract of a paper which was published recently.

1. Jeong, E.Y., C. Sopher, I.S. Kim & H. Lee 2001. Mutational study of the role of tyrosine-49 in the *Saccharomyces cerevisiae* xylose reductase. Yeast **18**:1081-1089.

The *xyl1* gene encoding xylose reductase was cloned from *Saccharomyces cerevisiae* and expressed in *Escherichia coli*. The purified enzyme readily carried out xylose reduction in vitro. It prefers NADPH as the coenzyme by about 80-fold over NADH. Compared to the native enzyme purified from *S. cerevisiae* [Kuhn et al., 1995], the recombinant xylose reductase displayed slightly higher (about 2-fold) affinities (K_m) for the substrate (xylose) and cofactor (NADPH), as well as a 3.9-fold faster turnover number (K_{cat}) and 7.4-fold greater catalytic efficiency (K_{cat}/K_m). The reason for the apparent

discrepancies in kinetic constants between the recombinant and native *S. cerevisiae* xylose reductases is not known. Replacement of Tyr49 by Phe in the recombinant enzyme led to greater than 98% loss of activity, suggesting that this residue plays a critical role in catalysis. Intrinsic enzyme fluorescence spectroscopic analysis showed that the wild type and the Y49F variant both bound the coenzyme NADPH with similar affinity. This supports the view that Tyr49 is involved in interaction with the substrate and not the cofactor during catalysis.

V. Industrial Microbiology Section, Kluyver Laboratory of Biotechnology, Delft University of Technology, The Netherlands. Communicated by Jack Pronk <j.t.pronk@tnw.tudelft.nl>.

Recent publications.

1. Bakker BM, Overkamp KM, van Maris AJ, Kotter P, Luttk MA, van Dijken JP, Pronk JT. 2001. Stoichiometry and compartmentation of NADH metabolism in *Saccharomyces cerevisiae*. FEMS Microbiol. Rev. **25**:15-37.

Metabolic compartmentation is an essential characteristic of eukaryotic redox metabolism. In this publication, we have attempted to review the current knowledge on metabolic

compartmentation of NADH oxidation in one of the most intensively studied eukaryotic systems: the yeast *Saccharomyces cerevisiae*.

2. Rodrigues F, Corte-Real M, Leao C, van Dijken JP, Pronk JT. 2001. Oxygen requirements of the food spoilage yeast *Zygosaccharomyces bailii* in synthetic and complex media. *Appl. Environ. Microbiol.* **67**:2123-2128

This manuscript is the result of a collaboration with our colleagues in Braga (Portugal). The ability to grow in the complete absence of oxygen is rare among yeasts. Since oxygen

availability might be a key parameter in food spoilage by yeasts, we have investigated the oxygen requirements of the food spoilage yeast *Z. bailii*.

3. Luttik MA, Kötter P, Salomons FA, van der Klei IJ, van Dijken JP, Pronk JT. 2000. The *Saccharomyces cerevisiae* *ICL2* gene encodes a mitochondrial 2-methylisocitrate lyase involved in propionyl-coenzyme A metabolism. *J. Bacteriol.* **182**:7007-7013.

For several years, *ICL2* has been an enigmatic yeast gene. Although *ICL2* shows strong sequence similarity with *ICL1*, Jürgen Heinisch and others showed that even multiple copies

cannot complement isocitrate-lyase-deficient mutants. In a joint effort with colleagues in Frankfurt (Germany) and Haren (the Netherlands) the 'real' identity of *ICL2* has now been revealed.

4. Bakker BM, Bro C, Kotter P, Luttik MA, van Dijken JP, Pronk JT. 2000. The mitochondrial alcohol dehydrogenase Adh3p is involved in a redox shuttle in *Saccharomyces cerevisiae*. *J. Bacteriol.* **182**: 4730-4737.

To our surprise, *S. cerevisiae* mutants lacking the 'internal' mitochondrial NADH dehydrogenase (Ndi1p) were still capable of fully respiratory growth on glucose. As part of our

ongoing collaboration with the Frankfurt group on yeast redox metabolism, we investigated the mechanism responsible for redox shuttling from the mitochondrial matrix to the cytosol.

VI. Institute of Molecular Biology, Bulgarian Academy of sciences, Department of Molecular Genetics, Sofia, Bulgaria. Communicated by P. Venkov.

The following are summaries of recently published paper from our group dealing with improvement and usage of a mutagenicity/carcinogenicity test based on *S. cerevisiae* cells.

1. L. Staleva, L. Waltscheva, E. Golovinsky, P. Venkov. 1996. Enhanced cells permeability increases the sensitivity of a yeast test for mutagens. *Mutation Res.* **370**:81-89

ts1 is a mutation which causes a general increase in permeability of *Saccharomyces cerevisiae* cells in an unspecific manner. The introduction of the *ts1* mutation under homozygous conditions into the D7 diploid strain enhanced the sensitivity of the test system described by Zimmermann et al. (1975). The newly constructed strain D7ts1 responded with a four to six times higher frequency compared to the D7 strain for all genetic endpoints induced with chemical mutagens (Ethyl methanesulfonate, Methyl methanesulfonate, hydroxyurea, benzpyrene). The increased sensitivity of D7ts1 is specific only for mutagens active

in yeast, since treatment of D7ts1 cells with 5-bromouracil or 5-bromouridine, known to be non-mutagenic in yeast, did not result in the induction of any of the measured genetic alterations. Five out of 14 water samples taken from the environment induced recombinogenic events in D7ts1, whereas all 14 water samples were without effect in D7 test system. We concluded that D7ts1 cells show a higher sensitivity in the detection of mutagenic or carcinogenic action because of their generally enhanced permeability due to the *ts1* mutation.

2. L. Staleva, R. Gugova, P. Venkov, L. Waltscheva, E. Golovinsky. 1998. Genotoxic effect of 4-aryl-1-(2-chloroethyl)-1-nitrosohydrazinecarboxamides on *Saccharomyces cerevisiae* cells. *J. Cancer Research Clin Oncol* **124**:321-325.

The study of some 4-aryl-1-(2-chloroethyl)-1-nitrosohydrazinecarboxamides with a *Saccharomyces cerevisiae* mutagenicity test of increased sensitivity defined two of them, 4-(4-bromobenzoyl)-1-(2-chloroethyl)-1-nitrosohydrazinecarboxamide and 4-(4-fluorophenyl)-1-(2-chloroethyl)-1-nitrosohydrazine carboxamideas typical cytostatic agents. At concentrations of 2-5 mg/ml the substances kill up to 60%-70% of cells without having any detectable

recombinogenic and mutagenic effects. At the same concentrations, lomustine, well known as a cytostatic reference, demonstrated recombinogenic and mutagenic activity on yeast cells. The advantage of the newly synthesized substances is that, in a certain concentration range, their biological activity is mainly cytotoxic without induction of recombinogenic and mutagenic events of surviving cells.

3. Venkov P., Topashka-Ancheva M., Georgieva M., Alexieva V., Karanov E 2000. Genotoxic effect of substituted phenoxyacetic acids. *Arch Toxicol* **74**:560-566

The potential toxic and mutagenic action of 2,4-dichlorophenoxyacetic acid has been studied in different test systems, and the obtained results range from increased chromosomal damage to no effect at all. We reexamined the effect of this herbicide by simultaneous using three tests based on yeast, transformed hematopoietic, and mouse bone marrow cells. The results obtained demonstrated that 2,4-dichlorophenoxyacetic acid has cytotoxic and mutagenic effects. The positive response of yeast and transformed hematopoietic was verified in kinetics and dose-response experiments. The analysis of metaphase chromosomes indicated a statistically

4. A. Terziyska, L. Waltschewa and P. Venkov 2000. A new sensitive test based on yeast cells for studying environmental pollution. *Environmental Pollution* **109**:43-52

Different tests based on yeast cells were developed for determination of mutagenic/cancerogenic action, however they all showed lower sensitivity compared to bacterial tests, the main reason for this being the limited permeability of yeast cells. We found that general permeability of *Saccharomyces cerevisiae* cells can be increased by mutation and on this bases we developed a more sensitive test. The aim of this study was to prove the applicability of our test, called D7ts1, in environmental studies. Soil, water and air samples were taken during 1998 from regions in Bulgaria with declared low, average or high pollution levels and investigated for presence of mutagenic/cancerogenic activities in the bacterial test of Ames, the yeast D7 test of Zimmermann and our new D7ts1 test. Results

proved indication of breaks, deletions, and exchanges after the intraperitoneal administration of 2,4-dichlorophenoxyacetic acid in mice. The study of phenoxyacetic acid and its differently chlorinated derivatives showed that cytotoxicity and mutagenicity are induced by chlorine atoms at position 2 and /or 4 in benzene ring. The mutagenic effect was abolished by introduction of a third chlorine atom at position 5. Thus 2,4,5-trichloro phenoxyacetic acid was found to have very weak, if any mutagenic effect; however, the herbicide preserved it toxic effect.

obtained evidenced the following conclusions: 1) The usage of D7ts1 test instead of D7 test permits a more clear measurement of positive samples and detects mutagenic/cancerogenic activities undetectable by D7 test. 2) All samples with positive Ames test were positive in the D7ts1 test, however some samples clearly positive in the D7ts1 were negative in the Ames test. Therefore, the simultaneous usage of D7ts1 and Ames tests in environmental studies is advantageous because detects dangerous for the human health activities to which bacterial cells does not respond. 3) Regions in Bulgaria declared clean were found to be polluted. Particularly troubled are the whole year positive data in the three tests for air samples from a "clean" region.

VII. Vietnam Collection of Industrial Microorganisms, Department of Microbiology, Food Industries Research Institute (FIRI), 301-Nguyen Trai, Thanh Xuan, Hanoi, Vietnam. Communicated by Vu Nguyen Thanh <thanh@firi.ac.vn>.

The Vietnam Collection of Industrial Microorganisms originated from private collections of Vietnamese researchers working in food-processing industry. In spite of very little resources, the collection has significantly contributed to the local fermentation industry and research development. Since 1998 the collection has received official sanction and constant funding from the state. Our mission for next few years will include: setting-up a standard identification capability, re-identification of all strains currently being used in fermentation and research activities, enriching the collection with new strains and isolates.

Publications.

1. V.N. Thanh, N.H. Giang, D.M. Hang, H. Lünsdorf, P.N. Golyshin. Double stranded RNA virus associated with killer yeast *Torulaspora delbrueckii*. International Workshop in Biology, Hanoi July, 2001.
 2. V.N. Thanh, M.S. Van Dyk, and M.J. Wingfield. *Debaryomyces mycophilus* sp. nov., a mold-dependent yeast isolated from wood-lice (submitted to FEMS Yeast Research).
 3. M. Hamamoto, V.N. Thanh and T. Nakase. *Bannoahahajimensis* gen. nov., sp. nov., and three related anamorphs *Sporobolomyces bischofia* sp. nov., *Sporobolomyces ogasawarensis* sp. nov. and *Sporobolomyces syzygii* sp. nov., yeasts isolated from plants in Japan. Int. J. Syst. Evol. Microbiol. (in press).
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Beside the understandable shortage in funding the collection suffers serious problems with the availability of type strains, which are almost unavoidable in most taxonomic studies. Thus, we would welcome and highly appreciate any suggestions in term of research collaboration, strain exchanges, and potential donors.

Current projects: Survey of yeasts associated with local traditional fermentation products. Survey of yeasts associated with tropical fruits. Study on unconventional killer yeasts and possible applications.

VIII. Institute for Fermentation, Osaka (IFO), Osaka 532-8686, Japan. Communicated by K. Ueda-Nishimura <nishimura-kumiko@ifo.or.jp>.

Recent publications.

1. Ueda-Nishimura, K. & Mikata, K. 2001 Reexamination of *Dipodascus* and *Geotrichum* strains by DNA-DNA hybridization. IFO Res. Commun. **20**: 92-96.
2. Mikata, K. 2001 Unique shape of the ascospores of *Pichia sporocuriosa*. IFO Res. Commun. **20**: 97-100.
3. Ueda-Nishimura, K. & Mikata, K. 2001 Reclassification of *Pichia scaptomyzae* and *Pichia galeiformis*. Antonie van Leeuwenhoek **79**: 371-375.
4. Mikata, K., Ueda-Nishimura, K. & Hisatomi, T. 2001. Three new species of *Saccharomyces sensu lato* van der Walt from Yaku Island in Japan: *Saccharomyces naganishii* sp. nov., *Saccharomyces humaticus* sp. nov. and *Saccharomyces yakushimaensis* sp. nov. Int. J. Syst. Evol. Microbiol. **51**: 2189-2198.
5. Ueda-Nishimura, K. & Mikata, K. 2002. Species distinction of the ascomycetous heterothallic yeast-like fungus *Stephanoascus ciferrii* complex: description of *Candida allociferrii* sp. nov. and reinstatement of *Candida mucifera* Kocková-Kratochvilová et Sláviková. Int. J. Syst. Evol. Microbiol. (in press).

The nucleotide sequences of the 18S rRNA gene (rDNA) from nine strains of heterothallic ascomycetous *Stephanoascus ciferrii* complex were determined, and the strains were separated into three groups by their sequences. 18S rDNA sequences were identical within the same group. In group A, the 18S rDNA sequences had no introns; in group B, the 18S rDNA had one group I intron, Sc1506-1 at position 1506n position 1506 had nineteen base differences but were very similar. Therefore, it is suggested that these introns existed in the common ancestor of groups B and C, and that they were vertically inherited. DNA similarity values showed that the strains within the same group were identical species. Group B included the isotype strains of *Stephanoascus ciferrii*; and in group C, the 18S rDNA had two

group I introns, Sc943 at position 943 and Sc1506-2 at position 1506. Sc1506-1 and Sc1506-2 were found in the type strains of *Candida ciferrii* and *Sporothrix catenata*; and this confirmed that group B strains correspond to *Stephanoascus ciferrii* and that *Candida ciferrii* and *Sporothrix catenata* are synonyms of *Stephanoascus ciferrii*. The single member of group C, IFO 10918^T, corresponds to the type strain of *Candida mucifera* and was independent of the other tested strains. Thus, *Candida mucifera* should be regarded as an independent species from *Stephanoascus ciferrii*. It is suggested that group A might be a new *Stephanoascus* species, but, since group A strains could not form ascii by themselves in this study, they are described as a new species *Candida allociferrii* sp. nov. (type strain IFO 10194^T).

IX. 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. In the press: J A Barnett & C F Robinow. 2002. A history of research on yeasts 4: cytology part I, 1890-1950. Yeast.
2. In preparation: J A Barnett & C F Robinow. A history of research on yeasts 4: cytology part II, after 1950.

X. Molecular Genetics and Evolution, Research School of Biological Sciences, Australian National University, P.O.Box 475, Canberra City, ACT, 2601 Australia. Communicated by G.D. Clark-Walker <DCW@rsbs.anu.edu.au>.

The following paper is in press.

1. G.D.Clark-Walker and X.J.Chen. 2001. Dual Mutations Reveal Interactions Between Components of Oxidative Phosphorylation in *Kluyveromyces lactis*. Genetics (In press).

Loss of mtDNA or mitochondrial protein synthesis cannot be tolerated by wild type *K. lactis*. The mitochondrial function responsible for p^o-lethality has been identified by disruption of nuclear genes encoding electron transport and F_o-ATP synthase components of oxidative phosphorylation. Sporulation of diploid strains heterozygous for disruptions in genes for the two

components of oxidative phosphorylation results in the formation of non-viable spores inferred to contain both disruptions. Lethality of spores is thought to result from absence of a transmembrane potential, ΔΨ, across the mitochondrial inner membrane due to lack of proton pumping by the electron transport chain or reversal of F₁F_o-ATP synthase. Synergistic

lethality, caused by disruption of nuclear genes, or ρ^0 -lethality can be suppressed by the *atp2.1* mutation in the β -subunit of F_1 -ATPase. Suppression is viewed to occur by an increased hydrolysis of ATP by mutant F_1 allowing sufficient electrogenic exchange by the translocase of ADP in the matrix for ATP in the cytosol to maintain $\Delta\Psi$. In addition, lethality of haploid strains with a

disruption of *AAC* encoding the ADP/ATP translocase, can be suppressed by *atp2.1*. In this case suppression is considered to occur by mutant F_1 acting in the forward direction to partially uncouple ATP production thereby stimulating respiration and relieving detrimental hyperpolarization of the inner membrane. Participation of the ADP/ATP translocase in suppression of ρ^0 -lethality is supported by the observation that disruption of *AAC* abolishes suppressor activity of *atp2.1*.

XI. Department of Applied Microbiology, Lund University, PO Box 124, 221 00 Lund, Sweden. Communicated by M.F. Gorwa-Grauslund <Marie-Francoise.Gorwa@tmb.lth.se>.

Research activities on yeast at the department of Applied Microbiology concern (i) the design of pentose-fermenting *Saccharomyces cerevisiae* strains via metabolic engineering and (ii) the expression of recombinant protein in different yeast systems.

Xylose fermentation. While *S. cerevisiae* can easily ferment the hexose fraction from wood hydrolysates, it cannot use the xylose-rich pentose fraction, due to the lack of efficient transport and pathway for xylose. New yeast strains are therefore being developed to produce ethanol from both hexose and pentose fractions [4]. The genes for xylose reductase (XR) and xylitol dehydrogenase (XDH) from the yeast *Pichia stipitis* were cloned into *S. cerevisiae* and it was shown that the ratio between XR, XDH and xylulokinase (XK) had a strong impact on the formation of xylitol, a major by-product [3]. In parallel, it was demonstrated that over-expression of XK enabled an increased ethanol yield by reducing the xylitol yield [5]. From these results, the genes for XR and XDH from *P. stipitis* and an additional copy of XK gene were cloned and stably integrated into *S. cerevisiae* and anaerobic ethanol formation from xylose was demonstrated for the first time [2]. A metabolic flux model under anaerobic conditions was developed on the obtained recombinant *S. cerevisiae* strain TMB3001 [9]. Possible bottlenecks for xylose uptake in the pentose-phosphate pathway (PPP) were investigated. Concomitant overexpression of lower PPP genes [6] did not enhance uptake of xylose. Study on xylulose

fermentation revealed that xylulose uptake could be increased by lowering the flux through oxidative PPP [1].

In parallel, we worked on the expression of bacterial xylose isomerases in *S. cerevisiae*. The gene *xylA* for xylose isomerase from the bacterium *Thermus thermophilus* was mutated to improve activity at 30°C and the modified enzymes were kinetically characterized [7]. Since xylose isomerase is inhibited by the xylitol produced from xylose by the homologous aldose reductase from *S. cerevisiae*, the gene encoding this enzyme was deleted to limit xylitol formation in *xylA* over-expressing strains [8].

Expression of heterologous proteins in yeast. The metabolic burden of different *S. cerevisiae* systems for the expression of xylanase II from *Trichoderma reesei* was investigated and it was shown that the effect of foreign gene expression was disproportionately large, with respect to the amount of protein produced [10]. The xylanase from *Cryptococcus albidus* was expressed in *P. stipitis* under the control of *PSADH2*-promoter, which is activated under oxygen limitation. Endo-1,4- β -xylanase was produced after a shift to anoxic conditions and enhanced by limited aeration after the shift [11]. The endo- β -1,4-mannanase from *Aspergillus aculeatus* was expressed in *Saccharomyces cerevisiae* and the recombinant enzyme was characterized [12].

List of recent published papers.

1. Eliasson A., E. Boles, B. Johansson, M. Österberg, J.M. Thevelein, I. Spencer-Martins, H. Juhnke and B. Hahn-Hägerdal. 2000. Xylulose fermentation by mutant and wild-type strains of *Zygosaccharomyces* and *Saccharomyces cerevisiae*. *Appl. Microbiol. Biotechnol.* **53**:376-382.
2. Eliasson A., C. Christensson, C.F. Wahlbom and B. Hahn-Hägerdal. 2000. Anaerobic xylose fermentation by recombinant *Saccharomyces cerevisiae* carrying *XYL1*, *XYL2*, and *XKS1* in mineral medium chemostat cultures. *Appl. Environ. Microbiol.* **66**:3381-3386.
3. Eliasson A., J.S. Hofmeyr, S. Pedler and B. Hahn-Hägerdal. 2001. The xylose reductase/xylitol dehydrogenase/xylulokinase ratio affects product formation in recombinant xylose-utilising *Saccharomyces cerevisiae*. *Enz. Microb. Technol.* **29**:288-297.
4. Hahn-Hägerdal B., Wahlbom C.F., Gárdonyi M., van Zyl W.H., Cordero Otero R.R. and Jönsson L.J. 2001. Metabolic engineering of *Saccharomyces cerevisiae* for xylose utilisation. *Adv. Biochem. Eng.* **73**:53-84.
5. Johansson B., C. Christensson, T. Hobley and B. Hahn-Hägerdal. 2001. Xylulokinase over-expression in two strains of *Saccharomyces cerevisiae* also expressing xylose reductase and xylitol dehydrogenase and

- its effect on fermentation of xylose and lignocellulosic hydrolysate. *Appl. Environ. Microbiol.* **67**:4249-4255.
6. Johansson B. and B. Hahn-Hägerdal. 2001. Over-production of pentose phosphate pathway enzymes using a new Cre/loxP expression vector for repeated genomic integration in *Saccharomyces cerevisiae*. Accepted for publication in *Yeast*.
 7. Lönn A., Gárdonyi M., van Zyl W, Hahn-Hägerdal B. and Cordero Otero R. 2001. Cold adaptation of xylose isomerase from *Thermus thermophilus* through random PCR mutagenesis. Accepted for publication in *Eur. J.Biochem.*
 8. Träff K., Otero Cordero R. R., van Zyl, W. H. and Hahn-Hägerdal, B. 2001. Deletion of the *GRE3* aldose reductase gene and its influence on xylose metabolism in recombinant strains of *Saccharomyces cerevisiae* expressing the *xylA* and *XKS1* genes. *Appl. Environ. Microbiol.* **67**(12). In press.
 9. Wahlbom C.F., A. Eliasson and B. Hahn-Hägerdal. 2001. Intracellular fluxes in a recombinant xylose-utilizing *Saccharomyces cerevisiae* cultivated anaerobically at different dilution rates and feed concentrations. *Biotechnol. Bioeng.* **5**:289-296.
 10. Görgens J.F., W.H. van Zyl, J.H. Knoetze and B. Hahn-Hägerdal. 2001. The metabolic burden of the PGK1 and ADH2 promoter systems for heterologous xylanase production by *Saccharomyces cerevisiae* in defined medium. *Biotechnol. Bioeng.* **73**:238-245.
 11. Passoth V.V. and B. Hahn-Hägerdal. 2000. Production of a heterologous endo-1,4-beta-xylanase in the yeast *Pichia stipitis* with an O(2)-regulated promoter. *Enzyme Microb. Technol.* **26**:781-784.
 12. Setati E.V., P. Ademark, W.H. van Zyl, B. Hahn-Hägerdal and Henrik Stålbrand. 2001. Expression of the *Aspergillus aculeatus* endo- β -1,4-mannanase encoding gene (*man1*) in *Saccharomyces cerevisiae* and characterization of the recombinant enzyme. *Protein Expres. Purif.* **21**:105-114.

XII. Department of Food Science & Technology, DalTech, P.O. Box 1000, Halifax, Nova Scotia Canada B3J 2X4. Communicated by A. Speers.

Recent publication.

1. Hsu J. W-C., Speers, R.A. and Paulson, A.T. 2001. Modeling of orthokinetic flocculation of *Saccharomyces cerevisiae*. *Biophys. Chem.* In press.

This study examined the flocculation behavior of two *Saccharomyces cerevisiae* strains expressing either *Flo1* (LCC1209) genotype or NewFlo (LCC125) phenotype in a laminar flow field by measurement of the fundamental flocculation parameter, the orthokinetic capture coefficient. This orthokinetic capture coefficient was measured as function of shear rate (5.95-223 /s) and temperature (5-45°C). The capture coefficients of these suspensions were directly proportional to the inverse of shear rate, and exhibited an increase as the temperature was increased to 45°C. The capture coefficient of pronase-treated cells was also measured over

similar shear rate and temperature range. A theory, which predicts capture coefficient values due to zymolectin interactions, was simplified from that developed by Long et al. (*Biophys. J.* **76**: 1112). This new modified theory uses estimates of: (1) cell wall densities of zymolectins and carbohydrate ligands; (2) cell wall collision contact area; and (3) the forward rate coefficient of binding to predict theoretical capture coefficients. A second model that involves both zymolectin interactions and DLVO forces was used to describe the phenomenon of yeast flocculation at intermediate shear ranges, to explain yeast flocculation in laminar flow.

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

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

1. J.B. Guerra, R.C. Araújo, C. Pataro, G.R. Franco, E.S.A. Moreira, L.C. Mendonça-Hagler and C.A. Rosa. 2001. Genetic diversity of *Saccharomyces cerevisiae* strains during the 24 h fermentative cycle for the production of the artisanal *cachaça*. *Lett. Appl. Microbiol.* **33**:106-111.

Aims: Characterization of yeast populations and genetic polymorphism of *Saccharomyces cerevisiae* strains collected during the short fermentative cycles from the spontaneous fermentations during the artisanal *cachaça* production. **Methods and Results:** The prevalent *S. cerevisiae* strains were analysed by PFG and RAPD-PCR using primers EI1 and M13. The molecular analysis have showed a high degree of genetic polymorphism among the strains within a 24 h fermentative cycle. **Conclusions:** The genetic diversity observed in the *S. cerevisiae* strains may be occurring due to the existence of a large number of individual genotypes within the species. The unique characteristics of the *cachaça* fermentation process probably allows for a faster detection of molecular polymorphisms of yeast strains than other types of fermentations. **Significance and Impact of the Study:** Spontaneous fermentations to produce *cachaça*, due to their characteristics, are an excellent model for the study of molecular diversity of *S. cerevisiae* strains during the production of fermented beverages.
2. J.P. Ramos, P. Valente, R.A. de Souza, C.A. Rosa and O. Leoncini. 2001. Heteroduplex mobility assay of the D1/D2 region of the 26S rDNA for differentiation of *Saccharomyces* species. *Lett. Appl. Microbiol.* **33**:206-210.

Aims: We present the HMA method for *Saccharomyces* differentiation using the PCR amplified D1/D2 26S rDNA. **Methods and Results:** This methodology is based on heteroduplex formation when two different DNAs are hybridized. We tested 11 type cultures of *Saccharomyces*, 27 different cultures of *S. cerevisiae* and four other ascomycetous genera. **Conclusions:** The method was capable to differentiating *Saccharomyces* species and was mainly very efficient for *S. cerevisiae* identification. HMA can probably be applied in other genera, where identification is sometimes difficult only by conventional traits, which are based on physiology and morphology. **Significance and Impact of the Study:** HMA provides a rapid and relatively simple molecular tool, contributing for yeast taxonomy.
3. E.R. Duarte, J.C.P. Resende, C.A. Rosa and J.S. Hamdan. 2001. Prevalence of yeasts and mycelial fungi in bovine parasitic otitis in the state of Minas Gerais, Brazil. *J. Vet. Med. B* **48**, (in press).

Infestations by rhabditiform nematodes and acarids of the genus *Raillietia* are considered the primary causes of external otitis in cattle in tropical regions. Recently, yeasts of the genus *Malassezia* have been associated with a relatively high percentage of otitis cases, but the occurrence of other yeasts and mycelial fungi has not yet been reported in the literature. This work studied the presence of fungi in the ear canal of 45 cattle with external parasitic otitis. The results were positive for yeasts of the genus *Malassezia* in 31 (89.9%) of the 45 cultures in Mycosel medium supplemented with olive oil. The 45 cultures in Sabouraud dextrose medium revealed the growth of seven (15.5%) yeasts of the genus *Candida*, five (11.1%) *Rhodotorula mucilaginosa*, two (4.4%) fungi of the genus *Aspergillus* and eight 'Mycelia sterilia'. Future studies may confirm and elucidate the importance of these agents in the aetiology of bovine otitis.
4. N.C.M. Gomes; C.A. Rosa; P.F. Pimentel; L.C.S. Mendonça-Hagler. 2002. Uptake of free and complexed silver ions by different strains of *Rhodotorula mucilaginosa*. *Braz. J. Microbiol.* (in press).
5. F.C.O. Gomes, C. Pataro, J.B. Guerra, M.J. Neves, S.R. Corrêa, E.S.A. Moreira and C.A. Rosa. 2001. Physiological diversity and trehalose accumulation in *Schizosaccharomyces pombe* strains isolated from spontaneous fermentations during the production of the artisanal Brazilian *cachaça*. Submitted for publication.
6. A.C.P. Teixeira, M.M.M. Souza, J.R. Nicoli, Y. Antonini, R.P. Martins, M.A. Lachance and C.A. Rosa. *Starmerella meliponinorum*, a new ascomycetous yeast species associated with stingless bees. Submitted for publication.

The following is the outline of a short graduate course given by Dr. Marc-André Lachance in my Department, August 16-17 of this year.

Yeast Molecular Systematics:

A. GENERAL OVERVIEW OF THE PRINCIPLES OF SYSTEMATICS

1. Taxonomy *versus* systematics
2. Operations of biological taxonomy
 - a. Description
 - b. Classification
 - c. Identification
3. Taxa, designations, and categories
4. Taxonomic epistemology: are species real?
 - a. Nominalism *vs* realism
 - b. Typology *vs* population thinking
5. Phenetics and cladistics
 - a. Opposing schools of thought
 - b. Sources of analytical methodologies
6. The dangers of molecular systematics: reductionism and holism
8. The quality of classifications
 - a. Good – bad

- b. Phylogenetic – artificial
- c. Taxon structure: Monophyly - paraphyly – polyphyly
- d. Taxonomic characters
 - i. Similarity *vs.* homology
 - ii. Character states in cladistics

B. MOLECULAR SYSTEMATICS

1. The importance of defining objectives
2. Types of biological information: Relational *vs.* descriptive
3. The use of protein information in determining relatedness
4. DNA based approaches
 - a. DNA base composition
 - b. DNA/DNA reassociation
 - c. RFLP, RAPD, satellite DNA, and related approaches
 - d. DNA sequencing
 - i. Methodology
 - ii. Analysis
 - e. PCR based applications
 - f. The future: microarrays and microchips

XIV. Institut für Angewandte Mikrobiologie, Universität für Bodenkultur, Nußdorfer Lände 11, A-1190 Vienna, Austria. Communicated by H. Prillinger.

The following abstract is a summary of an Internet publication which can be found under the following address:
<http://www.boku.ac.at/forumltb/issue1/paper3.html>

1. K. Lopandic, S. Zelger, L.K. Banszky, F. Eliskases-Lechner, & H. Prillinger. Phenotypic and Genotypic Identification of Yeasts from Milk Products.

A polyphasic approach including phenotypic (morphological, biochemical, physiological characterisation) and genotypic (RAPD-PCR, sequencing of genes coding for 26S and 18S rRNA) methods was used for the identification of yeasts isolated from different Austrian milk products. There were 514 yeast isolates in all, 461 ascomycetous and 53 basidiomycetous strains. By means of the applied techniques it was possible to identify 92% strains altogether. The following species were determined: *Candida catenulata*, *C. incospicua*, *C. intermedia*, *C. parapsilosis*, *C. pararugosa*, *C. pseudoglaebosa*, *C. saitoana*, *C. sake*, *C. sojae*, *C. zeylanoides*, *Clavispora lusitaniae*, *Debaryomyces fabryi*, *D. hansenii*, *Geotrichum candidum*, *Issatchenka orientalis*, *Kluyveromyces lactis*, *K. marxianus*, *Pichia fermentans*, *P. guilliermondii*, *Saccharomyces cerevisiae*,

S. unisporus, *Torulaspora delbrueckii*, *Yarrowia lipolytica*, *Cryptococcus curvatus*, *Rhodotorula mucilaginosa*, *Trichosporon cutaneum* and *T. ovoides*. The largest part of the microflora was represented by *D. hansenii* and *K. marxianus*, followed by *G. candidum*, *C. zeylanoides* and *Y. lipolytica*. The majority of the yeasts were isolated from fresh and sour curd cheese. The phylogenetic studies on the basis of 26S and 18S rDNA sequences as well as the RAPD-analysis showed that new species of the genera *Yarrowia*, *Rhodotorula*, *Cryptococcus* and *Trichosporon* could be characterised. The results described in this work clearly show that molecular identification is an excellent alternative approach as compared to the time consuming physiological tests.

Article in press.

2. W.Schweigkofler, K.Lopandic, O.Molnár & H.Prillinger. Analysis of phylogenetic relationships among Ascomycota with yeast phases using ribosomal DNA sequences and cell wall sugars. Organisms, Diversity, Evolution (in press).

Analysis of the monosaccharid composition of purified cell walls of unicellular and filamentous ascomycetous fungi shows three pattern: (1) the mannose glucose type (for most hemiascomycetous yeasts) (2) the mannose glucose galactose type (for several members of all three main ascomycetous clades) and (3) the mannose glucose galactose rhamnose type (for members of the Euascomycetes and the *Protomyces/Schizosaccharomyces* group). In order to estimate the usefulness of the carbohydrate pattern for phylogenetic analysis we compared them with a phylogenetic tree, which we

constructed based on 18SrRNA-gene sequences using the Neighbor-Joining Method. In contrast with the situation for basidiomycetous fungi, the Ascomycota show no fixed cell wall type for the three classes. Based on cell wall carbohydrates, sequence data and molecular characters the Hemiascomycetes appear as the first branch within the Ascomycota. A second clade, comprising the genera *Schizosaccharomyces*, *Pneumocystis*, *Taphrina*, *Protomyces*, *Neolecta* and *Saitoella* appears as a sister group of the Euascomycetes. We discuss the erection of a new class for this group of ascomycetous fungi for

which we propose the name *Protomycetes*.

The following is an invited lecture given at the 21th Yeast symposium at Lviv.

3. Prillinger, H, Lopandic, K., Suzuki, M., Sterflinger, K., Weber, E., and Oberwinkler, F. Molecular phylogeny and systematics of yeasts and yeast-like Fungi with special reference to the Asco- and Basidiomycota.

1965 Zuckerkandl & Pauling: Molecules as documents of evolutionary history. Sequence comparisons of informational macromolecules offer new insights in molecular phylogeny (J. Theoret. Biol. 8: 357-366). **1968** Kimura: Evolutionary rate at the molecular level. The neutral theory of molecular evolution (Nature 217: 624-626).

PHYLOGENETIC REVOLUTION: **1987** Woese: 16S rDNA Molecular phylogeny of Prokaryotes (Microbiol. Rev. 51: 221-271). **1990** Woese, Kandler & Wheelis: Life on this planet divides into three primary groupings: DOMAIN: Archea, Bacteria, Eukarya (Proc. Natl. Acad. Sci. USA 87: 4578-4579). **1996** Sogin, Morrison, Hinkle & Silberman: KINGDOMS of the Eukarya (Microbiologia 12: 17-28).

Systematics: (main goal: clarify natural relationships between organisms).

EUKARYOTE KINGDOMS (Crown group). **Heterokontobionta** (Chromista or Stramenopila: Oomycota, Hyphochytridiomycota, Labyrinthulomycota, Phaeophyceae, Bacillariophyceae, Chrysophyceae, Xanthophyceae, Chloromonadophyceae). **Chlorobionta** (Plantae: Chlorophyta, Bryophyta, Pteridophyta, Spermatophyta; **Prototheca**: achlorophyllous, unicellular, yeast-like). **Mycobionta** (Fungi, Eumycota: Chytridio-, Zygo-, Asco-, Basidiomycota; **Yeasts** are known from all four divisions, e.g. *Basidiobolus*, *Mycotypha*, *Ophiostoma*, *Ustilago*).

Zoobionta (Animalia: Metazoa and unicellular relatives).

Rhodobionta (Red algae). **Alveolobionta** (Alveolates: Ciliates, Dinophyta, Apicomplexans).

ASCOMYCOTA. Three distinct classes: Hemiascomycetes, Protomycetes, Euascomycetes (Cell wall sugars, 18S rDNA complete sequences, urease-activity). The Mannose Glucose and the Glucose, Mannose, Galactose pattern cannot be used to separate distinct classes or orders (Suzuki & al. 1999; J. Gen. Appl. Microbiol. 45: 229-238). Different from Berbee & Taylor (1993: Can. J. Bot. 71: 1114-1127) and Nishida & Sugiyama (1994: Mycoscience 35: 361-366) we have concentrated in our phylogenetic tree of complete 18S rDNA sequences on yeasts and dimorphic Proto- and Euascomycetes). The Protomycetes appear basal to the Euascomycetes and Basidiomycota in a neighbour-joining tree (distance estimation according to Jin & Nei 1990; Mol. Biol. Evol. 7: 82-102) using the one parameter evolution model of Jukes & Cantor (1969; In: Munro H.H. (ed.) Mammalian protein metabolism. Academic Press, New York, pp. 21-132) and the two parameter evolution model of Kimura (1980; J. Mol. Evol. 16: 111-120). The Hemiascomycetes are not monophyletic and occupy a basal position within the phylogenetic tree of the Ascomycota.

Dimorphism: Fundamental principle for all three classes of the Ascomycota and Basidiomycota and may be important for the rapid evolution of the Mycobionta. We have detected a yeast stage in the Euascomycetes *Acremonium strictum*, *Fusarium oxysporum*, and *Verticillium dahliae*. **Galactocandida:** This new

genus was introduced for *Candida* species exhibiting the glucose mannose galactose cell wall sugar pattern of the Stephanoascales sub-clade II. Three new species will be described. *G. mastotermitis* and *G. reticulitermitis* are from the hindgut of the lower termites *Mastotermes darwiniensis* and *Retculitermes santonensis*. *G. jahnoperii* was isolated from the aseptic fruiting body trama of the polypore *Jahnoporus hirtus*. **Eremothecium**. Based on complete sequences of the 18S rDNA *E. coryli* and the filamentous fungus *E. ashbyi* can be included in the family Saccharomycetaceae within the Saccharomycetales as already suggested by Prillinger & al. 1997; Yeast 13: 945-960). **Fucotaphrina**. *Taphrina vestergrenii* is so far the only representative of the Ascomycota having fucose in the qualitative and quantitative monosaccharide pattern of purified yeast cell walls. Based on fucose in its cell walls and complete 18S rDNA sequences we have placed *T. vestergrenii* in the new genus *Fucotaphrina*. Based on its occurrence on ferns (*Dryopteris filix-mas*) we consider *F. vestergrenii* as a missing link in the evolution from the Protomycetes to the Urediniomycetes. **Lecythophora**. Dimorphic species of the anamorphic genus *Lecythophora* exhibit the glucose, mannose, galactose, rhamnose cell wall sugar pattern. They form a monophyletic clade with the teleomorphic *Coniochaeta* species and appear phylogenetically related to the Sordariales. **Hypozyma**. Although the qualitative and quantitative cell wall sugar pattern of the anamorphic *Hypozyma* species resembles different species of *Taphrina*, *Lecythophora* and *Ophiostoma* no phylogenetic relationship could be detected using complete sequences of the 18S rDNA. The genus *Hypozyma* appears polyphyletic. *H. lignicola* is phylogenetically closely related with *Symbiotaphrina kochii*. *H. variabilis* shows affinities to *Calyptozyma arxii*. **Black Yeasts**. As already shown by Berbee (1996; Mol. Biol. Evol. 13: 462-470) black yeasts and **bitunicate Ascomycota** have a **polyphyletic** origin. From the museum of natural sciences in Vienna we have isolated a new *Exophiala* species which belongs to the Chaetothyriales and a new *Capnobotryella* species which belongs to the Dothideales.

BASIDIOMYCOTA. Three distinct classes: Urediniomycetes, Ustilaginomycetes, Hymenomycetes. In addition to complete sequences of the 18S rDNA and partial sequences of the 26S rDNA (D1/D2-region) the qualitative and quantitative monosaccharide pattern of purified cell walls can be used to separate distinct classes. In a neighbour-joining tree using distance estimation according to Jin & Nei (1990) the topology of the phylogenetic tree changes according to the evolution model. One parameter model of Jukes & Cantor (1969): The Urediniomycetes appear basal. Two parameter model of Kimura (1980) the Ustilaginomycetes are the basal group. Ultrastructural and physiological data on smut fungi are in favour of the Urediniomycetes as basal group within the Basidiomycota. **Asterotremella**. Based on complete sequences of 18S rDNA yeast isolates from the agarics *Asterophora*

lycoperdoides and *A. parasitica* are phylogenetically closely related with *Cryptococcus humicola*. *C. humicola* is phylogenetically distinct from the type species of the genus *Cryptococcus*, *C. neoformans*. We therefore have introduced the genus *Asterotremella* for basidiomycetous yeasts without arthrospores and affinities to the genus

Trichosporon. We interpret *A. lycoperdoides* and *A. parasitica* as sexual symbionts and missing links in the evolution from mycoparasitism to heterothallism. **Rhodotorula**. The genus *Rhodotorula* is extremely heterogeneous. Species of *Rhodotorula* cluster within the Urediniomycetes (e.g. Cystobasidiales: *R. minutula*; Microbotryales: *R. glutinis*) and Ustilaginomycetes (e.g. Microstromatales: *R. bacarum*; Ustilaginales: *R. acheniorum*). **Schizonella**. Smut fungi of the dimorphic genus *Schizonella* cluster within the Ustilaginales. We have isolated a new species from *Carex atrata*.

XV. National Collection of Agricultural and Industrial Microorganisms, H-1118, Budapest Somlo ut 14-16. Hungary. Communicated by G. Peter <gpeter@hoya.kee.hu>.

The following articles has been published since our last report:

1. Péter, G., Tornai-Lehoczki, J., Dlauchy, D. & Vitányi, G. 2000. *Pichia sporocuriosa* sp. nov., a new yeast isolated from rambutan. *Antonie van Leeuwenhoek* **77**: 37-42. (The abstract appeared earlier in the Yeast Newsletter)
2. Tornai-Lehoczki, J., Dlauchy, D. 2000. Delimitation of brewing yeast strains using different molecular techniques. *Int. J. Food Microbiol.* **62**: 37-45.

In general, the genetic characteristics, the phenotype and the microbial purity of the production brewing yeast strains are among the most important factors in maintaining a consistently good quality of products. Analysis of restriction fragment length polymorphism (RFLP) patterns of 18S rRNA-coding DNA was investigated to group ale and lager strains. All production brewing yeast strains showed the same RFLP pattern as the type strain and synonym type strains of *S. cerevisiae*, and were quite

different from the type and synonym type strains of *S. pastorianus*. Based on these data, all production brewing yeast strains investigated in this study appeared to belong to *S. cerevisiae*. Electrophoretic karyotyping and random amplified polymorphic DNA (RAPD) analysis appeared to be suitable methods for distinguishing not only the type and synonym type strain of *S. cerevisiae* and *S. pastorianus*, but also the ale and the lager strains.

XVI. Microbial Genomics and Bioprocessing Research Unit, NCAUR, ARS, USDA, 1815 N. University St., Peoria, IL 61604-3999, USA. Communicated by C.P. Kurtzman.

Recent publications.

1. C.P. Kurtzman, M.A. Lachance, H.V. Nguyen & H. Prillinger. 2001. (1485) Proposal to conserve the name *Kluyveromyces* with a conserved type (Ascomycota: Hemiascomycetes, Saccharomycetaceae). *Taxon* **50**:907-908.

Various molecular comparisons have shown the genus *Kluyveromyces* to be polyphyletic. If use of the genus name were restricted to the type species, *K. polysporus*, and related species, the economically important *K. lactis*/*K. marxianus* clade (*K. aestuarii*, *K. dozhanskii*, *K. lactis*, *K. marxianus*, *K. nonfermentans*, *K. wickerhamii*) would need to be renamed, resulting in considerable confusion among biotechnologists,

geneticists and others. Recent changes in the International Code of Botanical Nomenclature permit conservation of important names that would otherwise be lost to revisions. Consequently, this paper proposes to restrict the genus *Kluyveromyces* to the *K. lactis*/*K. marxianus* clade and to select *K. marxianus* as the new type species for the genus. Under this proposal, *K. marxianus* is designated a conserved type.

2. C. P. Kurtzman and J. Sugiyama. 2001. Ascomycetous Yeasts and Yeastlike Taxa. The Mycota VII Part A, Systematics and Evolution.

This book chapter discusses the ecology, economic importance and systematics of the ascomycetous yeasts and their relatives.

3. C.P. Kurtzman, C.J. Robnett, and E. Basehoar-Powers. 2001. *Zygosaccharomyces kombuchaensis*, a new ascoprogenous yeast from 'Kombucha tea'. *FEMS Yeast Research* **1**:133-138

A new ascoprogenous yeast, *Zygosaccharomyces kombuchaensis* sp. n. (type strain NRRL YB-4811, CBS 8849), is described; it was isolated from Kombucha tea, a popular

fermented tea-based beverage. The four known strains of the new species have identical nucleotide sequences in domain D1/D2 of 26S rDNA. Phylogenetic analysis of D1/D2 and 18S

- rDNA sequences places *Z. kombuchaensis* near *Z. lentinus*. The two species are indistinguishable on standard physiological tests used for yeast identification, but can be recognized from differences in RFLP patterns obtained by digestion of 18S-ITS1 amplicons with the restriction enzymes *Dde* I and *Mbo* I.
4. S.O. Suh, C.P. Kurtzman and M. Blackwell. 2001. The status of *Endomyces scopularum*--a filamentous fungus and two yeasts. *Mycologia* **93**(2):317-322
- Given the clouded history of species of *Endomyces* and the Endomycetales, we determined partial sequences of nuclear small and large subunits of the ribosomal RNA gene for three cultures of *Endomyces scopularum*, a parasitic fungus of agaric basidiocarps, in order to clarify the phylogenetic position of the species within the ascomycetes. As the result of sequence comparisons, the three cultures were divided into two distinct groups in phylogenetic trees. Strain CBS 131.86 is in an ophiostomatalean clade in the euascomycetes. By comparison, CBS 154.92 and CBS 155.92, which have identical sequences in both subunits of rDNA, are in the Saccharomycetales clade, *Candida fukazawae*, *C. sagamina*, and *C. fungicola*, inhabitants of basidiocarps, were the closest taxa to the two yeast cultures.
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- XVII. Institut für Pflanzengenetik und Kulturpflanzenforschung, Corrensstr. 3, D-06466 Gatersleben, Germany. Communicated by G. Kunze <kunzeg@ipk-gatersleben.de>.**
- Recent publications:
1. T. Wartmann, G. Gellissen & G. Kunze. 2001. Regulation of the *AEFG1* gene, a mitochondrial elongation factor G from the dimorphic yeast *Arxula adeninivorans* LS3. *Curr. Genet.* (in press).
- Oxygen influences the synthesis of mitochondrial proteins by alteration of the expression of mitochondrial genes as well as several nuclear genes. One of those nuclear localised genes is the *EFG1* gene that encodes the mitochondrial elongation factor G (MEF-G). This unique gene (*AEFG1*) has been isolated from the non-conventional dimorphic yeast *Arxula adeninivorans* LS3. The *AEFG1* gene comprises a ORF of 2274 bp, which corresponds to 757 amino acids. In the present study the regulation of *AEFG1* has been analysed for different morphological stages of *Arxula adeninivorans* and culture conditions. It could be demonstrated that the transfer of aerobic growing cultures to anaerobic conditions resulted in an accumulation of *AEFG1* transcript, correlated with an increase in AMEF-G protein concentration. Since this regulation occurs in budding cell culture growing at 30°C and in the both mycelia cultures grown at 45°C and at 30°C respectively, the oxygen level, but not the cultivation temperature or the morphologic stage influences the *AEFG1* regulation.
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- XVII. Department of Biology, Queen's University, Kingston, ON, Canada K7L 3N6. Communicated by J. Karagiannis <karagiaj@biology.queensu.ca>.**
- Recent publication:
1. J. Karagiannis and P.G. Young. 2001. Intracellular pH homeostasis during cell cycle progression and growth state transition in *Schizosaccharomyces pombe*. *J. Cell Science* **114**:2929-2941.
- Accurate measurement of intracellular pH in unperturbed cells is fraught with difficulty. Nevertheless, using a variety of methods, intracellular pH oscillations have been reported to play a regulatory role in the control of the cell cycle in several eukaryotic systems. In this report we examine pH homeostasis in *Schizosaccharomyces pombe* using a non-perturbing ratiometric pH sensitive GFP reporter. This method allows for accurate intracellular pH measurements in living, entirely undisturbed, logarithmically growing cells. In addition, the use of a flow cell allows internal pH to be monitored in real time during nutritional, or growth state transition. We can find no evidence for cell cycle related changes in intracellular pH. In contrast, all data is consistent with a very tight homeostatic regulation of intracellular pH near 7.3 at all points in the cell cycle. Interestingly, pH set point changes are associated with growth state. Spores, as well as vegetative cells starved of either nitrogen, or a carbon source, show a marked reduction in their internal pH as compared to logarithmically growing vegetative cells. In both cases, however, homeostatic regulation is maintained.
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- XVIII. Japan Collection of Microorganisms, RIKEN, Wako, Saitama 351-0198, Japan. Communicated by M. Hamamoto <hamamoto@jcm.riken.go.jp>.**
- The Japan Collection of Microorganisms (JCM) was established in 1980 and started to preserve microorganisms as a culture collection from 1982. Therefore, we had a symposium in celebration of the 20th anniversary of the JCM. Dr. C.P.

Kurtzman (ARS Culture Collection, USA) kindly presented a talk entitled "Culture Collection as Catalysts for Studies on Molecular Systematics of Yeasts" in the symposium. Some of the directors of the culture collections in Japan spoke about the perspective of operations of culture collection of microorganisms in the 21st Century. Now we have 10197 strains (3437 yeast and fungal, 6555 bacterial and 205 archaeal strains) in JCM, among whom 6485 strains are available for distribution. From 1984 we started to distribute our strains and about 3000 strains per year were

The following articles have appeared, or are in press.

1. Bai, F.-Y., Takashima, M. and Nakase, T. 2001. Description of *Bullera kunmingensis* sp. nov., and clarification of the taxonomic status of *Bullera sinensis* and its synonyms based on molecular phylogenetic analysis. FEMS Yeast Research **1**:103-109.

A ballistoconidium-forming yeast strain, CH 2.506, isolated from a semi-dried leaf of *Parthenocissus* sp. collected near Kunming City in Yunnan, China, was shown to be closely related to the non-ballistoconidium-forming species *Cryptococcus luteolus* (Saito) C.E. Skinner and the ballistoconidium-forming species *Bullera sinensis* Li by molecular phylogenetic analysis based on 18S rDNA sequencing. This strain was demonstrated to represent a distinct undescribed yeast species by internal transcribed spacer (ITS) region sequence and G+C content comparison and DNA-DNA relatedness, for which the name *Bullera kunmingensis* sp. nov. is proposed. Meanwhile, the

2. Bai, F.-Y., Takashima, M. and Nakase, T. 2001. Phylogenetic analysis of strains originally assigned to *Bullera variabilis*: descriptions of *Bullera pseudohuiaensis* sp. nov., *Bullera komagatae* sp. nov. and *Bullera pseudoschimicola* sp. nov. Int. J. Syst. Evol. Microbiol. **51**:2177-2187.

Twenty strains previously assigned to the species *Bullera variabilis* Nakase & Suzuki were reclassified using a molecular taxonomic approach. The strains were regrouped first by nucleotide sequence comparison of the rDNA internal transcribed spacer (ITS) regions, including the 5.8S gene. Phylogenetic positions of *B. variabilis* strains with different ITS region sequences were then analysed based on their 18S rDNA sequences. The taxonomic status of the original *Bullera variabilis* strains was clarified further by DNA-DNA hybridization experiments. Of the 20 strains studied, five remained in the species *B. variabilis*, six strains were reassigned to the species *Bullera mrakii* and three novel species were

3. Takashima, M., Sugita, T., Shinoda, T. and Nakase, T. 2001. Reclassification of the *Cryptococcus humicola* complex. Int. J. Syst. Evol. Microbiol. **51**:2199-2210.

Ten strains of the *Cryptococcus humicola* complex were reclassified on the basis of sequence analyses of 18S rDNA and internal transcribed spacer regions and DNA-DNA reassociation experiments. They were differentiated into seven species including

C. humicola. Five novel species are proposed: *Cryptococcus daszewskae* sp. nov. (type strain CBS 5123^T = JCM 11166^T =MUCL 30649^T), *Cryptococcus fragicola* sp. nov. (type strain JCM 1530^T =CBS 8898^T), *Cryptococcus longus* sp. nov. (type strain CBS 5920^T =JCM 11167^T =MUCL 30690^T), *Cryptococcus musci* sp. nov. (type strain JCM 1531^T =CBS 8899^T) and

distributed in recent years. We also started to publish the JCM strain catalogue once every 3 years since 1983 and will publish the eighth edition on January 2002. We can now accept your applications and inquiry by e-mail <curator@jcm.riken.go.jp> or fax (+81 48 462 4617). You can also get a wealth of information on microorganisms (yeasts, fungi, a yeast-like alga *Prototheca*, bacteria and archaea) from the catalogue on the JCM home page at <http://www.jcm.riken.go.jp/>.

taxonomic relationships among *Bullera sinensis* and its synonyms *B. derxii* Nakase & Suzuki and *B. alba* (Hanna) Derx var. *lactis* Li, were clarified on the basis of molecular phylogenetic analysis and DNA-DNA reassociation. *B. derxii* was confirmed to be conspecific with *B. sinensis*, while *B. alba* var. *lactis* was shown to represent a variety of *B. sinensis*. A new combination, *Bullera sinensis* Li var. *lactis* (Li) Bai, Takashima et Nakase, is therefore proposed. Comparative analysis of different types of molecular criteria employed in the present study suggested that when inferring phylogenetic relationships among sibling taxa, sequence data from ITS regions should be interpreted with caution.

proposed for eight of the nine remaining strains, namely *Bullera pseudohuiaensis* sp. nov. (one strain; type strain JCM 5984^T =AS 2.2203^T), *Bullera komagatae* sp. nov. (one strain; type strain JCM 5983^T =AS 2.2202^T) and *Bullera pseudoschimicola* sp. nov. (six strains; type strain JCM 3915^T =AS 2.2201^T). The remaining strain, JCM 6140, was closely related to *B. pseudoschimicola*. However, differences in ITS region sequences between strain JCM 6140 and strains of *B. pseudoschimicola*, and the intermediate DNA-DNA relatedness to representative strains of *B. pseudoschimicola* did not allow a definite taxonomic decision to be made for strain JCM 6140.

Cryptococcus pseudolongus sp. nov. (type strain JCM 9712^T =CBS 8297^T). A syntype of *Sporobolomyces albidus* JCM 1460^T is also revealed to be a distinct species; the name *Cryptococcus ramirezgomezianus* nom. nov. is therefore proposed for *Sporobolomyces albidus* Ramirez Gomez (type strain IJFM 502^T =CBS 2839^T =JCM 1460^T =NRRL Y-2478^T), since the name *Cryptococcus albidus* (Saito) C. E. Skinner has already been recognized for a distinct species within the genus *Cryptococcus*. Strains possessing either Q-9 or Q-10 have been reported to occur in *C. humicola*; however, after reclassification, the ubiquinone type of the species in each phylogenetic group was shown to be uniform, indicating that it is a useful criterion for the

taxonomy of the Trichosporonales.

4. Nagahama, T., Hamamoto, M., Nakase, T., Takami , H. and Horikoshi, K. 2002. Distribution and identification of red yeasts in deep-sea environments around the northwest Pacific Ocean. *Antonie van Leeuwenhoek* **80**:101-110.

We isolated 99 yeast strains including 40 red yeasts from benthic animals and sediments collected from the deep-sea floor in various areas in the northwest Pacific Ocean. Comparing the yeast isolates from animals and sediments collected from shallow locations, the proportion of red yeasts differed considerably, comprising 81.5% and 10.6% of the isolates from animals and sediments, respectively. All of the red yeast isolates belonged to the genera *Rhodotorula* and *Sporobolomyces*. On the basis of morphological and physiological characteristics, the isolates were identified as *R. aurantiaca*, *R. glutinis*, *R. minuta* and *R. mucilaginosa* of the genus *Rhodotorula*, and *S. salmonicolor* and *S. shibatanus* of the genus *Sporobolomyces*. Only *R. glutinis* and *R. mucilaginosa* were isolated from sediments. All of the others were isolated from animal sources. Phylogenetic analyses based

on internal transcribed spacer (ITS) regions and 5.8S rRNA gene sequences allowed us to establish the precise taxonomic placement of each of the isolates and thereby investigate the intraspecific relationships among the isolates. Twenty-two strains identified as members of *R. glutinis*, which showed a wide distribution in the deep-sea, and five isolates identified as *R. minuta*, which were isolated only from benthic animals, showed substantial heterogeneity within the species. The isolates phenotypically identified as *Sporobolomyces* species and *R. mucilaginosa* phylogenetically occupied the placements corresponding to these species. Some strains assigned to known species on the basis of phenotypic features should be regarded as new species as suggested by the results of molecular analysis.

5. Fungsin, B., Hamamoto, M., Arunpairojana, V., Sukhumavasi, J., Atthasampunna, P. and Nakase, T. 2002. *Kockovaella barringtoniae*, a new basidiomycetous yeast species isolated from a plant leaf collected in a tropical rain forest in Thailand. *Int. J. Syst. Evol. Micorbiol.* **52**(1). (in press).
6. Takashima, M. and Nakase, T. *Tilletiopsis derxii*, *Tilletiopsis oryzicola* and *Tilletiopsis penniseti*, three new species of the ustilaginomycetous anamorphic genus *Tilletiopsis* isolated from leaves in Thailand. *Antonie van Leeuwenhoek* (in press).
7. Nagahama, T., Hamamoto, M., Nakase, T. and Horikoshi, K. *Rhodotorula lamellibrachii* sp. nov., a new yeast species from a tubeworm collected at the deep-sea floor in Sagami Bay and its phylogenetic analysis. *Antonie van Leeuwenhoek* (in press).
8. Hamamoto, M., Thanh, V. N. and Nakase, T. *Bannoaa hahajimensis* gen. nov., sp. nov., and three related anamorphs *Sporobolomyces bischofiae* sp. nov., *Sporobolomyces ogasawarensis* sp. nov. and *Sporobolomyces syzygii* sp. nov., yeasts isolated from plants in Japan. (accepted by IJSEM).

XIX. Department of Soil Biology, Faculty of Soil Science, Moscow State University, Vorobyovy Hills, Moscow 119899, Russia. Communicated by I.P. Babjeva.

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

1. Babjeva I.P., Kartinzeva A.A., Maximova I.A., Chernov I.Yu. 1999. Yeasts in spruce forest of the Central Forest Reserve. *Vestnik MGU* (Proceedings of Moscow State University), ser. Soil Science, **4**: 45-49 (in Russian).

In spruce forests of central zone of Russia yeast fungi are found mainly on plant material and are usually scarce in soils. Some yeast species are found to be connected with certain kinds

of plant substrata. This phenomenon can be accounted for the yeasts ecological characteristics and coevolutional relations with plants and invertebrates.

2. Babjeva I.P., Lisichkina G.A., Maksimova I.A., Reshetova I.S., Terenina E.E., Chernov I.Yu. 2000. A new yeast species *Candida anutae* sp. nov., from the fruiting bodies of Agarics. *Mikrobiologija* (Microbiology) **69**(2): 225-228 (in Russian).

Among the yeasts isolated from the fruiting bodies of different species of agarics picked in forests near Moscow and Turku (Finland) in 1995-1998, populations of an earlier unknown species, morphologically similar to *Metshnikowia lunata* but

different from it by physiological characteristics and the absence of ascii with spores, were constantly found. Description of new species is given within the genus *Candida* Berkhoult.

3. Babjeva I.P., Lisichkina G.A. 2000. A new species of psychrophilic basidiomycetous yeasts *Leucosporidium fasciculatum* sp. nov. *Mikrobiologija (Microbiology)* **69**:801-804 (in Russian).
4. Terenina E.E., Chernov I.Yu. 2001. Taxonomic structure of yeast communities associated with invertebrates. *Mikologija i Phytopatologija (Mycology and phytopatology)* **35**(4): 65-73 (in Russian).

Yeast are very common in the intestinal tract of various forest inhabiting invertebrates in the Moscow Region. Ascomycetous yeasts are generally predominate in invertebrates while species of basidiomycetous affinity are the most widespread in background substrates such as plants, plant debris, and soil. The taxonomic composition of yeast community in the

intestinal tract of an invertebrate is shown to be determined first of all by its trophic type. Yeasts are especially abundant in phytophagous invertebrates. Ubiquitous ascomycetous yeast species are the most common in phylophagous invertebrates while specialized symbiotic yeast communities are formed in xylophages and mycophages.

XX. U.O. Microbiologia, Istituto Agrario di S. Michele, via Mach, 1. 38010 S. Michele all'Adige, Italy.
Communicated by A. Cavazza Agostino. <Cavazza@mail.ismaa.it>.

Every year the Istituto Agrario di S. Michele and Cavit winery cooperate in a program of commercial wine yeast quality control. The 2001 results can be found on the institute web page: www.ismaa.it. The checked parameters are: Yeast viability, presence of contaminants (non-*Saccharomyces* yeasts and Lactic Acid Bacteria). Kinetic parameters on 2 grape musts (latency, maximum fermentation rate, Total Fermentation Time) and wine composition after fermentation (acetic acid produced, residual sugars and acetaldehyde). The karyotypes of all yeast strains are also shown.

XXI. PROIMI, Avda. Belgrano y Pje. Caseros, T4001 MVB, S.M. de Tucumán, Argentina.
Communicated by J.F.T. Spencer <alragout@proimi.edu.ar>

The following mini-review was published recently.

1. J.F.T. Spencer, A.L. Ragout de Spencer, and C. Laluce. Non-Conventional Yeasts.

In the beginning there was yeast, and it raised bread, brewed beer, and made wine. After, not many days but many centuries and even millenia later, it was named *Saccharomyces cerevisiae*. After more years and centuries, there was another yeast, and it was named *Schizosaccharomyces pombe* and now there were two stars in the yeast heaven. In only a few more years there were other yeasts and then more, and more, and more. The era of the non-conventional yeasts had begun. The current book on yeast taxonomy (Kurtzman and Fell, 1998) lists more than 700

species. Undoubtedly among these species will be found many, possessing useful qualities and abilities, which will far outnumber those in both Wolf's groups and the others listed here. While the yeasts, *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe* have been used in biotechnological processes such as food and beverage manufacture for millennia, recently other yeast species, the nonconventional yeasts, have become increasingly important.

XXII. Departamento de Biotecnología, Universidad Autónoma Metropolitana, Iztapalapa, Apartado Postal 55-535, México D.F. 09340, Mexico. Communicated by Mariano García-Garibay and Lorena Gómez-Ruiz <jmgg@xanum.uam.mx and lcgr@xanum.uam.mx>.

The following are papers that will be published during the current year by our group.

1. A.R. Estrada-Godina, A.E. Cruz-Guerrero, P. Lappe, M. Ulloa, M. García-Garibay and L. Gómez-Ruiz. 2001. Isolation and identification of killer yeasts from *Agave* sap (aguamiel) and pulque. *World J. Microbiol. Biotechnol.* 17 (in press).

Wild killer yeasts have been identified as inhibitory to strains used as starters in the production of alcoholic beverages such as beer and wine; therefore, killer or killer-resistant strains have been sought for use in alcoholic fermentations. In the current paper a total of 16 strains belonging to six species were isolated. From two samples of *Agave* sap (aguamiel) the following yeast strains were isolated: *Candida lusitaneae* (1), *Kluyveromyces marxianus* var. *bulgaricus* (2), and *S. cerevisiae* (*capensis*) (1). Additionally, in seven samples of pulque (the fermented product), the species *Candida valida* (6 strains),

Saccharomyces cerevisiae (*chevalieri*) (4), *S. cerevisiae* (*capensis*) (1), and *Kluyveromyces marxianus* var. *lactis* (1) were found. The killer strains were *C. valida* and *K. marxianus* var. *lactis* from pulque and *K. marxianus* var. *bulgaricus* from aguamiel. One strain of *S. cerevisiae* (*chevalieri*) isolated from pulque which did not show killer activity was, on the other hand, resistant to other killer strains and it had a remarkable ethanol tolerance, suggesting that this strain could be used for alcohol production.

2. E. Barranco-Florido, M. García-Garibay, L. Gómez Ruiz and A. Azaola. 2001. Immobilization system of *Kluyveromyces marxianus* cells in barium alginate for inulin hydrolysis. *Process Biochem.* (in press).

A suspension of *Kluyveromyces marxianus* cells (256 mg/ml) with inulinase activity were immobilized in barium alginate treated with glutaraldehyde. After five runs of inulin hydrolysis the immobilized cells system had up to 85 % residual activity. When beads were heat treated at 65°C for 5 min, the hydrolysis rate of inulin was improved without affecting the stability of the system in five hydrolysis batches. Immobilized enzyme was thermostable at temperatures between 45 and 65°C. The system efficiency (η) was highly dependent on beads

diameter, reaching values up to 0.79 with a bead diameter of 1.43 mm. Inulin diffused into alginate matrix with a diffusion coefficient (D_E) of $2.5 \times 10^{-6} \text{ cm}^2/\text{s}$, resulting in apparent kinetic values of the immobilized enzyme of $K_m' = 0.522 \text{ mM}$ and $V_{max}' = 113.7 \mu\text{mol min}^{-1}$; while values for free enzyme were 0.15 mM and $250 \mu\text{mol min}^{-1}$. Thiele module values (Φ) were close to 1, indicating that the limitations of the system were due to diffusional resistance of substrate.

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

The following paper, whose abstract was given in the last issue, has now been published.

1. Kirschner, R., Sampaio, J.P., Gadinho, M., Weiss, M. and Oberwinkler, F. 2001. *Cuniculitrema polymorpha* (Tremellales, gen. and sp. nov.), a heterobasidiomycete vectored by bark beetles, which is the teleomorph of *Sterigmatosporidium polymorphum*. *Antonie van Leeuwenhoek*. **80**: 149-161.

The following paper was accepted for publication.

2. Valério, E., Gadinho, M. and Sampaio, J.P. *Sporobolomyces odoratus* sp. nov., a new species in the *Sporidiobolus ruineniae* clade. *FEMS Yeast Research*.

This report presents the description of a new *Sporobolomyces* species, *Sp. odoratus* sp. nov. The new species was characterized by DNA fingerprinting using the micro/minisatellite-primed PCR approach (MSP-PCR). A phylogenetic analysis using the D1/D2 region of the 26S rDNA revealed that *Sp. odoratus* belongs to a clade that includes *Rhodosporidium fluviale* and *Sporidiobolus ruineniae*. An

integrated comparison with related species is presented. A number of presumptive strains of *S. ruineniae* were also investigated using the MSP-PCR method and 26S rDNA sequence analysis. Mating experiments revealed, for the first time, that *S. ruineniae* includes heterothallic strains, besides those already known to be self-fertile.

XXIV. Research Institute for Viticulture and Enology, Matúškova 25 831 01 Bratislava, Slovakia.
Communicated by E. Minárik.

The following papers were recently published.

1. E. Minárik. 2001. Optimizing efficient conditions of active dry wine yeasts. *Vinič a víno* **1**:7-8 (in Slovak).

The following most important viewpoints and parameters of active dry wine yeast performance are summarized: 1. rapid ability to ferment and yeast reproduction, 2. low foaming, 3. 2-3 g/l residual saccharides in the wine after fermentation has finished, 4. optimum colour and tannin yields, 5. high alcohol

formation and tolerance against ethanol, 6. low volatile acid formation/0.3-0.4 g/l, 7. low substance formation combining SO₂, 8. low H₂ and mercaptan production, 9. not influencing malolactic fermentation.

2. E. Minárik. 2001. Red wine ageing on fine lees. **1**:104 (in Slovak).

Recent results of research confirmed differences in mannoprotein releasing by different yeast strains during alcoholic fermentation of grape must and their influence on wine stability and red wine colour. It is very important to consider the selection

of suitable yeast strain in ageing red wine "sur lies" with-regard to the release of mannoproteins during fermentation and autolysis of dead yeast cells.

3. E. Minárik. 2001. Influence of spontaneous alcoholic fermentation on the content of 2-phenylethanol. *Vinohrad* **39**:8-9 (in Slovak).

Spontaneous grape must fermentation shows increased 2-phenylethanol and glycerol formation as well as higher alcohols and esters, compared with musts fermented by pure yeast starters. In extreme cases even 278-280 mg/l 2-phenylethanol may be formed compared with only 20-40 mg/l

produced by active dry yeast cultures. As 2-phenyl-ethanol is engaged in the bouquet formation of the wine, views of some winemakers on benefits of spontaneous must fermentation are not quite unjustified. Further studies on this phenomenon should be carried out in order to bring out new knowledge in this aspect.

4. E. Minárik. 2001. Fermentation of white wines in barrels ageing on lees/sur lies. *Vinohrad* **39**:4-5 (in Slovak).

White wines ageing in barrels after alcoholic fermentation with occasional stirring become increased intensive in aroma. Taste and odour parameters show higher persistence, harmony of

individual constituents and more favourable white wine colour. Physicochemical influence of the system "sur lies" is considered.

5. E. Minárik. 2000. Contaminations by yeasts in wines during wine production and ageing. *Vinohrad* **38**:7-8 (in Slovak).

By modern techniques of contaminating yeast and yeast-like microorganisms control, methods of decontamination procedures in barrique wine production, perfect management and

elimination of contaminating yeasts, are possible, limiting thus undesirable influences on taste and typical varietal character of barrique wine. Results of recent studies are briefly described.

6. E. Minárik. 2001. Elimination of substances inhibiting alcoholic fermentation of grape must by Yeast ghosts. *Vinohrad* **39**:6-7.

Yeast ghosts recently represent a relatively new category of alcoholic fermentation activators of grape musts in wine production. They increase not only final alcohol level of the wine but enable utilization of residues of fermentable saccharides in last stage of fermentation by yeasts. They possibly also influence the decrease of volatile acid formation. Yeast ghosts

considerably accelerate fermentation start and the whole fermentation process. The International Office of Vine and Wine in Paris has recently recommended the use of yeast ghosts in winemaking to member countries, though they are not widely used at present in winery practice.

XXV. Department of Biology, Faculty of Medicine, Masaryk University, Jostová 10, 66243 Brno, Czech Republic. Communicated by Marie Kopecká <mkopecka@med.muni.cz>.

Recent publications.

1. Ishiguro, J., Shimada S., Gabriel, M., Kopecká, M. 2001. Characterization of a fission yeast mutant which displays defects in cell wall integrity and cytokinesis. *Genes Genet. Syst.* **76**:257-269.
2. Kopecká, M., Gabriel, M., Takeo, K., Yamaguchi, M., Svoboda,A., Ohkusu, M., Hata, K., Yoshida, S. 2001. Microtubules and actin cytoskeleton in *Cryptococcus neoformans* compared with ascomycetous budding and fission yeasts. *Eur. J. Cell Biol.* **80**:303 -311.

XXVI. Collection de Levures d'Intérêt Biotechnologique (CLIB), Laboratoire de Microbiologie et Génétique Moléculaire, INA-PG INRA, BP01, F-78850 Thiverval-Grignon, France. Communicated by Nguyen H.-V. <clib@grignon.inra.fr>.

The following have been recently published.

1. S. Casaregola, H-V Nguyen, G. Lapathitis, A. Kotyk, C. Gaillardin. 2001. Analysis of the constitution of beer yeast genome by PCR, sequencing and subtelomeric sequence hybridization. *Int. J. Syst. Evol. Microbiol.* **51**:1607-1618.
2. Addendum to the article "Genetic identification of *Saccharomyces bayanus* var. *uvarum*, a cider-fermenting yeast. *Int. J. Food Microbiol* **65**:163-167. (2001) communicated by Dr. Naumov in the previous issue of the Yeast Newsletter, p.4.

I was surprised to discover in the Yeast Newsletter the above article with my name as one of the authors. I had informed Dr. Naumov that I did not wish to be a co-author when he told me that the manuscript was accepted. In the work done I am responsible for the molecular identification of the strains used for genetic identification made by him but I disagreed with the suggested nomenclature for strains that I identified and published as *S. uvarum* (Nguyen et al., *Syst. Appl. Microbiol.* **23**:71-85 (2000)). I wrote to Prof. M. Jakobsen, Editor in Chief of International Journal of Food Microbiology asking him to publish a correction of the nomenclature in a future number of the journal.

In the December 2000 issue of the Yeast Newsletter, p. 57, Dr. Naumov used the incorrect name in articles 3 and 6, but the correct name in article 4. In the same issue (p. 72) I pointed out the difference between *S. uvarum* and *S. bayanus*, suggesting that they should not be considered synonyms. By now, publications on *S. uvarum* and *S. bayanus* are presented separately on the NCBI database (<http://www.ncbi.nlm.nih.gov>). There is a need to reinstate *S. uvarum* as a distinct species according to the rules of ICBN.

The proposal made by Naumov (2000) to treat *S. uvarum* as a variety of *S. bayanus* relied solely on low but significant (27%) inbred viability in a cross involving strain B19-3C, a derivative of *S. bayanus* CBS 380T, and *S. abuliensis* MCYC 623 (CBS 7001). These two strains exhibit 91% nDNA relatedness (Vaughan-Martini and Kurtzman, 1985). Separation of subspecies or varieties is normally done when DNA relatedness between two strains is less than 70% (Kurtzman (1987a)). In the cross between CBS 380 and B19-3C, reduced fertility was obviously due to chromosomal changes and chromosome loss (Kurtzman (1987b)). Ryu et al. (1986, 1998) reported a chromosomal translocation in strain B19-3C. We

found that its karyotype lacked three chromosomal bands compared to the *S. bayanus* type strain CBS380 from which it was derived or to *S. uvarum* (Nguyen et al., 2000).

References:

- Naumov G.I. 2000. *Saccharomyces bayanus* var. *uvarum* comb. nov., a new variety established by genetic analysis. *Microbiology* **69**:338-342. Translated from *Mikrobiologiya* 69:410-414.
- Vaughan-Martini, A.,Kurtzman, C.P. 1985. Deoxyribonucleic acid relatedness among species of *Saccharomyces sensu stricto*. *Int.J. Syst. Bact.* **35**:508-511.
- Kurtzman C.P. 1987a. Prediction of biological relatedness among yeasts from comparison of nuclear DNA complementary. *Stud Mycol*, **30**:459-468.
- Kurtzman C.P. 1987b. Molecular taxonomy of industrial yeasts. In: Eds. G.G Stewart, I. Russel, R.D. Klein and R.R. Hielsch (Eds) *Biological research on industrial yeasts*. Vol I, pp 27-45. CRC Press, Inc. Boca Raton, Florida.
- Ryu, S.-L., Murooka, Y., Kaneko, Y. 1996. Genomic reorganization between two sibling yeast species, *Saccharomyces bayanus* and *Saccharomyces cerevisiae*. *Yeast* **12**:757-764.
- Ryu, S.-L., Murooka, Y., Kaneko, Y. 1998. Reciprocal translocation at duplicated RPL2 loci might cause speciation of *Saccharomyces bayanus* and *Saccharomyces cerevisiae*. *Curr. Genet.* **33**:345-351.
- Nguyen, H.-V., Lépingle, A., Gaillardin, C. 2000. Molecular typing demonstrates homogeneity of *Saccharomyces uvarum* and reveals the existence of hybrids between *S. uvarum* and *S. cerevisiae*, including the *S. bayanus* type strain CBS 380. *Syst Appl Microbiol* **23**:71-85.

3. List of strains used in the Genolevures project with their corresponding CBS and ATCC numbers. You may find in databases sequences from the Genolevures project (FEBS Lett. 487 (1), Dec 2000; <http://cbi.labri.u-bordeaux.fr/Genolevures/Genolevures.php3>) where only CLIB numbers are given, the following table gives their corresponding CBS, ATCC numbers and species names:

CLIB	CBS	ATCC	Species
89	7504	20460	<i>Y. lipolytica</i>
179	379	599	<i>S. exiguum</i> T
182	3082	58438	<i>S. kluuyveri</i> T
189	4311	58439	<i>S. servazzii</i> T
197	767	32239	<i>D. hansenii</i> var. <i>hansenii</i> T
210	2539		derivative of <i>K. lactis</i> var. <i>lactis</i>
282	712		<i>K. marxianus</i> T
292	6340	56472	<i>K. thermotolerans</i> T
315	94	750	<i>C. tropicalis</i>
421	4732	34438	<i>P. angusta</i>
491	732	2623	<i>Z. rouxii</i> T
492	7064		<i>P. farinosa</i> var. <i>farinosa</i>
533	7001		derivative of <i>S. uvarum</i>

This last strain was described elsewhere as 623-6c is a derivative of strain CBS7001 (MCYC623), isolated and described as *S. abulensis* by Santa Maria (Biotaonomic study on yeast. Commun. Inst. Nac. Invest. Agrarias, 3:1-61, 1978), due to a

mistake in the original monograph it was always cited as *S. abulensis*. We have found it to be homologous to *S. uvarum* CBS395; in the Genolevures it was used under the name *S. bayanus* var. *uvarum* nomen invalidum.

XXVII. École Nationale Supérieure Agronomique de Montpellier. Ufr. de Microbiologie Industrielle et de Génétique des Microorganismes, Place Pierre-Viala - 34060 Montpellier Cedex. Communicated by G. Moulin <moulin@ensam.inra.fr>.

The following are publications (2000-2001) from my laboratory.

1. Dubreucq E., Ducret A. and Lortie R. 2000. Optimization of lipase catalyzed sorbitol monoester synthesis in organic medium. *J. Suf. Det.* **3**:327-333.
2. Boze, H., Laborde C., Chemardin P., Richard F., Venturin, C., Combarous Y., Moulin, G. 2001. High-level secretory production of recombinant porcine follicle-stimulating hormone by *Pichia pastoris*. *Process Biochemistry*, **36**:907-913.
3. Issaly N., Solsona O., Joudrier P., Gautier M.F., Moulin G., Boze H. 2001. Optimization of the wheat puroindoline-a production in *Pichia pastoris*. *J. Appl. Microbiol.*, **90**:397-406.

Obituary

Professor Robert R. Davenport, M.Sc., Ph.D., FIFST, F.I.Biol., FRIPH (1933-2001)

Professor Bob Davenport, a world authority on spoilage yeasts, died on 9th February 2001, following a long illness. He will be deeply missed by family, friends and colleagues, both academic and in industry.

Robert Raymond Davenport was born in 1933 into a farming family in Gloucester in the West of England, being the eldest of three children. Following National Service, 1952-1954, Bob started his academic career as a laboratory technician assisting research into bone marrow, in the Department of Anatomy at Bristol University. In 1958, he moved to the Long Ashton Research Station, an out-station of Bristol University, as a laboratory assistant in the Cider and Fruit Juices Section. Here he stayed for the next 22 years, perhaps the golden age of yeast research in cider ("hard cider" in the USA) and perry, working with colleagues such as Professor Fred Beech. Anyone lucky enough to visit Long Ashton

during their celebrated open days was struck by their commitment to their research, the friendliness of the researchers and the quality of their single apple-cultivar experimental beverages. Bob Davenport was awarded an M.Sc. in 1970 with a thesis entitled “Epiphytic yeasts associated with the developing grape vine”, and, together with Fred Beech published one of the definitive works on cider yeasts “The role of yeasts in cidermaking” in The Yeasts Vol. 3, A.H. Rose & J.S. Harrison (eds.) Academic press. In 1975, following publication of his monumental thesis “The distribution of yeasts and yeast-like organisms in an English vineyard”, Bob Davenport was awarded his Ph.D. by the University of Bristol. It is clear that during this period, Bob gained much of the insight into the ecology and environmental influences on yeasts that illuminated his later work into industrial spoilage yeast ecology. During the later years at Long Ashton, Bob Davenport’s research moved more towards the spoilage yeasts and in particular to *Zygosaccharomyces bailii*, a species with which he will always be associated in many people’s minds. Together with Dr. Sue Thomas he published what is still the best overview of *Z. bailii* spoilage and characteristics (Thomas & Davenport, 1985, Food Microbiol 2, 157).

With the closure of Long Ashton in 1986, Bob was transferred to the Institute of Food Research Reading, U.K., continuing to work on spoilage by yeasts and becoming widely recognised as a spoilage consultant to industry. From 1993, he worked at the IMI, International Mycological Institute at Kew. Leaving the IMI in 1995 and working as hard as ever, he set up the R.D. Consultancy at his home in Reading. He was in great demand by industry as a confidential consultant, identifying problems and their solutions. Bob Davenport was a scientist so involved in his work that effectively he never retired. Perhaps his greatest asset in this work was his realistic and practical approach. He was one of the few scientists able to link his academic knowledge to practical solutions for immediate use. Professor Davenport pioneered a unique “Forensic Approach” to food spoilage using the microbes present to predict spoilage, lapses of factory hygiene, likely sources of infection etc. This approach was only made possible through Bob Davenport’s depth of experience of spoilage. Bob always made it look so easy, often identifying yeasts with a high degree of accuracy just using a microscope and knowledge of where they had been found. Just as some gardener’s have “green fingers”, Bob’s knowledge of yeasts in industrial environments was extraordinary. Due to Bob’s ethics and consultant confidentiality, little of this knowledge has been published. The loss to science and industry is enormous.

In character, Bob was always warm and good-natured; a giant of a man, both in stature and character, a real gentleman. I will always recall visiting him at work in the wooden buildings at the bottom of his garden where he ran his Consultancy, surrounded by books, papers, scientific equipment, microscopes and photographic equipment. Seated comfortably in an armchair and drinking coffee, he talked with such enthusiasm about yeasts, their behaviour and oddities, techniques for isolation and recovery. Bob Davenport will not be forgotten.

Malcolm Stratford

International Commission on Yeasts

The 21st International Specialized Symposium on Yeasts (ISSY XXI) on “Biochemistry, Genetics, Biotechnology and Ecology of Non-conventional Yeasts”, held in Lviv, Ukraine, was a great success. Andrei Sibirny and co-workers are to be congratulated on presenting an interesting scientific programme for over 150 participants who also enjoyed the attractive town and the excellent social atmosphere of the meeting. A meeting’s report by Hans van Dijken will appear in FEMS Yeast Research Vol.1 Nr. 3. A limited number of abstract books is still available, requests may be sent to <gonchar@biochem.lviv.ua> or <lex.scheffers@tnw.tudelft.nl>.

The meeting of the International Commission on Yeasts in Lviv, on August 22, 2001, was attended by nineteen Commissioners.

It was agreed that the following Symposia will be arranged under the auspices of ICY:

Lex Scheffers, Chair

ISSY XXII (2002) South Africa, Pilanesberg, 25-28 March, 2002. “Yeast Fermentations and other Yeast Bioprocesses”. Chair: James C. du Preez.

ISSY XXIII (2003) Hungary. Preliminary title: “Interactions between Yeasts and other Organisms”. Chair: Tibor Deák.

ISY XI (2004) Brazil. General yeast symposium. Chair: Leda Cristina Mendonça-Hagler

ISSY XXIV (2005) Spain. Preliminary title: “Morphogenesis of Dimorphic Fungal Species: Basic and Applied Aspects”. Chair: Rafael Sentandreu.

ISSY XXV (2006) Finland. Preliminary title: “Physiology of Yeasts and Metabolic Engineering”. Chair: Merja Penttilä.

The next meeting of the International Commission on Yeasts will be held during ISSY XXII in South Africa, during lunch on Tuesday 26 March, 2002, 12.30-14.00 h.

For matters regarding ICY, please contact:

<lex.scheffers@tnw.tudelft.nl>

Recent Meetings

29th Annual Conference on Yeasts of the Czech and Slovak Commission for Yeasts Smolenice, Slovakia, May 23-25, 2001

The 29th Annual Conference on Yeast organized by the Czech and Slovak Comission for Yeasts and the Institute of Chemistry of the Slovak Academy of Sciences brought together 65 scientists from Czech and Slovak Republics and also few participants from the neighboring Hungary. The program consisted of plenary lectures given in three different sessions and poster presentations as shown below.

Plenary lectures in the session Membrane phenomena

Kopecká M., Gabriel M., Svoboda A., Takeo K., Yamaguchi M.: Cytoskeleton in the cell cycle of *Cryptococcus neoformans* and the effects of cytoskeletal inhibitors
Raclavský V., Drivinya A., Hrušková P., Takeo K.: *Cryptococcus neoformans* is able to escape the Rylux BSU and Congo red antifungal action
Šesták S., Farkaš V.: "In situ" assays of enzymes in cells permeabilized by osmotic shock
Hrušková-Heidingsfeldová O., Pichová I.: Effect of concentration of ammonium ions on the expression of secreted aspartic proteinase by *Candida albicans*
Certík M.: Physiological regulation of lipid biosynthesis in yeasts

Plenary lectures in the session Molecular biology and genetics

Špírek M., Sulo P.: Evolution of yeast *Saccharomyces* taxonomic classification
Puta F., Lebduška P., Ambrožková M., Skrútný M., Fuková I., Folk P.: Transcription coregulator snw - functional analysis in yeast
Pichová A., Hlavatá L., Sauerová E., Laun P., Madeo F.: Yeast mother cells of *Saccharomyces cerevisiae* show markers of apoptosis
Hlavatá L., Vondráková D., Sauerová E., Pichová A.: The role of *RAS2* gene in aging of yeast *Saccharomyces cerevisiae*
Dudáš A., Marková E., Chovanec M., Brozmanová J.: DNA double-strand break repair in the yeast *Saccharomyces cerevisiae*
Flegelová H., Vojtíšková K., Novotná D., Janderová B.: Characterization of *S. cerevisiae* *GRF18* mutants with lowered sensitivity to K1 or K2 killer toxin
Malínská K., Janatová I., Mikulík K., Hašek J.: The rpg1p localization and phosphorylation in *Saccharomyces cerevisiae*
Kutejová E., Durcová G., Gach A., Janata J., Perecko D.: Structure and substrate specificity of the mitochondrial Lon protease
Simonics T., Maráz A.: Cloning of a *Schizosaccharomyces pombe* gene, having role in sulphate utilization
Hostinová E., Solovicová A., Gašperík J.: Raw starch degrading glucoamylase produced by food-born yeast *Saccharomycopsis fibuligera*

Plenary lectures in the session Biotechnology

Malík F.: Pure wine yeast cultures in strategies of enological moderna
Šmogrovicová D.: New trends in beer fermentation

Fiala J., Cíková H., Vernerová J.: Lager brewing yeast under HGB conditions
Bláha M., Šavel J., Mareš M., Dostálk P.: Verification of the quality of beer recovered from spent yeast
Márová I., Breierová E., Slovák B., Kocí R., Pokorná J., Omelková J.: Comparison of carotenoid production by some yeast strains under stress conditions

Posters

Flegelová H., Janderová B.: The *Saccharomyces cerevisiae* cells cultivated at extreme temperature or in presence of ethanol exhibit lowered sensitivity to killer toxin K1
Raclavský V., Novotná J., Kafková L., Kolář Z.: *Saccharomyces cerevisiae* *slt2Δ* mutant is sensitive to the cdk-inhibitor bohemine
Poliačková D., Kolarov J., Šabová L.: Is oxidative stress the major cause of BAX induced lethality in *K. lactis*?
Kozovská Z., Šubík J.: Transformed wine yeast strain exhibiting multidrug resistance
Obernauerová M., Kiprichová S., Dobiašová M., Dókušová M., Šubík J.: Isolation of the *PEL1/PGS1* homologue from *Kluyveromyces lactis*
Takáčová M., Sklenář P., Breunig K. D., Gbelská Y., Šubík J.: Complementation of mutations in PDR gene network of *Saccharomyces cerevisiae* using *Kluyveromyces lactis* genomic bank
Sklenář P., Breunig K. D., Šubík J.: Construction and properties of the PDR3-GAL4 chimeric transcription factor
Liptajová D., Sojáková M., Šimoncicová M., Borovský M., Šubík J.: Etiology of vulvovaginal candidosis and susceptibility to antimycotics of clinical yeast isolates
Dostál J., Hrušková-Heidingsfeldová O., Hamal P., Pichová I.: Secretion of aspartic proteinases by clinical isolates of *Candida*
Sauerová E., Vondráková D., Hlavatá L., Pichová A.: Single point mutation in ras2 gene of *Saccharomyces cerevisiae* can suppress the dominant RAS2^{Val19} mutation
Vlčková V., Baborová I.: Antimutagenic activity of homoisoflavonoids on *Saccharomyces cerevisiae*
Špírek M., Poláková S., Škutová D., Sulo P.: Yeast organelle engineering. How the alien mitochondria and nuclei get together
Fekete V., Másiarová E., Sulo P.: Mitochondrial signalization under the extreme condition. Is the ADP/ATP translocator mutant in combination with p^c lethal? How to rescue "death" cells
Marková E., Vlasáková D., Dudáš A., Chovanec M., Brozmanová J.: Overexpression of the *E. coli* RecA protein facilitates DNA double strand break repair in the yeast *S. cerevisiae*
Holoubek A., Vecer J., Brodská B., Sigler K.: H⁺-ATPase-containing proteoliposomes reconstituted from the plasma membrane of *S. pombe* and *S. cerevisiae*
Krasowska A., Chmielewska L., Gapa D., Pokorný M., Holoubek A., Bilinski T., Sigler K.: Sensitivity of different yeast species to hydrophobic, amphiphilic and hydrophilic oxidants

- Havelková M., Unger E.: Leptomycin B: a powerful antibiotic tool for studying nuclear transport
- Szabo R., Štofaníková V.: Role of nitrogen source sensing in the regulation of hyphal growth in *Yarrowia lipolytica*
- Czakó-Vér K., Koós Zs., Antal J., Grama L., Pesti M.: Hereditological analysis of chromium-tolerant mutants of *Schizosaccharomyces pombe*
- Farkas N., Pesti M., Belágyi J.: Changes induced by hexavalent chromium in the plasma membrane of Cr(VI)-sensitive and resistant mutants of *Schizosaccharomyces pombe* strains
- Gazdag Z., Emri T., Farkas N., Zoltán M., Belágyi J., Pócsi I., Pesti M.: Analysis of the kinetics of Cr(VI) reduction in fission yeast
- Novák A., Vágvölgyi Cs., Fekete-Forgács K., Pesti M.: Isoenzyme and RAPD-PCR analysis and adhesion to acrylic of the hybrids of *Candida albicans* morphological mutants
- Šandula J., Kriková L., Duracková Z., Machová E., Kogan G.: Free radical scavenging activity of yeast cell-wall mannans
- Slaniniaková B., Jungová O., Pátková J.: Technological properties of autochthonous wine yeast
- Pátková J., Slaniniaková B., Jungová O.: Parameters for characterisation of wine variety
- Sláviková E., Košíková B., Mikulášová M.: Biodegradation of waste lignin products by soil-inhabiting yeast organism
- Švanová L., Breierová E., Augustín J., Sasinková V., Stratilová E.: Differences in the production of polygalacturonases by yeasts in their exponential phase of growth in physiological and stressed conditions of cultivation
- Szufnarowska M., Breierová E., Dzúrová M., Stratilová E.: *Aureobasidium pullulans* from several sources – the production of polygalacturonases and their properties
- Breierová E., Juršíková P., Sasinková V.: The protective role of polysaccharides toward the toxicity of Cd²⁺ and Ni²⁺ ions for yeast cells
- Certik M., Breierová E., Šimonciková P., Šajbidor J.: Characterization of yeast lipids affected by salt stress
- Certik M., Breierová E., Juršíková P., Šajbidor J.: Lipid structural changes of yeasts grown under heavy metals presence
- Omelková J., Breierová E., Márová I.: The influence of oxidative and osmotic stress on the morphological features of the red yeasts
- Slovák B., Kocík R., Márová I., Pokorná J., Drdák M.: Oxidative stress influences content and composition of carotenoids produced by yeast strain *Rhodotorula glutinis*
- Dostálek P., Palmi P., Patzak M., Vymetalová V.: Cadmium determination in yeast cell by fluorescent probe
- Gregor T., Breierová E., Fišera M., Juršíková P.: Accumulation of Cd²⁺ and Ni²⁺ ions by *Aureobasidium pullulans*
- Vadkertiová R., Sláviková E.: The occurrence of yeasts in the grass-grown soils
- Bartek P., Kolarova N.: Enzymatic galactosylation of polysaccharides by galactosyltransferases from the yeast *Cryptococcus laurentii*

The jubilee 30th Annual Yeast Meeting will be held again in the Smolenice Castle in May 2002 and its program will be devoted to the following areas of yeast research: Yeasts and Problems of Current Medicine, Yeast Apoptosis and Molecular Biology and Genetics of Yeasts. The Committee of the Czech and Slovak Commission for Yeasts plans to invite to the jubilee conference all retired members of the Committee as well as several guest speakers from abroad to join the important celebration of the traditional activities of Czech and Slovak Yeast researchers.

Communicated by Peter Biely

Forthcoming Meetings

22nd International Specialised Symposium on Yeasts:

Yeast Fermentations and other Yeast Bioprocesses

(Organised under the auspices of the International Commission for Yeasts)

Pilanesberg National Park, South Africa, 25-28 March 2002

The theme of ISSY 22 is the biotechnological applications of yeasts. The symposium will be hosted at the Kwa Maritane Game Lodge in the Pilanesberg National Park, 175 km northwest of Johannesburg. The session topics are: Systematics, yeast preservation and starter cultures; Alcoholic beverages and industrial alcohol; Yeasts in food production; Non-food products and processes; Indigenous fermentations; Physiology and regulation of yeast bioprocesses; Metabolic engineering for improved or novel bioprocesses.

Invited speakers: Sakkie Pretorius, University of Stellenbosch, South Africa (Challenging traditional winemaking practices with yeast biotechnological innovations); Morten Kielland-Brandt, Carlsberg Laboratory, Denmark (Amino acids in yeast fermentations); Jens Nielsen, Technical University of Denmark (The role of yeast in modern biotechnology); Clete Kurtzman, National Center for Agricultural Utilization Research, Peoria, USA (Impact of gene sequence analysis on yeast

systematics and on biotechnology); Linda Bisson, University of California, Davis, USA (Eradication of yeast fermentation arrest during wine production: solving an age-old problem with contemporary technologies); Katherine Smart, Oxford Brookes University, UK (Prediction of fermentation performance); Virgílio Loureiro, Instituto Superior de Agronomia, Portugal (Food spoilage yeasts: a technological approach); Peter Raspor, University of Ljubljana, Slovenia (How much minerals yeast can accumulate and tolerate: the physiological and technological constraints); Lene Jespersen, The Royal Veterinary and Agricultural University, Denmark (Occurrence and taxonomic characteristics of strains of *Saccharomyces cerevisiae* predominant in African indigenous fermented foods and beverages); Han de Winde, DSM Life Sciences, The Netherlands (Functional genomics in industrial fermentation; yeast from model to application); Thomas Kjeldsen, Novo Nordisk A/S, Denmark (Protein engineering of insulin for

secretory expression in yeast).

Please note the following deadlines:

25 January 2002: Early registration & accommodation booking

Chair, Organising Committee:

James du Preez

University of the Free State

South Africa

E-mail: dpreezjc@sci.uovs.ac.za

Website: <http://www.uovs.ac.za/faculties/nat/issy22>

7th International Mycological Congress
August 11-17 2002 - Oslo, Norway

Clete Kurtzman and Teun Boekhout have organized for this congress a symposium entitled **Molecular Systematics and Ecology of Yeasts**. Molecular genetic comparisons have radically changed our views on the definition of a species and the criteria for circumscription of genera and higher orders of classification. In turn, the new approaches to systematics affect our perceptions of species ecology and biodiversity. The speakers will address species and genus concepts, classification of ascomycetous and basidiomycetous yeasts from multigene comparisons and the effect that this work is having on the ecology of yeasts as well as on studies of plant and human pathogenic species.

To view the **first circular** and **registration** details, contact

IMC7 Congress Secretariat
P.O.Box 24 Blindern
N-0314 Oslo
Norway

Email: IMC-7@bio.uio.no
<http://www.uio.no.conferences/imc7/>

VIIIth International Fungal Biology Conference
Guanajuato, Gto. Mexico, December 02-06, 2002

This Conference continues the series of specialized conferences devoted to the analysis of cellular and molecular aspects of fungal growth, development, differentiation, and morphogenesis. Begun in 1966 under the banner of Fungal Spore Conference, these truly international gatherings have been held at various sites. The last two Conferences took place in, Konstanz, Germany in 1996 and in Gröningen, The Netherlands in 1999. The Eighth Conference returns the series to the American Continent. We have selected the historic colonial city of Guanajuato in the central México as the meeting place for scholars and students interested in Fungal Biology.

Guanajuato. The City of Guanajuato is located in the Mexican central plateau, about 350 Km N.W. from México City. In December its weather is dry, fresh at night and warm during the day. Its original fame came after discovering silver in the place, which gave rise to a rush of miners around 1550-1560. Guanajuato soon became one of the most important and richest cities in the New Spain as well as the main world silver producer

28 February 2002: Late registration & accommodation booking

(Only a limited number of delegates can be accommodated; therefore early registration is recommended. Delegates are also advised to make their airline reservations as soon as possible due to cutbacks in international flights.)

Secretariat:

Ms Marianne Oosthuizen

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Fax: +27-51- 4041705

E-mail: marianne@proper.co.za

Speakers and Topics: Steve Oliver, Manchester, UK, What makes a species? Redundancy, recombination and reproductive isolation. Clete Kurtzman, Peoria, IL, USA, Genus relationships in the Saccharomycetales from multigene analyses. Álvaro Fonseca, Quinta da Torre, Portugal, Molecular approaches to the reappraisal of species diversity within the ascomycete genus *Taphrina*. André Lachance, London, ON, Canada, Biogeography of floricolous yeasts: is everything everywhere? Jack Fell, Key Biscayne, FL, USA, Molecular ecology of basidiomycetous yeasts in tropical marine habitats. Teun Boekhout, Utrecht, The Netherlands, Systematics of the human pathogen *Cryptococcus neoformans* in the genomics era. Rich Calderone, Washington, D.C., USA, Functional genomics in *Candida albicans* for drug target design.

during the XVIII Century. The city of Guanajuato has been named by UNESCO part of the the cultural heritage of mankind , due to its picturesque location, the number of beautiful palaces, historic buildings, museums and churches erected during the colonial period, its attractive pedestrian alleys, and the underground streets and avenues that cross the city to facilitate traffic. The City has one of the oldest universities in México, and is famous for its cultural atmosphere and the Cervantes International Festival that every autumn converts its three theaters, alleys and squares in the scenario for all kind of activities with leading artists from all over the world.

Guanajuato is served by the Bajío international airport, located about 30 km from the city, with direct flights from main Mexican cities, including México City as well as US cities Los Angeles, Chicago, Dallas, Houston and Atlanta. By road, comfortable coaches connect Guanajuato to México City and other important cities in the country.

Secretariat. Jesús Aguirre; José Ruiz-Herrera, Organizers

International Scientific Committee. Ralph Dean, Louise Glass, Neil Gow, Wilhelm Hansberg, Regine Kahmann, Robert Roberson, Rafael Sentandreu, Hans Sietsema.

Tentative Program. 1. Fungus-host interactions. 2. Secretion and extracellular enzymes. 3. Signal transduction. 4. Non-conventional yeasts. 5. Fungal structure. 6. Fungal growth, development and differentiation. 7. Genetics and Genomics. 8. Fungal pathogenesis. 9. Secondary metabolism. 10. Fungal cell wall synthesis and structure. 11. Sexual development. 12.

Biotechnology. 13. Evolution and phylogenetics. 14. Fungal cytoskeleton. 15. Regulation of fungal metabolism.

Further information. If you are interested in attending the meeting and wish to receive further information, please check our Web page at: www.ira.cinvestav.mx/Cur-even/fungal.htm, or send an email to the address: pre-reg@ira.cinvestav.mx, with the following information: Name, Full address, Tel./Fax Numbers, E-mail, Intention to present a communication, General topic.

1st FEMS Congress

29 June to 3 July, 2003 - Ljubljana, Slovenia

From 29 June to 3 July, 2003, FEMS will hold the 1st European Congress of Microbiology in Ljubljana, Slovenia. This congress will bring together a wide range of microbiologists for a thematic congress with several specialist sessions running in parallel and plenary sessions enhancing trans-disciplinary exchange. Ljubljana offers an excellent range of hotel accommodation in a convenient central European location. Slovenia, as an EU approaching country, is a vibrant, active country currently attracting a great deal of commercial interest. In addition the country is an attractive holiday location with well developed tourist facilities offering the opportunity to combine

business with pleasure.

The Congress Committees are about to be established and are working to assemble an attractive programme covering a wide range of microbiological topics in both theory and practice. A professional trade exhibition and a number of poster sessions will support the programme. We intend to bring together microbiologists from academia, industry, governmental and other organisations to make this a pattern-setting event for future European meetings.

Check future issues of the Yeast Newsletter for updates.

Brief News Items

Postdoctoral positions in Yeast Genetics and Physiology Carlsberg Research Center

This ad was first posted in Nature in November 2001. Evaluation of applications was to begin after 20 November 2001. Prospective applicants should inquire on whether the positions have yet to be filled.

At the Carlsberg Research Center, postdoctoral positions are available for fundamental and applied research on brewing yeast transcriptome analysis (1 position), yeast stress physiology (2 positions) and amino acid uptake and sensing (1 position). Expected duration 2-3 years, as given below. Applications preferably by e-mail: Professor Morten C. Kielland-Brandt, Department of Physiology, Carlsberg Laboratory, Gamle Carlsberg Vej 10, DK-2500 Copenhagen Valby, Denmark, <http://www.crc.dk/yeast/>, e-mail mkb@crc.dk, phone 3327 5331, fax 3327 4765. Applications preferably by 20 November 2001. Contracts are renewed at the shifts of calendar years. Salary similar to similar positions at Danish universities, i.e., at least DKK26,249 per month plus 10% pension deposit.

Stress Resistance in Yeast. Yeast is able to sense and to respond to environmental changes at many levels, including fine-tuning of its gene expression profile. Stressful environmental conditions affect metabolism, growth and viability, and cannot always be avoided in industrial applications of yeast. Ethanol, rapid changes of the osmolarity of the growth medium, low pH, high temperature and starvation for energy can all negatively affect the yeast, and in particular combinations of these factors can be stressful. Insight into the molecular mechanisms that enable yeast to sense and respond to stressful growth conditions has increased with the characterisation of stress-responsive genes and signal transduction pathways mediating the expression. Stress responses are complex, and yeast seems to respond to different stressful conditions by functionally overlapping

components.

The aim of this project is to investigate and exploit how yeast senses and responds to stressful environmental changes. Environmental conditions thought to be important in industrial applications, like osmotic stress, ethanol stress and forced internal acidification, will be studied individually and in combinations. These objectives will be pursued in laboratory yeast strains and in brewer's yeast by i) generating stress-resistant strains by means of mutagenesis and over-expression, ii) identification of the genetic alterations responsible for the stress resistance and iii) studying the importance of the identified components in the stress response. One postdoc position within an EU-contract on High-Gravity Stress with other European academic and industrial partners is open immediately. In addition, we foresee that an internally funded postdoc position will be available in a few months. Both positions are expected to have a duration of three years.

EST-SAGE analysis of beer fermentation. One postdoc position is open immediately to work on the genome and transcriptome of allotetraploid brewing yeast with an expected duration of two years. The main technologies are EST and SAGE. An important basis for the project is our finding in the early 1980-ies that lager brewing yeast is allopolloid (a species hybrid), which is an important fact to consider in breeding efforts, see e.g., Hansen, J. & M.C. Kielland-Brandt: *Nature Biotechnol.* 14, 1587-1591, 1996. It may be necessary to keep a minor part of the findings secret, but we foresee to publish the large majority of the results.

Amino acid uptake and sensing. One postdoc position is open immediately to work on amino acid uptake and sensing with an expected duration of three years. Both fundamental aspects on

amino acid sensing and brewery-oriented aspects of amino acid uptake will be dealt with. A few years ago we discovered that a plasma membrane protein, Ssy1p, with strong homology to amino acid permeases, but without detectable transport activity, is an essential component of a system that senses extracellular amino acids, thereby inducing transcription of several amino acid permease genes. Potencies of amino acids vary by at least 3 orders of magnitude; L-leucine can be sensed at a concentration

a few micromolar (Didion et al.: J. Bacteriol. 178, 2025-2029, 1996; Mol. Microbiol. 27, 643-650, 1998). Other components of the system are Ptr3p, Ssy5p, Stp1p and Stp2p. In an international collaboration, using a new, efficient system for screening and selection, we have found a dominant, constitutive mutant of SSY1. An important part of the further approach will be to look for dominant mutants in which other components of the system are changed.

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Postdoctoral Position

Institute of Microbiology - Duesseldorf University

Starting as soon as possible but not later than March 2002, we have an opening for a Postdoc position at the Institute of Microbiology, University of Duesseldorf, Germany, in the general area of yeast genetics, molecular biology and physiology. In a 3-years EU-funded project together with nine different laboratories and industry, we aim to engineer the yeast *Saccharomyces cerevisiae* into a more reliable system for expression of heterologous membrane transporters, e.g. sugar, ammonium, neurotransmitter, amino acid and ion transporters from mammals and plants. The novel yeast strains will be used to develop new types of screening assays for identifying chemical

compounds that act selectively on the transporters of interest.

A challenging position is offered with the opportunity to interact with colleagues in related and complementary fields of membrane transport research. The applicant is expected to collaborate closely with the other scientific and industrial partners. The preferred background of the applicant is in yeast molecular biology with some experience in membrane transport or heterologous expression systems. The initial salary starts at about 40.000 EUR p. a., depending on scientific credentials and age.

Applications (preferably by e-mail) to:

Dr. Eckhard Boles
Institut fuer Mikrobiologie
Universitaet Duesseldorf
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The Yeast Newsletter in the Library of Congress

Dr. J. Lederberg, who has a keen interest in the history of yeast research, was reassured to discover that the Yeast Newsletter has been deposited in the Library of Congress, in Washington DC, since 1951. [Editor's note: I have assiduously continued to mail each issue to the Library of Congress, as suggested by Herman Phaff in 1987, when he handed over the editorship to me.] Here are the catalogue entries.

Yeast

Type of Material: Serial (Periodical, Newspaper, etc.)
Brief Description: Yeast
London, Ontario, Canada
International Commission on Yeasts and Yeast-like
Microorganisms
1951-
ISSN: 0513-5222

Yeast

LC Control Number: sf 81003104
Type of Material: Serial (Periodical, Newspaper, etc.)
Brief Description: Yeast.
Davis, Calif.
International Commission of Yeasts and Yeast-like
Microorganisms.
v. 28 cm.
Began in 1951?
ISSN: 0513-5222