
Y E A S T

A Newsletter for Persons Interested in Yeast

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

Associate Editors

Peter Biely
Institute of Chemistry
Slovak Academy of Sciences
Dúbravská cesta 9
842 38 Bratislava, Slovakia

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

Yasuji Oshima
Department of Biotechnology
Kansai University
Suita, Osaka 564, Japan

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

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Editorials

Change in the Editorial Board

Dr. B.J.M. Zonneveld informed the International Commission on Yeasts of his intention to resign as Associate Editor of the Yeast Newsletter. Dr. Patrizia Romano has agreed to join the editorial board in her role as coordinator of the Yeasts in Foods and Beverages Network. She informs us that much interest has been expressed by researchers world-wide. As a first project, the group wishes to collect and publish a series of protocols for recovery and identification of yeasts in foods and beverages.

ISSY-19, Braga, Portugal

Dr. Cecília Leão and her organizing committee are to be congratulated for the excellent symposium held at the University of Minho, in Braga, Portugal. Participants agreed that Dr. Leão and her team put together an outstanding scientific program complemented by sumptuous social events. The symposium was characterized by an attention to detail, technical efficiency, discretion, and a sense of esthetics in programs, books of abstracts, room decorations, organization posters, schedule booklets, etc. Our congratulations and gratitude to Dr. Leão and her team.

THE YEASTS Is Out of Print

We are informed that due to its tremendous success, the Fourth Edition of THE YEASTS, A TAXONOMIC STUDY (Kurtzman and Fell, Editors) has been sold out for some time, not even a year after its long awaited publication. We trust that Elsevier will see fit to produce a second printing of this important monograph at their earliest convenience.

I wish all our readers a happy and scientifically prosperous New Year!

M.A. Lachance
Editor

I. All-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>.

The following papers have been published recently.

1. Golubev, W.I. and Nakase, T, 1998. Mycocinogeny in the genus *Bullera*: killer activity of *Bullera unica* and intragenetic killer-sensitive relationships. *Mikrobiologiya* **67**(2):225-230 (English translation: 184-188).

The killer toxin secreted by the type strain of *Bullera unica* is sensitive to proteases and high temperature and has fungicidal activity in a pH range of 3.5-6.0. Ascomycetous yeasts are insensitive to this toxin except for members of Lipomycetaceae and two species of *Debaryomyces*. *B. unica* mycocin displays activity against

basidiomycetous yeasts, primarily those of tremellaceous affinity. The killer phenotype of *B. unica* type strain was incurable. Using all known *Bullera* killers intragenetic killer-sensitive relationships were studied. The results are discussed from taxonomic viewpoint.

2. Okunev, O.N., Matys, V.Yu. and Golubev, W.I. 1998. Fungal phytases: screening of isolates, selection of active producers and enzyme application. Abstr. Int. Conf. "Biocatalysis -98: Fundamentals and Application" (June 13-18, Puschino), 48.
3. Puchkov, E.O., Yurkova, T.V. and Golubev, W.I. 1998. Effects of *Cryptococcus humicolus* killer toxin upon *Cryptococcus terreus* envelope: combined fluorometric and microscopic studies. *Biochim. Biophys. Acta* **1381**(1):61-67.

Killer toxin (mycocin) produced by *Cryptococcus humicolus* 9-6 induced interaction of the fluorogenic dyes, ethidium bromide, propidium iodide, and hemimagnesium 8-anilino-1-naphtalenesulfonate, with the sensitive strain of *Cryptococcus terreus* VKM Y-2253. The toxin also made the cells susceptible to cetyltrimethylammonium bromide and leaky for K ions. When excited at 360 nm, cell-bound ethidium (propidium) fluorescence was enhanced by 8-anilino-1-naphtalenesulfonate, and cell-bound 8-anilino-1-naphtalenesulfonate fluorescence was quenched by ethidium (propidium), indicating energy transfer from

8-anilino-1-naphtalenesulfonate to ethidium (propidium). These results suggest that at least a portion of the probe molecules had the same binding site, possibly the cytoplasmic membrane. The parameters of kinetics of mycocin action were evaluated fluorometrically. They were found to be identical for all probes and depended on mycocin concentration. The fluorescence increment of ethidium and 8-anilino-1-naphtalenesulfonate upon binding to mycocin-treated cells correlated with the fraction of stainable cells and viability.

4. Puchkov, E.O., Kulakovskaya, T.V. and Golubev, W.I. 1998. Envelope of *Cryptococcus terreus* as a target for *Cryptococcus humicolus* killer toxin (mycocin). *Folia Pharm. Univ. Carolinae* **23** (Suppl.):38-41.

In the envelope of *Cryptococcus terreus*, cell wall and/or capsule are the most probable primary permeability barrier for ethidium, propidium, anilino-1-naphtalenesulfonate and cetyltrimethylammonium. The mycocin affect the cytoplasmic

membrane, cell wall and/or capsule and inhibits unspecific esterases in *Cr. terreus* cells. The perturbations in the envelope of sensitive yeast induced by the *Cr. humicola* mycocin are related to the fungicidal action of this killer toxin.

II. Department of Environmental Biology, University of Guelph, Room 3218, Bovey Building, Guelph, Ontario Canada N1G 2W1. Communicated by H. Lee <hlee@uoguelph.ca>.

The following are abstracts of articles that were published recently.

1. Lohmeier-Vogel, E.M., C.R. Sopher & H. Lee. 1998. Intracellular acidification as a mechanism for the inhibition by acid hydrolysis-derived inhibitors of xylose fermentation by yeasts. *J. Industr. Microbiol. Biotechnol.* **20**:75-81.

The main degradation products (furfural, hydroxymethylfurfural, acetate) derived from acid hydrolysis of hemicellulosic materials inhibit growth on and fermentation of xylose by *Pachysolen tannophilus* and *Pichia stipitis* with the latter yeast being the more sensitive. The inhibitory effect was more severe

when the inhibitors were present together in the medium. Agarose immobilization partially protected the yeasts from the deleterious effects of these compounds. Intracellular deenergization and acidification may be the mechanism by which these compounds exert their toxic effect on the yeast cells.

- Lee, H. 1998. The structure and function of yeast xylose (aldose) reductases. *Yeast* **14**:977-984.

Yeast xylose (aldose) reductases are members of the aldo-keto reductase family of enzymes which are widely distributed in a variety of other organisms. In yeasts, these enzymes catalyze the first step of xylose metabolism where xylose is converted to xylitol. In the past 16 years, xylose reductases from yeasts able to ferment or utilize xylose have been isolated and studied mainly because of their importance in xylose bioconversions. In recent years, genes encoding xylose reductases from several yeasts have been cloned and sequenced. A comparison of the primary sequences of yeast

xylose reductases with the much better characterized human aldose reductase and human aldehyde reductase reveals that the yeast enzymes are hybrids between aldo-keto reductases and the short chain dehydrogenases/reductases (SDR) families of enzymes. Why this is so and its evolutionary significance is presently not known. This short review will critically examine the structure and function information that can be gleaned from the sequence comparison. Several interesting questions arise from the sequence comparison and these can provide fruitful areas for further investigations.

III. Department of Applied Microbiology and Food Science, University of Saskatchewan, 51 Campus Drive, Saskatoon, Canada S7N 5A8. Communicated by W. M. Ingledew <ingledew@sask.usask.ca>.

The following papers have been published since our last report.

- N.V. Narendranath, S.H. Hynes, K.C. Thomas and W.M. Ingledew. 1997. Effects of lactobacilli on yeast-catalysed ethanol fermentations. *Appl. Environ. Microbiol.* **63**:4158-4163,

Normal gravity (22 - 24° Plato) wheat mashes were inoculated with five industrially important strains of Lactobacilli at ~10⁵, 10⁶, 10⁷, 10⁸ and 10⁹ CFU/ml in order to study their effects on yeast growth and ethanol productivity. *Lactobacillus plantarum*, *L. paracasei*, *Lactobacillus* # 3, *L. rhamnosus* and *L. fermentum* were used. Controls with yeast but no bacterial inoculation, and additional treatments with bacteria alone inoculated at ~ 10⁷ CFU/ml of mash were included. Decreased ethanol yields were due to the diversion of carbohydrates for bacterial growth and the production of lactic acid. As higher numbers of these bacteria were produced (depending on the strains), 1-1.5 % w/v lactic acid resulted in the case of homofermentative organisms. *L. fermentum*, a heterofermentative organism, produced only 0.5 % w/v lactic acid. When *L. plantarum*, *L. rhamnosus* and *L. fermentum* were inoculated at ~ 10⁶ CFU/ml, approximately 2 per cent decrease in the final ethanol concentration

was observed. Fewer initial numbers (only 10⁵ CFU/ml) of *L. paracasei*, or *Lactobacillus* # 3 were sufficient to cause over 2 per cent decreases in the final ethanol concentrations measured as compared to the control. Such effects after an inoculum of only 10⁵ CFU/ml may be due to the higher tolerance to ethanol of these latter two bacterial species, the more rapid adaptation (shorter lag phase) of these two industrial isolates to fermentation conditions, and/or to their more rapid growth and metabolism. When up to 10⁹ CFU of bacteria/ml were present in mash, approximately 3.8 - 7.6 per cent reduction in ethanol concentration occurred depending on the strain. Production of lactic acid and a suspected competition with yeast for essential growth factors in the fermenting medium were the major reasons for reductions in yeast growth and final ethanol yield when lactic acid bacteria were present.

- S. Wang, K.C. Thomas, W.M. Ingledew, K. Sosulski and F.W. Sosulski. 1997. Rye and triticale as feedstock for Fuel ethanol production. *Cereal Chem.* **74**:621-625.

Normal gravity rye and triticale mashes, containing 20-21 g dissolved solids per 100 ml of mash liquid, were fermented with active dry yeast at 27°C. Fermentations were completed within 48 hr for rye, and within 72 hr for triticale. Supplementation of mashes with urea at a concentration of 8 mM accelerated rates of sugar consumption and fermentation, and reduced fermentation time from 48 hr to 36 hr for rye, and from 72 hr to 48 hr for triticale. Rye fermented faster than triticale, due to its higher level of free

amino nitrogen. Ethanol yields were 356-363 L/tonne of 14% moisture rye grain, and 362-367 L/tonne of 14% moisture triticale. Fermentation efficiencies, which were 90-91% for triticale, and 91-93% for rye, and ethanol yields were comparable to those obtained from wheat, and were not affected significantly by urea supplementation. The replacement of wheats by less expensive crops such as rye and triticale would provide good economic opportunities and alternatives for the fuel alcohol industry.

- S. Wang, K.C. Thomas, W.M. Ingledew, K. Sosulski and F.W. Sosulski. 1997. Production of fuel alcohol from rye and triticale by very-high-gravity (VHG) fermentation. *Appl. Biochem. Biotechnol.* **69**:157-176.

Very-high-gravity (VHG) rye and triticale mashes containing about 28.5 g dissolved solids per 100 ml of mash supernatant were prepared by adjusting water to grain ratios to 2:1. Because of high viscosity which develops during mashing, it was necessary to pretreat ground rye-water slurries with viscosity-reducing enzymes. There were no viscosity problems during the preparation of triticale mashes. Fermentations were conducted at 20°C, with and without 16 mM urea as a nitrogenous supplement. All fermentations were completed within 120-144 hr. Supplementation with urea shortened the times

required for completion of fermentation by 33% for triticale and by 40% for rye. The fermentation efficiencies for both grains ranged between 90-93%. These values are comparable to those reported for wheat, implying competitiveness of rye and triticale as fermentation feedstocks to replace wheat. The final ethanol yields were 409 L for rye and 417-435 L for triticale per tonne (dry basis). For a given size of fermentation vessel, 33% more grain was used in the very-high-gravity fermentation process than in normal gravity fermentation. This resulted in a 35-56% increase in ethanol

concentration in the beer when fermentors were filled to a constant volume. The corresponding reduction in water use by about 1/3 would result in savings in energy consumption in mash heating, mash cooling, and ethanol distillation. Fermentation efficiencies

and final ethanol yields obtained per unit weight of grain fermented were not significantly different from the normal gravity fermentations.

4. S. Wang, K. Sosulski, F. Sosulski, and M. Ingledew. 1997. Effect of sequential abrasion on starch composition of five cereals for ethanol fermentation. *Food Research Internat.* **30**: 603-609.

Five cereals were abraded successively on a Satake abrasive test mill to enhance the starch contents of the pearled grains to improve fermentation efficiency of fuel ethanol plants. Tempering the cereals to 12.5% and 15.0% moisture had no consistent effects on rates of grain mass removal by abrasion but final starch losses to the abraded fines could be minimized by optimizing grain moisture content. Increasing abrasion time from 10 to 55 sec increased the average grain mass removal from 3.5 to 19.5% in four hullless cereals but 70 sec of abrasion was needed to remove 32.6% of two-row

barley to obtain a comparable starch content in the pearled grain. On a dry basis, the pearled grain starch levels were: Canadian Prairie Spring (CPS) red wheat (73.4%); CPS white wheat (72.2%); two-row barley (71.0%); fall rye (70.4%); and triticale (69.1%), which represented, on average, a 12% increase in starch content over those of the original grains. Ethanol yields per tonne of fermentation feedstock were increased by 6.5-22.5% due to abrasion, which would significantly increase ethanol plant throughput and grain fermentability.

5. D. Bayrock, and W.M. Ingledew. 1997. Fluidized bed drying of baker's yeast: moisture levels, drying rates, and viability changes during drying. *Food Research Internat.* **30**:407-415.

Moisture levels, drying rates, and yeast cell viabilities (survival curves) were determined for two commercial compressed baker's yeasts dried in a modified fluidized bed dryer. Unlike the survival curves seen for classical thermal death kinetics, the survival curves for both compressed yeasts stabilized each at a different, distinct viability as temperatures were changed in the fluidized bed dryer. The D and Z values describing drying kinetics were determined from survival curves. The D values obtained for both compressed yeasts showed a linear relationship with drying temperature. At 80°C, compressed yeast #1 exhibited a D value of 4.20 minutes

while the D value for compressed yeast #2 was 8.34 minutes. The Z value for compressed yeast #1 was 28.4°C and for compressed yeast #2 it was 27.5°C. It was determined that the viability of the compressed yeasts in the fluidized bed dryer was not significantly affected by the drying temperature during the warming-up and constant-rate drying periods (when moisture contents were greater than 15%). Mechanisms of death for fluidized bed drying appear to be very different from that reported for spray drying as evidenced by the ~200 fold difference in D values at the same temperature.

6. D. Bayrock, and W. M. Ingledew. 1997. Mechanism of viability loss during fluidized bed drying of baker's yeast. *Food Research Internat.* **30**:417-425.

Saccharomyces cerevisiae was dried in a modified Pulvis dryer and also exposed to moist heat in classical thermal death time experiments. The results showed that dehydration and not moist heat, dry heat, or oxidation was responsible for viability decreases during fluidized bed drying. It was determined that the viabilities of the compressed yeasts placed in the dryer were not significantly affected by the drying temperature during the warming-up and constant-rate drying periods (when moisture contents were greater than 15%). At moisture contents below 15%, when compressed yeasts were in the falling-rate drying period, viability decreased sharply. Phantom thermal death time curves were constructed in order to distinguish between the kinetics of death due to moist heat

(classical thermal death time experiments) and the kinetics of death due to dehydration in the fluidized bed dryer. For both compressed yeasts below 50°C, moist heat D (thermal) values were found to be greater than the dryer dD (dehydration) values which indicates that at such temperatures, the death of both compressed yeasts due to moist heat is less vital than death due to dehydration. With temperatures above 50°C, moist heat was more responsible for killing the compressed yeasts than dehydration. When dried in the presence of trehalose (a known dehydration protectant), compressed yeast #2 showed 30% higher viability than the same yeast dried without trehalose.

IV. Department of Biology, Faculty of Medicine, Masaryk University, Jostova 10, 662 43 Brno, Czech Republic. Communicated by M. Kopecka <mkopecka@med.muni.cz>.

I was invited to serve as Guest Professor at CHIBA University. From July 1, 1998 to January 31, 1999, I will be at the Research Center for Pathogenic Fungi and Microbial Toxicoses, Chiba University, 1-8-1 Inohana, Chuo-ku, Chiba 260, Japan. The following were published recently.

1. Svoboda A. & Slaninova I. 1997. Colocalization of microtubules and mitochondria in the yeast *Schizosaccharomyces japonicus* var. *versatilis*. *Can. J. Microbiol.* **43**:945-953.

2. Gabriel M., Horky D., Svoboda A. & Kopecka M. 1998. Cytochalasin D interferes with contractile actin ring and septum formation in *Schizosaccharomyces japonicus* var. *versatilis*. *Microbiology (UK)* 144 (in the press).
3. Kopecka M. & Gabriel M. 1998. The aberrant positioning of nuclei and the microtubular cytoskeleton in *Saccharomyces cerevisiae* due to improper actin function. *Microbiology (UK)* 144:1783-1797.

V. Gifu Pharmaceutical University, 5-6-1, Mitahora-higashi, Gifu 502, Japan. Communicated by M. Miyata.

Recent publications.

1. M. Miyata, M. Matsuoka, & T. Inada 1997. Induction of sexual co-flocculation of heterothallic fission yeast (*Schizosaccharomyces pombe*) cells by mating pheromones. *J. Gen. Appl. Microbiol.* 43:169-174.

Heterothallic fission yeast (*Schizosaccharomyces pombe*) cells preincubated with sex pheromone, P- or id-factor of the obverse mating-type cells, in mannose synthetic medium (MSM) results in remarkably increased sexual co-flocculation with obverse mating-type cells almost without time lag, *i.e.*, within 10 min. By contrast, comparable flocculation requires over 1 h if untreated control cells are mixed with obverse mating-type cells. The agglutinin of P cells is more inducible than that of M cells. These pheromonal inductions of sexual co-flocculation are inhibited by the addition of cycloheximide or tunicamycin during preincubation

but not by chloramphenicol or hydroxyurea. These results demonstrate that, in addition to (a) the repression of cell division (G1 arrest) and (b) the activation of cell wall autolytic processes (mating-specific elongation of cells: formation of their conjugation tubes), mating pheromones of fission yeast have another important role; (c) to induce sexual co-flocculation (agglutinability). Using our experimental system of preincubation with sexual pheromones, we show that M-agglutinin is heat-stable and its induction is inhibited by tunicamycin, but that P-agglutinin is heat-labile and its induction is only partially inhibited by tunicamycin.

2. M. Miyata, H. Doi, H. Miyata & B.F. Johnson. 1997. Sexual co-flocculation by heterothallic cells of the fission yeast *Schizosaccharomyces pombe* modulated by medium constituents. *Antonie van Leeuwenhoek* 71:207-215.

Novel simple synthetic media for inducing sexual co-flocculation in a short time after mixing heterothallic fission yeast (*Schizosaccharomyces pombe*) cells of h⁻ and h⁺ were devised; The most effective of these, mannose synthetic medium (MSM), contains 0.4% mannose as a carbon source in addition to galactose, KH₂PO₄ (pH 4.0) and 4 vitamins. The addition of galactose to the medium suppressed the asexual self-flocculation but rather promoted the sexual co-flocculation. By transferring and mixing h⁻ and h⁺ cells grown in malt-extract broth plus galactose into MSM, these

heterothallic strains were revealed to be sexually ready through a long period of the log to stationary phases. Furthermore, a variety of C sources and NH₄Cl at various concentrations in various media were examined for their effects upon sexual co-flocculation, conjugation and sporulation; it was found that the sugar concentration strictly affected the progress of the sequence of sexual reproduction at 26 °C but not 30 °C and that sexual co-flocculation of the heterothallic strains was induced only under lower concentrations of C and N source than that for the homothallic one.

VI. Department of Microbiology & Biochemistry, University of the Orange Free State, P.O. Box 339, Bloemfontein 9300, South Africa. Communicated by J.P. van der Walt.

Submitted publication

1. Van der Walt, J.P., M.Th. Smith & H.J. Roelijmans. Four new species in *Lipomyces*. *Systematic and Applied Microbiology* (Submitted January 1998)

Four new species in *Lipomyces*, *L. doorenjongii*, *L. kockii*, *L. yamadae* and *L. yarrowii* are described. In terms of nuclear genome comparison the genus comprises three species-clusters. A key to nine species now assigned to the genus is given. The delimitation of the species by (i) rRNA nucleotide sequence analyses

of the 18S and 25S subunits and (ii) nDNA homology determinations do not invariably lead to the recognition of congruent taxa. The family of the Lipomycetaceae nevertheless, provides an illuminative model for phylogenetic study in terms of more appropriate ribosomal analyses of both its teleomorphic and anamorphic members.

VII. Division of Industrial Microbiology, Department of Food technology and Nutritional Sciences, Wageningen Agricultural University, P.O. Box 8129, 6700 EV Wageningen, The Netherlands. Communicated by J.C. Verdoes <Jan.Verdoes@algemeen .im.wau.nl>.

The following papers have appeared recently.

1. Wery, J., Verdoes, J.C. and van Ooyen A.J.J. (1998). Efficient transformation of the astaxanthin-producing yeast *Phaffia rhodozyma*. *Biotechnology Techniques* **12**: 399-405.

An efficient transformation system for the astaxanthin-producing yeast *Phaffia rhodozyma* was developed based on electroporation that routinely yields approximately 1000 transformants per μg of plasmid DNA. The high transformation efficiency depends on vector integration in the ribosomal DNA (rDNA) and the presence of the homologous glycolytic

glyceraldehyde-3-phosphate dehydrogenase (*gpd*) promoter and terminator to drive the expression of the transposon Tn5 encoded kanamycin resistance gene (Km^{R}) as a selective marker. Using this system stable transformants were obtained, carrying multiple plasmid copies. Plasmid copy number could be markedly increased by deletion of the *gpd* terminator from the transforming plasmid.

2. Weijers, C.A.G.M., Botes, A.L., van Dyk, M.S. and de Bont, J.A.M. 1998. Enantioselective hydrolysis of unbranched aliphatic 1,2-epoxides by *Rhodotorula glutinis*. *Tetrahedron: Asymmetry* **9**: 467-473.

Epoxyde hydrolase catalysed resolution of aliphatic terminal epoxides has been demonstrated for the hydrolysis of a homologous range of unbranched 1,2-epoxyalkanes by the yeast *Rhodotorula glutinis*. Both enantioselectivity and reaction rate were strongly influenced by the chain length of the epoxide used. Enantioselectivity

showed an optimum in the hydrolysis of 1,2-epoxyhexane ($E = 84$). Resolution of (\pm)-1,2-epoxyhexane resulted in (*S*)-1,2-epoxyhexane (e.e. > 98%, yield = 48%) and (*R*)-1,2-hexanediol (e.e. = 83%, yield = 47%).

VIII. The International Centre for Brewing and Distilling, Heriot-Watt University, Edinburgh, Scotland. Communicated by G.G. Stewart <G.G.Stewart@hw.ac.uk>.

The International Centre for Brewing and Distilling (ICBD) was established at Heriot-Watt University in 1988 as a successor to the brewing school that had been in existence since 1971. Interest in brewing education and related areas at Heriot-Watt, however, stretch back much further than this to the turn of the century. The British School of Malting and Brewing at the University of Birmingham closed in the mid-1980s, and the ICBD is now the sole surviving institution in the United Kingdom that provides a package of courses, research, and consultancy for the malting and brewing industries. In addition, the location of the ICBD in Scotland makes it ideally situated to serve the Scotch Whisky industry. The ICBD is a partnership between Heriot-Watt University and the brewing, malting and distilling industries. In addition, to provide important funding, the industries nominate executives and senior technical managers to the Board of Management, the Course Committee and the Research Committee. They also provide visiting lecturers and opportunities for detailed site visits and discussions. The ICBD is based on the self-contained Heriot-Watt campus at Riccarton. The campus is close to Edinburgh Airport. The ICBD is housed in the Biological Sciences building. Although it shares many facilities with other sections of the Department of Biological Sciences, the ICBD has its own suite of laboratories, a micro-malting plant, a 2.5 hL brewery, and two glass pot stills which provide the correct proportions of copper to prevent flavour problems. The brewing pilot plant was designed for maximum versatility and has the capacity for studies on all aspects of the malting, brewing and distilling processes using a range of malted and unmalted cereals to produce mature beers and distilled spirits.

The Centre offers a number of degrees and diplomas:

- BSc Honours Degree in Brewing and Distilling - Four year course.
- Postgraduate Diploma in Brewing and Distilling - Nine months.
- MSc Degree in Brewing and Distilling - One year.
- Postgraduate Diploma in Malting and Brewing - Two to five years, by distance learning.
- MSc Degree in Malting and Brewing - Two to five years, by distance learning.
- MPhil and PhD Degrees - Research Degrees.

The curriculum for the BSc Honours Degree in Brewing and Distilling is modular, and was designed with input from industry members of the Course Committee. This degree includes modules on malting, brewing and distilling science and technology, chemical engineering, packaging technology, applied microbiology, molecular biology, biochemistry, production management and basics of business, plus a research project in the fourth year. Between years three and four, the students are required to undertake a period of at least eight weeks industrial training in a malting, brewing or distilling company. The Postgraduate Diploma and Masters courses contain the same core lectures, laboratory work, industrial lectures and tours, and experience in the pilot plant as is contained in the BSc curriculum. This postgraduate courses are augmented with additional tutorials and a postgraduate lecture series given by members of the industry.

The research programme considers a number of areas: Raw materials, High gravity brewing, Answering biochemical questions with molecular biology techniques, Fermentation performance and its influence on beer and fermented wash flavour, Beer stability (flavour, colloidal and foam).

Some recent yeast related publications from the Centre are as follows:

1. Crumplen, R.M., Slaughter, J.C. and Stewart, G.G. 1996. Characteristics of maltose transporter activity in an ale and lager strains of the yeast *Saccharomyces cerevisiae*. *Lett. Appl. Microbiol.* **23**:448-452.
2. Stewart, G.G. 1996. Yeast performance and management, *The Brewer* **82**:211-215.
3. Stewart, G.G. 1996. Brewing technology for the future. *The Brewer* **82**:348-353.
4. Rees, E.M.R. and Stewart, G.G. 1997. The effects of magnesium and calcium on yeast fermentation performance with high gravity worts, *J. Inst. Brew.* **103**:287-292.
5. Stewart, G.G., Bothwick, R., Bryce, J., Cooper, D., Cunningham, S., Hart, C. and Rees, E. 1997. Recent developments in high gravity brewing. *Tech. Quart. Master Brewers Assoc. Amer.* **34**:264-270.
6. Lyness, C.A., Steele, G.M. and Stewart, G.G. 1997. Investigating ester metabolism: characterisation of the *ATFI* gene in *Saccharomyces cerevisiae*. *J. Amer. Soc. Brew. Chem.* **55**:141-146.
7. Lyness, C.A. and Meaden, P.G. 1997. Expression of the *STA* glucoamylase gene of *Saccharomyces cerevisiae* in brewer's yeast. *J. Inst. Brew.* **103**:35-39.
8. Doyle, A. and Slaughter, J.C. 1998. Methionine and sulphate as competing and complimentary sources of sulphur for yeast during fermentation. *J. Inst. Brew.* **104**: 147-156.
9. Rees, E.M. and Stewart, G.G. 1998. Strain specific response of brewer's yeast strains to zinc concentrations in conventional and high gravity words. *J. Inst. Brew.* **104**:221-228.
10. Younis, O.S. and Stewart, G.G. 1998. Sugar uptake and subsequent ester and higher alcohol production by *Saccharomyces cerevisiae*. *J. Inst. Brew.* **104**:255-264.
11. Gent, DP and Slaughter, J.C. 1998. Intracellular distribution of amino acids in an *slp1* vacuole-deficient mutant of the yeast *Saccharomyces cerevisiae*. *J. Appl. Microbiol.* **84**:752-758.
12. Hayashida, Y., Nishimura, K. and Slaughter J.C. 1998. The importance of the furanones HDMF and HEMF in the flavour profile of Japanese barley miso and their production during fermentation. *J. Sci. Food Agr.* **78**:88-94.
13. Tessier, W.D., Meaden, P.G., Dickinson, F.M. and Midgley M. 1998. Identification and disruption of the gene encoding the K⁺-activated acetaldehyde dehydrogenase of *Saccharomyces cerevisiae*. *FEMS Microbiol. Lett.* **164**:29-34.
14. Stewart, G.G., Lyness, C.A. and Younis O.S. 1999. The control of ester synthesis during wort fermentation. *Tech. Quart. Master Brew. Assoc. Amer.* **36**: in press.

IX. Department of Plant Pathology, University of Wisconsin, Madison, WI 53716, U.S.A. Communicated by J.W. Buck <jwbuck@facstaff.wisc.edu>.

The following thesis was defended recently.

1. J.W. Buck. 1998. Adhesion of the yeast *Rhodosporidium toruloides* to barley leaves and the relevance of adhesion to colonization. Ph.D. dissertation. The University of Wisconsin-Madison.

The phylloplane mycoflora in temperate zones includes several genera of yeasts that provide a natural buffer against foliar plant pathogens and individual yeast isolates have biological control

potential. While yeasts are the main fungal colonizers of leaves, essentially nothing is known about how they attach to surfaces or the role of adhesion in colonization. To explore adhesion, we chose

as a model system the basidiomycetous yeast *Rhodosporidium toruloides* (anamorph *Rhodotorula glutinis*), a common phylloplane epiphyte with biocontrol potential.

We obtained non-adherent fungal mutants after chemical mutagenesis with methane-sulfonic acid ethyl ester (EMS). Sixteen attachment-minus (Att⁻) mutants were identified by three methods: screening capsule-minus colonies for loss of adhesive ability, enrichment for mutants unable to attach to polystyrene, and selection for reduced fluorescence of fluorescein-Concanavalin A stained cells by fluorescence-activated cell sorting (FACS). None of the 16 mutants attached to polystyrene or barley leaves. Hapten competition assays indicated that Con A bound to mannose residues on the cell surface. Adhesion of wild-type *R. toruloides* was transient; non-adhesive cells subsequently became adhesive with bud development. All Att⁻ mutants and non-attaching wild-type cells lacked polar regions of intense staining with FITC-Con A and India ink. Lectin, enzyme, and chemical treatments showed that the polar regions consisted of alkali-soluble materials, including mannose residues. Tunicamycin treatment reduced wild-type adhesion indicating that the mannose residues could be associated with glycoproteins. These results suggest that compounds, including mannose residues, localized at sites of bud development, mediate adhesion.

Cell surface charge and hydrophobicity, and adhesion to polystyrene, glass, and barley, were assessed to determine which physicochemical forces mediate attachment. Cells were grown under conditions promoting (excess carbon) or not promoting (excess nitrogen) capsule production. Hydrophobicity was measured by adhesion to xylenes and surface-charge characteristics were assessed by attachment to either DEAE- (positive) or CM- (negative) Sephadex ion-exchange beads. Hydrophobicity and adhesiveness of non-encapsulated, wild-type *R. toruloides* decreased from mid-log to late stationary phase. Encapsulated wild-type *R. toruloides* were more hydrophobic and more adhesive than non-encapsulated cells. However, two encapsulated Att⁻ mutants were more hydrophobic than the wild-type and adhesion of *R. toruloides* was similar on polystyrene and less hydrophobic glass surfaces. Adhesion of wild-type yeast to barley and polystyrene was correlated with attachment to CM-Sephadex beads, indicating a positive cell surface charge.

None of the 16 Att⁻ mutants exhibited a positive cell surface charge and wild-type yeast cells that did not attach to CM-Sephadex did not adhere to either polystyrene or barley. Wild-type *R. toruloides* attached to CM-Sephadex beads by the poles of the cells, which correlated with regions of intense staining with both India ink and FITC-Con A. In aggregate these results suggest that adhesion is due to localized, positive charge and not hydrophobic interactions.

We investigated, by observing adhesion kinetics of *R. toruloides*, whether adhesion to the phylloplane can be described by the standard 2-phase model, with weak, non-specific attachment followed by stronger, time-dependent adhesion. While 50-60% of the cells adhered in short-term assays (up to 3 h), less than 10% were adherent after 10 d. Ten Att⁻ mutants, deficient in phase I attachment, did not adhere after 5-d incubations, further suggesting a lack of stronger, phase II adhesion. Long-term (5-d) adhesion was similar for two isolates of *R. toruloides* and the ubiquitous leaf-surface fungus *Aureobasidium pullulans*. Adhesion of *R. toruloides* was significantly greater to a waxless barley mutant than to the wild-type cv. Bonus after long-term incubations. Application of exogenous nutrients (dilute yeast carbon base) to resident, wild-type *R. toruloides* populations resulted in both a rapid re-colonization to the apparent carrying capacity of the leaves and increased the total adherent populations. Att⁻ mutants re-colonized barley segments, when supplied with nutrients, even after more than 99% of the cells had been removed by agitation. Therefore, adhesion of *R. toruloides* cells to leaves was not required for subsequent colonization of the phylloplane.

Conclusions: We conclude that compounds, including mannose residues, localized at sites of bud development, mediate adhesion of *R. toruloides* to both polystyrene and barley leaf surfaces. Adhesion is transient and influenced by culture conditions. It results from a localized, positive charge and not hydrophobic interactions. *R. toruloides* becomes less adhesive in long-term assays suggesting a lack of stronger, phase II adhesion mechanisms. Overall, these data suggest that the frequency of yeast emigration from leaf surfaces, microbial growth rates, and leaf-surface characteristics are major factors influencing colonization of leaf surfaces.

X. Centraal Bureau voor Schimmelcultures, Yeast Division, Kluiver Laboratory TUD, Julianalaan 67, 2628 BC Delft, The Netherlands. Communicated by M.Th. Smith <CBS.YeastDiv@stm.TUdelft.nl>.

The catalogues of the CBS collections can be consulted on the www at <http://www.cbs.knaw.nl/www/cbshome.html>.

The following articles have appeared, are accepted or in press.

1. R. Schiemann, A. Glasmacher, E. Bailly, R. Horr , E. Molitor, C. Leutner, M.T. Smith, R. Kleinschmidt, G. Marklein and T. Sauerbruch. 1998. *Geotrichum capitatum* septicaemia in neutropenic patients: case report and review of the literature. *Mycoses* **41**:113-116.

We report a case of systemic infection with *Geotrichum capitatum* in a patient with acute myeloid leukaemia. Three days before death, the patient developed acute renal failure, probably caused by occlusion of glomerula with hyphae of *G. capitatum*. Up until now, prophylaxis and treatment of infections caused by

Geotrichum capitatum have not been established. However, the prophylactic administration of high-dose itraconazole and the therapeutic use of liposomal amphotericin B are subjects of discussion.

2. M.T. Smith & G.A. Poot. 1998. *Dipodascus capitatus*, *Dipodascus spicifer* and *Geotrichum clavatum*: Genomic characterization. Antonie van Leeuwenhoek, (in press).

The G+C contents of 25 strains of *Dipodascus capitatus*, *Dipodascus spicifer* and *Geotrichum clavatum* were found to be heterogeneous on basis of derivative graphs of the melting profiles. Strains showing similar derivative graphs of the melting curve exhibited high levels of DNA homology (80-100%); strains showing

dissimilar derivative graphs exhibited low levels of DNA homology (5 to 45%). Being considered separate taxa on basis of these parameters, *D. capitatus*, *D. spicifer* and *G. clavatum* could be identified by a combination of the key characteristics growth on xylose, cellobiose, salicin and arbutin.

3. J.P. van der Walt, M.T. Smith, and H.J. Roelijmans. Four new species in *Lipomyces*. Syst. Appl. Microbiol. (in press).

See abstract under Dr. van der Walt's communication.

4. M.L. Kerkmann, G. Schönian, K.D. Paul, K. Ulbrich, M.T. Smith, M. Schuppler. Red pigmented *Candida albicans* in cystic fibrosis. J. Clin. Microbiol. (in press).

5. E. Guého, T. Boekhout, H.R. Ashbee, J. Guillot, A. van Belkum, J. Faergemann. The role of *Malassezia* species in the ecology of human skin and as pathogens. Med. Mycol. **36**: Supplement I, 220-229.

The new taxonomic structure of the lipophilic genus *Malassezia* was presented with key characteristics for the seven described species. Among techniques used for epidemiological surveys, the pulsed field gel electrophoresis (PFGE) was found to be of little value in contrast to randomly amplified polymorphic DNA (RAPD).

Immunological studies still yielded conflicting results but at least the immunomodulatory capacity of *Malassezia* yeasts appeared to be related to the cell wall lipids. A review of *Malassezia* infections together with the present consensus for their prevention and treatment was also made.

6. W.H. Müller, R.C. Montijn, B.M. Humbel, A.C. van Aelst, E.J.M.C. Boon, T.P. van der Krift and T. Boekhout. 1998. Structural differences between two types of basidiomycete septal pore caps. Microbiology **144**:1721-1730.

The septal pore cap (SPC) of *Trichosporon sporotrichoides* CBS 8245 is vesicular-tubular, connected with flat-tubular endoplasmic reticulum (ER), and stains densely with zinc/iodine/osmium tetroxide, as does the ER. The SPC of *Schizophyllum commune* CBS 340.81 is more complex, about 600 nm in diameter, with perforations of 80-120 nm diameter, and stains less densely with zinc/iodine/osmium tetroxide than the ER. In high-pressure frozen and freeze-substituted hyphae of *T. sporotrichoides* the ER is present parallel to the dolipore septa, and electron-dense material occurs opposite the septal pore channel; the SPC rarely showed smooth vesicular-tubular membranes, suggesting that this is an ephemeral function of the SPC. The SPC of *S. commune* has a smooth outer and inner membrane, which enclose a matrix with a palisade-like substructure. A thin layer of electron-dense material covers the inner surface of the SPC of *S. commune*, from which

beaded filamentous structures connect the SPC and the pore-occluding material. These filamentous structures may maintain the intracellular position of the SPC and possibly play a role in plugging the septal pore channel. The septal pore swellings of *T. sporotrichoides* contain more 1,6-glucan than the septum, and intracellular glucans are also present near the septal pore channel. This cytosolic 1,6-glucan in *T. sporotrichoides* may serve as a matrix to keep the tubular membranous structures of the SPC together. In contrast, 1,6-glucan is not observed in the SPC and in the pore-occluding material of *S. commune*, and hyphal septa of this species show less labelling of 1,6-glucan than the septal swelling. The evolutionary transition from simple to more complex types of SPCs may have resulted in a requirement for different components to maintain the morphological integrity and cell biological function.

7. W.H. Müller, J.A. Stalpers, A.C. van Aelst, T.P. van der Krift, T. Boekhout. 1998. Field emission gun-scanning electron microscopy of septal pore caps of selected species in the *Rhizoctonia* s.l. complex. Mycologia **90**:170-179.

Techniques of freeze-fracturing and cytoplasmic maceration were combined to reveal the septal pore cap in some members of the *Rhizoctonia* s.l. complex by the use of field emission gun-scanning electron microscopy. Perforate septal pore caps were observed in *Rhizoctonia solani*, *Aquathanatephorus pendulus*, *Ceratobasidium cornigerum*, and *Waitea circinata*, showing a successive decrease in cap size. *W. circinata* revealed an irregular distribution of holes in the cap. Imperforate septal pore caps were observed in *Epulorhiza anaticula* and *Sebacina vermifera*. The cap of *E. anaticula* was connected to tubular endoplasmic reticulum,

while the cap of *S. vermifera* was connected to plate-like endoplasmic reticulum. Scanning microscopy is a useful method to complement our knowledge of the septal pore cap as it shows the septal pore cap in situ in relation with other organelles. Septal pore caps present within the *Rhizoctonia* s.l. complex can be subdivided into: (i) a perforate septal pore cap with either regularly or irregularly distributed holes; (ii) an imperforate septal pore cap connected with tubular endoplasmic reticulum or with plate-like endoplasmic reticulum. Our results sustain the current classification of the investigated species in four orders.

8. T. Boekhout, J.W. Fell, C.P. Kurtzman, and E.A. Johnson. Proposal to reject the name *Rhodomycetes dendrorhous* (Fungi), Basidiomycota). Taxon (in press).
9. T. Boekhout, M. Kamp, and E. Guého. Molecular typing of *Malassezia* species with PFGE and RAPD. Med. Mycol. (in press).
10. J.W. Fell, H. Roeijmans and T. Boekhout. Cystofilobasidiales, a new order of basidiomycetous yeasts. Int. J. System. Bacteriol. (in press).

XI. Center for Biological Research (CIBNOR). Unit of Marine Pathology. P.O. Box 128, La Paz 23000. B.C.S., México. Communicated by N.Y. Hernández-Saavedra <nhernan@cibnor.mx>.

The following are summaries of theses completed at the Centro de Investigaciones Biológicas del Noroeste (CIBNOR).

1. Hernández-Saavedra, N.Y. (PhD). Characterization of the superoxide dismutase enzyme type copper-zinc from the marine yeast *Debaryomyces hansenii*, and cloning of the encoding sequence from cDNA.

Copper-zinc superoxide dismutase (SOD-1) is a ubiquitous eukaryotic enzyme with a variety of important effects on respiring organisms. This enzyme has been studied on several species of eukaryotic and non eukaryotic organisms. However, the Cu-Zn SODs from molds and yeast have not been studied extensively. Today, the only fungi species from which the SOD-1 has been characterized is *Saccharomyces cerevisiae*, although the nucleotide sequences from *Schizosaccharomyces pombe*, *Neurospora crassa*, and *Aspergillus japonicus* are included in data banks. We have isolated a cytosolic Cu-Zn SOD from *Debaryomyces hansenii* showing a subunit mass of 15.6 kDa. The preparation was found to be heterogeneous by IMAC chromatography, native- and IEF-electrophoresis (showing two pI ranges: 5.14 to 4.0 and 1.6 to 1.8), suggesting the existence of two Cu-Zn SOD enzymes in *Deb. hansenii*. Differences in specific activity and amino acid composition of two Cu-Zn SOD IMAC fractions (32 and 37) support this conclusion. Major differences are observed in the number of histidine and tyrosine residues. Dh SOD-1 (IMAC 32, ~17300 U/mg of protein) has 6 histidines, whereas Dh SOD-2 (IMAC 37, ~8 000 U/mg of protein) has only 3. The number of tyrosine residues in Dh SOD-1 are 2 whereas in Dh SOD-2 they are increased to 5. We suggest that the 3 tyrosine residues could play the role of histidines at the active site of Dh SOD-1. Under stress conditions, promoted by ClO₂ concentration, a differential expression of two Dh SOD genes was noticed.

The enzyme preparation had a remarkably strong stability from pH 6 to 7, surviving boiling periods of 10 minutes without losing more than 60% activity. On western blots these enzymes were recognized by antibodies raised in rabbits against *Deb. hansenii* extracts, but only a weak cross-reaction was detected using

antibodies generated against either *Sacch. cerevisiae* or bovine erythrocyte Cu-Zn SODs. When mice were immunized with acrylamide gel slices containing the Dh SOD enzymes, a severe effect on the animals' health was observed (~25% lower weight compared to controls). In sequencing analysis, a peptide obtained by trypsin digestion was found to correlate 85% in homology with *Sacch. cerevisiae* Cu-Zn SOD. The pure Dh SOD-1 is 300% more active than bovine and yeast Cu-Zn SOD used as a reference. Considering all these properties, we concluded that the cytosolic Cu-Zn SOD preparation from *Deb. hansenii* could be an interesting alternative source for such an enzyme. A gene (*dh sod-1*) encoding one of the Cu-Zn SOD types of the marine yeast *Deb. hansenii* was cloned using mRNA and RT-PCR technique. Two identical clones containing fragments of 470 bp were obtained. From nucleotide sequence, we determined that both clones contained a 462 bp coding region, which encodes a 154-amino acid protein with a predicted molecular mass of 15.92 kDa. The encoding region in the *dh sod-1* gene has no introns. When partially digested genomic DNA was used as a template on PCR amplification, no PCR products larger than 470 bp were obtained. The amino acid composition deduced from the sequence of *dh sod-1* corresponds to that of IMAC 32, suggesting that the cloned gene corresponds to one form Cu-Zn SOD in *Deb. hansenii*. Comparing the deduced amino acid sequence with the cytosolic superoxide dismutases from *N. crassa* and *Sacch. cerevisiae* ~70% of homology was observed. Lower homologies (55 - 65%) were obtained with the corresponding enzyme of other eukaryotic organisms. However, despite that, the obtained encoding clones (*dh sod-1*) contain all the necessary information to produce a functional protein, when an expression experiment was done in a bacterial system, recombinant products were not detected.

2. García González, A. (PhD). Use of the superoxide dismutase from *Debaryomyces hansenii* on animal inflammation models.

New natural products useful in the treatment of disease with low secondary effects and low cost are among the goals of modern biomedical research. In our laboratory, we have been working with an extract with high superoxide dismutase activity from the marine yeast *Debaryomyces hansenii*, and we have evaluated the anti-inflammatory effect of the SOD obtained from *Debaryomyces hansenii* (Dh-SOD), and compared it with that of bovine erythrocytes

(Be-SOD). Adjuvant induced arthritis (AIA) did not showed any significant anti-inflammatory effect of the yeast enzyme, but when we used carrageenan-induced edema (CIE), the results showed a similar anti-inflammatory effect among the yeast enzyme, Be-SOD, and naproxen (a commercial anti-inflammatory drug). Comparing the anti-inflammatory effect of Dh-SOD and Be-SOD on collagen-induced arthritis (CIA) and mice-ear edema (MEE) models, we

have observed that both enzymes have a good anti-inflammatory effect in the preventive (edema development) and therapeutic (tissue damage) approach and that both enzymes decreased the incidence and intensity of arthritis, with the yeast SOD more effective. No secondary effects were observed on animals, and induction of

anticollagen antibodies was not affected by the enzyme administration. A commercial cream added with yeast SOD also showed the same anti-inflammatory effect as the nonsteroid anti-inflammatory aceclofenaco in a cream base, yet, their mode of action seems to be different.

3. Ramírez-Orozco, M. (MS). Cell yield and super-oxide dismutase evaluation in *Debaryomyces hansenii*.

The present work comprised the study of growth kinetics for the strain C-11 identified as *Debaryomyces hansenii*, and for a particular molecule, the superoxide dismutase enzyme. Culture medium was optimized diminishing the concentrations of the yeast extract and peptone nutrients, making it a cheaper culture medium without affecting the biomass production. We found that optimal concentrations of yeast extract and peptone were 0.15% and 0.3% respectively. The effect of physical factors on biomass production

was established. *Deb. hansenii* strain C-11 cultured at pH 5.0, 500 r.p.m., 30°C, and 5 L/min of filtered air supplied as oxygen source, yield approximately 50% on the basis of carbon source. We found a significant increase on the SOD activity when molecular oxygen, copper or glycerol were to the chemical reactor. This enzyme shows a 3.5 fold increase on activity when glycerol was used as carbon source, while an increase of 4 folds was obtained when either, oxygen or copper were supplied to the culture medium.

4. Sánchez-Paz, J.A. (Bch.). Evaluation of electrophoretic techniques for the characterization and taxonomic determination of marine yeast from the Western coast of Baja California Sur, Mexico.

Classification and identification of yeast and yeast like organisms, relies heavily on morphological and physiological characteristics, however the problem of using such criteria frequently suffers from lack of sensitivity and specificity. Whole-cell protein electrophoresis, restriction endonuclease analysis of genomic DNA (RFPL) and random amplified polymorphic DNA (RAPDs) were

used to distinguish different strains of marine yeast collected on the west coast of Baja, California Sur, México. Taken together, these results indicate that such techniques will be useful tools for routine identification of marine yeast. Noteworthy, of the assayed techniques, RAPDs proved to be the most reliable for taxonomic purposes.

The following are summaries of papers published recently, in press or submitted.

5. Hernández-Saavedra N. Y., Egly, J. M. and Ochoa, J. L. 1998. Cloning and Sequencing of a cDNA encoding a copper-zinc superoxide dismutase enzyme from the marine yeast *Debaryomyces hansenii*. *YEAST*, 14(6):573-581.

Cu-Zn superoxide dismutase (SOD-1) is a ubiquitous occurring eukaryotic enzyme with a variety of important effects on respiring organisms. A gene (*dhsod-1*) encoding a Cu-Zn superoxide dismutase of the marine yeast *Debaryomyces hansenii* was cloned using mRNA by the RT-PCR technique. The deduced amino acid sequence shows ~ 70% homology with that of cytosolic superoxide dismutase from *Saccharomyces cerevisiae* and *Neurospora crassa*,

as well as lower homologies (between 55 and 65%) with the corresponding enzyme of other eukaryotic organisms, including human. The gene sequence encodes a protein of 153 amino acids with a calculated molecular mass of 15.92 kDa, in agreement with the observed characteristics of the purified protein from *Deb. hansenii*.

6. Ramírez-Orozco, M., Hernández-Saavedra, N. Y. Ascencio Valle, F., Acosta González, B., and Ochoa, J.L.. 1998. Cell yield and superoxide dismutase activity of the marine yeast *Debaryomyces hansenii* under different culture conditions. *J. Marine Biotechnol.* (In press).

The effect of aeration, pH, stirring rate, and temperature on the biomass production and superoxide dismutase (SOD) activity of the marine yeast *Debaryomyces hansenii* strain C-11 was determined. The cell biomass yield was approximately 50% in a seawater formulated medium using glucose as the carbon source.

The SOD activity increased with application of a pulse of molecular oxygen or 0.8 mM sulfate copper into the chemical reactor. The SOD enzyme had an activity of 400 U/mg of protein in a crude extract produced under such conditions, the best activity ever reported for this enzyme in a crude preparation.

7. Hernández-Saavedra N.Y., and Ochoa, J.L. Copper-zinc superoxide dismutase from the marine yeast *Debaryomyces hansenii*. (Submitted to Yeast).

We have isolated the cytosolic form of Cu-Zn SOD from *Deb. hansenii*. This enzyme has a dimer structure (30.91 kDa) with a subunit mass of 15.9 kDa. The preparation was found to be heterogeneous by IF electrophoresis with two pI ranges; 5.14 to 4.0 and 1.6 to 1.8. The enzyme preparation had a remarkably strong stability at pH 7.0 surviving boiling periods of 10 minutes without losing activity. On western blots this enzyme was recognized by antibodies raised in rabbits against *Deb. hansenii* extracts, whereas only a weak cross-reaction could be detected using antibodies

generated against either *Saccharomyces cerevisiae* or bovine erythrocyte Cu-Zn SODs. In sequencing analysis, a peptide obtained by trypsin digestion was found to correlate 100% in homology with the carboxyl terminal end of the human EC-SOD. Considering all these properties, we may conclude the cytosolic SOD of *Deb. hansenii* could be an interesting alternative in biomedical applications. Future studies include cloning and regulation of the Cu-Zn SOD gene from this particular marine yeast strain.

8. Ramírez-Orozco M, Hernández-Saavedra Norma Y. and Ochoa, J.L. Chemical sterilization using chlorine dioxide in the biomass production of the marine yeast *Debaryomyces hansenii* and its effect on Cu-Zn SOD activity. (Submitted to J. Biotechnol.).

In this work, we propose the use of chlorine dioxide, ClO₂, as an alternative for the chemical sterilization of a culture medium employed to obtain cell biomass of the marine yeast *Debaryomyces hansenii*, and to prevent growth of contaminant microorganisms during culturing. The marine yeast can tolerate high concentrations of ClO₂ (some resistant colonies can growing up to 130 mg/mL of the agent, as shown by sensidisc technique), while other

microorganisms commonly found in seawater cannot. This observation was confirmed in a liquid culture media using seawater as solvent, showing that the fermentation proceeds, and is completed, without the growing of contaminant microorganisms in the presence of ClO₂ and not affecting the Cu-Zn SOD activity in *Deb. hansenii* cultured under such conditions.

XII. Division of Infection and Immunity, Institute of Biomedical and Life Sciences, Joseph Black Building, University of Glasgow, Glasgow, G12 8QQ, Scotland. Communicated by L.J. Douglas <J.Douglas@bio.gla.ac.uk>.

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

1. Baillie, G. S. and Douglas, L. J. 1998. Effect of growth rate on resistance of *Candida albicans* biofilms to antifungal agents. *Antimicrob. Agents Chemother.* **42**:1900-1905.
 2. Baillie, G. S. and Douglas, L. J. 1998. Iron-limited biofilms of *Candida albicans* and their susceptibility to amphotericin B. *Antimicrob. Agents Chemother.* **42**:2146-2149.
 3. Hawser, S. P., Baillie, G. S. and Douglas, L. J. 1998. Production of extracellular matrix by *Candida albicans* biofilms. *J. Med. Microbiol.* **47**:253-256.
 4. Vardar-Unlu, G., McSharry, C. and Douglas, L. J. 1998. Fucose-specific adhesins on germ tubes of *Candida albicans*. *FEMS Immunol. Med. Microbiol.* **20**:55-67.
 5. Baillie, G. S. and Douglas, L. J. Role of dimorphism in the development of *Candida albicans* biofilms. *J. Med. Microbiol.*, in press.
 6. Baillie, G. S. and Douglas, L. J. *Candida* biofilms and their susceptibility to antifungal agents. In: *Biofilms. Methods in Enzymology*, in press.
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XIII. Institute of Molecular Biology, Odense University, DK-5230 Odense M, Denmark. Communicated by J. Friis.

1. A.K. Overgaard, L. Kierklo, H. Christiansen, P. Chrois, L. Rasmussen, and J. Friis. Resumption of rapid proliferation from lag phase in cultures of *Saccharomyces cerevisiae* in poor nutrient conditions. Effect of surface and intracellular signaling mechanisms. Cell Biol. (In press).

Saccharomyces cerevisiae was inoculated into a dilute synthetic minimal medium with glycerol as the carbon source. The number of live cells in the cultures was determined by colony counts on agar plates. Untreated control cells had doubled in number about once at the end of the first week and had gone through eight doublings already at the end of the second week. Addition of either 8-bromo-cyclic guanosine monophosphate, 8-bromo-cGMP, or human recombinant insulin, made the cells go through 12 and 10 doublings, respectively, already during the first week. In contrast, 8-bromo-cyclic adenosine monophosphate, 8-bromo-cAMP, had only slight stimulating effects on cell multiplication, but if it was

combined with phorbol-1 2-myristate-1 3-acetate, PMA, the cells went through about 12 doublings during the first week. Addition of LY 83583, an inhibitor of soluble guanylate cyclase, prevented cell proliferation. Further addition of 8-bromo-cGMP bypassed this inhibition. Singly, bradykinin or PMA did not affect cell multiplication. However, when these two compounds were combined, the cells went through about 10 doublings during the first week. Neither bradykinin, nor PMA had any releasing effect on the inhibition of LY 83583. These results indicate the existence of several routes leading to cell proliferation in wildtype *S. cerevisiae* cells.

XIV. Japan Collection of Microorganisms, The Institute of Physical and Chemical Research (RIKEN), Wako, Saitama 351-0198, Japan. Communicated by M. Hamamoto <hamamoto@ulmus.riken.go.jp> and T. Nakase <nakase@ulmus.riken.go.jp>.

The seventh edition of JCM Catalogue of Strains will be published on January 1999. The catalogue covers 2260 yeast and fungal (including a yeast-like alga *Prototheca*), 3640 bacterial and 108 archaeal strains. It includes five main sections as follows:

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3. Culture collection designation index;
4. Media formulations;
5. Literature references.

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The following articles have been published recently.

1. Caûete-Gibas, C. F., Takashima, M, Sugita, T. & Nakase, T. 1998. Three new species of *Kockovaella* isolated from plants collected in the Ogasawara Islands. J. Gen. Appl. Microbiol. **44**:11-18.

Five strains of unknown ballistoconidogenous yeasts, which were isolated from plant leaves collected in the Ogasawara Islands, Japan, were taxonomically studied. They represent three different species of the genus *Kockovaella* based on morphological, physiological and biochemical characteristics, analysis of small

subunit ribosomal RNA gene sequences, and DNA-DNA reassociation experiments. Three new species, *Kockovaella machilophila* (1 strain), *Kockovaella phaffii* (3 strains) and *Kockovaella schimae* (1 strain) are proposed for these five strains.

2. Takashima, M. & Nakase, T. 1998. *Bullera penniseticola* sp. and *Kockovaella sacchari* sp. nov., two new yeast species isolated from plants in Thailand. Int. J. Syst. Bacteriol. **48**:1025-1030.

Two strains of ballistoconidium-forming yeasts, isolated from plants collected in the south-east seacoast of Bangkok, Thailand, were described. The strains (K-272T and K-337T) were assigned to the genera *Bullera* and *Kockovaella*, respectively, based on morphological and chemotaxonomic characteristics. Phylogenetically, strain K-227T is close to *Bullera hanna*, and

strain K-337T is close to *Kockovaella thailandica* and *Kockovaella imperatae*. These two strains represent new species based on DNA-DNA reassociation experiments. *Bullera penniseticola* Takashima et Nakase sp. nov. and *Kockovaella sacchari* Takashima et Nakase sp. nov. are proposed for K-227T (= JCM 9857T) and K-337T (=JCM 9858T), respectively.

3. Sugita, T. & Nakase, T. 1998. *Trichosporon japonicum* sp. nov. isolated from the air. *Int. J. Syst. Bacteriol.* **48**:1425-1429.

A yeast strain isolated from the air in Japan was found to represent a new species, and was named *Trichosporon japonicum*. This species produced arthroconidia and appressoria. *T. japonicum* formed a cluster with the appressorium-forming species *Trichosporon inkin* and *Trichosporon ovoides* in a phylogenetic

tree constructed using small-subunit rDNA sequences. However, they had low relatedness to each other in DNA-DNA hybridization experiments. *T. japonicum* is distinguished from both *T. inkin* and *T. ovoides* by its ability to assimilate inulin, and its inability to assimilate L-rhamnose. JCM 8357T is the type strain of the species.

4. Suzuki, M. & Nakase, T. 1998. Cellular neutral sugar compositions and ubiquinone systems of the genus *Candida*. *Microbiol. Cult. Coll.* 14, No. 2 (in press).

XV. Alkomohr Biotech Ltd., and Department of Biosciences, University of Helsinki, Biocentre, Viikinkaari 9, FIN-00710 Helsinki, Finland. Communicated by M. Korhola.

The following papers have appeared in print since my latest communication.

1. Codón, A.C., Benítez, T. and Korhola, M. 1997. Chromosomal reorganization during meiosis of *Saccharomyces cerevisiae* baker's yeasts. *Curr. Genet.* **32**:247-259.

The genomic constitution of two *S. cerevisiae* baker's yeasts and their meiotic products have been analyzed by pulse-field gel-electrophoresis, hybridization with specific gene probes, marker segregation, and flow cytometry. The parental strains have chromosomal patterns substantially different from those of laboratory strains used as controls. This pattern is partly the result of there being more than one copy of homologous chromosomes of different size, as judged by Southern-blot hybridization carried out with specific gene probes. Flow cytometry indicated that the strains have a 2.7 C DNA content. Tetrad analysis showed disomy for some chromosomes and tetrasomy for others. When two complete tetrads were subjected to molecular analysis the results confirmed instances of segregation of homologous chromosomes of different size. However, the presence of chromosomal bands absent in the parentals

and the disappearance of chromosomal bands present in the parental strains were frequently seen. This result was attributed to two different phenomena: (1) the presence of multiple Ty1 and Ty2 transposable elements which seem to undergo interchromosomal translocation together with amplification, giving rise to differences in chromosomal size; (2) the presence of multiple Y' subtelomeric regions, giving rise to asymmetrical homologous recombination and, as a consequence, differences between the size of the recombinant chromosomes and the non-recombinant parental chromosomes. Chromosomal reorganization occurs with a very high frequency during meiosis. By contrast, mitosis is very stable, as judged by the reproducible electrophoretic karyotype shown by the parental strains in successive generations.

2. Codón, A.C., Benítez, T. and Korhola, M. 1998. Chromosomal polymorphism and adaptation to specific industrial environments of *Saccharomyces* strains. *Appl. Microbiol. Biotechnol.* **49**:154-163.

Several industrial *Saccharomyces* strains, including baker's, wine, brewer's and distiller's yeasts, have been characterized with regards to their DNA content, chromosomal polymorphism and homologies with the DNA of laboratory strains. Measurement of the DNA contents of cells suggested that most of the industrial yeasts were aneuploids. Polymorphisms in the electrophoretic chromosomal pattern were so large that each strain could be individually identified. However, no specific changes relating to particular group were observed. Hybridization using different probes from laboratory strains was very strong in all cases, indicating that all industrial strains possess a high degree of DNA homology with laboratory yeasts. Probes *URA3*, *CUP1*, *LEU2*, *TRP1*, *GAL4* or *ADC1* demonstrated the presence of one or two bands, two especially in

baker's strains. Also, results indicate that all hybridized genes are located on the same chromosomes both in laboratory and industrial strains. Translocation from chromosome VIII to XVI seems to have occurred in a distiller's strain, judging by the location of the *CUP1* probe. Finally, when the *SUC2* probe is used, results indicate a very widespread presence of the *SUC* genes in only baker's and molasses alcohol distiller's strains. This clearly suggests that amplification of *SUC* genes is an adaptive mechanism conferring better fitness upon the strains in their specific industrial conditions. The widespread presence of Ty1 and Ty2 elements as well as Y' subtelomeric sequences could account for the inter- and intrachromosomal changes detected.

XVI. Institut für Pflanzengenetik und Kulturpflanzenforschung, Corrensstr. 3, D-06466 Gatersleben, Germany. Communicated by G. Kunze.

Recent publications.

1. K. Tag, M. Lehmann, Chiyui Chan, R. Renneberg, K. Riedel & G. Kunze. 1998. *Arxula adenivorans* LS3 as suitable biosensor for measurements of biodegradable substances in salt water. J. Chem. Technol. Biotechnol. (in press.).

A microbial amperometric sensor based on the yeast *Arxula adenivorans* was tested to determine its suitability for measuring biochemical oxygen demand (BOD) in salt water. The viability of cells immobilised onto the sensor membrane was hardly influenced up to 10% (w/v) NaCl in the sample, although the solubility of O₂ was affected. NaCl concentrations higher than 10% (w/v) caused extreme decrease in the O₂ solubility and deactivated the sensor. This outcome depended on the substrates used, e.g., alanine-, galactose- and acetic acid-sensor signals were influenced by any salt concentration whereas glucose-, glycerol-, maltose- and arginine- sensor signals were influenced only by higher salt

concentrations. Sensor signals from yeast extract as well as glucose correlated with the quantity of these substances and with the salt concentration contained in the water. This correlation was linear up to 10% (w/v) NaCl and 0.125% (w/v) yeast extract or up to 10% (w/v) NaCl and 0.125% (w/v) glucose in the sample. The sensor signals are therefore influenced only by NaCl-determined solubility of O₂ and not by the physiological parameters of the immobilized cells. However, an increase of yeast extract- or glucose-concentrations in the presence of NaCl caused physiological effects on the sensor cells.

2. K. Kaletta, I. Kunze, G. Kunze & M. Köck. 1998. The peptide HDEF as a new retention signal is necessary and sufficient to direct proteins to the endoplasmic reticulum. FEBS Letters **434**: 377-381.

The key feature of tomato RNase LX localized solely outside the vacuole is the C-terminal peptide HDEF which is very similar to known endoplasmic reticulum (ER) retention signals. For functional testing of the ability of HDEF, different constructs including the complete RNase LX, two truncated forms without HDEF and the truncated chitinase FB7-1ΔVTP C-terminally flanked by HDEF, were expressed in *Saccharomyces cerevisiae*. The

majority of RNase and chitinase, both containing HDEF, accumulates within the ER. However, the truncated constructs without the peptide are released into the medium. We provide compelling evidence that peptide HDEF at the C-terminus of secretory plant proteins is a new ER retention signal in yeast and most likely in plants.

XVII. Department of Molecular Genetics and Microbiology, Robert Wood Johnson Medical School, University of Medicine and Dentistry of New Jersey, 675 Hoes Lane, Room 705, Piscataway, New Jersey 08854-5635, U.S.A. Communicated by M.J. Leibowitz.

1. Z. Tallóczy, S. Menon, L. Neigeborn, and M.J. Leibowitz. 1998. The *[KIL-d]* cytoplasmic genetic element of yeast results in epigenetic regulation of viral M double-stranded RNA gene expression. Genetics **150**:21-30.

[KIL-d] is a cytoplasmically inherited genetic trait that causes killer virus-infected cells of *Saccharomyces cerevisiae* to express the normal killer phenotypes in *a/a* cells, but to show variegated defective killer phenotypes in *a* or *α* type cells. Mating of *[KIL-d]* haploids results in "healing" of their phenotypic defects, while meiosis of the resulting diploids results in "resetting" of the variegated, but mitotically stable, defects. We show that *[KIL-d]* does not reside on the double-stranded RNA genome of killer virus. Thus, the *[KIL-d]* effect on viral gene expression is epigenetic in nature. Resetting requires nuclear events of meiosis, since *[KIL-d]* can be cytoplasmically transmitted during cytoduction without

causing defects in killer virus expression. Subsequently, mating of these cytoductants followed by meiosis generates spore clones expressing variegated defective phenotypes. Cytoduction of wild-type cytoplasm into a phenotypically defective *[KIL-d]* haploid fails to heal, nor does simultaneous or sequential expression of both *MAT* alleles cause healing. Thus, healing is not triggered by the appearance of heterozygosity at the *MAT* locus, but rather requires the nuclear fusion events which occur during mating. Therefore, *[KIL-d]* appears to interact with the nucleus in order to exert its effects on gene expression by the killer virus RNA genome.

**XVIII. Research Institute for Viticulture and Enology, Matúškova 25, 833 11 Bratislava, Slovakia.
Communicated by E. Minárik.**

The following papers appeared recently.

1. Minárik, E. 1998. Lack of nutrients in grape must (in Slovak). *Vinohrad (Bratislava)* **36(4)**:86-87.

Complete alcoholic fermentation in grape must occurs only when wine yeasts show increased viability at the end of fermentation. Combined assimilable nitrogen sources and oxygen addition to grape must in the course of fermentation cause an increase of viability and

biomass formation provided that the addition is performed at the right moment. Maximum concentrations of oxygen and assimilable nitrogen additions are proposed. A brief review of recent results in viticultural countries are given.

2. Minárik, E. and Jungová, O. 1998. Novel activation of alcoholic grape must fermentation. *Kvasný průmysl (Prague)* **44(9)**:244-245.

A combined preparation Fermaid® "E" containing important yeast nutrients (NH₄)₂HPO₄, (NH₄)₂SO₄, thiamine, and yeast ghosts stimulates wine yeasts in alcoholic fermentation of grape must. It guarantees smooth fermentation start, course and completion

even at unfavourable fermentation conditions, particularly at yeast nutrients deficiency. Practical aspects for wine practice are elucidated and discussed.

XIX. Department of Microbiology, Technical University of Denmark - DTU, Building 301, DK-2800 Lyngby, Denmark. Communicated by J. Piškur <jp@im.dtu.dk>.

1. Z. Gojkovic, S. Paracchini & J. Piškur. 1998. A new model organism for studying the catabolism of pyrimidines and purines. In: Griesmacher *et al.* (Eds), *Purine & Pyrimidine Metabolism in Man IX*, Plenum Press, NY.

Several different mutants of *Saccharomyces kluyveri* with presumed defects in the pyrimidine or purine catabolic pathway

have been isolated. Currently efforts are being made to isolate the corresponding genes by complementation using a genomic library.

2. J. Piškur, S. Smole, C. Groth, R.F. Petersen & M.B. Pedersen. 1998. Structure and genetic stability of mitochondrial genomes vary among yeasts of the genus *Saccharomyces*. *Int. J. Syst. Bacteriol.* **48**:1015-1024.

Several yeast species/isolates belonging to the genus *Saccharomyces* were examined for the organization of their mtDNAs and ability to generate petite mutants. A general characteristic for all of the mtDNAs tested was that they were very A+T-rich. However, restriction patterns and inducibility of petite mutations revealed a great diversity in the organization and genetic behaviour of mtDNAs. One group of yeasts, *Saccharomyces sensu stricto*, contains mtDNA ranging in size from 64 to 85 kb. mtDNAs from these yeasts contain a high number of restriction sites that are recognized by the enzymes *HaeIII* and *MspI*, which cut specifically in C+C clusters. There are three to nine *ori/rep* sequences per genome. These yeasts spontaneously generate respiration deficient

mutants. Ethidium bromide (Et-Br), at low concentrations, induces a majority of cells to give rise to petites. A second group of yeasts, *Saccharomyces sensu lato*, contains smaller mtDNAs, ranging in size from 23 to 48 kb, and probably only a few intergenic G+C clusters and no *ori/rep* sequences. These yeasts also generate petite clones spontaneously, but Et-Br, even when present at high concentrations, does not substantially increase the frequency of petites. In most petite clones from these yeasts only a small fragment of the wild-type molecule is retained and apparently multiplied. A third group, represented by *Saccharomyces kluyveri*, does not give rise to petite mutants either spontaneously or after induction.

3. I. Masneuf, J. Hansen, C. Groths, J. Piškur & D. Dubourdieu. New hybrids between *Saccharomyces sensu stricto* yeast species found amongst wine and cider production strains. *Appl. Environ. Microbiol.* **64**: in press.

Two yeast isolates, a wine-making yeast first identified as *S. uvarum* and a cider-making yeast are characterized for their nuclear and mitochondrial genomes. Electrophoretic karyotyping analyses, RFLP maps of PCR-amplified *MET2* gene fragments and the sequence analysis of a part of the two *MET2* gene alleles found, supports the notion that these two strains constitute hybrids between *S. cerevisiae* and *S. bayanus*. The two hybrid strains have completely different restriction patterns of mtDNA as well as

different sequence of the *OLII* gene. The sequence of the *OLII* gene from the wine hybrid strain appears to be the same as for the *S. cerevisiae* gene whereas the *OLII* gene of the cider hybrid strain is equally divergent from both putative parents, *S. bayanus* and *S. cerevisiae*. Finally some fermentative properties are examined, and one phenotype is found to reflect the hybrid nature of these two strains. The origin and nature of such hybridization events are discussed.

4. R.F. Petersen. 1998. Genome dynamics and evolution of the mitochondrial and nuclear genomes in *Saccharomyces sensu lato* species. Ph.D. Thesis, May 1998.

The genus *Saccharomyces* contains several species which belong to two groups, *sensu stricto* and *sensu lato*. In this dissertation the structure and dynamics of the *sensu lato* genomes have been studied. The size and organization of the mitochondrial (mtDNA) and nuclear genomes vary among these yeasts and differ from *sensu stricto* yeasts. While the coding parts of the mtDNA are greatly conserved among all *Saccharomyces* species, significant variations are observed in the size, intergenic sequences, introns and gene order. The mtDNAs of the *sensu lato* group exhibit sizes below 50kb and they seem not to contain a large number of introns and extensive intergenic regions. On the other hand, mitochondrial genomes of the *sensu stricto* group are large, more than 64kb, and they have accumulated biologically active GC-clusters. Comparison of the gene orders suggests that two different molecular mechanisms have formed the modern mitochondrial genomes. In *sensu lato* a

mechanism based on inversions, possibly employing short intergenic sequences, has primarily operated during evolution of the *sensu lato* mitochondrial genomes. In comparison, a transposition mechanism seem to have operated in *sensu stricto*. Also the nuclear genomes have been significantly remodeled in evolution. The *sensu lato* yeasts have heterogeneous nuclear genomes and the number of chromosomes vary from seven to 16. The sizes of *sensu lato* chromosomes are larger than 500kb while *sensu stricto* contain also smaller chromosomes. There has been a tendency in the evolution of the *Saccharomyces* nuclear genomes towards a higher number of chromosomes as well as the appearance of relatively small ones. The nuclear chromosomes of *sensu lato* type strains were partially mapped for the *S. cerevisiae* chromosome III genes. While some of these genes are physically coupled in some *sensu lato* yeasts, others seem to have "jumped" around the genome.

XX. Institute of Applied Microbiology, Muthgasse 18, A-1190 Vienna, Austria. Communicated by H.J. Prillinger <H.Prillinger@iam.boku.ac.at>.

Recent publications and theses.

1. H. Prillinger, O. Molnar, F. Eliskases-Lechner, K. Lopandic. Phenotypic and genotypic identification of yeasts from cheese. Antonie van Leeuwenhoek (in press).

Eighty-five yeast strains isolated from different cheeses of Austria, Denmark, France, Germany, and Italy were identified using physiological methods and genotypically using random amplified polymorphic DNA polymerase chain reaction (RAPD-PCR) analysis. Good congruence was found between the phenotypic and genotypic data for 39 of the isolates. However, 26 isolates of *Geotrichum* could only be identified to the species level using the genotypic methods and 7 isolates were correctly identified to the genus level only using phenotypic identification methods. The phenotypic identification did not agree with the genotypic data for 14 yeast isolates. Using ubiquinone analysis, yeast cell wall sugars and the diazonium blue B test 5 incorrectly identified isolates with phenotypic methods could be identified genotypically. In addition the 7 isolates identified only to the genus level by the phenotypic methods and the 26 *Geotrichum* strains were identified to the species

level using the polyphasic molecular approach mentioned above. Eleven strains remained unidentified. The 76 identified yeast isolates were assigned to 39 species, the most frequent assignments were made to *Debaryomyces hansenii*, *Geotrichum candidum*, *Issatchenkia orientalis*, *Kluyveromyces lactis*, *K. marxianus*, *Saccharomyces cerevisiae*, *Yarrowia lipolytica*, and *Candida catenulata*. It is proposed that *Debaryomyces hansenii* (Zopf) Lodder et Kreger-van Rij and *Debaryomyces fabryi* Ota should be reinstated. The RAPD-PCR data reinforced the view that the species *Galactomyces geotrichum* is heterogeneous with all of the *Geotrichum* isolates from cheese products being assigned *G. geotrichum* group A sensu M. T. Smith. It is suggested that the name *Geotrichum candidum* be conserved for this rather common species.

2. W. Schweigkofler. Molecular identification and characterization of endophytic and latent pathogenic fungi from grapevines (*Vitis vinifera* L.) in Austria and south Tyrol. PhD-thesis.

The presented work is subdivided into two main parts: 1) Isolation of endophytic and latent pathogenic fungi from wooden parts of symptomless grapevines (*Vitis vinifera*) from different growing areas in Austria and south Tyrol. 2) Use of molecular markers (sequences of ribosomal genes, RAPD-PCR) for identifying fungal isolates, estimation of genetic variability and phylogenetic relationships.

1) Symptomless grapevines contain a highly variable mycoflora: strains belonging to 26 different genera were isolated (mainly filamentous fungi imperfecti: Hyphomycetes, Coelomycetes). Ascomycota occur seldomly, filamentous basidiomycota are lacking at all. The dimorphic fungi

Aureobasidium pullulans and *Sporothrix* sp. and the dimorphic basidiomycetous yeasts *Rhodotorula glutinis*, *Cryptococcus macerans*, and a probably new *Sporobolomyces* species were isolated as well. The predominant endophytes are *Aureobasidium pullulans*, *Alternaria alternata* and several species of *Cladosporium* (mainly *C. herbarum* and *C. cladosporioides*). Beside saprophytes and weak pathogens also the causal agents of known grapevine diseases were isolated (e. g. *Phomopsis viticola*, *Verticillium dahliae*). Air borne fungi with dispersal of conidia and ascospores (*Alternaria* spp., *Cladosporium* spp., *Phoma* spp.) are predominant on plant organs above ground; roots inhabit numerous soil borne strains (*Fusarium* spp., *Cylindrocarpon* spp., *Verticillium dahliae*) which

invade their hosts via hyphal contact. No significant difference of the fungal flora from different *Vitis*-cultivars was found.

2) The genetic variability of *Verticillium dahliae*- and *Aureobasidium pullulans* - strains from different host plants and geographic regions was determined using RAPD (= Random Amplified Polymorphic DNA) PCR. *V. dahliae* isolates cluster in two distinct groups: one specific for the host plant *Brassica napus* (oilseed rape), the other group comprising isolates from very different host plants, including grapevine. The genetic variability within the *A. pullulans*- strains came up to 30%, but showed no correlation to the host plants. No correlation of the RAPD pattern of both fungi with the geographic origin of the isolates was observed. For the estimation of the phylogenetic position of *Verticillium dahliae* and *Holleya sinecauda* (= *Eremothecium sinecaudum*) the 18SrRNA genes were sequenced and used for the construction of phylogenetic trees. The deuteromycetous species *V. dahliae* clusters in the ascomycetous order of the Hypocreales. The dimorphic plant pathogen *H. sinecauda* falls into the family Saccharomycetaceae (Hemiascomycetes). Analyses of the ITS1 and ITS2 (Internal Transcribed Spacer) regions corroborated these results. These molecular data contradict the close relationship of *H. sinecauda* to the Metschnikowiaceae which was postulated using morphological criteria. Partial sequences of the 18S- and 26SrRNA gene were determined from eight filamentous, one dimorphic and 27 yeast species. Because of very poor sequence information available on GenBank, only a few filamentous isolates were identified on genus using the Blast server (e.g. MA532 = *Fusarium* sp., MA884 = *Pleospora herbarum*). Based on partial sequences of the 18SrRNA

gene the dimorphic isolate was integrated into the deuteromycetous genus *Sporothrix*, closely related to the ascomycetous family of the Ophiostomataceae. Yeast isolates were identified in many cases on genus level by comparing 18SrRNA gene sequences with the GenBank. A higher resolution (on species level) was obtained by comparing the 26SrRNA partial sequences with the data sets of Prof. Fell (University of Miami, FL.) and Dr. Kurtzman (NCAUR, Peoria, IL.). The yeast strains HA1035 (isolated from the higher basidiomycete *Jahnporus hirtus*) and HB167 and HB174 (isolated from termites) were identified as new species. The 3' end of the 26SrRNA gene and the 5' end of the IGS1 (Intergenic Spacer 1) was sequenced from four *Saccharomyces* and five *Eremothecium* strains, showing a high variability also within closely related strains. A phylogenetic analysis of the Ascomycota using 18SrRNA sequence information and calculation of phylogenetic trees with the programs Neighbor-Joining and Maximum Likelihood showed a division into the Euascomycetes, Hemiascomycetes and a rather heterogenous clade, which includes *Schizosaccharomyces*, *Protomyces* and *Taphrina* among others. This clade corresponds largely to the Archiascomycetes *sensu* Sugiyama et Nishida. However, in contrast to these authors, our analyses suggest this clade to be derived in comparison to the more primitive Hemiascomycetes. Therefore we erect a new class for this clade, which we call Protomycetes. In summary, both the diversity of endophytic fungi from grapevine as well as the usefulness of molecular methods for the characterization of these microorganisms were proved through this study.

3. K. Lopandic. Genotypic identification and comparative analysis of cell wall sugars from Asco- and basidiomycetous yeasts. Ph.D. thesis.

The cell wall carbohydrate composition of 140 ascomycetous and basidiomycetous strains have been analyzed with the aim to evaluate this analysis in predictions of phylogenetic relatedness among yeasts. Investigated yeasts show altogether 6 carbohydrate patterns. Basidiomycetous yeasts exhibit three carbohydrate profiles that correspond to the three classes already described on the basis of sequencing the 18S rRNA gene. Thus, glucose, mannose and galactose are present in the cell walls of Ustilaginomycetes, mannose, glucose, galactose and fucose have been detected in Urediniomycetes, whereas Hymenomycetes contain xylose in addition to glucose and mannose. Within the ascomycetous yeasts three carbohydrate patterns have also been found, but opposing to basidiomycetous yeasts, no correlation of specific cell wall carbohydrate composition with estimated classes has been found. Thus, the majority of Hemiascomycetes contain exclusively glucose and mannose, but in certain number of the strains galactose is present as well. Glucose, mannose and galactose have also been determined in Euascomycetes and Archiascomycetes, however, a number of Euascomycetes and Archiascomycetes contain rhamnose in addition. On the basis of these results it could be concluded that analysis of cell wall carbohydrate composition has limited potential in prediction of phylogenetic relationships among yeasts, but it is a useful criterion for the characterization of yeast strains.

In this study the characterization of 18 newly isolated strains from lichens was performed by several procedures including determination of cell wall carbohydrate composition, analysis of genomic DNA by RAPD method (Random Amplified Polymorphic DNA), partial sequencing of the gene coding for 18S rRNA and

sequencing of the ITS1 and ITS2 regions. Analysis of the cell wall carbohydrate composition has shown that all investigated strains contain glucose, mannose and xylose. Presence of xylose in the cell walls as well as the ubiquinone Q10 in mitochondrial membrane and the reproduction by sterigmatoconidia (Prillinger et al., 1997) have indicated that the strains belong to the genus *Fellomyces*. To investigate phylogenetic relationships of isolated strains with already known *Fellomyces* species (*F. polyborus*, *F. horovitziae*, *F. fuzhouensis*, *F. penicillatus*), the partial sequencing the 18S rRNA (385 bp) was performed. High sequence homologies have been found among strains (differences are in the range up to 4%) supporting the close phylogenetic relationship of the new strains with the genus *Fellomyces*. Further characterization of genomic DNA have been performed by RAPD-method, which relies on the amplification of random DNA fragments. An evaluation of the method had been performed using the type strains of the genus *Metschnikowia*. Analyses of 10 *Metschnikowia* species have shown that the strains of different species have <30% DNA fragments in common, whereas the strains within a single species exhibit >50% common DNA fragments. Genomic DNA of the newly isolated strains and 4 already known *Fellomyces* species have been analyzed by RAPD method. On the basis of the estimated criteria for the distinguishing among species it has been shown that newly isolated strains belong to 5 groups, different than already determined type strains of the genus *Fellomyces*. Hence, it has been concluded, on the basis of morphological, chemotaxonomic and genotypic investigations that these strains represent 5 new species of the genus *Fellomyces* that have been named: *F. borneensis*, *F. chinensis*,

F. lichenicola, *F. sichuanensis* and *F. thailandicus*. Furthermore, the ITS1 and ITS2 regions of 5 newly determined strains and an already known strain *F. polyborus* have been sequenced to determine the level of variability of these sequences among species. Differences within ITS1 regions have been in the range of 0-40%,

whereas the differences of 1.7-29% have been found within ITS2 regions. These results have indicated that the ITS1 and ITS2 sequences could not be used as a criterion for distinguishing among strains at the species level.

4. Prillinger, H., Kraepelin, G., Lopandic, K., Schweigkofler, W., Molnar, O., Weigang, F., and Dreyfuss, M.M. 1997. *System. Appl. Microbiol.* **20**:572-584.

XXI. National Collection of Yeast Cultures, Institute of Food Research, Norwich Research Park, Colney, Norwich NR4 7UA, UK. Communicated by I.N. Roberts <ian.roberts@bbsrc.ac.uk>.

Recent news from the collection

The NCYC strain database is now available live on the web at <http://www.ifrn.bbsrc.ac.uk/ncyc/>.

Other new services being offered include rapid PCR fingerprinting of strains of *Saccharomyces cerevisiae*. Contact Chris Bond or Steve James for details.

Recent publications.

1. H. Steels, C.J. Bond, M.D. Collins, I.N. Roberts, M. Stratford and S.A. James. 1999. *Zygosaccharomyces lentus* sp. nov., a new member of the yeast genus *Zygosaccharomyces* Barker. *Int. J. Syst. Bacteriol.* (In Press).

Successful defense of a doctoral dissertation.

2. S.A. James. 1998. Molecular systematics of ascomycetous yeasts. PhD Thesis. University of East Anglia.

At present, yeasts are characterised, classified and identified by morphological, physiological and biochemical criteria. However, the reliability of using such criteria for yeast characterisation is being questioned, and is recognised as not reflecting the evolutionary relationships between these organisms. Knowledge of such relationships amongst bacteria has been revolutionised with the advent of small-subunit rRNA sequence analyses. In this study, such an approach was used to elucidate the relationships between species of the ascomycetous genera *Saccharomyces*, *Torulaspota* and *Zygosaccharomyces*.

The 18S rRNA gene sequences of twelve strains of the genus *Saccharomyces* were also determined. Results showed that the *Saccharomyces sensu stricto* (viz: *S. bayanus*, *S. cerevisiae*, *S. paradoxus* and *S. pastorianus*) formed a distinct phylogenetic group, while the *Saccharomyces sensu lato* (viz: *S. barnettii*, *S. castellii*, *S. dairensis*, *S. exiguus*, *S. rosinii*, *S. servazzii*, *S. spencerorum*, *S. transvaalensis*, *S. unisporus*) and *S. kluyveri* displayed marked phylogenetically heterogeneity, being interdispersed with members of other ascomycetous genera (e.g. *Kluyveromyces*, *Torulaspota* and *Zygosaccharomyces*).

The 18S rRNA gene sequences for the type strains of all current species belonging to the genera *Torulaspota* and *Zygosaccharomyces* were determined by DNA-direct sequencing of rDNA-PCR fragments. Analysis of the sequence data revealed that the species of the two genera were phylogenetically intermixed and formed four distinct lines: the '*Torulaspota delbrueckii* group' (viz: *T. delbrueckii*, *T. pretoriensis*, *T. globosa*, *Z. microellipsoides* and *Z. mrakii*), the '*Zygosaccharomyces bailii* group' (viz: *Z. bailii*, *Z. bisporus*, *Z. mellis* and *Z. mrakii*), the species pair of *Z. cidri* and *Z. fermentati*, and the species *Z. florentinus*.

In the course of this study, *Saccharomyces martiniae* sp. nov. and *Saccharomyces kunashirensis* sp. nov. were described. The sequences of the Internal Transcribed Spacer (ITS1 and ITS2) region for all species of the genera *Saccharomyces*, *Torulaspota* and *Zygosaccharomyces* were determined. Results showed that this spacer region was ideal for use in species delineation, being far more variable than the 18S rRNA gene. Consequently, species-specific PCR primers for *T. delbrueckii*, *Z. bailii*, *Z. bisporus* and *Z. rouxii* were developed.

XXII. Toulouse Levure Club. Centre de Bioingenierie Gilbert Durand, UMR-CNRS 5504, Lab Ass. INRA. Communicated by J.M. François <fran_jm@insa-tlse.fr>.

Five yeast groups participated in a yearly meeting, exchanging results, techniques, tips, abstracts books from International Meetings., etc.

Group Biogenesis of RNA, M. Caizergues-Ferrer, Yeast Ribosomes Biogenesis Laboratory, LBME du CNRS, 118 route de Narbonne, 31062 TOULOUSE cedex. <ferrer@ibcg.biotoul.fr>.

Recent publications.

1. Lafontaine, D., Bousquet-Antonelli, C., Henry, Y., Caizergues-Ferrer, M. & Tollervey, D. 1998. The box H+ACA snoRNAs carry Cbf5p, the putative rRNA pseudouridine synthase. *Genes Dev.* **12**:527-537.
2. Sicard, H., Faubladiere, M., Noaillac-Depeyre, J., Léger-Silvertre, I., Gas, N. & Caizergues-Ferrer, M. 1998. The role of the *S. pombe* gar2 protein in nucleolar structure and function depends on the concerted action of its highly charged N-terminus and its RNA binding domains. *Mol. Cell Biol.* **9**:2011-2023.
3. Kiss-Laszlo, S., Henry, Y. & Kiss, T. 1998. Sequence and structural elements of methylation guide snoRNAs essential for site-specific ribose methylation of pre-rRNA. *EMBO J.*, **17**:797-807.
4. Henras, A., Henry, Y., Bousquet-Antonelli, C., Noaillac-Depeyre, J., Gelugne, JP. & Caizergues-Ferrer M. Nhp2p and Nop10p are essential for the function of H/ACA snoRNP. *EMBO J.* (in press).
5. Qu, L., Henras, A., Lu, Y., Zhou, H., Zhou, W., Zhu, Y., Henry, Y., Caizergues-Ferrer, M., & Bachellerie, JP. Seven novel methylation guide snoRNAs are processed from a common polycistronic transcript by Rat1p and RNaseIII in yeast. *Mol. Cell. Biol.* (in press).

Theses.

6. C. Bousquet-Antonelli (March 1998). Caractérisation des partenaires fonctionnels de la protéine Gar1p de *S. cerevisiae*.
7. H. Sicard (December 1998). Etude des domaines fonctionnels de la protéine nucléolaire gar2 de la levure *S. pombe*.

Group Cell cycle, B. Ducommun, Université Paul Sabatier, Institut de Pharmacologie et de Biologie Structurale, IPBS - CNRS UPR9062, 205, route de Narbonne, 31077 TOULOUSE Cedex. <ducommun@ipbs.fr>
Focus: Cell cycle regulation in *Schizosaccharomyces cerevisiae*. Role of cdk's and cdc25 inhibitors.

Recent publication.

1. Pelloquin, L., Belenguer, P., Menon, Y. & Ducommun, B. 1998. Identification of a fission yeast dynamin-related protein involved in mitochondrial DNA maintenance. *Biophys. Biochem. Res. Commun.* **251**: 720-726.

Group Yeast and fungi metabolism - fermentation technology and process control. J.M. François (metabolism) and G. Goma (fermentation), Molecular Microbial Physiology and Biochemical Engineering groups, Centre de Bioingénierie Gilbert Durand, Département de Génie Biochimique et Alimentaire, INSA Toulouse. <fran_jm@insa-tlse.fr> or <Goma@insa-tlse.fr>.

Recent publications.

1. Alexandre, H., Plourde, L., Charpentier, C. & François, J. 1998. Lack of correlation between trehalose accumulation, cell viability and intracellular acidification as induced by various stress in *Saccharomyces cerevisiae*. *Microbiology*, **144**:1103-1111.
2. Dallies, N., François, J. & Paquet, V. 1998. A new method for quantitative determination of polysaccharides in the yeast cell wall. Application to the cell wall defective mutants of *Saccharomyces cerevisiae*. *Yeast* **14**:1297-1306.
3. Hajjaj, H., Blanc, P.G., Goma, G. & François, J. 1998. Sampling techniques and comparative extraction procedures for quantitative determination of intra- and extracellular metabolites in filamentous fungi. *FEMS Microbiol Lett.* **164**:195-200.

4. Martin, H., Dagkessamanskaia, A., Satchanska, G., Dallies, N. & François, J. 1998. *KNR4*, a suppressor of *Saccharomyces cerevisiae* cwh mutants, is involved in the transcriptional control of chitin synthase genes. Microbiology (in press).
5. Hajjaj, H., Kláébé, A., Loret, M.O., Goma, G., Blanc, P.G. & François, J. 1998. Biosynthetic pathway of citrinin in the filamentous fungus *Monascus ruber* as revealed by ¹³C nuclear magnetic resonance. Appl. Env. Microbiol. (in press).
6. Parrou, J.L., Enjalbert, B., Plourde, L., Bauche, A., Gonzalez, B. & François, J. 1998. Dynamic responses of reserve carbohydrate metabolism under carbon and nitrogen limitations in *Saccharomyces cerevisiae*. Yeast (in press).
7. Antonelli, R., Estevez, L. & Denis-Duphil, M. 1998. Carbamyl-phosphate synthetase domain of the yeast multifunctional protein Ura2 is necessary for aspartate transcarbamylase inhibition by UTP. FEBS Lett. **422**:170-174.

Theses.

8. R. Antonelli (March 1998). Étude de la protéine multifonctionnelle Ura2 chez *Saccharomyces cerevisiae*.
9. H. Hajjaj (February 1998). Mécanismes régulant la voie de biosynthèse des polycétides (pigments rouges et toxines) chez un champignon filamenteux, *Monascus ruber*.
10. H. Martin (November 1998). Étude des mécanismes moléculaires et cellulaires impliqués dans l'assemblage de la paroi chez la levure *Saccharomyces cerevisiae*.

Group Yeast as a tool for heterologous production of molecules. G. Loison, Sanofi Recherche, Genetics and Cell Biology Laboratory, Microbiology Department, Labege Innopole Voie N°1, BP137, 31376 Labege France <gerard.loison@tls1.elfsanofi.fr>.

Publications.

1. Paul, S. Silve, N. de Nys, P.H. Dupuy, C. Labit-Le Bouteiller, J. Rosenfeld, P. Ferrara, G. Le Fur, P. Casellas & G. Loison. 1998. Both the immunosuppressant SR31747 and the antiestrogen tamoxifen bind to an emopamil-insensitive site of mammalian delta8-delta7 sterol isomerase. J. Pharmacol. Exp. Ther. **285**:1296-1998
2. Silve, P.H. Dupuy, P. Ferrara & G. Loison. 1998. Human Lamin B receptor exhibits sterol C14-reductase activity in *Saccharomyces cerevisiae*. Biochim. Biophys. Acta **1392**:233-244.

XXIII. Department of Biological Sciences, Tata Institute of Fundamental Research, Homi Bhabha Road, Mumbai, India. Communicated by A. Sadhu.

Sugar transport in the Fission yeast *Schizosaccharomyces pombe* is recently being studied, and seems to be much different from that in *S. cerevisiae*. The process of sugar transport in pombe is not governed by as many genes as in *S. cerevisiae*. Only two genes have so far been reported - one by Milbradt & Hofer (Microbiology 140: 2617-2623,1994), and the other from our laboratory. The isolation of two Sugar Transport Defective mutants (std1/8 & std 1/23) was reported from our lab a few months back

(J. Bact. Vol.180, No.3.p. 674-679). The mutants also failed to grow on other disaccharides. The mutations were found to be allelic and complemented the YGS4 and YGS5 glucose symporter mutants. In continuance of this work, we are interested in knowing how exactly are these two genes involved in the process of sugar uptake. To proceed along this line, we are engaged in isolating suppressors of the aforesaid mutation and studying them.

XXIV. Department of Microbiology & Molecular Genetics, Oklahoma State University, 36 Life Science East, Stillwater, Ok 74078-0289, U.S.A. Communicated by H.S. Vishniac.

Recent publications.

1. Vishniac, H. S. 1998. The ecology of extremophile yeasts. Proc. SPIE Conference on Instruments, Methods, and Missions for Astrobiology **3441**:68-74.
2. Vishniac, H. S. Psychrophilic Yeasts. In Enigmatic Microorganisms and Life in Extreme Environments, J. Seckbach, ed. (In Press).

XXV. Institut für Biologie IV (Mikrobiologie) der RWTH Aachen, Worringer Weg, 52074, Aachen, Germany. Communicated by V. Passoth <volkmar.passoth@tmb.lth.se>.

The following dissertation was defended recently. The thesis was done under the supervision of Prof. U. Kliner.

1. V. Passoth.¹ About the regulation of ethanol formation in the xylose fermenting yeast *Pichia stipitis*. (Zur Regulation der Ethanolbildung in der Xylose vergärenden Hefe *Pichia stipitis*).

¹Present address: Applied Microbiology, Lund Institute of Technology/Lund University, P.O. Box 124, S-221 00 Lund, Sweden.

The regulatory mechanisms governing fermentation and respiration in the Crabtree-negative, xylose fermenting yeast *Pichia stipitis* are very different from that described for *Saccharomyces cerevisiae*. While *S. cerevisiae* has the ability to repress the respiratory chain, *P. stipitis* appears to be strictly respirative. Activity of the respiratory chain was not repressed by either high concentrations of fermentable sugars or by oxygen limitation. However, fermentation was not induced by high sugar concentrations, but was inactivated under aerobic conditions.

In *P. stipitis* the activity of pyruvate dehydrogenase (PDH) was constitutive. In contrast, pyruvate decarboxylase (PDC), alcohol dehydrogenase (ADH), and aldehyde dehydrogenase (AldDH) were induced by a reduction in the oxygen tension. It was demonstrated that the pyruvate decarboxylase is not induced by a signal from

glycolysis. The PDH has a Michaelis-Menten kinetics and shows similar K_m values as described in other eukaryotes. PDC shows allosteric kinetics but contrary to *S. cerevisiae*, it was not inhibited by phosphate.

Two *ADH*-genes (*PsADH1* and *PsADH2*) were isolated by complementation of a *S. cerevisiae* Adh-mutant. The sequences showed homologies to zinc-dependent *ADH*-gene family. *PsADH2* encodes for the ADH-activity that is responsible for ethanol formation during oxygen limitation. This gene is regulated at the transcriptional level. Moreover, also in cells grown on ethanol, only *PsADH2*-transcript was found. The *PsADH1*-transcript was only detected under aerobic conditions on fermentable carbon sources at a low level.

XXVI. Yeast Research Group, School of Molecular and Life Sciences, University of Abertay Dundee, Dundee, DD1 1HG Scotland. Communicated by G.M. Walker <g.walker@tay.ac.uk>.

Activities of the Group.

Yeast research in the School of Molecular and Life Sciences at the University of Abertay Dundee is concerned with investigations of both fundamental and applied aspects of yeast cell physiology. Research projects deal with regulation of nutrition, growth, cell division, metabolism and cell death in yeasts of industrial, medical and environmental importance. Collaborative projects have been undertaken with industry (e.g., BP Chemicals, Glare, ICI, Quest International, Bass Brewers, Scottish Courage, Lallemand Inc, Scottish Crop Research Institute, Sri Lanka Sugarcane Research Institute) and academia (e.g., Heriot-Watt University, Oxford-Brookes University, University of Perugia, Italy).

Current Research Projects. Cell Physiology of Industrial Yeasts. Stress responses in brewing and winemaking yeasts. Role of metal ions in regulating fermentation in alcohol-producing yeasts. Expression of the Crabtree Effect in non-*Saccharomyces* yeasts. Steady-state perturbations in baker's yeast physiology. Heavy metal biosorption/bioremediation by industrial yeasts. **Killer Yeast Biotechnology.** Potential of yeast killer toxins as novel antimycotic pharmaceuticals. Killer yeasts and their toxins as biocontrol agents against plant pathogenic and wood biodeteriogenic fungi.

For further information on the work of the Yeast Research Group at Abertay, including possible collaborative ventures, please contact Dr. Walker at the address above.

Recent publications.

1. Walker, G.M. 1994. Crit. Rev. Biotechnol. **14**:311-354.
2. Hodgson, V.J., Walker, G.M. & Button, D. 1994. FEMS Microbiol Lett. **120**:201-206.

3. Walker, G.M. *et al.* 1995. In: Proc. Aviemore Conf. Malting, Brewing & Distilling. Ed. I. Campbell and F.G. Priest. Institute of Brewing (London) pp. 185-189.
4. Walker, G.M., McLeod, A.H. & Hodgson, V.J. 1995. FEMS Microbiol. Lett. **127**:213-222.
5. Hodgson, V.J., Button, D. & Walker, G.M. 1995. Microbiology **141**:2003-2012.
6. Walker, G.M. & Maynard, A.I. 1995. Enz. Microb. Technol. **18**:455-459.
7. Walker, G.M. *et al.* 1996. J. Amer. Sec. Brew. Chem. **54**:13-18.
8. Walker, G.M. & Maynard, A.I. 1997. J. Indust. Microbiol. **18**:1-3.
9. Chandrasena, G., Staines, H. & Walker, G.M. 1997. J. Amer. Sec. Brew. Chem. **55**:24-29.
10. Walker, G.M. 1998. Yeast Physiology and Biotechnology. J. Wiley & Sons. Walker, G.M. 1998 J. Amer. Sec. Brew Chem. **56**: in press.
11. G. Walker. 1998. Yeast Physiology and Biotechnology. Wiley, 362 pp.

Yeast Physiology and Biotechnology redresses the balance by linking key aspects of yeast physiology with yeast biotechnology. Individual chapters provide broad and timely coverage of yeast cytology, nutrition, growth and metabolism - important aspects of yeast cell physiology which are pertinent to the practical uses of yeasts in industry. The final chapter reviews traditional, modern and emerging biotechnologies in which roles of yeasts in the production of industrial commodities and their value in biomedical research are fully discussed. Relevant aspects of classical and modern yeast genetics and molecular biology are fully integrated into the appropriate chapters. This up-to-date and fully referenced book is aimed at advanced undergraduate and postgraduate bioscience students, but will also prove to be a valuable source of information for yeast researchers and technologists.

Contents.

Introduction to yeasts: The world of yeasts. Importance of yeasts to mankind. Yeast physiology and biotechnology.

Yeast cytology: Introduction. General cellular characteristics of yeasts. Cytological methods for yeasts. Yeast cell architecture and function. Yeast nutrition: Introduction. Yeast nutritional requirements. Nutrient acquisition by yeasts.

Yeast growth: Introduction. Cellular growth of yeast. Population growth of yeast. The physicochemical environment and yeast growth. Biotic factors influencing yeast growth. Yeast cell death.

Yeast metabolism: Introduction. Carbon and energy metabolism. Nitrogen metabolism. Phosphorus and sulphur metabolism. Specialized metabolism.

Yeast technology: Introduction. Applied molecular genetics of yeast. Developments in yeast technologies. Yeasts in biomedical research.

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XXVII. Laboratory of Dairy Science, Institute of Food Science, Swiss Federal Institute of Technology, ETH, 8092 Zürich, Switzerland. Communicated by M.T. Wyder <marie-therese.wyder@ilw.agrl.ethz.ch>.

Successful defense of doctoral dissertation:

1. Wyder, M.T. 1998. Identification and characterisation of the yeast flora in kefir and smear ripened cheese - contribution of selected yeasts to cheese ripening. Ph.D. Dissertation, Diss. ETH No. 12842.

Yeasts are widely dispersed in the environment and, thus, are encountered also in several dairy products as part of the typical microflora, or else as contaminants. They are known for their high tolerance towards low pH and temperature as well as for their ability to ferment carbon sources to mainly ethanol and carbon dioxide. In kefir which undergoes both a lactic acid and an alcoholic fermentation, yeasts are responsible for the development of a distinctive prickling taste. A large number of cheese varieties are characterised by a specific surface microflora composed of moulds, yeasts, micrococci and coryneform bacteria. Yeasts contribute to the metabolism of lactic acid with the consequence of increased pH. As contaminants in fermented milk products, they may cause

blowing of the packages. The importance of yeasts in the production and spoilage of dairy products is indisputable. There is, thus, a need for a rapid and accurate identification technique. Furthermore, not much is known about the direct contribution by yeasts to cheese ripening.

In the present study, the yeast flora of five kefir grains and three smear ripened cheese varieties was investigated using non-traditional methods. Electrophoretic karyotyping in the contour-clamped homogeneous electric field and amplification of the ITS region with subsequent restriction analysis were used to characterise and group isolates from kefir, whereas for the characterisation and grouping of isolates from cheese only the ITS method was applied.

The use of PCR and subsequent restriction analysis proved to be a simple, rapid and reliable technique to differentiate yeasts at the species level. Isolates from cheese were then tested in cheese curd slurries in order to select four species for the production of foil ripened Raclette cheeses. The objective was to gain more knowledge on their direct contribution to ripening and flavour development.

The composition of the kefir yeast flora with totally five different species was strongly dependent on the production procedure. In grains with at least two different species *Yeast U* always dominated and when absent, *Saccharomyces unisporus* was predominant. But during fermentation, the lactose fermenting yeasts *Kluyveromyces marxianus* and *Candida kefir* could catch up with *Yeast U*. When kefir was taken as inoculum, *K. marxianus* or *C. kefir* outnumbered all other mostly lactose negative species, if absent, however, *S. unisporus* dominated. Repeated subculturing (22 °C for 24 h) always led to a decrease of the yeast count.

It was not possible to identify *Yeast U* with certainty. This yeast was found, according to its biochemical characteristics, to be close to either *S. bayanus* or *S. pastorianus* which, however, could not be confirmed by analysing the ITS region. Since the species occurs fairly often in grains, its identity would be of high interest.

A total of 12 species were isolated from the surface and the inside of Limburger, Münster and Tête de Moine cheeses. In spite of applying cultures of *Debaryomyces hansenii* and *Galactomyces geotrichum* on the surface of Limburger and Münster, most of the yeasts found originated from spontaneous contamination. The predominant species in all investigated cheese varieties was *D. hansenii* regardless of whether it had been added as a starter to the smear or of whether it originated, in case of Tête de Moine, from spontaneous contamination. The second most frequent species was *Gal. geotrichum* followed by *Yarrowia lipolytica*. The soft cheeses Limburger and Münster made with pasteurised milk revealed a homogeneous yeast flora with a maximum of 3-5 species. The composition inside and on the surface of the cheeses was very much alike. The yeast flora in the inner part of Tête de Moine which is made with raw milk, was quite heterogeneous with a spectrum of seven species. However, the surface flora composed of mainly *D. hansenii* was not affected by the inside flora. The counts of

100–1000 CFU/g inside the cheeses were always much lower than on the surface. According to the results obtained with cheese curd slurries, most yeasts isolated from the smear ripened cheeses could be divided into two groups. One group was characterised by the ability to ferment glucose, to utilise lactate, to increase pH and not showing proteolytic activity. The resulting odour of the slurries was mainly acidic, fruity or fermented. The second group was composed of non fermenting species which utilised lactate but did not affect pH. They were proteolytic yielding a cheesy aroma. For the production of Raclette model cheeses, *Pichia jadinii* was chosen from the first group, and *Gal. geotrichum* and *Y. lipolytica* from the second group. The fourth species was *D. hansenii* B, isolate from Tête de Moine, which combined properties of both groups.

In the mature Raclette model cheeses ripened in foil, hardly any yeasts could be detected as a consequence of primarily anaerobic conditions in and around the cheeses. Thus, no lactic acid degradation occurred. In contrary, yeasts led to a moderate increase of lactic acid concentration. This might be attributed to a stimulation of the lactic starter as a result of released metabolism products by yeasts. Furthermore, yeasts may have affected syneresis and consequently the final lactic acid content. Lipid metabolism seemed to be characterised either by esterase (*Gal. geotrichum* and *D. hansenii* B) and/or lipase activity (*P. jadinii* and *Y. lipolytica*). As far as proteolysis is concerned, all but *Y. lipolytica* revealed peptidase activity resulting in smaller breakdown products and free amino acids. Except for *Gal. geotrichum*, they also enhanced formation of biogenic amines. Finally, yeasts influenced the sensory quality of cheese significantly. *Y. lipolytica* was able to improve overall sensory characteristics. All other species, however, influenced the flavour rather negatively.

Gal. geotrichum, *P. jadinii* and *D. hansenii* are species often applied in cheese production. From the results obtained with the Raclette model cheeses, it can be concluded that yeasts should always be applied in combination. In order to improve flavour, *Y. lipolytica* should never be missing. If biogenic amine formation needs to be suppressed, *Gal. geotrichum* might be of benefit. However, since hardly any yeasts were detectable in the mature cheeses, the action of yeasts can most probably be attributed to enzymes released after cell lysis.

XXVIII. Department of Microbiology, Swedish University of Agricultural Sciences, P.O. Box 7025, S-750 07 Uppsala, Sweden. Communicated by J. Schnürer <Johan.Schnurer@mikrob.slu.se>.

The following thesis has been defended recently.

1. Petersson, S. 1998. Yeast/mold interactions during airtight storage of high-moisture feed grain. Doctor's dissertation. ISSN 1401-6249, ISBN 91-576-5505-7.

The yeast *Pichia anomala* (Hansen) Kurtzman, isolated from cereals, was evaluated in terms of its potential as a biocontrol agent. In vitro, *Pichia anomala* was antagonistic in a dose-dependent manner against a variety of spoilage molds in the Ascomycetes, Deuteromycetes, and Zygomycetes. *Pichia anomala* prevented the growth of *Penicillium roqueforti* in high moisture winter wheat, barley, and oats during malfunctioning airtight storage in laboratory experiments as well as in pilot-scale (0.2 m³) experiments. The atmosphere in the silos and the high levels of *Pichia anomala* in the wheat grain contributed to the growth suppression of *Penicillium roqueforti*, which is the most important spoilage fungi in

airtight-stored cereals. Inhibition by *Pichia anomala* was least pronounced at the optimum temperature (21 °C) and water activity (0.95) for growth of *Penicillium roqueforti*. *Pichia guilliermondii*, which has been used to control citrus molds, and baker's yeast, *Saccharomyces cerevisiae*, were less efficient in suppressing the growth of *Penicillium roqueforti* in wheat: Even at the highest inoculated yeast level, only a slight growth reduction was observed.

Pichia anomala grew equally well on barley, oat, rye, and wheat (winter and spring variety). Rye cv. Motto and two lots of spring wheat cv. Dragon were resistant to infection by *Penicillium roqueforti*. The barley cultivars Maud and Mentor also showed

some degree of resistance. *Pichia anomala* was shown to reduce ochratoxin A accumulation in co-culture with *Penicillium verrucosum* in vitro and in wheat. The level of yeast needed to reduce mycotoxin accumulation was lower than the level needed to reduce growth of the mold isolates. In contrast to earlier findings with other yeast-mould co-cultures, no stimulation of mycotoxin

accumulation in the tested co-cultures was observed *Pichia anomala* should have great potential as a biocontrol agent in airtight systems used for storing high-moisture grains, particularly when used together with grain cultivars that exhibit endogenous resistance to *Penicillium roqueforti*.

**XXIX. Department of Food Science and Technology, University of California, Davis, CA 95616, USA.
Communicated by H.J. Phaff <hjphaff@ucdavis.edu>.**

The following paper was accepted recently.

1. H.J. Phaff, W.T. Starmer, C.P. Kurtzman. 1999. *Pichia lachancei* sp. nov., associated with several Hawaiian plant species. Int. J. Syst. Bacteriol. (In press).

A description is given of *Pichia lachancei*, a new species of yeast that occurs in association with several Hawaiian plant species of the genera *Tetraplasandra*, *Cheirodendron*, and *Clermontia*. The new species is heterothallic and occurs in nature in the haploid as well as the diploid state. Upon conjugation of complementary mating types zygotes are formed that reproduce by budding as diploid cells. When placed on sporulation medium,

four hat-shaped spores are produced which are rapidly released from the ascus. Phylogenetic analysis showed that *P. lachancei* is most closely related to *Pichia rhodanensis* and *P. jadinii*. The diploid type strain of *P. lachancei*, isolated from rotting bark of *Tetraplasandra hawaiiensis* on the island of Hawaii, is strain UCD-FST 79-9 = ATCC 201914 = CBS 8557 = NRRL Y-27008.

**XXV. Department of Plant Sciences, University of Western Ontario, London, Ontario N6A 5B7.
Communicated by M.A. Lachance <lachance@julian.uwo.ca>.**

The following articles, whose abstracts appeared in the previous issue of the Yeast Newsletter, are now in print.

1. Rosa, C.A. and M.A. Lachance. 1998. The yeast genus *Starmerella* gen. nov. and *Starmerella bombicola* sp. nov., the teleomorph of *Candida bombicola* (Spencer, Gorin & Tullock) Meyer & Yarrow. Int. J. Syst. Bacteriol. **48**:1413-1417.
2. Lachance, M.A., C.A. Rosa, W.T. Starmer, B. Schlag-Edler, J.S.F. Barker, and J.M. Bowles. 1998. *Wickerhamiella australiensis*, *Wickerhamiella cacticola*, *Wickerhamiella occidentalis*, *Candida drosophilae*, and *Candida lipophila*, five new related yeast species from flowers and associated insects. Int. J. Syst. Bacteriol. **48**:1431-1443.
3. Rosa, C., M.A. Lachance, W.T. Starmer, J.S.F. Barker, J.M. Bowles, and B. Schlag-Edler. *Kodamaea nitidularum*, *Candida restingae*, and *Kodamaea anthophila*, three new related yeast species from ephemeral flowers. Int. J. Syst. Bacteriol. **49**:309-318.
4. Lachance, M.A., C.A. Rosa, W.T. Starmer, and J.M. Bowles. 1998. *Candida ipomoeae*, a new yeast species related to large-spored *Metschnikowia* species. Can. J. Microbiol. **44**:718-722.

The following publications were accepted recently.

5. Lachance, M.A., J.M. Bowles, W.T. Starmer, and J.S.F. Barker. 1999. *Kodamaea kakaduensis* and *Candida tolerans*, two new yeast species from Australian *Hibiscus* flowers. Can. J. Microbiol. (Accepted).

Two new yeast species were isolated from flowers of *Hibiscus* species in Eastern and Northern Australia. *Kodamaea kakaduensis* is heterothallic, haploid, and similar to other *Kodamaea* species and to *Candida restingae*. Buds are often produced on short protuberances, and a true mycelium is formed. The new species

differs from others by the assimilation of trehalose, melezitose, and xylitol, and is reproductively isolated. The cells of *Candida tolerans* are small and a pseudomycelium is formed. The carbon and nitrogen assimilation pattern is reminiscent of that of *Zygosaccharomyces rouxii* but the two are not closely related.

Sequences of the D1/D2 domain of large subunit ribosomal DNA confirm the membership of *K. kakaduensis* in the genus *Kodamaea* and indicate that *C. tolerans* belongs to the *Clavispora-Metschnikowia* clade, with a moderate relatedness to *Candida mogii*.

The type strains are *K. kakaduensis*, UWO(PS) 98-119.2 (h⁺, holotype, CBS 8611) and UWO(PS)98-117.1 (h⁻, isotype, CBS 8612); *C. tolerans*, UWO(PS) 98-115.5 (CBS 8613).

6. Rosa, C.A., R.P. Martins, E.M. Viana, Y. Antonini, and M.A. Lachance. 1999. *Candida batistae*, a new yeast species associated with solitary digger nesting bees in Brazil. *Mycologia* (In press).

Several strains the new yeast species *Candida batistae* have been isolated from larval provisions, larvae, and pupae of the solitary bees *Diadasina distincta* and *Ptilotrix plumata* in Minas Gerais, Brazil. *Candida batistae* was the predominant species in the yeast community associated with this habitat, where it often co-occurred with a yeast form of *Mucor* sp. It is thought that these two organisms play a role in the maturation of beebread. The new asexual ascomycetous yeast is related to *Starmerella bombicola* and other

floricolous *Candida* species, as evidenced by their nutritional profiles and the sequences of the D1/D2 domains of their large subunit ribosomal DNAs. Identification characteristics are similar to those of *S. bombicola*, but the species can be separated on the basis of some physiological characters and absence of mating with haploid strains of that species. The type culture is strain UFMG-Y-192 (CBSCBS 8550).

Paper presented recently.

7. Lachance, M.-A. 1998. The role of rDNA sequencing in exploring the biodiversity of yeasts associated with flowers. 19th ISSY, University of Minho, Braga, Portugal.

rDNA sequencing played a major role in the description of several new yeasts from flowers. (1) Varieties of the giant-spored *Metschnikowia continentalis* from Convolvulaceae conjugate freely, but inter-varietal ascospores are rarely formed. Conjugation with *M. hawaiiensis* occurs with only one mating type combination and produces sterile asci. *M. hibisci* forms conjugation tubes when mixed with these. This speciation gradient helps to calibrate rDNA divergence. D1/D2 domains of the varieties differ at 4 positions, and these differ at 22 positions from *M. hawaiiensis*. *M. hibisci* has a shorter D2 with little similarity to the others. *Candida ipomoeae* differs by 11 positions from the giant-spored species, but its origin is elusive. (2) The D1/D2 domains of three *Wickerhamiella* species differ by 2-5%. Two *Candida* species had not been classified in the past due to their lack of diagnostic traits.

Sequencing showed that *C. lipophila* is closely related to *W. occidentalis* and that *C. drosophilae* is basal to these and to *W. cacticola* and *W. australiensis*. Whether the teleomorphs represent a separate genus is not resolvable by sequence comparison. For lack of a better alternative, a "lumper" attitude was adopted. (3) Three species form a tight clade with *Kodamaea ohmeri*. *Candida restingae* and *K. nitidulidarum* are sister species from cactus flowers, and *K. anthophila* is from *Ipomoea* and *Hibiscus* species. (4) The D1/D2 sequence of a new heterothallic yeast similar to *Wickerhamiella* is identical to that of *Candida bombicola*. As the latter is not related to any known teleomorphs, the genus *Starmerella* was proposed. The general conclusion is that the use of rDNA sequencing in ecological systematics is revolutionizing yeast biodiversity research.

Minutes of the Business Meeting, International Commission for Yeasts - ICY 19th ISSY, Braga, Portugal, 30 August 1998

Commissioners in attendance: Graham Fleet (Chair), Australia; Sally A Meyer (Vice Chair) & Clete Kurtzman, USA; Leda Mendonça-Hagler, Brazil; H. Verachtert, Belgium; André Lachance, Canada; A.Y. Gibriel, Egypt; Matti Korhola, Finland; Tibor Deak & Anna Maraz, Hungary; Alesandro Martini & Patrizia Romano, Italy; Hans van Dijken & Lex Scheffers, The Netherlands; Isabel Spencer-Martins & Maria Loureiro-Dias, Portugal; T. Lachowicz, Poland; Inna Bab'eva, Gennadi Naumov, Russia; Peter Raspor, Slovenia; Bernard Prior, South Africa; Barbel Han-Hagerdal, Sweden; Andrei Sibirny, Ukraine; David Berry, UK; Invited participant: Cecilia Leão, Portugal.

1. Graham Fleet called the meeting to order, welcomed everyone and thanked them for attending. He read the names of those Commissioners that had sent their apologies for not attending the meeting. Apologies were received from: J. du Preez, South Africa; I. Russell, Canada; B. Johnson, Canada; P. Venkov, Bulgaria; J. Stenderup, Denmark; J. Gancedo, Spain; M. Beckers,

Latvia; P. Sandven, Norway; E. Minárik, Slovakia; H. Neujahr, Sweden; P. Biely, Slovakia; H. Prillinger, Austria; M. Kopecka, Czech Republic; N. Pamment, Australia; K. Watson, Australia; R. Sentandreu, Spain.

2. The **Minutes** of the last meeting were approved as published in the Yeast Newsletter, December 1997.

3. **Commissioner list.** Graham Fleet outlined progress in updating the list of Commissioners. Following from the last meeting of ICY Commissioners (Bled, 1997) a working party consisting of Sally Meyer, Graham Stewart and Graham Fleet was set up to address the long-overdue task of updating this list. Letters were written to all commissioners who were identified to be inactive, to determine their future commitment. If no response was received within three months, then this name would be removed from the list of Commissioners and replacements sought. As a consequence of this initiative, some commissioners reconfirmed their commitment, others indicated their willingness to retire and others did not respond

and will be removed from the list.

Resignations: T. Hirano, Japan; H. Verachtert, Belgium; T. Enari, Finland; M. Shepherd, New Zealand; J. Gancedo, Spain; B.J. Zonneveld, The Netherlands; F. Sherman, USA.

Commissioners to be removed from the list: L. Ming-Xia, China; C. Emeis, Germany; W. Nordheim, Germany; P. Weber, Germany; T. Subbaiah, India; Y. Fukuzawa, Japan; K. Iwata, Japan (the new Japanese Commissioner, Professor A. Toh-e has confirmed that these former Japanese commissioners are no longer active or contactable); O. Kappeli, Switzerland.

New commissioners: The following new commissioners were nominated and approved: C. Leão, Portugal; J. Thevelein, Belgium; A. Toh-e, Japan; I. Pretorius, S. Africa.

Nominated but confirmation and acceptance required: M. Foda, Egypt; M. Penttila, Finland; S. Inge-Vechtomov, Russia.

4. Report on ISSY 19. Cecilia Leão spoke on the organisation of ISSY-19. Money was a concern. It took a year to find money to support the symposium. She noted that her University was very helpful and supportive. It was acknowledged by several individuals that money is always a problem in industry/university sponsorship. It was suggested that, for each symposium, a small percentage of the registration fee be retained to serve as seed money for future symposia. Some commissioners thought that there may be administrative hurdles associated with this and may be more trouble than it is worth. Something to think about for the future. Everyone agreed that the ISSY 19 was well-organised and Cecilia and her committee did an excellent job.

5. Future symposia

ISSY 20. Graham opened the discussion of future meetings and reminded everyone that ISSY 20 will take place in Smolenice, Bratislava, Slovakia in May 1999. Peter Biely is organising the symposium. The topic is **Yeast Cell Surfaces and Membrane Phenomena**. The first circular is available. It was recognised that ISSY 20 will coincide with the conference on **Yeast genetics and molecular biology** to be held in Italy. After some discussion, it was suggested that Graham Fleet write to Peter Biely to see if the date of ISSY 20 could be changed to avoid this clash. However, it was pointed out that the venue, Smolenice Castle, was already booked and it would be difficult to change that since it is a venue in great demand. Also, it was noted that this Castle would accommodate only about 80-90 participants, and that ISSY 20 would not be aiming to attract large numbers – only a very specialised group of scientists, few of which would be involved with the Yeast Genetics symposium (Graham Fleet has since contacted Peter Biely and confirmed these circumstances. It is not possible to change the dates of ISSY 20 and he was advised that the clash of dates had little impact on the speakers so far invited to ISSY 20; ISSY 20 would proceed as scheduled and advertised).

ISSY 21 will take place 19-22 August 2001 at the University of Lviv, Ukraine. Andrei Sibirny will organise this symposium. Andrei suggested the topic **Biochemistry, genetics, biotechnology and ecology of non-conventional yeasts**. There was discussion about the term ‘non-conventional yeasts’. It was recommended by the meeting that the title be changed to ‘Biochemistry, genetics, biotechnology and ecology of non-*Saccharomyces* yeasts’.

ISSY 22 in the year 2002. Bernard Prior volunteered to organise a symposium in South Africa in either April or July. It will be held at a game reserve. The topic suggested is **Yeasts**

and Fermentation. Bernie informed the commissioners that the air fare is competitive.

ISSY 23. Tibor Deak foreshadowed the possibility of a meeting on **Saprophytic Yeasts** in 2003.

ISY 10 in the year 2000. Lex Scheffers gave a detailed report on the on-going organisation of the next ISY that will take place 27 August-1 September 2000 at Papendal, Arnhem, The Netherlands. The title is **The Rising Power of Yeast in Science and Industry**. ISY 10 will bring together scientists from all disciplines involved in the study of yeasts and yeast-like organisms: physiologists, geneticists, taxonomists, molecular biologists, biotechnologists, food microbiologists and medical mycologists. First Announcements will be sent out in January 1999. Promotion posters have been sent out.

There was vigorous discussion about the balance between the subject topics to be covered at ISY 10, as outlined in the report. In particular, numerous commissioners felt that the proposed plenary sessions gave too much emphasis on the molecular aspects of yeast biology, with the exclusion of topics covering taxonomy, ecology, and the applied and medical aspects of yeast biology. It was noted that the ISY series of symposia are general symposia and should give a balanced cover of all aspects of yeast biology and technology. It was emphasised by Lex Scheffers (Secretary) and Hans van Dijken (Chair) of the ISY 10 organising committee that the current proposal was a draft for discussion and that Commissioners should convey their ideas and suggestions to them for better balancing of the program. **PLEASE DO SO**. It was agreed that Graham Fleet would communicate with Lex and Hans on the matter of finding an appropriate balance in the ISY 10 program. Generally, the commissioners were impressed with the advance attention and planning given by the organising committee of ISY 10.

ISSY 11. Clete Kurtzman suggested the possibility of having ISSY 11 in 2005 in conjunction with the Mycological Society of America in Chicago, IL, USA. An alternative is to have an ISSY on systematics/phylogeny with this group. ISY 11 in the year 2005 would take us out of our usual 4 year cycle. Clete was to explore the options further and advise ICY commissioners in due course.

The IUMS Congress will meet in Sydney in August 1999. The ICY has organised a symposium on **Biodiversity** within this Congress. Participants and topics include: Sandro Martini/taxonomy; André Lachance/ecology; Isabel Spencer-Martins/physiology; Barbel Han-Hagerdal/metabolism; Gennadi Naumov/genetics; Ken Watson/diversity of stress response.

6. Yeast Newsletter. André Lachance discussed the current status of the Yeast Newsletter. There are 420 readers in 55 countries. The YNL is solvent. Subscription fees are being paid. Dr. Hirano (Japan) has resigned from his position as Associate Editor and is to be replaced by Dr. Oshima (Japan). The ICY formally thanks Dr. Hirano for his contribution. Dr. Zonneveld (The Netherlands) will also vacate his position as Associate Editor and he is thanked for his contribution.

7. Network for food and beverage yeast. Patrizia Romano reported on the organisation of a network for ‘yeast people’ where information can be dispersed about methods of isolation and identification, enumeration, workshops. Researcher interested in this network should contact Patrizia.

8. New business: Clete Kurtzman brought up the topic of ‘Names of Common Use’. This is a relatively ‘new’ concept in mycology in which the names accepted in recent taxonomic

treatments are considered valid and not subject to change (even if previous names were discovered). For the yeasts the currently accepted names are in the new edition of THE YEASTS, A TAXONOMIC STUDY. Clete said he wants to consult with several 'taxonomic authorities' to consider the impact this would have before he brings a formal proposal to the ICY. The other topic he discussed was that the recently adopted International Code now allows yeast type material to be lyophilised.

9. Graham thanked all the Commissioners for attending the business meeting and thanked Cecilia for providing lunch. Cecilia

informed the commissioners that there was a small gift for each member, a packet of postcards from the different regions of Portugal. She was thanked by all and given round of applause for organising an outstanding ISSY.

10. **Next meeting.** The next meeting of commissioners will be held during ISSY 20, May 1999, Smolenice, Bratislava, Slovakia.

11. **Meeting adjourned** at 3:00 pm.

Sally Meyer (Vice-Chair) and Graham Fleet (Chair)

Recent meetings

27th Annual Conference on Yeasts of the Czech and Slovak Commission for Yeasts Smolenice, Slovakia, May 13-15, 1998

The 27th Annual Conference on Yeasts, organized by the Czech and Slovak Commission for Yeasts and the Institute of Chemistry, Slovak Academy of Sciences, took place in the Smolenice Castle, the Congress Center of the Slovak Academy of Sciences, May 13-15, 1998. The meeting was attended by 27 participants from the Czech Republic, 34 participants from Slovakia, 4 participants from Hungary, and by two distinguished guests from other countries who gave plenary lectures in English. The presence of three plenary speakers was generously sponsored by the Center for Advancement, Science and Technology (SARC) in Bratislava. The program consisted of a block of three plenary lectures in English and three plenary sessions in Czech and Slovak, the two closely related languages of recently separated nations, and a poster session. The meeting covered the following three subjects: Yeast Cell Biology, Molecular Biology and Genetics and Yeasts and Stress. The last subject was on the program for the first time. The lectures were complemented by 34 posters. The titles of all contributions are listed below:

Plenary lectures of foreign guests

- J. Thevelein (Belgium). Nutrient-induced signal transduction and its importance in industrial application of yeasts.
- V. Pölös (Hungary). Development of wine yeast cultures in the Research Institute for Viticulture and Enology.
- D. Haltrich, B. Nidetzky, K. Kulbe (Austria). Technological application of NAD(P)H-dependent xylose reductase from *Candida tenuis*.

Plenary lectures in cell biology

- Kopecká, M. Gabriel. Actin cytoskeleton and yeast cell wall construction.
- V. Raclavský. Signaling towards cell wall synthesis in *Saccharomyces cerevisiae*.
- A. Pichová. Cell morphology and septum formation in *Schizosaccharomyces pombe*.
- D. Vondráková, A. Pichová. Yeasts as a model organisms for the study of aging process.

Plenary lectures in the session Yeasts and Stress

- K. Sigler, N. Stadler, B. Brodská, D. Gášková. Oxidative stress and yeast membrane.
- D. Gášková, B. Brodská, K. Sigler. The influence of various *Saccharomyces cerevisiae* membrane affecting stressors on membrane potential.
- E. Kutejová, A. Kocifajová, D. Pereško. ATP-dependent proteases and stress.
- S. Šesták, V. Farkaš. The effect of osmotic shock and ultrasonication to plasma membrane integrity and glucan synthase activity in *Saccharomyces cerevisiae*.
- E. Breierová, E. Stratilová. The influence of hyperosmosis on the production and the structure of extracellular yeast glycoproteins.
- J. Vernerová, J. Havlíček. Cold shock – stress factor of brewing yeast.
- J. Šajbidor, F. Malík. The influence of ethanol stress on the content and lipid composition in four isolates of wine yeasts.
- D. Šmugrovičová, Z. Dömény, J. Šajbidor, J. Pátková. Effects of immobilization on the ethanol tolerance of brewery yeasts.

Plenary lectures in molecular biology and genetics

- J. Brozmanová, V. Vlčková, I. Fridrichová, Z. Mikulovská, J.A.P. Henriques. DNA Repair of oxidative damage in the yeast *Saccharomyces cerevisiae*.
- V. Vlčková, E. Farkašová, M. Chovanec, J. Brozmanová. Capability of the *E. coli ada* gene to increase resistance of yeast to toxic and mutagenic effects of alkylating agents.
- P. Griaè, S.A. Henry. Regulation of yeast *Saccharomyces cerevisiae* phospholipid biosynthesis.
- I. Hikkel, Y. Gbelská, J. Šubík. The role of SPT genes in yeast transcription.
- Šabová, B. Sokolíková, J. Kolarov. Transcriptional regulation and function of genes encoding ADP/ATP translocator in yeast *Saccharomyces cerevisiae* – new function for the *AAC1* isogene ?

List of posters

- Havelková, E. Unger. Localization of calmodulin in *Yarrowia lipolytica*.
- M. Pesti, J. Belagyi. Perturbations induced by Cr^{VI} in protoplast membrane. An EPR study.
- B. Brodská, D. Gášková, J. Plášek, K. Sigler. The measurement of yeast membrane potential changes by means of new fluorescent method.
- J. Fiala, P. Patáková, C. A. Kent, M. Rychtera, M. Al-Rubeai. Applications of flow cytometry for monitoring of *Saccharomyces cerevisiae* yeast fermentation.
- R. Szabo. Dimorphism in *Yarrowia lipolytica* is regulated by metabolic status of the cell.
- J. Pátková, Z. Ciesarová, D. Šmogrovičová, Z. Dömény. Ethanol fermentation at high cell concentrations.
- P. Bafrncová, T. Foltin, I. Sláviková, D. Šmogrovičová. Selection of yeast strain for alcohol fermentation using high glucose concentrations.
- H. Miková, M. Rosenberg, L. Krištofiková. Parameters involved in the process of conversion of fumarate on malate by yeast *Endomyces magnusii*.
- S. Šilhár, E. Panghyová, P. Farkaš, J. Sadecká. Use of yeasts and fungi yeasts in the production of aromatic compounds in the simple broths.
- T. Foltin, S. Baxa, S. Šilhár. The influence of pressure changes upon the viability of yeasts in various media.
- S. Baxa, T. Foltin, S. Šilhár, V. Sitkey, V. Sasinková. The influence of physical-chemical characteristics of solvent for purification process of *Saccharomyces* sp.
- R. Kollár, G. Ashwell, H. Yeh, E. Cabib. Yeast cell-wall proteins contain together with mannans and glucans possibly another type of glycosylation.
- N. Kolarova, P. Capek. Production of acidic extracellular polysaccharides by yeasts *Cryptococcus laurentii*.
- N. Kolarova, P. Capek. Galactoglucoxylomannan of yeasts *Cryptococcus laurentii*.
- J. Šandula, E. Machová, G. Kogan. Glycoproteins of yeast cell walls
- M. Slaninová, A. Slivková. Analogy of *uvs11* mutant *Chlamydomonas reinhardtii* with *rad9* mutant *Saccharomyces cerevisiae* in connection of the important cell processes.
- M. Slaninová, T. Pinterová, V. Vlèková. Genetic changes in yeast induced by green algae bioactivated nitrosoamines
- E. Farkašová, G.P. Margison, J. Brozmanová. The influence of Ada alkyltransferase on toxic and mutagenic effects of dibromoalkanes in yeast *Saccharomyces cerevisiae*.
- D. Lacková, J. Šubík. The use of the mutated *PDR3* gene as a dominant selectable marker in transformation of prototrophic yeast strains.
- V. Džugasová, K. Horváthová, M. Obernauerová, M. Vachová, M. Žáková, J. Šubík. *PEL1p* influences the cardiolipin content of *S. cerevisiae*, is localized in mitochondria and homologues of its gene are detectable in various yeast species.
- L. Tomáška, J. Nosek. Regulation of the mitochondrial telomere-binding protein of the yeast *Candida parapsilosis*: Effect of phosphorylation by cAMP-dependent protein kinase on its DNA-binding properties *in vitro*.
- M. Špirek, A. Šoltésová, A. Horváth, P. Sulo. Xenomitochondrial cybrids among *Saccharomyces*.
- S. Betina, M. Wésolowski-Louvel. Identification of gene *RAG4*, product of which takes part on regulation of genes encoding glucose transporters of *Kluyveromyces lactis*.
- O. Kinclová, H. Sychrová. Function analysis of gene *NHA1* product in yeast *Saccharomyces cerevisiae*.
- R. Raková, J. Hašek. Deletion and ts mutation of *S. cerevisiae* essential gene *RPG1* is suppressed by *S. pombe* gene *eno1*.
- Z. Storchová, A.P. Rojas Gil, V. Vondrejs. The influence of inactivation of various genes on mutagenesis induced by specific starvation in *Saccharomyces cerevisiae*.
- A.P. Rojas Gil, P. Èejka, L. Hlavatá, V. Vondrejs. Characterisation of papillae on *Saccharomyces cerevisiae* *BJC3* colonies.
- M. Bartùnik, V. Vondrejs. The sensitivity of individual cells to zymocins.
- I. Horová, M. Kuthan, B. Janderová, Z. Palková. Mutants defective in communication between colonies.
- A. Tomšíková. New yeast pathogens – agents of nosocomial mycoses.
- V. Stollárová. The influence of some anthropogenic factors on composition of natural yeast species.
- E. Sláviková, R. Vadkertiová. Yeasts and yeast-like organisms isolated from soil.
- L. Kremnický, M. Vršanská, P. Biely. Structure of signal molecules triggering the synthesis of polysaccharide-degrading enzyme systems in yeasts.

The 28th Annual Conference on Yeasts will be organized by the Czech and Slovak Yeast Commission in the year 2000. The year 1999 is reserved for the 20th International Specialized Symposium on Yeasts (20th ISSY) which will be organized by the Commission in the Smolenice Castle, May 23-27, 1999. See "Forthcoming Meetings".

Communicated by Peter Biely

19th ISSY - Yeast in the Production and Spoilage of Food and Beverages 30 August to 3 September 1998, University of Minho, Braga, Portugal

The Department of Biology of the University of Minho was responsible for the organisation of the 19th ISSY - International Specialized Symposium on Yeasts held, from 30 August to 3 September 1998, in Braga. It was promoted by the International Commission for Yeasts being one of the most important events for the scientific community working on Yeasts. The Symposium

constituted a point of convergence of the scientific and technologic international community focusing on the different areas of Yeast in the Production and Spoilage of Food and Beverages. It was the second time that such a meeting was held in Portugal. The first was organized in Lisboa by Prof. Van Uden in 1986 (11th ISSY). It was both a privilege and an honour for the University of Minho

to be the organiser of such an important scientific event.

The scientific programme, supervised by an international advisory committee, consisted of five plenary lectures of wide interest, eight sessions including 29 lectures and 14 small oral presentations and 138 poster presentations focusing on the following topics: Food ecology and differential diagnosis of yeasts. Food-identification methods. Genetic improvement of food-processing yeasts.

The Congress was an enormous success with a total of 238 participants from all over the world. We had the presence of international renowned researchers and we would like to emphasise the attendance of many young scientists. We believe that this Symposium was extremely important for the general scientific

borne yeasts: metabolism and regulation. Alcoholic beverages and ethanol tolerance. Yeast activity in fermented food. Yeast in food spoilage: quality control in the production chain. Stress in food environments: yeast physiology and molecular biology. Molecular typing and rapid

community becoming a great space of discussion and implementation of future international collaborations. The selection of papers presented at the 19th ISSY is being implemented for publication in the journal of Food Technology and Biotechnology.

Cecília Leão

Workshop: Biology and Phylogeny of Basidiomycetous Yeasts and Fungi 3-4 October 1998, Vigo, Spain

Organizing committee: Jack Fell (jfell@rsmas.miami.edu), June Kwon-Chung (JKCHUNG@atlas.niaid.nih.gov), Teun Boekhout (T.Boekhout@STM.Tudelft.nl). Local Organizer: Pilar Calo-Mata (calomata@chusuk.arrakis.es). Sponsor: US Pharmaceuticals, Pfizer, Inc. The purpose of this workshop was to address the many problems facing yeast systematists working on basidiomycetous yeasts. A number of leading and active researchers on various disciplines of basidiomycete biology were invited to discuss the current position of their research and consider the important future directions. The two-day meeting was organized around four themes: 1. phylogeny; 2. the generic level; 3. the species problem; and 4. issues at the strain level. Topics 1 and 2 were discussed on the first day.

David Hibbett (Harvard University, USA) gave a detailed account of the diverse aspects of the reconstruction of phylogeny based on molecular data and the applications of phylogeny to taxonomy and evolutionary biology. He discussed some of the problems of rDNA-based phylogenies, such as intragenomic heterogeneity, rate heterogeneity, taxon- and data sampling and long branch attraction, with examples from the Homobasidiomycetes. The use of multiple sequences, including protein coding sequences (e.g. elongation factor 1- α , β -tubulin and RNA polymerase subunit 2) is currently being explored but is also faced with difficulties in data analysis. 'Supertree' approaches are being developed to combine the many available small phylogenetic studies using molecular data.

Eric Swann (University of Minnesota, USA) gave an overview on the Urediniomycetes. This class is supported by 18S rDNA data, 5S secondary structure, cell wall biochemistry, and ultrastructure of septal pore and spindle pole body. The controversy between the morphologically and parasitically different groups of the Heterogastridiales, Microbotryales and Sporidiales (nom. nud.), which also largely differ in their life cycles, is not yet settled and deserves further attention.

Franz Oberwinkler (University of Tübingen, Germany) showed some examples of new heterobasidiomycetes, raised some critical remarks with respect to conflicting results on comparative morphology versus molecular phylogenies and addressed the issue of co-evolution. He discussed examples such as the phylogenetic placement of the gasteromycetes within the agarics and the long standing issue of the molecular divergence between smuts in the

Ustilaginales and those in the Microbotryales.

Tom Bruns (University of California, Berkeley, USA) nicely covered the generic issue. He addressed some of the current problems in classification (information on evolutionary relationships; the use of unique, simple and stable names; how to handle para- and polyphyletic taxa; the use of the traditional linnaean system) and defined the ideal attributes of a genus (monophyletic, recognizable, and group a reasonably large number of species). He analysed the advantages and disadvantages of the use of a rank-free classification. Bruns advocated the use of the linnaean system at the genus level (and below) and the classification of higher taxa according to their evolutionary relationships (ideally resulting in monophyletic groups).

Jack Fell (University of Miami, USA) addressed the issue of the many polyphyletic and paraphyletic anamorphic yeast genera, illustrated with rDNA sequence data from *Cryptococcus*, *Rhodotorula*, *Bensingtonia*, *Sporobolomyces* and others. He raised the question of whether to create many small monophyletic anamorphic taxa or to consider those anamorphic taxa just as being artificial, but practical groupings.

Chee-jeen Chen (Food Industry Research & Development Institute, Taiwan) gave a comprehensive overview of his recent work in the genus *Tremella* and discussed its polyphyletic nature based on an integrated analysis of morphological, ecological and molecular characteristics. Álvaro Fonseca (New University of Lisbon, Portugal) presented recent results on basidiomycetous yeast-like fungi with auricularioid basidia to be classified in the genera *Occultifur* and *Kondoa*. He advocated the need for an integrated approach to the study of dimorphic basidiomycetes (studied by either fungal or yeast systematists) in order to set up a robust classification system that adequately reflects their biological nature. He also raised the issue of the difficulties in defining taxonomic ranks above the species level for anamorphic taxa based on molecular data. The most important conclusions of this first day were: continue the integration of morphological, physiological, ecological and molecular data; be aware of the existence of sampling problems; consider the issue of rank-free classification versus linnaean classification; go for more characters in the yeasts, e.g. teleomorphs; pursue the isolation and description of new taxa. The second day was devoted to the species problem and to some practical aspects of dealing with strains and isolates.

Rytas Vilgalys (Duke University, USA) gave an overview of issues related with the species concept. He nicely demonstrated the relation between the biological species concept (BSC) and the phylogenetic species concept (PSC) with his research on *Pleurotus*. It seems that phylogenetic groups based on molecular data do not always agree with intersterility groups (ISG), but may rather be geographical variants within ISG (allopatric speciation). Using the PSC these geographic variants should be considered to represent species.

Teun Boekhout (CBS Yeast Division, The Netherlands) gave an overview of the more than 22 species concept existing in the literature, and illustrated some of the problems facing yeast taxonomists. He showed that this century's evolution in taxonomic activity, measured as the number of basionyms described in time, displayed some interesting trends. Initially this curve appears to be exponential, followed by a first period with a linear relationship; after the very distinct second world war dip, a second linear period followed with a somewhat lower slope, but the eighties and nineties showed a dramatic decrease in number of species described. He concluded that since the full inventory of yeast species is far from completed this trend most likely reflects the worldwide decrease of taxonomic activity. He suggested that there might be a molecular renaissance soon.

Austin Burt (Imperial College London, UK) nicely illustrated some new concepts at the species level, namely the use of molecular data to distinguish between clonal and recombining natural populations. Using multiple molecular markers, the morphological species investigated appeared to consist of two or more genetic species. If this is true there are more species in nature than those discovered so far. An interesting observation was that the two varieties of *Cryptococcus neoformans* seem to represent separate genetic units, which may be called species. Also the serotypes seem to be genetically isolated to some extent.

Nils Hallenberg (University of Göteborg, Sweden) discussed the biological species concepts (BSC) illustrated with examples from the corticioid basidiomycetes. Some of the problems related with the BSC are that mating is regulated by only a small number of genes, and genetic isolation can be caused by relative 'simple' genetic mechanisms if there is a selective advantage. He also discussed some the possible mechanisms of speciation.

Finally, Wouter Middelhoven (University of Wageningen, The Netherlands) presented some of his latest research on the yeast genus *Trichosporon* to illustrate the difficulties in defining species of anamorphic taxa based on physiological data.

In the session on strains, June Kwon-Chung (Natl. Institutes of Health - Bethesda, USA) gave a talk on issues related with strain characterization. In her opinion the term strain is a genetic annotation which should be used with care. Apparently, it is still very difficult or even impossible to unequivocally characterize strains or isolates by molecular means. This is very important. Not only in medical mycology, but also in industrial applications. There is a great need for stable cryopreservation methods to store strains or isolates without inducing any genomic change.

The last two speakers (Tim Anke, University of Kaiserslautern, Germany) and Eric Johnson (University of Wisconsin-Madison, USA) presented overviews on the applied use of basidiomycetes. Tim Anke concentrated on secondary metabolites from mushrooms, whereas Eric Johnson concentrated on the yeasts, particularly the species *Phaffia rhodozyma*. It became clear from both presentations that the basidiomycetes are important sources for bioactive compounds, pigments, anti-oxidants, lipids, acids, etc.

Some of the relevant conclusions of this second day were that the species and generic concepts as applied in the yeast domain are not clear, particularly for the anamorphs; the advantage of the phylogenetic species concept is that both recombining and clonally reproducing taxa can be included; finally, ecological, geographical and phylogenetic relationships of the basidiomycetous yeasts are underexplored, as is their applied potential. This two-day meeting produced valuable interactions between the participants, particularly between the 'filamentous' and the 'yeast' people. It was also an excellent opportunity for pondering future research efforts and has hopefully opened the way for future meetings of this kind. Last but not least, the meeting was only made possible due to the local organization of Dr. Pilar Calo-Mata (Instituto de Investigaciones Marinas, CSIC, Vigo, Spain) who did an excellent job, the financial support from Pfizer, June Kwon-Chung who arranged the finances, and Jack Fell for logistics.

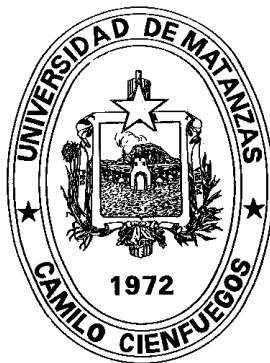
Álvaro Fonseca <amrf@mail.fct.unl.pt>
and Teun Boekhout.

Forthcoming meetings

Second International Alcohol Production Workshop (IAPW'99)
Matanzas University, Cuba, March 29th to April 1st 1999

Aims. To promote the scientific exchange dealing with the current advances in the field of ethanol production and Yeast Biotechnology. To foment the development of alternative biotechnological methods in alcohol production and wastewater treatment systems. To contribute to take reliable actions in order to improve the international cooperation in these fields.

Topics For Discussion. Ethanol and yeast production. Biochemistry and Microbiology of alcohol and Yeast Production. Technological and Biotechnological alcoholic fermentation. Treatment and reutilization of



alcohol industrial wastes and wastewaters. Present and perspective of fuel ethanol. Biotechnology of yeast. Application of electricity and magnetism in yeast and fermentation studies. Production of alcoholic beverages. Alcohol and health problems. Alcoholism.

Official Languages. Although the official language will be Spanish, papers may also be presented in English. Translation services will be offered on request during the sessions and activities of the meeting. Each presentation should last 15 min. plus 10 for questions.

Registration. The registration fees for taking part in the workshop will be \$100,00 USD. The registration must be paid in cash at the Matanzas University. Request for participation and a brief summary (English or Spanish) not more than 300 words, should be sent before February 15th, 1999. Information could be received in floppy disks 3 1/2".

Mailing posters. In the light of any probable reason, such as work, distance or others, which might keep the interested from attending the event, there is still another effective way of registration: that of mailing your papers in poster-like form either stored in diskettes or in standard-sized packages by registered mail. These posters will properly be presented in a special session to that end. In this case, the registration fees will just be \$50.00 USD which must in turn be posted to the event's organizing committee by check, bank transfer, or registered mail. You will actually receive detailed information on the materials and papers presented all through the event by air mail. Those taking this choice should send application via Fax or E-mail.

Accommodation. The University of Matanzas offers full lodging facilities at the University Motel with air conditioned rooms, radio and TV-sets, air conditioned snack-bar, restaurant, international telephone, souvenir shop, transportation, etc. The following lodging variants are offered: A tourist package for seven days for \$240.00 USD or fifteen days for \$450.00 USD that includes transfer in – out, welcome cocktail, lodging and meals (breakfast and dinner), tour around the city, an excursion to Varadero beach, use of the University facilities and farewell dinner.

For further information on accommodation, contact:

Lic. Humberto Suárez
Gerente Turismo Especializado
Fax (53)(52) 53101
Phone (53)(52) 61934

For further general information, please contact:

Prof. Dr. Marcelo Marcet
Chairman of the Organizing Committee
Department of Chemistry and Chemical Engineering
Matanzas University
Matanzas 44740, Cuba

or

Ing. Carlos Martin
Department of Applied Microbiology
University of Lund/Lund Institute of Technology
P.O.Box 124
S-221 00 Lund, Sweden

Social activities. During the free time, a group of social activities will be held for attendants and companions. Also a special activity will be organized in which different alcoholic beverages will be presented. (Equipments, instruments and new technologies could be also presented). This presentation will be performed by national or international producers or by official firms or companies established in Cuba.

Matanzas and its University. The University of Matanzas was founded in 1972. It is located in the outskirts of Matanzas, the capital city of the province with the same name. The city of Matanzas is situated in the northwest part of Cuba, 100 kilometers away from Havana and about 25 kilometers from the world-wide known Varadero beach, the most relevant tourist resort in Cuba. The city of Matanzas could be reached through the international airports in Varadero and Havana. There are speedways that connect both cities with Matanzas.

The foundation of the city dates back to October 12, 1693 with the name of "Villa de San Carlos y San Severino de Matanzas". Known by many as "The City of Many Bridges and Rivers", Matanzas cradels a rich cultural tradition and beautiful landscapes. Its heights and plains, the fertility of it's soils, the famous Yumuri slope mouth and its exclusive flora and fauna, turn it to be one of the most attractive places in Cuba. Recreated by painters and sang by poets and musicians, Matanzas posseses many museums and places of extraordinary importance for their antique values and cultural richness, among them: Gener y Del Monte Library, the French Pharmacy founded in the XIX Century, San Pedro Apostle Church, the Ermita de los Catalanes de Monserrat, as well as many historical and unique landscapes in Playa Girón and Ciénega de Zapata. Many sugar mills are located in Matanzas province. Sugarcane molasses are the main feedstock for alcohol production. There are in Matanzas two alcohol distilleries which produce the ingredients for the famous Havana Club Rum..

Fax 53 52 5 3101
Phone 53 52 6 1432
or 53 52 2569 (private)
Email <umcc@reduniv.edu.cu>
or <elektron@ceniai.inf.cu >

Fax +46 46 222 4203
Phone +46 46 222 8331
E-mail <Carlos.Martin@tmb.lth.se>

20th International Specialized Symposium on Yeasts Yeast Cell Surfaces and Membrane Phenomena Smolenice, Slovak Republic, May 23-27, 1999

Invitation. You are cordially invited to participate in the 20th ISSY "Yeast Cell Surfaces and Membrane Phenomena" organized by Czech and Slovak Yeast Commission of the Czechoslovak Society for Microbiology, and Institute of Chemistry, Slovak Academy of Science, under the auspices of the Federation of European Microbiological Societies (FEMS). FEMS will provide financial support for a limited number of young scientists to attend the meeting. The symposium will take place in Smolenice near Bratislava, Slovakia. This is the fourth time the ISSY is organized in Smolenice castle. The 1st ISSY was held in Smolenice in 1971, the 9th ISSY took place here in 1983, while the 14th ISSY was hosted in 1990. The castle of Smolenice is located at the foothills of the Small Carpathian mountains, a picturesque surroundings well known for its viticulture.

Organizing Committee. Peter Biely (Chairman); Libor Ebringer, Mária Vršanská (Vice-Chairpersons); Grigorij Kogan (Secretary); Mária Cziszárová (Treasurer); Nadezda Kolarova, Elena Sláviková.

Program Committee. Peter Biely, Vladimír Farkaš, Grigorij Kogan, Marie Kopecká, Karel Sigler, Július Šubík. Foreign members: Hans van Dijken, Graham H. Fleet, Bernard Prior and Peter Raspor.

Peter Biely (Chair)
Institute of Chemistry, Slovak Academy of Sciences
Dubravská cesta 9
84238 Bratislava, Slovakia

or

Grigorij Kogan (Secretary)
Institute of Chemistry, Slovak Academy of Sciences
Dubravská cesta 9
84238 Bratislava, Slovakia

Scientific Program: The program of the symposium will consist of invited plenary lectures, oral contributions and poster sessions on the following topics: Membrane Transport, Biogenesis and Structure of Cell Wall and Yeast Cytoskeleton.

Speakers that confirmed plenary lectures: D.C. Amberg (USA), M. Breitenbach (Austria), H. Bussey (Canada), E. Cabib (USA), D.C. Drubin (USA), G. Fleet (Australia), A. Goffeau (Belgium), J. Hasek (Czech Republic), M. Hofer (Germany), B.A. Prior (South Africa), R. Sentandreu (Spain), C.W. Slayman (USA), K. Sigler (Czech Republic), J. Subík (Slovakia), W. Tanner (Germany), T. Toda (UK)

Location and Accommodation: All scientific events will take place in the castle facilities. All participants will be accommodated and catered in the castle, as well. The transportation from Bratislava to Smolenice and back will be organized by the symposium service.

Further Information: The second circular will be distributed in December 1998 and will include information on fees, scientific and social programs, as well as on transportation, registration and instructions for the abstract submission.

To obtain the second circular, contact:

Phone +421 7 5941 2754
Fax +421 7 5477 5565
Email <chempbsa@savba.sk>

Phone +421 7 5941 2740
Fax +421 7 5477 5565
E-mail <kogan@savba.sk>

Nineteenth International Conference on Yeast Genetics and Molecular Biology, Rimini, Italy, 26-30 May, 1999

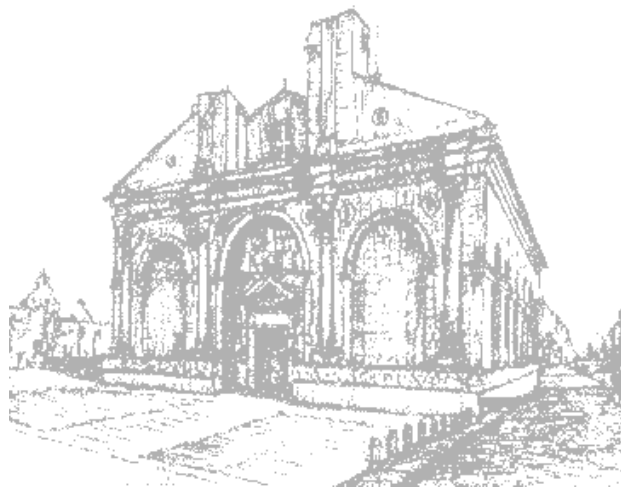
Second announcement and call for abstracts. Deadlines: Abstract receipt deadline 10 January 1999. Early registration 10 January 1999. Hotel accommodation 15 March 1999.

Steering committee: Piotr P. Slonimski (Chairperson), R.C. von Borstel (Secretary), Gerald R. Fink (Member-at-large), Hennie J.J. van Vuuren (Chair of the XVIII Conference), Laura Frontali (Chair of the XIX Conference).

Invitation. On behalf of the Organising and Scientific Committees of this Congress, it is my pleasure to invite you to attend the XIX International Conference on Yeast Genetics and Molecular Biology. The Conference programme encompasses a broad range of topics structured on plenary lectures, symposia, workshops and

poster sessions. The Congress will be held in Rimini at the Palacongressi, Italy's largest Conference Centre. The participants will have the opportunity to attend high level scientific events and to visit a large scientific exhibition. Posters will be exhibited for the duration of the Conference and there will be plenty of time for informal discussions. Organised tours will give participants and accompanying persons the possibility to visit some of Italy's finest beauties such as Ravenna and Urbino. We hope you will be able to join us in Rimini and enjoy both the Conference and the beauty of our country.

Laura Frontali



Scientific program. Plenary lectures will take place in the Introductory and Closing sessions of the Conference. Symposia will be held in the morning Plenary sessions. 4/5 speakers are foreseen for each Symposium. The following topics will be discussed:

1. Cell cycle and checkpoints
2. DNA replication, recombination and repair
3. Signal transduction
4. General metabolic regulation
5. Transcriptional and post-transcriptional control
6. Nucleo-mitochondrial interactions*
7. Targeting, translocation and secretion
8. Telomeres, silencing and aging
9. Genomics

*dedicated to the 50th Anniversary of the discovery of the “petite” mutation

Posters will be exhibited throughout the conference. The titles of Poster sessions are indicated below:

1. Gene expression
2. Chromosome structure and function
3. Metabolism and metabolic regulation
4. Meiosis and sporulation
5. Organelles
6. Biotechnology and industrial applications
7. Cell cycle and DNA replication
8. Recombination and repair
9. Biodiversity in yeasts
10. Signal transduction and stress response
11. Intracellular dynamics and transport
12. Cell wall and morphogenesis
13. Non conventional yeasts

Workshops on the above topics will be organized and will take place during three afternoons and one evening. Abstracts to be presented orally during workshops will be selected by the conveners.

List of invited speakers. R. Allshire, P.O. Brown, A.M. Carr, G.D. Clark-Walker, T.G. Cooper, J.F.X. Diffley, G.R. Fink, F. Foury, E. Gilson, A. Goffeau, L. Guarente, R. Hagenauer-Tsapis, M. Hall, L. Hartwell, E. Hurt, A. Hyman, M. Johnston, C. Koehler,

P. Legrain, A. Levitzki, P. Linder, S. Lindquist, V. Lundblad, S. Moreno, A. Murray, A. Nicolas, J. Nosek, P. Novick, Z. Palkova, T. Petes, J.T. Pronk, S. Roth, C. Schüller, B. Séraphin, W. Seufert, P.P. Slonimski, F. Thoma, J. Verdière, M.P. Yaffe.

The following **Satellite Meetings** are foreseen: *Kluyveromyces* - to be held on 24-25 May. EUROFAN - to be held on 30-31 May. Details will be given on the web site.

Call for abstracts. Deadline for abstract receipt 10 January, 1999. Instructions: Abstracts should be submitted in one of the following ways: 1) directly on the web site: <http://www.icgeb.trieste.it/yeast99/> 2) by sending a diskette with a text file Word 6.0 or earlier version to the Organizing Secretariat O.I.C. s.r.l., Via La Marmora 24, I-50121 Florence, Italy. In this case please indicate address, telephone, fax, e-mail of the corresponding author and choice of poster session. Before sending the abstract please check the following: The abstract should be typed as a Word 6.0 file (or earlier version) in Times New Roman 10 point font size. The abstract text alone should contain about 1200 characters. Each abstract should not exceed 29 lines altogether, including blank lines, lines should not exceed 17 cm. in length. Indication of the choice of Poster session is necessary. Keep to the following order in the text: poster session, title (bold), authors (bold, last name preceded by initials), affiliation and address with e-mail, abstract. Save the text in Word as an .rtf (Rich Text Format, Interchange Format) file. Illustrations should be attached to the abstract in question as an .eps file. Abstracts will be published in Current Genetics. Receipt of the abstracts will be notified soon thereafter, while their acceptance will be notified by 28 February, 1999. Abstracts not accompanied by the registration fee will not be considered.

Poster presentations. Posters will be exhibited throughout the Conference. The hours during which the authors need to be present at the posters will be notified in the final program. Instructions for posters. 1. Size of poster boards: width 90 cm / height 140 cm. 2. Posters should contain a heading which includes Title, Names of Author(s) and Affiliation. 3. Materials for mounting posters will be provided.

Oral presentations. Authors of the posters selected for oral presentation during the workshops will be notified by the Conveners. The list of the Conveners will be available on the web site. Overhead projection and single slide projection will be available.

Registration Fees. All payments in Italian Lire (VAT included).

	before 10 January 99	after 10 January 99
Delegate	750	1.000.000
Student	450	600
Accompanying persons	100	100

The registration fee for delegates or students includes personal badge, access to all scientific sessions and to the exhibition, congress bag, final programme and abstract book, opening ceremony, get-together reception, working lunches on 26, 27, 28, 29 and 30 May. Students must provide a proof of status. The registration fee for accompanying persons includes personal badge, access to the

opening ceremony and get-together reception. Scientific sessions are not open to accompanying persons.

Working lunches. Lunch facilities will be available in the congress area for all participants. Tickets for the working lunch on 26, 27, 28, 29 and 30 May are included in the registration fee for delegates and students.

Official letters of invitation. Official letters of invitation will be sent on request. We point out that this procedure cannot grant any financial support.

Passport and visas. Non-European citizens are strongly advised to make their own arrangements in due time with respect to entering Italy. The Congress Secretariat is available to provide whatever documentation may be necessary.

Climate and clothing. The weather in Italy in May is sunny and pleasant during the day and cool at night. Temperature ranges from 15-26 °C (60-80 °F). We suggest you also bring a raincoat and an umbrella. Clothing is informal for all occasions.

Banking. Money can be easily exchanged at all main banks in downtown Rimini. Normal banking hours are: Monday through Friday 8.20-13.20 and 14.30-15.30. Banks are closed on Saturday and Sunday. Some automatic exchange machines are located outside the major banks to provide a 24-hour service. A bank point will be operating at the congress center. Many hotels and shops accept credit cards and foreign currencies as US Dollars, German Marks etc., although the exchange rate may be slightly less favourable.

Scientific and technical exhibition. Companies and research institutions will have the opportunity to present their activities and specific know-how. The exhibition will take place at the Palacongressi and will be open throughout the Conference. Prospective participants should contact the Conference Secretariat.

Travel information. By Air: The nearest international airport is Bologna, with connections to all major European cities. Other international airports are Venice, Rome and Milan. By Rail: Rimini is connected by rail to many European and to all important Italian cities. It can be reached from Bologna (1 hr.), Venice (3,5 hrs.), Milan (3 hrs.) and Rome (4 hrs.). Bus no. 7 connects the railway station to the Palacongressi. By Car: The motorway A14 Adriatica is the main motorway linking Rimini to Northern and Southern Italy (tollgate Rimini Sud). The use of seat belts is mandatory in Italy. All road distances and speed limits are indicated in kilometers. The timetable of the most important flight connections and train service will be available on the web site: <http://www.icgeb.trieste.it/yeast99/> from December 1998.

Hotel accommodation. Deadline for hotel reservation: 15 March, 1999. O.I.C. s.r.l. is the official travel agency and will take care of the hotel reservations for the participants to the Nineteenth International Conference on Yeast Genetics and Molecular Biology. Rooms will be allocated on a first come, first served basis. As single rooms are limited, the O.I.C. s.r.l. will have to assign double rooms for single use if necessary. The price in this case will be slightly

lower than the double room rate and will be indicated on the voucher. After the deadline no guarantee can be given that your request will be satisfied or that the rates listed will be applied, although O.I.C. s.r.l. will make every effort to meet the participant's requirements. Prices are per room and per day, and quoted in Italian Lire. Bed/breakfast include break-fast, service charges and local taxes. Half board rates include dinner. This arrangement is economically advisable; Rimini hotels are generally appreciated for the quality of their food. All rooms are with private bathroom or shower. Camping sites are also available in the Rimini area. For information please contact. It is possible to book using the electronic form available on the Web Site: <http://www.icgeb.trieste.it/yeast99/> (payment by credit card).

Reservations cannot be confirmed until the deposit is received. Telephone reservations cannot be accepted. A voucher containing all reservation data will be sent to you on receipt of the appropriate hotel deposit. The deposit minus the handling fee will be forwarded to the hotel and credited to the delegate's hotel account. The balance due will be paid directly to the hotel on checking out. Any change regarding a reservation should be made through O.I.C. s.r.l. (no later than 30 days prior to arrival) and not directly with the hotel. Requests for accommodation before 24 May and/or after 31 May, 1999 will be accepted depending on room availability at the time of the request.

Social programme. Welcome Party, 25 May, 1999 - 19.00. To welcome the participants to the XIX International Conference on Yeast Genetics and Molecular Biology the Organizing Committee will offer a welcome party at the Palacongressi after the Opening Lecture by Lee Hartwell. Free of charge for registered congress participants and accompanying persons. **Social Dinner,** 29 May, 1999 - 21.00. A farewell dinner will be organised at the Restaurant "Casa Zanni" at Villa Verucchio, a medieval town in the countryside 15 km. from Rimini, where you will be able to taste and enjoy the typical cuisine of the Romagna region. Transportation from the Palacongressi will be provided. Cost: Lit. 70.000 (VAT included)

Optional tours. Deadline for reservation: 15 March, 1999. Each tour will be conducted by bilingual guides (Italian/English). All full day tours include lunch. The organizers reserve the right to cancel tours with less than 30 participants, or change the date of a tour for logistic reasons. Reservations will be accepted on a first come, first served basis. Early booking is recommended, as on-site availabilities cannot be guaranteed. Please fill in correctly the Optional Tours section of the Registration Form. It is not recommended to bring children under 5 on tours. The rates include the guide, transportation and entrance to museums. Any cancellation must be notified in writing to the Congress Secretariat O.I.C. Refund of the fee, less a 30% administrative charge, can be requested until 15 April, 1999. No refund will be made after that date.

Details and registration forms

Tel +39-055-50351
Fax +39-055-5001912
E-mail oic@dada.it
Web <http://www.icgeb.trieste.it/yeast99/>

Conference Secretariat
O.I.C. s.r.l.
Via A. La Marmora 24
I-50121 Florence, Italy

Chair

Prof. Laura Frontali
Dipartimento di Biologia Cellulare e dello Sviluppo
University of Rome "La Sapienza"
Piazzale Aldo Moro 5
I-00185 Rome, Italy

Tel +39-06-4953950
Fax +39-06-49912351
E-mail frontali@axcasp.caspur.it

Secretary

Prof. Carlo V. Bruschi
International Centre for Genetic Engineering and
Biotechnology
Area Science Park, Padriciano 98
I-34012 Trieste, Italy

Tel +39-040-375-7304
Fax +39-040-375-7343
E-mail bruschi@icgeb.trieste.it

IX ICM - IXth International Congress of Mycology
International Union of Microbiological Societies
16-20 August 1999, Sydney Convention Centre, Darling Harbour, Sydney Australia



The IXth International Congress of Mycology of the IUMS will be held jointly with the IXth International Congress of Bacteriology & Applied Microbiology. The organization of the Mycology Congress is chaired by J.I. Pitt. The main topics covered in the Mycology symposia, many of which will be held concurrently with Bacteriology symposia are as follows. Systematics and phylogeny, Molecular taxonomy, Medical mycology, Ecology and biodiversity, Genetics, Molecular genetics, Physiology,

The IUMS Congress Secretariat
GPO Box 128
Sydney NSW001
Australia

Biotechnology, Biocontrol, Biodegradation, Pathology, Toxins, Morphology and ultrastructure, Lichens, Food water and air, Applied mycology, Other.

Abstracts may be submitted on any mycological topic. Please choose one of the topic areas above to facilitate sorting. Most submitted mycology papers will be given as poster presentations. A small number of submitted abstracts will be selected for oral presentation in symposia listed in the Congress Program. The deadline for submission of abstracts is March 5 1999. Electronic submission is preferred.

Forms are available at www.tourhosts.com.au/iums.

Yeast researchers will be particularly interested in a symposium on Biodiversity in Yeasts, chaired by G.H. Fleet, and dealing with the following topics. M. Martini (University of Perugia, Italy) "The taxonomic and morphological diversity in yeasts". M.A. Lachance (University of Western Ontario, Canada) "The ecological diversity of yeasts". I. Spencer-Martins (New University of Lisbon, Portugal) "Yeast physiological diversity". B. Hahn-Hagerdahl (Lund University, Sweden) "The diversity of metabolic pathways in yeasts". K. Watson (University of New England, Armidale, N.S.W.) "Diversity of the stress response in yeasts". G. Naumov (State Scientific Research Institute for Genetics and Selection of Industrial Microorganisms, Moscow) "Genetic diversity in yeasts".

It is recommended that interested persons consult the WWW site below to obtain general information on the Congress.

For further details, contact

Tel +61 2 9262 2277
Fax +61 2 9262 3135
WWW www.tourhosts.com.au/iums
Email iums@tourhosts.com.au/iums

Yeasts 2000 - Tenth International Symposium on Yeasts
Sunday, 27 August - Thursday 31 August 2000. Papendal, Arnhem, The Netherlands

The 10th International Symposium on Yeasts will bring together scientists from all disciplines involved in the study of yeasts and yeast-like organisms: physiologists, geneticists, taxonomists, molecular biologists, biotechnologists, food microbiologists and

medical mycologists. The Symposium will be structured for optimal interaction between scientists working in these fields, thus stimulating new developments in yeast research in the third millennium. Further information will follow in due course.

Brief News Items

Query to Old Yeast Hands - re: Winge Festschrift

Can any of you point me to the reference to a "Winge Festschrift" published in 1955 (1956?), in which David Catcheside

participated. (David, alas, died in 1994).
Please reply to:

<lederberg@mail.rockefeller.edu>

Graduate Student Seeks Postdoctoral Position
Investigating Molecular Aspects of Yeast Adhesion and Aggregation

I am interested in pursuing postdoctoral research in a laboratory working on molecular aspects of yeast adhesion and aggregation. I obtained my Honours B.Sc. in Genetics, at the University of Western Ontario, London Ontario, Canada, and my M.Sc. from the Department of Microbiology and Immunology, also at the University of Western Ontario. I am currently completing my Ph.D. in the Faculty of Medical Sciences, Laboratory for Materia Technica,

The University of Groningen, Groningen, the Netherlands (expected completion date, Dec. 1, 1999). I have experience with DNA, RNA and protein gels, Northern hybridization, and sequencing; molecular typing of bacteria; yeasts and bacteria adhesion assays; yeasts and bacteria coaggregation assays; determination of cell surface physico-chemical properties, including XPS, FTIR, contact angles, zeta potentials, and MATH. For more information, contact:

Kevin Millsap
Laboratory for Materia Technica
Bloemensingel 10
9712 KZ Groningen
The Netherlands

Tel +31 50 3633140 (work)
+31 50 5714941 (home)
Fax +31 50 3633159
Email k.w.millsap@med.rug.nl

Postdoctoral fellowship Available - *Btn1p* in yeast

A Postdoctoral position is available immediately to study the function of *Btn1p* in yeast. *Btn1p* is the yeast homologue to the human *Cln3p* which when defective is responsible for the most common childhood neurological disorder, Batten disease. We are studying *Btn1p* with the awesome power of yeast genetics, and have demonstrated that deletion of *BTN1* (*btn1-D*) is complemented

by human *CLN3* (Pearce and Sherman, 1998 [PNAS 95, 6915-6918]). We are looking for a highly motivated individual with experience in yeast molecular biology to contribute to the research and understanding of this devastating disease. This postdoctoral fellowship is offered by David A Pearce, in the laboratory of Fred Sherman. For more information please contact:

David A Pearce
Department of Biochemistry and Biophysics
University of Rochester School of Medicine
601 Elmwood Avenue
Rochester, NY 16642