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A Newsletter for Persons Interested in Yeast

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Editorial

Ninth International Symposium on Yeasts, Sydney, Australia, 25-30 August 1996

Final preparations are underway for the Ninth International Symposium on Yeasts organized under the patronage of the International Commission on Yeasts. The program, given in detail in the December 1995 issue of the Yeast Newsletter, promises to be of the greatest interest to all yeast researchers. Graham Fleet informs me that some 200 persons have already registered, and that a similar number of poster abstracts have been received. The ISY 9 will be held simultaneously with the 10th International Biotechnology Symposium, allowing participation in both symposia for those who wish to do so. Anyone interested in late registration should contact the Organizing Committee at the earliest opportunity (see the *Forthcoming Meetings* section of the Newsletter). I am very much looking forward to seeing our readers in Sydney.

M. A. Lachance
Editor

I. Unité de Mycologie, Institut Pasteur, 25, rue du Dr. Roux, 75724 Paris Cedex 15, France. Communicated by É. Guého.

The following publications have appeared recently or are in press.

1. J. Guillot,¹ R. Chermette,¹ & É. Guého. 1994. Prevalence of the genus *Malassezia* in the Mammalia. *J. Mycol. Méd.* **4**:72-79.

¹Unité de Parasitologie-Mycologie, École nationale vétérinaire d'Alfort, 7, Avenue du General de Gaulle, 94104 Maisons-Alfort Cedex, France;

An investigation into the carriage of *Malassezia* yeasts on the skin of 271 domestic and 85 wild Mammals has been carried out. Ear samples were collected with sterile cotton tipped swabs. Skin was sampled by rubbing a piece of sterile fitted carpet. Sabouraud's glucose agar with addition of 0.05% chloramphenicol and 0.05% cycloheximide, and the same medium with 1% olive oil were used for the cultures. The plates were incubated for one week at 32°C and 37°C, respectively. Among the 356 animals examined representative of 40 different species, 122 (34%) had *Malassezia* yeasts on the skin, especially in the external ear canal (27%). The prevalence of lipophilic yeasts was found particularly important in some animal species (29% of cows, 36% of cats, 57% of pigs, 66% of dogs and 75% "pachyderms"). For these species, the presence of

yeasts seemed to be correlated with the amount of lipids on the skin. In contrast, no *Malassezia* yeasts were recovered from Rodents, Lagomorphs and Insectivora. Most of the strains isolated from domestic and wild Carnivora (33 dogs, 18 cats, 2 bears, 2 fennecs and 2 ferrets) belonged to the non lipid dependent species *Malassezia pachydermatis*. On the other hand, lipid dependent strains, hitherto correlated to the anthropophilic species *Malassezia furfur*, were recovered from 23 pigs, 7 elephants, 3 chimpanzees, 2 rhinoceros, 2 sheep, 1 cow, 1 cheetah and 1 okapi. No dermatological lesions were observed at the time of sampling, except for 18 dogs, 12 cats, 2 fennecs, 2 ferrets and 1 okapi suffering from otitis externa. The possible etiological role of *Malassezia* yeasts in animal cutaneous diseases is discussed.

2. E. Guého, J. Faergemann,¹ C. Lyman² & E.J. Anaissie³. 1994. *Malassezia* and *Trichosporon*: two emerging pathogenic basidiomycetous yeast-like fungi. *J. Med. Vet. Mycol.* **32** (Supplement 1):367-378.

¹University of Gothenburg, Gothenburg, Sweden;

²National Cancer Institute, Bethesda, Maryland;

³University of Texas, M.D. Anderson Cancer Center, Houston, Texas, USA.

3. J. Guillot, E. Guého & R. Chennette. 1995. Confirmation of the nomenclatural status of *Malassezia pachydermatis*. *Antonie van Leeuwenhoek* **67**:173-176.

Malassezia strains from dogs and rhinoceros all proved identical using mol % G+C and nDNA/DNA reassociation experiments. The use of the name *Malassezia pachydermatis*,

originally described for a strain isolated from a rhinoceros, is thus justified for non lipid-dependent strains of other sources.

4. J. Guillot & É. Guého. 1995. The diversity of *Malassezia* yeasts confirmed by rRNA sequence and nuclear DNA comparisons. *Antonie van Leeuwenhoek* **67**:297-314.

One hundred and four *Malassezia* strains (52 isolated from humans and 52 from animals) were compared using large subunit (LSU) ribosomal RNA sequence similarity and nuclear DNA complementarity. Eight groups of strains were recognized as genetically distinct species. Each taxon was confirmed by a homogeneous mol % G+C and percentages of DNA/DNA reassociations higher than 85%. The non-lipid-dependent *Malassezia* yeasts were maintained as the unique taxon *M. pachydermatis*. In contrast, lipid-dependent strains were shown to be distributed among seven species: *M. furfur*, *M. sympodialis* and *M. species 1-5*. These taxa matched remarkably well with

morphological and serological differences documented by previous investigators. The LSU rRNA sequences allowed a further intraspecific resolution with most of genomic taxa represented by several closely related sequences: *M. pachydermatis* counted up to seven sequences, *M. furfur* four sequences, *M. species 1* comprised three sequences and *M. species 2* and *M. species 5* two sequences. Three species, *M. sympodialis*, *M. species 3* and *M. species 4*, displayed a unique type of sequence. Thus, the present report demonstrates the usefulness of sequencing for both taxonomic and epidemiological purposes.

5. J. Guillot, É. Guého, & M.C. Prévost. 1995. Ultrastructural features of the dimorphic yeast *Malassezia furfur*. *J. Mycol. Méd.* **5**:86-91.

The ultrastructure of the dimorphic yeast species *Malassezia furfur* was investigated by transmission electron microscopy. **Materials and methods.** Yeast-mycelial conversion of the strain CBS 7019 was obtained at 32°C on Löwenstein-Jensen medium supplemented with 1% olive oil. Cells were fixed with 1.5% aqueous KMnO₄, post-fixed with 1% OsO₄, and stained with uranyl acetate. **Results and discussion.** Both yeasts and filaments exhibited the thick and multilamellar cell wall typical of basidiomycetous fungi. In contrast, the cell wall was confirmed to

be crossed by an electron-translucent band facing a helicoidal invagination of the plasma membrane, unique to our knowledge in the world of fungi. *M. furfur* was able to form true septate branching hyphae. The thick cross walls appeared composed of fibrillar material with no pore or micropore-like structures. These non-functional septa are reminiscent of similar features described in *Entyloma nymphaeae*, *Trichosporon pullulans* and a few Ustilaginales. A phenomenon of endosporulation was also observed.

6. E. Guého, G. Midgley,¹ & J. Guillot. In press. The genus *Malassezia* with description of four new species. *Antonie van Leeuwenhoek*.

¹Department of Medical Mycology, St. John's Institute of Dermatology, St. Thomas' Hospital, Lambeth Palace Road, London SE1 7EH, UK.

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

The following papers have been published since our last report.

1. K.C. Thomas, S.H. Hynes & W.M. Ingledew. 1995. Practical and theoretical considerations in the production of high concentrations of alcohol by fermentation. *Process Biochem.* **31**:321-331.

Three methods are described for the preparation of low-viscosity, very high gravity (350 or more grams dissolved solids per liter) mashes from different grains. These mashes when fermented to completion yield high concentrations of ethanol. The first mashing method involves adjustment of the water to grain ratio, and hydrolysis of viscosity-causing polymers using enzymes prior to starch gelatinization. It can be used to mash ground grain or starch enriched fractions (dry milling) or to mash starch slurries obtained through wet milling. The second method is a double mashing procedure where a normal gravity mash is first prepared, most of the insoluble materials removed and the extract thus obtained (basal mash) is then used as make-up water to prepare a second mash from the same or a different grain. Adjuncts containing high concentrations of fermentable sugars are used in the third general method to raise the sugar content of normal gravity grain mashes to

very high gravity (VHG) levels. Theoretical considerations show that VHG fermentation technology promotes considerable savings of water and increased plant productivity and therefore results in reduced fermentation and distillation costs per liter of ethanol. Formulae have been derived to permit calculation of the amounts of grain and water required to prepare mashes with predetermined concentrations of dissolved solids and insoluble materials. Practical and theoretical methods are described to determine the quantity of the liquid portion of the mash before and after fermentation. Using these values, the efficiency of mashing and fermentation can be calculated. A theoretical method is also presented to predict the maximum concentration of ethanol in fermented mash. These calculations, unlike those used currently in industry, take into account changes in the weight and volume of mash during fermentation.

2. N.C. L'Anthoën & W.M. Ingledew. 1995. Heat resistance of bacteria in alcohol-free beer. *J. Amer. Soc. Brew. Chem.* **54**:32-36.

Two of the most heat resistant beer lactic acid bacterial spoilage organisms found to date, *Pediococcus acidilactici* and *Lactobacillus delbrueckii*, and two human pathogens, *Escherichia coli* O157:H7 and *Salmonella typhimurium* (both associated with recent apple cider outbreaks of diarrhea and hemolytic uremic syndrome), were tested for heat resistance in commercial alcohol-free (0.5% v/v) beer and commercial 5% v/v alcohol beer. Using the attenuated dilution blank adaptation of the multiple point method, known cell concentrations were inoculated into the two types of beer held at a variety of selected heating temperatures. Survival curves, D values, and subsequently phantom thermal death time curves, and Z values were determined. Decimal reduction times at 60°C (D60), a common temperature for beer pasteurization, were

obtained by extrapolation. Both lactic acid bacteria showed an increased resistance to heat of 4 to 7 fold in alcohol-free beer compared to 5% alcohol beer, and pathogens demonstrated as much as 3 to 17 times more heat resistance in alcohol-free beer. Results illustrated a significant difference in the time-temperature relationship required to achieve microbiological stability of alcohol-free beers compared to 5% alcohol beer. Although pathogens tested are not at all heat resistant, and do not survive pasteurization temperatures even over short time intervals, many of these organisms were found to grow in the alcohol-free beer tested (but not in normal beer), and therefore industry is cautioned about production of such alcohol-free products by filtration or as draught beer.

3. K.C. Thomas, and W.M. Ingledew. 1995. Production of fuel alcohol from oats by fermentation. *J. Indust. Microbiol.* **15**:125-130.

Very high gravity (>30 g dissolved solids per 100 ml) mashes were prepared from hulled and hullless oats and fermented at 20°C with active dry yeast to produce ethanol. Excessive viscosity development during mashing was prevented by hydrolyzing β -glucan with crude preparations of " β -glucanase" or "Biocellulase". Both these preparations possessed endo-b-glucanase activity. By using these enzymes and by decreasing the water to grain ratio, very high gravity mashes with low viscosity were prepared. Unlike wheat and barley mashes, oat mashes contained sufficient amounts of

assimilable nitrogen to promote a fast rate of fermentation. The free amino nitrogen (FAN) content of oat mash could be predicted by the equation $\text{mg FAN/L} = 8.9n$ where n is the number of grams of dissolved solids in 100 ml of mash supernatant. Ethanol yields of 353.2 ± 3.7 liters and 317.6 ± 1.3 liters were obtained per tonne (dry weight basis) of hullless (59.8% starch) and hulled (50.8% starch) oats respectively. The efficiency of conversion of starch to ethanol was the same in normal and very high gravity mashes.

4. A.M. Jones, K.C. Thomas & W.M. Ingledew. 1995. VHG fermentation: fuel alcohol production from wheat mashes fortified with sugar adjuncts. *Int. Sugar J.* **97**:606-610.

The fermentation of very high gravity (VHG) wheat mashes prepared using soluble sugar adjuncts was assessed with and without nutrient supplementation. In the presence of nutrient supplementation, similar rates of fermentation and high ethanol yields were achieved from VHG wheat mashes and from mashes

made with a basal wheat mash and a variety of sugar adjuncts or freeze-dried wheat hydrolysate. These data suggest that the use of sugar adjuncts could alleviate some of the practical difficulties encountered during preparation and fermentation of high carbohydrate grain mashes.

5. K.C. Thomas, A. Dhas, B.G. Rossnagel & W.M. Ingledew. 1995. Production of fuel alcohol from hullless barley by VHG technology. *Cereal Chem.* **72**:360-364.

Very high gravity mashes (> 30 g dissolved solids per 100 mL) were prepared from an experimental hullless barley (SB 90354) and fermented with active dry yeast. A maximum ethanol concentration of 17.1 % (v/v) was realized in fermented mash and a total ethanol yield of 443 liters per tonne of barley (dry weight basis) was obtained. To prevent excess viscosity during mashing it was necessary to hydrolyze b-glucan in ground barley using crude preparations of β -glucanase or Biocellulase. While both these preparations possessed an endoglucanase activity, no measurable exoglucanase activity was detected. A typical mash prepared at a water to grain ratio of 3:1 and without hydrolysis of β -glucan had a

viscosity of 2480 Brabender Units (BU) while the viscosities of the mashes prepared after hydrolysis of β -glucan with β -glucanase or Biocellulase were 560 BU and 240 BU respectively. Hydrolysis of b-glucan not only reduced the viscosity of the barley mash but also released water bound and trapped by the β -glucan gel. The free amino nitrogen (FAN) content of the barley mashes was high compared to wheat mashes and about 80% of this FAN was taken up by yeast. In spite of the high FAN content of the mash, an exogenously added nitrogen supplement stimulated yeast growth and fermentation.

6. W.M. Ingledew, A.M. Jones, R.S. Bhatti, & B.G. Rossnagel. 1995. Fuel alcohol production from hull-less barley. *Cereal Chem.* **72**:147-150.

Hullless barleys were easily milled, mashed and fermented to ethanol. Problems experienced with viscosity were quickly eliminated by addition of b-glucanase, but even without enzyme, viscosity decreased over the duration of fermentation. Barley mashes prepared by adding 0.33 kg grain to 1 L of water fermented slightly faster than corresponding wheat mashes, and over 10% v/v alcohol was obtained (94% of theoretical). Distillers' hullless barley grains

collected at end-fermentation had similar protein contents to dried grains from wheat fermentations and were higher in protein than dried grains obtained from hulled barley. They were correspondingly lower in non digestible fiber. The following thesis has been completed. Mrs. L'Anthoën is now in Brittany, France.

7. N.C. L'Anthoën. 1995. Heat resistance of bacteria in alcohol-free beer. MSc Thesis. Department of Applied Microbiology. University of Saskatchewan. Saskatoon SK, Canada S7N 5A8.

Other News: Mr. Akihito Yokoyama, a research scientist from the Kirin Breweries, Technology Development Department, Yokohama, Japan spent two years in this laboratory studying very high gravity brewing technology and returned to Kirin in October, 1995.

III. Department of Applied Biology, The University of Hull, Hull HU6 7RX, England. Communicated by P.J. Large <p.j.large@appbiol.hull.ac.uk> Homepage: <http://www.hull.ac.uk/php/abspjl/>.

Recent publication.

1. Murphy, C.A., Large, P.J., Wadforth, C., Dack, S.J. & Boulton, C.A. 1996. Strain-dependent variation in the NADH-dependent diacetyl reductase activities of lager-brewing and ale-brewing yeasts. *Biotechnol Appl Biochem* **23**:19-22.

Significant differences were observed in the zymogram patterns of NAD(+)-dependent ethanol dehydrogenase and acetoin dehydrogenase activity in seven strains of brewer's yeast examined by nondenaturing PAGE. Bottom-fermenting (lager) strains contained quite different activity bands of acetoin dehydrogenase activity compared with top-fermenting (ale) strains. These differences were confirmed when cell-free extracts of ale yeasts were heated at 55°C. This destroyed most of the diacetyl reductase activity, while leaving acetaldehyde reductase and other reductase activities unaffected. In contrast, heating cell-free extracts of lager

yeasts at 55°C inactivated diacetyl reductase activity and the other reductase activities at the same rate, and more slowly than with ale strains. Similar distinctions between the two types of yeast could be made by examining the effect of heat on the ratio (activity of the various substrates with NADH as electron donor)/(activity with reduced acetylpyridine-adenine dinucleotide as electron donor). The data show that the acetoin dehydrogenase/diacetyl reductase enzyme present in ale-yeast strains differs in mobility and heat-stability from that of lager strains, and that both can be distinguished from the major alcohol dehydrogenase activity bands.

IV. Department of Biological and Biomedical Sciences, University of Ulster at Coleraine, Coleraine, Co. Londonderry BT52 1SA, Northern Ireland. Communicated by R.T. Moore.

On 27 March I gave an invited paper entitled "Evolutionary trends in the fungi" as part of the main symposium (Evolution of Microbial Life) of the 134th meeting of Society for General Microbiology, University of Warwick, and on 27 Aug I will present an invited paper entitled "Evolutionary trends in the higher fungi" as

part of the symposium on Evolution and Biodiversity of Prokaryotes and Eukaryotes of the 8th International Congress for Culture Collections, Veldhoven, 25-29 August. For the past year I have been establishing and managing the Internet home page of the British Mycological Society <http://www.ulst.ac.uk/faculty/science/bms>.

The following reports are scheduled to appear this year.

1. Moore, R.T. 1996. An inventory of the phylum Ustomycota. *Mycotaxon* **59**:1-31.
2. Moore, R.T. 1996. The dolipore/parenthesome septum in modern taxonomy. M B. Sney, S. Jabali-Hare, S. Neate & G. Dijst (Eds.). *Rhizoctonia* Species. [in press]. Kluwer: Dordrecht.
3. Moore, R.T. 1996. Evolutionary trends in the Fungi. In D. McL. Roberts, P. Sharpe, G. Alderson, & M. Collins, (Eds.). *Evolution of Microbial Life. Soc. Gen. Microbiol. Symp.* **54**:(in press). Cambridge University Press, Cambridge.
4. Moore, R.T. 1996. Cytology and ultrastructure. In C.P. Kurtzman & J. Fell (Eds.). *The Yeasts, a Taxonomic Study. Ed. 4* (in press). Elsevier, Amsterdam.
5. Moore, R.T. 1996. Lalaria Moore. In C.P. Kurtzman & J. Fell (Eds.). *The Yeasts, a Taxonomic Study. Ed. 4* (in press). Elsevier, Amsterdam.
6. Moore, R. T. 1996. Evolutionary trends in the higher fungi. In *Culture Collections to Improve the Quality of Life. ICC-8* (Veldhoven): (4pp-in press).

V. 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>.

The following papers have been published recently.

1. Pearson, B M, Carter, A T, Furze, J, Roberts, I N 1995 A novel approach for discovering retrotransposons: characterization of a long-terminal-repeat element in the spoilage yeast *Pichia membranaefaciens* and its use in strain identification. *Int. J. Syst. Bacteriol.* **45**:386-389.
2. Glayzer, D C, Roberts, I N, Archer, D B and Oliver, R P 1995 The isolation of ANT-1, a transposable element from *Aspergillus niger*. *Mol. Gen. Genetics* **249**:432-438.
3. Roberts, I N, Bond, C J, Clark, S Y, Collins, M D, Furze, J M, James, S A 1995 Phylogenetic analysis of yeasts at the NCYC. European Culture Collections Organisation Meeting ECCO XIV, Gozd Martuljek, Slovenia.
4. Roberts, I N and Wildman, H G 1995 The diverse potential of yeast. *Bio/technology* **13**:1246
5. James, S A, Collins, M D, Roberts, I N 1996 Use of an rRNA internal transcribed spacer region to distinguish phylogenetically closely related species of the genera *Zygosaccharomyces* and *Torulasporea*. *Int. J. Syst. Bacteriol.* **46**:189-194.
6. James, S A, Collins, M D, Roberts, I N 1996 Phylogenetic interrelationship amongst yeasts. British Yeast Group Meeting, Bedford, UK.
7. Cai, J, Roberts, I N and Collins, M D 1996 Phylogenetic relationships among members of the ascomycetous yeast genera *Brettanomyces*, *Debaryomyces*, *Dekkera* and *Kluyveromyces* as deduced by small sub-unit ribosomal RNA gene sequences. *Int. J. Syst. Bacteriol.* (April 96)

VI. Mycothèque de l'Université Catholique de Louvain, Université Catholique de Louvain, 3 Place Croix du Sud, B-1348 Louvain-la-Neuve, Belgium. Communicated by V. Robert <robert@mbla.ucl.ac.be>.

The following abstract will be presented at ISY IX, Sydney, Australia, 25-30 August 1996.

1. V. Robert, P. Evrard. 1996. Allev 2, an automated yeast identification system.

A new identification system called Allev 2 has been developed as a complete package including miniaturized tests (growth and fermentation tests in microplates), an automated reading system and a new Windows version of the Allev identification software. Allev 2 uses the conventional taxonomic criteria (96 tests and morphological features) and recognizes almost all described yeast species (693) as well as possible new species when the similarity level is very low. Unlike many rapid identification systems the database is fully updatable. New species can be added to the reference database on the basis of the original publication where

conventional characteristics are described. Allev 2 uses a microplate reader to reduce the operator's subjectivity and the need for high skill. Allev 2 allows further testing and identification of 300 to 500 isolates per month and per operator, which is far more efficient compared to conventional auxanographic identification methodology. Yeast species identification takes 3 to 10 days depending on the strain. Ecological, environmental or clinical studies involving a large number and a great diversity of yeast isolates could be improved by this automated yeast identification system.

Recent publications.

1. S. Hermann-Le Denmat, M. Sipiczki & P. Thuriaux. 1994. Suppression of yeast A polymerase III mutations by the *URP2* gene encoding a protein homologous to the mammalian ribosomal protein S20. *J. Mol. Biol.* **240**:1-7.

URP2 was cloned as a multicopy suppressor of several temperature-sensitive mutations defective in RNA polymerase III-dependent transcription, but without effect on mutations affecting RNA polymerase I or II. This single-copy gene encodes a hydrophilic polypeptide of 121 amino acid residues with a predicted molecular mass of 13.9 kDa and a basic isoelectric point of 9.7. *URP2* is a highly expressed gene, judging from its abundant messenger DNA and strong codon bias. The Urp2p protein is essential for cell growth, as shown by the lethal phenotype of the *urp2::HIS3* null allele. Given its striking similarity to the S20 ribosomal polypeptide of rat (55% identical residues), Urp2p is in all likelihood the yeast form of this polypeptide. Both proteins are

significantly related to S10 a component of the small ribosomal subunit of *Escherichia coli* that is known to operate as a transcriptional elongation factor. The latter observation suggests that the suppressor effect of *URP2* may be due to a direct involvement of Urp2p in RNA polymerase III-dependent transcription. Alternatively, the overexpression of Urp2p could bypass a partial preribosomal RNA processing defect associated with RNA polymerase III mutants. *URP2* was assigned to the left arm of chromosome VIII, and maps between *DUR3* and *YLF1*. The latter gene product has homology to the *E. coli gpl* gene product, and may define a new family of putative GTP-binding proteins.

2. I. Miklós, M. Sipiczki and Z. Benkö. 1994. Osmotolerant yeasts isolated from Tokaj wines. *J. Basic Microbiol.* **6**:379-385.

Yeasts growing in "Tokaj Aszu" wine and in "Aszu essence" were isolated and characterized. They proved to be physiological races of *Saccharomyces cerevisiae* and showed high osmotolerance, which was an inherited feature rather than the result of adaptation. No correlations were found between the osmotolerance and the ethanol tolerance or the cell size and morphology. Yeasts in "Aszu

essence" are usually undesirable contaminants that impair the quality of the essence. The isolate characterized in this work exhibited physiological parameters very similar to those of the "Tokaj Aszu" strain, which make it a potent competitor of other yeasts in Aszu fermentation. However, the high thermosensitivity of its cells offers a possibility to eliminate them selectively.

3. M. Sipiczki. 1995. Phylogenesis of fission yeasts. Contradictions surrounding the origin of a century old genus. *Antonie van Leeuwenhoek* **68**:119-149.

The phylogenesis of fungi is controversial due to their simple morphology and poor fossilization. Traditional classification supported by morphological studies and physiological traits placed the fission yeasts in one group with ascomycetous yeasts. The rRNA sequence comparisons, however, revealed an enormous evolutionary gap between *Saccharomyces* and *Schizosaccharomyces*. As shown in this review, the protein sequences also show a large gap which is almost as large as that separating *Schizosaccharomyces* from higher animals. Since the two yeasts share features (both cytological and

molecular) in common which are also characteristic of ascomycetous fungi their separation must have taken place later than sequence differences may suggest. Possible reasons for the paradox are discussed. The sequence data also suggest a slower evolutionary rate in the *Schizosaccharomyces* lineage than in the *Saccharomyces* branch. In the fission yeast lineage two ramifications can be supposed. First *S. japonicus* (*Hasegawaea japonica*) branched off, then *S. octosporus* (*Octosporomyces octosporus*) separated from *S. pombe*.

4. M. Molnar, J. Bahler, M. Sipiczki & J. Kohli. 1995. The *rec8* gene of *Schizosaccharomyces pombe* is involved in linear element formation, chromosome pairing and sister-chromatid cohesion during meiosis. *Genetics* **141**:61-73.

The fission yeast *Schizosaccharomyces pombe* does not form tripartite synaptonemal complexes during meiotic prophase, but axial core-like structures (linear elements). To probe the relationship between meiotic recombination and the structure, pairing, and segregation of meiotic chromosomes, we genetically and cytologically characterized the *rec8-110* mutant, which is partially deficient in meiotic recombination. The pattern of spore viability

indicates that chromosome segregation is affected in the mutant. A detailed segregational analysis in the *rec8-110* mutant revealed more spores disomic for chromosome III than in a wild-type strain. Aberrant segregations are caused by precocious segregation of sister chromatids at meiosis I, rather than by nondisjunction as a consequence of lack of crossovers. In situ hybridization further showed that the sister chromatids are separated prematurely during

meiotic prophase. Moreover, the mutant forms aberrant linear elements and shortened meiotic prophase. Meiotic chromosome pairing in interstitial and centromeric regions is strongly impaired in *rec8-110*, whereas the chromosome ends are less deficient in pairing.

We propose that the *rec8* gene encodes a protein required for linear element formation and the different phenotypes of *rec8-110* reflect direct and indirect consequences of the absence of regular linear elements.

5. M. Molnar & M. Sipiczki. 1995. Two novel genes involved in the sexual development of *Schizosaccharomyces pombe*. *Curr. Genet.* **28**:447-453.

We isolated two sterile mutants of *Schizosaccharomyces pombe*. One of them was mapped close to *ste13* (8cM). Since it turned out to be allelic with the hitherto unmapped *ral2*, its linkage with *ste13* localizes *ral2* on the right arm of chromosome II. The other mutants defines a novel class-I ste gene, *ste15*, closely linked

to *ste7* (4cM) on chromosome I. *ste15* is conjugation-specific and acts upstream of *pat1* and *ras1*. During its genetic analysis, a phenotypic suppression of *ste12-N9* was observed which was caused by mutations in the unlinked gene *ssw1*.

6. I. Farkas, É. Bakó, A. Muranyi, T. Zeke, M. Sipiczki & P. Gergely. 1995. Quantitation of phosphatase 1 and 2A in extracts of the budding yeast and fission yeast. *Int. J. Biochem. Cell. Biol.* **8**:767-773.

Serine/threonine protein phosphatases are also involved in the control of cell division. The aim of the present study was to compare the activity of protein phosphatase 1 (PP1) and 2A (PP2A) in cell extracts of the budding and fission yeast, made at different phases of growth. The activities of PP1 and PP2A toward phosphorylase were similar in extract of *S. cerevisiae*. In *S. pombe* extracts PP1 was responsible for more than 80% of the phosphorylase phosphatase activity. Ammonium sulfate-ethanol treatment increased the specific activity of the phosphatases and the percentage of PP2A in

S. cerevisiae extracts. No increase in the proportion of PP2A was observed upon the same treatment of *S. pombe* extracts. The above results were confirmed by fractionation of PP1 and PP2 activities on a heparin-Sepharose column. The proportion of PP1 and PP2 activities did not change significantly during exponential cell growth but cells from stationary phase exhibited lower phosphatase activities. These results may indicate a lower level of expression of the PP2A genes in *S. pombe* and/or differences the structure in the holoenzymes or their regulators in the two genera.

VIII. Research Institute for Viticulture and Enology, 833 11 Bratislava, Matuskova 25, Slovakia. Communicated by E. Minárik.

The following are summaries of recently published papers and those submitted for publication.

1. Jungová, O. & Minárik, E. 1996. Wine yeast gene bank and its importance (in Slovak) *Vinohrad* **34**:37-40.

The Yeast Collection of the Research Institute for Viticulture and Enology in Bratislava (RIVE 28) represents an important yeast gene bank utilized in the wine industry. Results of examination of basic technological properties of 471 yeast strains of the species

Saccharomyces cerevisiae had been confirmed: 63.5 % of strains fermented grape must up to 13-15 vol% alcohol. 12 % of examined strains showed better results in winery practice than the verified strain Bratislava 1.

2. Malík, F., Sitorová, S., Vollek, V., Linczényiová, K. 1996. Microbiological and cultivation properties of wine yeasts *Saccharomyces cerevisiae* of the FV series. *Biologia (Bratislava)* **51** (in press).

This work contributes to a more perfect knowledge of the life of ethanol tolerant yeast strains isolated from secondary habitats in Slovakia. Microbiological and cultivation properties of wine yeast *S. cerevisiae* FV 1 - FV 5 had been evaluated. Microscopic properties of particular strains (size, shape, cell balance, type of pseudomycelium, sporulation activity and qualitative representation

of 3-4 spore asci) is, with the exception of little deviations mutually similar; cultivation characteristics were identical in all 5 strains. During growth, no strain showed ring or film formation. Some differences were observed in the morphology of macrocolonies, diameter, type of the macrocolony, shape, margin and profile.

3. Malík, F., Sitorová, S., Vollek, V., Linczényiová, K. 1996. Technological properties of ethanol tolerant wine yeast *Saccharomyces cerevisiae* FV species. *Kvasný průmysl (Prague)* **42**(5):168-169 (in Slovak).

Technological properties of natural isolates of alcohol tolerant wine yeast strains *Saccharomyces cerevisiae* of the FV Series had been examined for their application possibilities in the process of sparkling wine production. Fermentation properties of tested strains in conditions of primary grape must fermentation correlated with

properties of reference strains. Yield coefficients were in the range $Y_{P/S} = 0.47-0.48$. Investigations in secondary fermentation conditions revealed the same satisfying properties of the strains tested ($Y_{P/S} = 0.41-0.44$). The strain *S. cerevisiae* FV 3 may be considered from the viewpoint as the most efficient.

4. Malík, F., Satko, J., Vollek, V. 1996. *Zygosaccharomyces rouxii* - contaminant of concentrated grape must (in Slovak). *Kvasný průmysl (Prague)* **40** (in press).

From concentrated grape must a contaminating yeast strain was isolated that was identified as the species *Zygosaccharomyces rouxii*. Basic morphological and basic biochemical properties (acidification power, respiratory activity) of this osmotolerant strain

were determined. Finally, it was confirmed that the strain is not suitable for primary and secondary fermentations of grape must and wine.

5. Malík, F., Sitorová, S., Vollek, V., Linczényiová, K. 1996. Some biochemical properties of wine yeasts *Saccharomyces cerevisiae* of the FV Series. *Biologia (Bratislava)* **51** (in press).

The contribution presents results of the evaluation of some biochemical properties/fermentative utilization of saccharides (oxidative utilization of saccharides and nitrogen sources acidification and respiration) of ethanol tolerant strains of *S. cerevisiae* of the series FV. Fermentation and assimilation properties of the strains were nearly identical. They differed little in the rate and depth of fermentation of a particular saccharide solution. All strains fermented entirely maltose and sucrose, raffinose to 1/3 (yeasts of the

2nd fermentation type). Based on results of previous experimental activities, auxanograms and zymograms the strains of the FV series belong to the species *S. cerevisiae* Hansen. The acidifying strength of strains tested was in correlation with technologically important wine yeast strains. All the strains showed the respiration chain linked with oxidative phosphorylation. Compared with the *S. cerevisiae* DT XII strain, their respiration activity was very satisfactory.

6. Volleková, A., Malík, F., Vollek, V., Linczényiová, K. 1997. Characterization of yeasts isolated from red wine surface film. *Folia Microbiologica (Prague)* (accepted for publication).

We have isolated 6 morphologically different axenic yeast strains from the film surface of red wine. Based on morphological, physiological and biochemical characteristics we have identified the investigated strains as follows: the isolates No. 1-4 are morphologically different strains of the anamorph basidiomycetous film-forming yeast *Candida humicola* (Daszewska) Diddens et

Lodder, syn. *Apiotrichum humicola* (Daszewska) von Arx & Weijman. The isolates No. 5 and No. 6 belong to the genus *Saccharomyces* of the associated species *S. cerevisiae* (No. 5 originally *S. bayanus*, No. 6 *S. capensis*). They do not participate in the surface film formation.

IX. European Brewery Convention, P.O. Box 510, 2380 BB Zoeterwoude, The Netherlands. Communicated by M. van Wijngaarden.

The following publications are available from the European Brewery Convention.

1. EBC Monograph XXIV. Immobilized Yeast Applications in the Brewing Industry. EBC Symposium held in Espoo, Finland, 1995. 260 pp., numerous figures and tables, DEM 88.00, postage not included.

Interest in immobilized yeast applications in the modern sense rose in the 1970's in brewing research, following the interest in continuous fermentations in the 1960's. The continuous systems in the 1960's mostly failed due to instability, inflexibility and sensitivity to contamination and mutation. This all led to inferior beer quality. In the 1980's immobilization systems achieved

increased interest and the immobilized yeast systems were considered to revitalize the continuous fermentation concept. The EBC Symposium on Immobilized Yeast Applications in the Brewing Industry is a natural continuation of the valuable work of the EBC Biochemistry Subgroup for Immobilized Enzymes/Cell Systems. The added value of the Symposium is this Monograph, in which all

presentations and discussions are published. The top scientists and most experienced practical brewers of the new technology have been immobilized in Espoo for the EBC Symposium to describe the

present status and future promises of immobilized systems to elucidate the topic from several angles.

Order by Fax from to the publisher (Fachbuchhandlung Hans Carl, Postfach 990153, D-90268 Nürnberg, Germany).
Fax: +49-911-952 8547. Order number 749.

Other EBC Monographs

- Monograph II. Barley and Malting, Zeist 1975, 304 pp., DEM 74.00
Monograph III. Computerized Process Control, Strasbourg 1976, 432 pp., DEM 84.00
Monograph IV. Noise Abatement, Copenhagen 1977, 252 pp., DEM 64.00
Monograph VIII. Brewing Economics and Technical Management, Frankfurt 1982, 280 pp., DEM 78.00
Monograph IX. Biotechnology, Nutfield 1983, 294 pp., DEM 84.00
Monograph XI. Wort Production, Maffliers 1986, 288 pp., DEM 82.00
Monograph XII. Brewers Yeast, Helsinki 1986, 254 pp., DEM 76.00
Monograph XIII. Hops, Weihenstephan 1987, 288 pp., DEM 82.00
Monograph XIV. Water in the Brewing Industry, Zoeterwoude 1988, 214 pp., DEM 72.00
Monograph XV. Plant Biotechnology, Helsinki 1989, 168 pp., DEM 66.00
Monograph XVI. Separation Processes, Leuven 1990, 276 pp., DEM 84.00
Monograph XVII. Packaging, Bremen 1990, 220 pp., DEM 74.00
Monograph XVIII. Wort Boiling & Clarification, Strasbourg 1991, 234 pp., DEM 78.00
Monograph XIX. Waste Reduction in Brewery Operations, Rhleinfeld 1992, 216 pp., DEM 78.00
Monograph XX. Instrumentation and Measurement, Copenhagen 1992, 214 pp., DEM 78.00
Monograph XXI. Process Hygiene, Nutfield 1994, 183 pp., DEM 68.00
Monograph XXII. Hops, Zoeterwoude 1994, 302 pp., DEM 86.00
Monograph XXIII. Malting Technology, Andernach 1994, 216 pp., DEM 78.00

All EBC Monographs are published in English.

X. Biochemisches Institut der Universität Freiburg, Hermann-Herder-Straße 7, D-79104 Freiburg im Breisgau, Germany. Communicated by H. Holzer.

The following paper is in press.

1. S. Nwaka, B. Mechler, & H. Holzer. In press. Deletion of the *ATH1* gene in *Saccharomyces cerevisiae* prevents growth on trehalose. FEBS Lett.

The biological function of the yeast trehalases (EC 3.2.1.28) consists of down-regulation of the concentration of trehalose via glucose formation by trehalose hydrolysis. While it is generally accepted that the cytosolic neutral trehalase (encoded by the *NTH1* gene) is responsible for trehalose hydrolysis in intact cells, very little is known about a role of the vacuolar acid trehalase and the product of the recently described neutral trehalase gene *YBR0106* (*NTH2*). We have analyzed the role of the acid trehalase in trehalose hydrolysis using the *ATH1* deletion mutant (*Δath1*) of *Saccharomyces cerevisiae* (M. Destruelle et al. (1995) *Yeast* **11**, 1015-1025) deficient in acid trehalase activity under various nutritional conditions. In contrast to wild-type and a mutant deficient in the neutral trehalase (*Δnth1*), the *Δath1* mutant does not grow on

trehalose as a carbon source. Experiments with diploid strains heterozygous for *Δath1* show a gene dosage effect of the *ATH1* gene for growth on trehalose. The necessity of acid trehalase for growth on trehalose is supported by the finding that acid trehalase activity is induced during exponential growth of cells on trehalose while no such induction is measurable during growth on glucose. Our results show that the vacuolar acid trehalase Ath1p is necessary for the phenotype of growth on trehalose, i.e. trehalose utilization, in contrast to cytosolic neutral trehalase Nth1p which is necessary for intracellular degradation of trehalose. For explanation of the necessity of vacuolar acid trehalase and not cytosolic neutral trehalase for growth on trehalose, the participation of endocytosis for uptake of trehalose from medium to the vacuole is discussed.

**XI. Institut für Pflanzengenetik und Kulturpflanzenforschung, Correnstr. 3, D-06466 Gatersleben, Germany.
Communicated by G. Kunze.**

Recent publications.

1. H. Rösel & G. Kunze. 1996. Identification of a group-I intron within the 25S rDNA from the yeast *Arxula adeninivorans*. Yeast (in press).

The 25S rDNA of the yeast *Arxula adeninivorans* LS3 has been cloned from a genomic library and sequenced. This DNA could be localized on chromosome I from *Arxula adeninivorans* and comprised 3,790 bp. The DNA sequence from this rDNA of the strain LS3 is very similar to the 25S rDNA of *Candida albicans* (91.7%), *Saccharomyces cerevisiae* (90.5%), *Schizosaccharomyces pombe* (83.8%) and *Mucor racemosus* (79.2%). Additionally a 411

bp insertion could be localized within the 25S rDNA. This intervening sequence, which is devoid of any long open reading frame, is a group-IC intron as revealed from its site of insertion, predicted secondary structure, and its self-splicing capability. The *Arxula* intron is intermediate in structure and sequence between the ribosomal introns of *Tetrahymena thermophila* and *Candida albicans*.

2. I.A. Samsonova, G. Kunze, R. Bode & F. Böttcher. 1996. A set of genetic markers for the chromosomes of the imperfect yeast *Arxula adeninivorans*. Yeast (in press).

The nuclear genome of the anamorphic yeast *Arxula adeninivorans* was analysed by benomyl-induced haploidization of parasexual hybrids marked with 32 auxotrophic mutations and pulsed field gel electrophoresis followed by DNA hybridization. Twenty seven genes have been arranged into four linkage groups by haploidization, 15 genes belong to group 1, six to group 2, and three

genes each to groups 3 and 4. Five genes could be localized by DNA hybridization on three out of four separated chromosomes. The gene *LYS2* of the largest linkage group I and the 25S rDNA were identified on the largest chromosome, the *GAA* and the *TEF7* gene on chromosome 2, and the *ILV7* gene of linkage group 4 on the smallest chromosome.

**XII. Institute of Chemistry, Slovak Academy of Sciences, Bratislava, Slovakia. Communicated by P. Biely
<chempbsa@savba.savba.sk>.**

The following papers have been published recently.

1. P. Biely & E. Sláviková. 1994. New search for pectolytic yeasts. *Folia Microbiol.* **39**:485-488.
2. E. Breierová. 1994. Cryoprotection of psychrophilic yeast species - by use of additives with cryoprotective media. *Cryo-Letters* **15**:191-197.
3. J. Haplová, V. Farkaš., M. Hejtmánek., R. Kodousek., J. Malínský. 1994. Effect of the new fluorescent brightener Rylux BSU on morphology and biosynthesis of the cell walls in *Saccharomyces cerevisiae*. *Arch. Microbiol.* **161**:340-344.
4. B. Košíková & E. Sláviková. 1994. The study of lignin effect on the growth of yeasts. *Drevarsky vyskum* **1-2**:33-38.
5. J. Šajbidor, M. Lamačka, E. Breierová, A. Chrastina, P. Pokreisz, M. Certík. 1994. Effect of salt stress on fatty acid alterations in some strains of *Dipodascopsis* and *Dipodascus* spp.: *World J. Microbiol. Biotechnol.* **10**:184-186.
6. E. Sláviková & B. Košíková. 1994. Inhibitory effect of lignin by-products of pulping on yeast growth. *Folia Microbiol.*, **39**:241-243.

7. R. Vadkertiová & E. Sláviková. 1994. Yeasts from sediments and soil along the lake Jakubov. *Biologia (Bratislava)* **49**:841-847.
8. A. Grabińska-Loniewska, E. Sláviková, E. Pajor. 1995. *Geotrichum capitatum*, a new isolate degrading phenol. *Acta Mycol.* **30**:207-211.
9. O. Molnár, R. Messner, H. Prillinger, U. Stahl, E. Sláviková. 1995. Genotypic identification of *Saccharomyces species* using random amplified polymorphic DNA analysis. *System. Appl. Microbiol.*, **18**:136-145.
10. N. Kolarová, M. Grešík, I. Šmondřková. 1995. Isolation and characterization of glycoproteins from the yeast *Cryptococcus laurentii*. *Chemical Papers-Chemicke Zvesti* **49**:214-218.
11. Z. Kossaczka, J. Drgoňová., B. Podobová., V. Betina, V. Farkas. 1995. Accumulation of Golgi-specific mannosyl-transferases in *Candida albicans* cells grown in the presence of Brefeldin A. *Can. J. Microbiol.* **41**:971-977.
12. M. Tomáška, P. Gemeiner, I. Materlín., E. Šturdík., G. Handříková. 1995. Calcium pectate gel beads for cell entrapment: A study of the stability of *Kluyveromyces marxianus* whole-cell lactase entrapped in hardened calcium pectate and calcium alginate gel beads. *Biotechnol. Appl. Biochem.* **21**:347-356.
13. M. Tomáška, M. Stredanský, P. Gemeiner., E. Šturdík. 1995. Improvement of the thermostability of β -galactosidase from *Kluyveromyces marxianus*. *Process Biochem.* **30**:649-652.
14. E. Sláviková & R. Vadkertiová. 1995. Yeasts and yeast-like organisms isolated from fish-pond water. *Acta Microbiologica Polonica* **44**:181-189.
15. E. Sláviková & R. Vadkertiová. 1995. Yeast population in the water of a polluted fish-pond. *Czech Mycol.* **48**:145-154.
16. R. Vadkertiová & E. Sláviková. 1995. Killer activity of yeasts isolated from the water environment. *Can. J. Microbiol.* **41**:759-766.
17. I.Y. Vereshchagin, T.A. Korolenko, J. Šandula. 1994. Protective effect of β -1,3-carboxymethylglucan in acute massive blood loss. *Patologičeskaja fiziologija i eksperimentalnaja terapija.* **42**:33-35.
18. M. Hofer, M. Pospíšil, J. Boháček, I. Pipalová, J. Šandula. 1995. Enhancement by carboxymethylglucan of early cellular damage in 1-Gy-irradiated mice. *Folia Biologica.* **41**:112-117.
19. M. Hofer, M. Pospíšil, S. Viklicka, I. Pipalová, J. Hola, J. Netíková, J. Šandula. 1995. Effects of post-irradiation carboxymethylglucan administration in mice. *Int. J. Immunopharm.* **17**:167-174.
20. M. Hofer, M. Pospíšil, I. Pipalová, J. Hola, J. Šandula. 1995. Haemopoiesis enhancing effect of repeatedly administered carboxymethylglucan in mice exposed to fractionated irradiation. *Folia Biologica* **41**:249-256.
21. D. Chorvatovičová & J. Šandula. 1995. Effect of carboxymethyl-chitin-glucan on cyclophosphamide induced mutagenicity. *Mutation Research* **346**:43-48.
22. E. Machová, G. Kogan, J. Alföldi, L. Šoltés, J. Šandula. 1995. Enzymatic and ultrasonic depolymerization of carboxymethylated β -1,3-D-glucans derived from *Saccharomyces cerevisiae*. *J. Appl. Polym. Sci.* **55**:699-704.

23. J. Šandula, E. Machová, V. Hříbalová. 1995. Mitogenic activity of particulate yeast β -1,3-D-glucan and its water soluble derivatives. *Int. J. Biol. Macromol.* **17**:323-326.
24. P. Biely, K. Heinrichová, M. Kružiková. 1996. Induction and inducers of the pectolytic system in *Aureobasidium pullulans*. *Curr. Microbiol.* **32**:000-000.
25. L. Kremnický, E. Sláviková, D. Mislovičová, P. Biely. 1996. Production of extracellular β -mannanases by yeasts and yeast-like microorganisms. *Folia Microbiol.* **41**:000-000.

XIII. Department of Biology, Indiana University, Bloomington, IN 47405, U.S.A. Communicated by M. Celerin <mcelerin@bio.indiana.edu>.

The following manuscript is in press.

1. M. Celerin, J. M. Ray,^{2,3} N.J. Schisler,⁴ A.W. Day,¹ W.G. Stetler-Stevenson² & D.E. Laudenbach.¹ Fungal Fimbriae are Composed of Collagen. *EMBO J.*

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⁴Zoology Department, University of Western Ontario, London, Ontario, Canada N6A 5B7.

Fungal fimbriae are surface appendages that were first described on the haploid cells of the smut fungus *Microbotryum violaceum*. They are long (1 to 20 μ m), narrow (7 nm) flexuous structures that have been implicated in cellular functions such as mating and pathogenesis. Since the initial description, numerous fungi from all five phyla have been shown to produce fimbriae on their extracellular surfaces. The present study analyzes the protein component of *M. violaceum* fimbriae. The N-terminus and three internal amino acid sequences were determined. All four show a strong similarity to sequences which are characteristic of the collagen gene family. Enzymatic digests and immuno-chemical analyses support this finding. Based on these results, it is suggested

that the proteinaceous subunits of fimbriae should be termed fungal collagens. Previously, collagen has been found only among members of the kingdom Animalia where it is the principal component of the animal extracellular matrix and is the most abundant animal protein. The unexpected finding of collagen in the members of the Mycota suggests that it may have evolved from a common ancestor that existed prior to the divergence of fungi and animals. Further, native fungal fimbriae can function as a mammalian extracellular matrix component. They can act as a substratum which permits animal cells to adhere, spread, and proliferate in a manner similar to animal collagens. The implications of this finding to both phylogeny and pathology are discussed.

XIX. BRF International, Lyttel Hall, Nutfield, Surrey, RH1 4HY, United Kingdom. Communicated by J.R.M. Hammond & J.M. Pye.

The following is a summary of some of our current research.

1. J.R.M. Hammond & J.M. Pye. Acetate ester synthesis: the role of alcohol acetyl transferase.

Many factors are known to influence acetate ester production in brewery fermentations. These include wort composition, yeast strain and pitching rate, fermentation temperature and pressure, fermenter agitation and degree of oxygenation. The effects of these parameters on the activity of Alcohol Acetyl Transferase (AAT), the enzyme responsible for ester synthesis, has not previously been determined in any great detail. Variation in AAT activity during fermentation has now been examined. Additionally the enzyme has been partially-purified and characterised. AAT has a very high molecular weight and may be part of a multi-enzyme complex. It is

able to synthesise ethyl, propyl and iso-amyl acetates. Concentration/activity curves for these alcohols, however, show a much greater affinity of AAT for 2- and 3-methyl butanol (isoamyl alcohols) than for other alcohols. The two isoamyl alcohols compete for the same active site in the enzyme whereas ethanol and 3-methyl butanol probably react at different sites but with cross-inhibition. Conditions giving disproportionate levels of ester formation arise because there is an increase in AAT level. Control of acetate ester levels depends upon controlling the levels of AAT and of precursor alcohols.

XV. Department of Viticulture and Enology, University of California, Davis, Ca 95616, U.S.A. Communicated by R.E. Kunkee.

The following text and reference book has been published recently by members of our department.

1. R.B. Boulton, V.L. Singleton, L.F. Bisson & R.E. Kunkee. 1996. Principles and Practices of Winemaking. Chapman & Hall, 115 Fifth Ave., New York, NY 10003, 600 pages, USD\$150.

A partial quotation from the dust cover: ... in depth discussion of every aspect of the wine production process, from the selection of grapes and preparation of the must and juice, through aging and the bottling and storage of finished wines. Of special interest to YNL readers will be the presentations having to do with yeasts.

Chapter 4, entitled "Yeast and Biochemistry of Ethanol Fermentation", has 30 Figures and 14 Tables (including a table of species of wine-related yeast). Content:

Definition, Origins and Identification of Wine-Related Yeast;
Natural Grape and Winery Flora;
Fermentation Inoculation Practices;
Yeast Morphology and Cellular Organization;
Yeast Nutrition and Growth Characteristics;
Fermentation Biochemistry;
Fermentation Kinetics;

End Products of Yeast Metabolism;
Nitrogen Metabolism during Fermentation;
Problem Fermentations, Ethanol Tolerance;
Fermentation Bouquet and Other Volatile Esters.

Chapter 9 deals with "Microbiological Spoilage of Wine and Its Control." Partial content:

Origins of Wine Spoilage Microorganisms;
Diagnosis of Spoilage as Microbiological;
Kinds of Microbiological Spoilages of Wine, Identification of Wine Spoilage Microorganisms;
Spoilage by Molds and Yeast.

Two other chapters (8 and 10) have to do with some other aspects of yeast, namely microbiological stabilization of wine, winery sanitation, sterile filtration, and bottling. For enological researchers, there is also a chapter devoted to "Preparation, Analysis and Evaluation of Experimental Wines" (Chapter 16).

XVI. Dipartimento di Biologia, Difesa e Biotecnologie Agro-Forestali, Università della Basilicata, Via Nazario Sauro 85, 85100 Potenza, Italy. Communicated by P. Romano & G. Suzzi.

Recent publications.

1. Romano, P., Suzzi, G. 1996. Minireview: origin and production of acetoin during wine yeast fermentation. Appl. Environ. Microbiol. **62**:309-315.

The paper presents a minireview of acetoin production in wine yeasts. It deals with production in wine yeasts (*Saccharomyces cerevisiae*, *Kloeckera apiculata*, *Hanseniaspora guilliermondii*,

Zygosaccharomyces spp., *Saccharomycodes ludwigii*), concentrations in wine and factors affecting the production, biochemistry and genetics.

2. Suzzi, G., Romano, P., Vannini, L., Turbanti, L., Domizio, P. 1996. Cell-recycle batch fermentation using immobilized cells of flocculent *Saccharomyces cerevisiae* wine strains. World J. Microbiol. Biotechnol. **12**:25-27.

Five, highly flocculent strains of *Saccharomyces cerevisiae*, isolated from wine, were immobilized in calcium alginate beads to optimize primary must fermentation. Three cell-recycle batch fermentation (CRBF) of grape musts were performed with the biocatalyst and the results compared with those obtained with free

cells. During the CRBF process, the entrapped strains showed some variability in the formation of secondary products of fermentation, particularly acetic acid and acetaldehyde. Recycling beads of immobilized flocculent cells is a good approach in the development and application of the CRBF system in the wine industry.

3. Suzzi, G., Romano, P., Westall, F., Vannini, L. 1996. The flocculation of wine yeasts: biochemical and morphological characteristics in *Kloeckera apiculata*. *Antonie van Leeuwenhoek* (in press).

The floc-forming ability of flocculent strains of *Kloeckera apiculata*, isolated from musts, was tested for susceptibility to proteinase and sugar treatments. Three different flocculent phenotypes were discriminated by protease digestion, whereas the inhibition of flocculation by sugars distinguished two definite patterns: one mechanism of flocculation involved a

galactose-specific protein and the other a broad-specificity lectin. SEM and TEM observation of the cell surface of two different *Kloeckera* strains revealed fine fibrils and a diffuse structure at the point of contact in one strain, and thick masses of mucus on the cell wall of the other strain.

4. Romano, P., Suzzi, G., Brandolini, V., Menziani, E., Domizio, P. 1996. Determination of 2,3-butanediol in high and low acetoin producers of *Saccharomyces cerevisiae* wine yeasts by automated multiple development (AMD). *Lett. Appl. Microbiol.* **22** (in press).

High performance thin layer chromatography with automated multiple development was used to determine 2,3-butanediol levels in wine produced by high and low acetoin-forming strains of

Saccharomyces cerevisiae. An inverse correlation between acetoin and 2,3-butanediol content was found suggesting a leaky mutation in acetoin reductase of the low 2,3-butanediol producing strains.

5. Polsinelli, M., Romano, P., Suzzi, G., Mortimer, R.K. 1996. Multiple strains of *Saccharomyces cerevisiae* on a single grape vine. *Lett. Appl. Microbiol.* (in press).

On the basis of the levels of secondary product formation four different phenotypes were represented among the 28 strains of *Saccharomyces cerevisiae* isolated. The genetic analysis indicated that four different strains, representing each phenotypic class, were

derived, one from the other, by mutation. The spontaneous fermentation of a Malvasia must was dominated by different strains of *Saccharomyces cerevisiae* at different stages of fermentation.

6. Romano, P., Suzzi, G., Domizio, P., Fatichenti, F. 1996. Secondary products formation as a tool for discriminating non-*Saccharomyces* wine strains. *Antonie van Leeuwenhoek* (in press).

A total of 78 strains of non-*Saccharomyces* yeasts were isolated: 30 strains of *Kloeckera apiculata*, 20 of *Candida stellata*, 8 of *Candida valida* and 20 of *Zygosaccharomyces fermentati*. The diversity of yeast species and strains was monitored by determining the formation of secondary products of fermentation, such as acetaldehyde, ethyl acetate and higher alcohols. Within each species, the strains were distinguishable in phenotypes through the production of different amounts of by-products. In particular, a great

variability was found in *Candida stellata*, where six different phenotypes were identified by means of the production of acetaldehyde, ethyl acetate, isobutanol and isoamyl alcohol. At different stages of the spontaneous fermentation different phenotypes of the non-*Saccharomyces* yeasts were represented, characterized by consistent differences in some by-products involved in the wine bouquet, such as acetaldehyde.

XVII. Planta Piloto de Procesos Industriales Microbiológicos (PROIMI), Avenida Belgrano y Pasaje Caseros, 4000 S.M. de Tucuman, Argentina. Communicated by J.F.T. Spencer.

The following are descriptions of projects underway at PROIMI.

1. A.L. Ragout, H. Borsetti, A. Martini, A.V. Martini, and J.F.T. Spencer. Yeasts isolated from sour bread starters.

Raising of some sour breads depends on the natural yeast and bacterial flora. We are investigating the yeast flora of starters ("sponges") used in making salt-rising bread, which is made without addition of commercial yeast. If the starter is incubated at 37°C, microbial action begins after 4-6 hr, and examination of the mix reveals heavy growths of bacteria and yeasts. We will determine the upper limits of temperature and the pH range for growth of the starter

culture. Enough acid is formed to reduce the pH far enough to kill the yeasts if the starter is allowed to incubate longer. We intend to determine the range of species of *Saccharomyces*-like yeasts participating in the flora of the sponge, and possibly isolate desirable temperature- and acid-tolerant strains of industrially useful yeasts.

2. A.L. Ragout, H. Borsetti and J.F.T. Spencer. Continuous culture of mixtures of the flocculent yeast *Saccharomyces diastaticus* NCYC 625 and *Lactobacillus* species.

Mixed cultures of lactic acid bacteria and yeasts are commonly found as fermenting and raising agents in Indian and other Oriental fermented foods, as well as in speciality breads such as white sour dough breads, sour rye breads, and salt-rising breads. Studies of the nature of the interaction of the lactic acid bacteria and the yeasts in the foods are rare. We have investigated the interaction between the yeast *Saccharomyces diastaticus* (*Saccharomyces cerevisiae* NCYC 625 and species of *Lactobacillus* in continuous culture. We will determine the effect on total biomass yield, product (acid) formation and utilization, effects of pH, and of embedding of the bacteria in the yeast flocs. We will use a carbon source which was

not utilized by the yeast (lactose) but which was utilized and converted to lactic acid by the bacteria. This by-product was utilized by the yeast for growth and biomass formation, so that the metabolism of the yeast was dependent on the activity of the other member of the mixed culture, the bacteria. The effect of embedding of the bacteria in the yeast flocs was determined using a non-flocculent yeast strain for comparison. We will determine the effect on the bacteria in this simulated immobilizing system to investigate the effect on the performance of the bacteria in immobilized cultures.

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

The following publications of my group are in press at the moment or have been published recently.

1. O. Molnar, R. Messner, H. Prillinger, K. Scheidel, U. Stahl¹, H. Silberhumer² & W. Wunderer³. 1995. Genotypic identification of *Saccharomyces* strains used in beverage industry by means of Random Amplified Polymorphic DNA-Polymerase Chain Reaction (RAPD-PCR). *Mitteilungen Klosterneuburg* **45**:113-122 (In German).

¹Technische Universität Berlin, Institut für Mikrobiologie und Genetik, Gustav Meyer-Allee 25, D-13355 Berlin,.

²Oesterreichisches Getraenkeinstitut, Michaeler-strasze 25, A-1180 Wien.

³Hoehere Bundeslehranstalt und Bundesamt für Wein und Obstbau, Wienerstrasze 74, A-3400 Klosterneuburg.

53 strains of *Saccharomyces* species identified originally by phenotypic characteristics in different wine and brewery institutes of Austria and Germany were investigated by Random Amplified Polymorphic DNA-Polymerase Chain Reaction (RAPD-PCR) analysis genotypically using type strains of *Saccharomyces bayanus*, *S. cerevisiae*, *S. pastorianus*, and *S. paradoxus*. Six strains were redesignated to *S. bayanus*, 42 to *S. cerevisiae*, and five to *S.*

pastorianus, respectively. In contrast to phenotypic characters genotypic identification using RAPD-PCR analysis guarantees species specificity. In comparison with n-DNA hybridization RAPD-PCR analysis turned out to be a simple and reliable method to separate *Saccharomyces* strains at the species and sub-species level. In addition this method may be used for strain improvement or the detection of killer phenotypes.

2. O. Molnar, H. Prillinger, K. Lopandic, F. Weigang¹, & E. Staudacher.² 1996. Analysis of coenzyme Q systems, monosaccharide patterns of purified cell walls, and RAPD-PCR patterns in the genus *Kluyveromyces*. *Antonie van Leeuwenhoek* (in press).

¹Hewlett-Packard GmbH; Lieblgasse, A-1222 Wien, Austria.

²Universität für Bodenkultur, Institut für Chemie, Gregor Mendel Straße 33, A-1180 Wien, Austria.

Analysis of the coenzyme Q system and the monosaccharide pattern of purified cell walls were used for species characterization in the genus *Kluyveromyces*. All the type strains of the genus possess coenzyme Q-6 and the mannose-glucose ("Saccharomyces type") cell wall sugar pattern. With the help of Random Amplified Polymorphic DNA-Polymerase Chain Reaction analysis 17 species were separated: *K. aestuarii*, *K. africanus*, *K. bacillisporus*, *K. blattae*, *K. delphensis*, *K. dobzhanski*, *K. lactis* (anamorph *Candida sphaerica*), *K. lodderae*, *K. marxianus* (syn. *K. fragilis*, *K.*

bulgaricus, *K. cicerisporus*, anamorphs *Candida macedoniensis*, *C. pseudotropicalis*, *C. kefyri*, *K. phaffii*, *K. piceae*, *K. polysporus*, *K. sinensis*, *K. thermotolerans* (syn. *K. veronae*, anamorph *Candida dattila*), *K. waltii*, *K. wickerhamii*, *K. yarrowii* (anamorph *Candida tannotolerans*). A strain of *K. drosophilorum* showed with the type strain of *K. lactis* only 63% similarity. The strain originally described as the type strain of *K. cellobiovorus* nom. nud. was excluded from the genus (Q-9), and found to be conspecific with the type strain of *Candida intermedia*.

3. H. Prillinger, R. Messner, H. Koenig,¹ R. Bauer,² K. Lopandic, O. Molnar, P. Dangell, F. Weigang,³ T. Kirisits,⁴ T. Nakase⁵ & L. Sigler.⁶ 1996. Yeasts associated with termites: a phenotypic and genotypic characterization and use of coevolution for dating evolutionary radiations in Asco- and Basidiomycetes. System. Appl. Microbiol. In press.

¹Universität Ulm, Institut für Angewandte Mikrobiologie, D-89069 Ulm, Germany.

²Universität Tübingen, Institut für Spezielle Botanik/Mykologie, Auf der Morgenstelle 1, D-72076 Tübingen, Germany.

³Hewlett-Packard GmbH; Lieblgasse, A-1222 Wien, Austria

⁴Universität für Bodenkultur, Institut für Forstentomologie, Forstpathologie und Forstschutz, A-1190 Wien, Austria.

⁵Japan Collection of Microorganisms, RIKEN, Wako 351-01, Japan.

⁶Microfungus Collection and Herbarium T6G 2EI, Devonian Botanic Garden, University of Alberta, Edmonton, Alberta, Canada.

Thirty-nine yeast isolates or dimorphic fungi were obtained from the hindgut of the lower termites *Mastotermis darwiniensis* (Mastotermitidae), *Zootermopsis angusticollis*, *Z. nevadensis* (Hodotermitidae), *Neotermes jouteli* (Kalotermitidae), *Reticulitermes santonensis*, *Heterotermes indicola* (Rhinotermitidae) and the roach *Cryptocercus punctulatus*. Using PPD-PCR the 39 yeast isolates were assigned to 13 different species. Commonly yeast species were specific to the termite species isolated from. There were only two yeast species which were found in different species of lower termites. Based on phenotypic characters *Debaryomyces hansenii* showed a high score in four species. The qualitative and quantitative yeast cell wall monosaccharide composition, the ubiquinone system, partial sequencing of 18S ribosomal DNA (bases 1273 to 948; numbering according to the gene of *Saccharomyces cerevisiae*), and the ultrastructure of septal pores indicate that 11 yeast species belong to

the Endomycetales. Although ascospores were lacking, two of these species were identified to belong to the genus *Debaryomyces*. One remaining yeast isolate was identified as a *Sporothrix anamorph* representative for the filamentous Ascomycetes (Ophiostomataceae s. str.); the second species showed affinities to the Basidiomycetes in particular to the genus *Trichosporon*. Comparing an additional 18S rDNA fragment (bases 595 to 993) and RAPD-PCR data using different species type strains of the genus *Sporothrix*, the filamentous ascomycete was genotypically identified as *Sporothrix albicans*, although phylogenetically closely related to *S. schenckii* var. *schenckii* and *Ophiostoma stenoceras* remains genotypically distinct. An emended species description of *S. albicans* is presented. Evidence is provided that the yeasts isolated from the hindgut can be considered symbionts.

4. K. Lopandic, H. Prillinger, O. Molnar, G. Gimenez-Jurado.¹ 1996. Molecular characterization and genotypic identification of *Metschnikowia* species. System. Appl. Microbiol. In press.

¹Portuguese yeast culture collection, Gulbenkian Institute of Science, Apartado 14, 2781 Oeiras Codex, Portugal.

Ten species currently described in the genus *Metschnikowia* (*M. agavae*, *M. australis*, *M. bicuspidata*, *M. gruessii*, *M. hawaiiensis*, *M. krissii*, *M. lunata*, *M. pulcherrima*, *M. reukauffii*, *M. zobellii*) were examined for their cell wall carbohydrate composition and ubiquinone type. Glucose and mannose are the only

carbohydrate components identified, and Q-9 is the main coenzyme-Q system. The RAPD-PCR fingerprinting supported the separation of the genus in ten species. According to the molecular features a proper position for the genus *Metschnikowia* among ascomycetous yeasts is proposed.

XIX. Escola Superior de Agricultura Luiz de Queiroz, Universidade de São Paulo and Fermentec s/c Itda., R. Treze de Maio, 768 s/43, CEP-13400-900, Piracicaba-SP, Brasil. Communicated by H.V. Amorim.

The following is a recently completed research project.

1. Basso, L. C., Alves, D. M. G. and Amorim H.V. 1996. Yeast trehalose contents as a biotechnological parameter for fuel alcohol fermentation.

The process of fuel alcohol production is *per se* a stressing condition for yeasts. In this context, the participation of trehalose, a well-known stress protector carbohydrate becomes an important parameter related to cell viability. However, in spite of the widespread knowledge not only about the trehalose metabolism, but also of some aspects of its biotechnological applications, there are a lack of information about this disaccharide in yeasts under industrial fermentation conditions. The present report shows the trehalose mobilization during alcoholic fermentation with baker's yeast as well

as with industrial strains at the end of fermentation, when they were submitted to a thermic treatment to consume this carbohydrate. The trehalose content was also measured in the yeasts under different physical, chemical and microbiological fermentation conditions with yeast cell reuse. The role of trehalose in keeping high cellular viability and its meaning as a biotechnological parameter in the industrial process of fuel alcohol fermentation with cell recycle, are drawn.

XX. École Nationale Supérieure Agronomique de Montpellier, Chaire de Microbiologie Industrielle et de Génétique des Microorganismes. Communicated by P. Galzy.

Recent publications.

1. Gueguen Y., Chemardin P., Arnaud, A., and Galzy, P. 1995. Comparative study of extracellular and intracellular β -glucosidase of a new strain of *Zygosaccharomyces bailii* isolated from fermenting agave juice. J. Appl. Bacteriol. (GBR) **78**:270-280.
2. Gueguen Y., Chemardin P., Pommères P., Arnaud A. and Galzy P. 1995. Enzymatic synthesis of dodecyl- β -D-glucopyranoside catalysed by *Candida molischiana* 35M5N β -glucosidase. Bioresource Technol. (GBR) **53**:263 -267.
3. Janbon G., Magnet R., Arnaud A. and Galzy P. 1995. Cloning and sequencing of the β -glucosidase gene from *Candida molischiana* strain 35M5N. Gene (NLD) **165**:109-113.
4. Venturin C., J. Zulaika, H. Boze, G. Moulin, P. Galzy. 1995. Purification and properties of an alcohol dehydrogenase (HU ADHII) from *Hanseniaspora uvarum* K5. J. Appl. Bacteriol. **79**:79-86.
5. Venturin C., H. Boze, G. Moulin, P. Galzy. 1995. Influence of oxygen limitation on glucose metabolism in *Hanseniaspora uvarum* K₅ grown in chemostat. Biotechnol. Letters **17**:537 -542.

XXI. Department of Biology, Carleton University, 587 Tory Building, 1125 Colonel By Drive, Ottawa, Ontario Canada K1S 5B6. Communicated by B.F. Johnson.

1. B.F. Johnson, B.Y. Yoo¹ & G.B. Calleja. 1995. Smashed fission yeast walls structural discontinuities related to wall growth. Cell Biophysics **26**:57-75.

¹Department of Biology, University of New Brunswick, Fredericton, New Brunswick, Canada.

²Diliman Institute, UP Campus, Diliman, Lunsod Quezon, The Philippines.

Twenty-three samples of fission yeast cells (*Schizosaccharomyces pombe*) were smashed by shaking them with glass beads. The samples represented all phases of the culture cycle, with the lag and log phases emphasized. Ruptured walls of the smashed cells were observed by phase-contrast and electron microscopy. Ruptures were tabulated with respect to their magnitudes and locations. Ruptures occurred not at random, nor at sites directed by geometry, but predominated in certain definable wall regions. These discontinuities were correlated with morphogenetic activities of the cell. Thus, the extensile end was

found to be most fragile through most of the culture cycle. Also fragile was the nonextensile end, its edge more than its middle. Further, the data were applied to the testing of predictions from extant models (Johnson endohydrolytic softening model and Wessels presoftened-posthardened and crosslinking model) for hyphal tip extension. The frequency of rupture at the extensile (old) end of the cell was qualitatively predicted by both models; the frequency at the nonextensile (new) end was not predictable by either. Rupture frequencies and characteristics at other regions conformed to predictions by one or the other model, but rarely by both.

2. M.M. Kekez, P. Savic & B.F. Johnson. 1996. Contribution to the biophysics of the lethal effects of electric field on microorganisms. Biochim. Biophys. Acta **1278**:79-88.

The proposed model assumes that the criteria leading to the lethal breakdown of microorganisms suspended in a continuous medium depend on two parameters: (a) the applied electric field must exceed the critical field of membrane to create holes and (b) the Joule energy (deposited in the membrane) must exceed the minimum value beyond which the cell can not recover. The first parameter initiates (reversible) breakdown and the second one, the completion

of the (irreversible) electrical breakdown leading to death of the cell. The number of cells surviving the electric field treatment is related to statistical distribution of cell size. Comparison between theory and the experimental results of Kinoshita and Tsong (1977); Hülshager et al. (1980, 1981, 1983); Rosembera and Korenstein (1990) and others is given.

3. M. Miyata,¹ H. Doi,¹ H. Miyata,² & B.F. Johnson. In press. Sexual co-flocculation by heterothallic cells of the fission yeast *Schizosaccharomyces pombe* modulated by medium constituents. Antonie van Leeuwenhoek.
¹Gifu Pharmaceutical University, 6-1, Mitahora-higashi 5 chome, Gifu 502 Japan.
²Nagoya Economic University. 61-1, Aza-Uchikubo, Inuyama 484, Japan.

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 this medium suppressed the asexual self-flocculation but rather promoted the sexual flocculation. By using 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 at 30°C and that sexual co-flocculation of the heterothallic strains was induced only under lower concentrations of C- and N-sources than that for the homothallic one.

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

The following poster will be presented at the 9th ISY in Sydney Australia.

1. Lachance, M.A. 1996. Non-specific, haustorium-mediated yeast predation in *Candida azyma*.

In the course of a study of the yeast communities associated with Australian *Hibiscus* and *Ipomoea* species, several strains identified as *Candida azyma* (van der Walt, Johannsen et Yarrow) Meyer et Yarrow were isolated from flowers and floricolous insects in both plant species. Upon mixing these strains in pairs to assess possible sexuality, a single isolate was found to interact with all others, not by conjugation, but by formation of haustoria and destruction of the cells that were penetrated. This phenomenon, which shows some similarity to the special cell contacts described in *Arthroascus javanensis* by Kreger-van Rij & Veenhuis (1973. J. Bacteriol. **113**:350-356), was investigated further. The predatory strain of *C. azyma* attacks a broad array of ascomycetous and basidiomycetous yeasts. The yeast-like organism *Aureobasidium pullulans* and the mould *Penicillium chrysogenum* are susceptible prey, but *Schizosaccharomyces pombe* or *Prototheca* sp. are not attacked. Induction of haustorium formation apparently requires

physical contact with the prey, which must be viable. The haustoria are ca. 250 nm in outside diameter, with a ca. 100 nm lumen. After penetration, the distal portion of the haustorium swells and typically contains membranous structures. Predatory activity is most intense on agar media and weaker or incomplete in liquid media. The phenomenon occurs most intensely on unbalanced (*e.g.* corn meal, YNB without carbon, YCB without nitrogen, etc.) media, but not on complete, rich formulations (YM). On certain poor media, predation enhances the growth yield of the predator. Under favourable conditions, using a *Metschnikowia* species as prey, the predator:prey ratio increased by one order of magnitude in 20 hours. Successive transfers of a mixture caused a gradual increase of the predator:prey ratio from a value of 1:10 to a maximum of ca. 100:1. Under the same conditions, a non-predatory strain of *C. azyma* stabilized at a ratio of ca. 1:1 with the target organism.

Recent meeting

25th Annual Conference on Yeasts of the Czech and Slovak Commission for Yeasts, Smolenice, Slovakia, April 24-26, 1996.

Czechoslovakia disappeared from the map of Europe for the second time 4 years ago. Despite its division in two separate countries, the Czech Republic and the Slovak Republic, close contacts and cooperation between Czech and Slovak yeast researchers continue. The 25th Annual Conference on Yeasts was attended by 35 scientists from Czech Republic and 37 scientists from Slovakia. Two guests were from neighbouring countries. The oral program was divided into the following sessions: 1. Yeasts in Industry; 2. Cell biology; 3. Molecular biology. Twenty two plenary lectures were complemented by 44 posters. Below are listed the contributions of the 25th Annual Conference.

Plenary lectures on industrial yeasts

- O. Bendová: Current trends in the research of brewing yeasts.
P. Kohoutová, I. Hollerová: The yeast collection of the Institute of brewing and malting.
B. Janderová: Genetic analysis of industrial strains of *Saccharomyces cerevisiae*.
Z. Ciesarová, D. Šmogrovičová: Ethanol tolerance of yeasts and some of its application aspects.
M. Rychtera, L. Paulová, J. Votruba, K. Melzoch, J. Fiala: Optimization of the continual cultivation of the yeast *Saccharomyces cerevisiae*.
V. Vala: Production of yeasts on sulfite liquors.
A. Tomšíková: Newly-seen yeast pathogens.
G. Kogan: Use of yeast chitin in industry and medicine.
L. Kremnický, P. Biely: B-Mannanolytic system of *Aureobasidium pullulans*.

Plenary lectures in cell biology

- E. Streiblová: Progress in elucidation of intracellular motility of yeasts.
M. Kopecka, M. Gabriel, A. Svoboda: Study of nuclei and cytoskeleton in actin mutants of yeasts.
M. Gabriel, D. Horký, A. Svoboda, M. Kopecka: Morphological and functional restitution of the aktin contractible ring in *Schizosaccharomyces japonicus* var. *versatilis* after removal of cytochalasine B.
A. Kotyk: The function of H⁺-ATPase from plasma membrane of *Saccharomyces cerevisiae*.
K. Sigler, N. Stadler, M. Hofer: Oxidative inactivation of the membrane H⁺-ATPase in yeasts.
A. Matějčková, H. Sychrová: Expression of the amino acid transporter in *Candida albicans* and *Saccharomyces cerevisiae*.
J. Horák: Mechanisms of the degradation of the transport proteins in *Saccharomyces cerevisiae*.

Plenary lectures in molecular biology

- J. Šubík: The role of transcription factors in the pleiotropic resistance against inhibitors in the yeast *Saccharomyces cerevisiae*.
J. Sulo: The fate of the mitochondrial intron in nucleus and cytoplasm.
I. Zeman, J. Kolarov: Expression of genes coding for the ADP/ATP translocator in aerobic yeast strains.
A.A. Sibirny: Mechanisms of peroxisome biogenesis and degradation in methylotrophic yeasts.
I. Hapala, A. Hunáková: Biogenesis of sterols in yeasts: mechanism and control of the uptake of external sterols.
O. Ondřejíčková, M. Čerňáková: Cytotoxic effect of chlorinated phenol derivatives on *Saccharomyces cerevisiae*.

Posters

- V. Stollárová: The composition of yeast population on fruits.
E. Sláviková, R. Vadkertiiová: Tolerance of certain yeast species to reduced water activity of the environment.
R. Vadkertiiová, E. Sláviková: Tolerance of certain yeast species to ions of heavy metals.
B. Zikánová, V. Vondrejs: Isolation and characterization of the resistant strains to cations of selected metals in the autogenomic library of *Saccharomyces cerevisiae*.
E. Breierová, M. Kačuráková, E. Stratilová: Relationship between the cryoprotectivity and composition of yeast extracellular polymers.
Z. Ciesarová, D. Šmogrovičová, J. Šajbidor, M. Lamacka: Yeast stress and ergosterol.
A. Tomšíková: Preponderance of *Candida* species in humans during years 1955-1995.
O. Janousková, V. Vondrejs: Effect of the medium on the sensitivity of *Saccharomyces cerevisiae* to zymocine K1.
J. Šandula, E. Machová, J. Kohut: Use of sonication in the extraction and modification of biologically active components of the yeast cell walls.
E. Machová, J. Šandula: Depolymerization of skeletal polysaccharides of yeasts and fungi.
H. Miková, M. Rosenberg, L. Křištofiková: Effect of detergents on the production of L-malic acid by yeasts.
R. Zeman, F. Adámek: Effect of vitamins and ions on the production of phenylacetylcarbinol in *Saccharomyces cerevisiae*.
E. Michálková, A. Vikartovska, L. Welward, P. Gemeiner: *Trigonopsis variabilis* with activity on cephalosporine C.
D. Šmogrovičová, Z. Ciesarová: Activity of yeast amylases.
E. Stratilová, E. Breierová, A. Malovíková, R. Vadkertiiová: Adaptability of the methylotrophic yeast *Candida boidinii* on media containing pectin substances.

- E. Stratilová, E. Breierová, E. Machová, R. Vadkertiová: Localization of pectolytic enzymes produced by *Aureobasidium pullulans*.
- P. Biely, M. Vršanská, L. Kremnický: Relation between the production of xylanases and mannanases in the strains of *Aureobasidium pullulans*.
- E. Sláviková, B. Košíková: Production of biomass of *Sporobolomyces roseus*, *Candida tropicalis* and *Saccharomyces cerevisiae* in the presence of the lignin component of a beechwood prehydrolysate.
- I. Jančová, N. Kolarová: β -Galactosidase of the yeast *Cryptococcus laurentii*.
- P. Bartek, N. Kolarová: Effect of nucleoside pyrophosphatase inhibitors on the galactosyltransferase activity of the yeasts *Cryptococcus laurentii*.
- J. Gabriel, V. Havlíček, L. Stadníková, M. Pospísek, P. Valíček: Antimicrobial metabolites of *Eleutherine subaphylla*.
- V. Raclavský, R. Novotný: Localization of chitin in the cell walls of *Saccharomyces cerevisiae* exposed to Rylux BSU.
- M. Havelková, E. Unger, I. Hönes: Effect of cytochalazine on the cells of *Yarrowia lipolytica*.
- P. Vavříčková, J. Hašek: F-Actin distribution in tubulin mutants of *Saccharomyces cerevisiae*.
- I. Pokorná, A. Svoboda: Microtubules and mitochondria in the life cycle of the yeast *Schizosaccharomyces japonicus* var. *versatilis*.
- M.V. Gonchar, L.B. Kostyryk, M.M. Maidan, A.A. Sibirny: Cytochrome C peroxidase from methylotrophic yeast *Hansenula polymorpha*.
- V.M. Ubiyovok, A.A. Sibirny: Biosynthesis, transport and physiological role of glutathione in the methylotrophic yeast *Hansenula polymorpha*.
- A.P. Rojas, Z. Storchová, V. Vondrejs: Effect of osmotic stabilization on vitality of *rad6-1* mutants of *Saccharomyces cerevisiae*.
- A.P. Rojas, V. Vondrejs: Qualitative and quantitative analysis of the formation of papillae under different conditions of the starvation for adenin in *Saccharomyces cerevisiae*.
- F. Cvrčková, V. Žárský, M. Patočka: Identification of plant homologues of yeast morphogenetic genes.
- E. Farkašová, M. Dérerová, M. Chovanec, V. Vlčková, J. Brozmanová: Comparison of the function of *psa4* and *RAD52* genes in the repair of the alkylation damage of DNA.
- M. Slaninová, V. Vlčková, J. Brozmanová: Effect of the *E. coli* RecA protein on *psa4-1* and *rad51* repair deficient mutants of *Saccharomyces cerevisiae*.
- V. Vlčková, M. Slaninová, M. Zavodna: Effect of metabolically activated metaphenylenediamine on genetic changes in *Saccharomyces cerevisiae*.
- M. Osuský, L. Kováč: Transfer of the mitochondrial genome between the species of the genus *Saccharomyces*.
- M. Janitor, M. Obernauerová, J. Šubík: Role of the gene *PEL1* in the metabolism of phospholipids of the yeast *Saccharomyces cerevisiae*.
- Y. Gbelská, I. Hikkel, S. Vrobelová, J. Šubík: Preparation and properties of the cytochrome c deficient mutant of *K. lactis*.
- I. Janatová, E. Meilhoc, J.M. Masson: Resistance to phleomycine as a reporter system in the yeast *Schwanniomyces occidentalis*.
- J. Nosek, L. Tomaska: Study of the dynamics of mitochondrial telomers of the yeast *Candida parapsilosis*.
- L. Adamíková, J. Nosek, L. Tomaska: Preparation of the transformation system for a multinuclear yeast: isolation of *URA3* gene and *ARS* sequences from *Endomyces magnusii*.
- V. Reiser: MAP kinase pathway of *Saccharomyces cerevisiae* activated by osmotic stress.
- L. Valášek: The absence of the yeast MAP110p causes a defect in the cell separation.
- J. Paleček: Protein interactions of the yeast *MAP110p*.
- L. Šabová, I. Bekesiová, K. Luciaková, S. Betina, J. Kolarov: Analysis of the *ROX1*-independent repressor region of the anaerobic isogene coding for ADP/ATP translocator in the yeast *Saccharomyces cerevisiae*.

At a meeting of the Committee of the Commission held during the conference it was agreed to hold the 26th Annual Conference on Yeasts during May 21-23, 1997, again in the Smolenice Castle near Bratislava. The Czech and Slovak Commission on Yeast also plans to organize an International Specialized Symposium on Yeasts which would be dedicated to Yeast Cell Surfaces and Membrane Phenomena. A tentative date of the meeting is spring of 1999.

Communicated by Peter Biely

Forthcoming meetings

International Union of Microbiological Societies Congresses 1996: 8th International Congress of Bacteriology and Applied Microbiology Division and 8th International Congress of Mycology Division, Jerusalem, Israel, August 18-23, 1996

Please contact the Secretariat if you require any information or assistance. Address all correspondence to:

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ISY IX - Ninth International Symposium on Yeasts, Sydney, Australia, 25-30 August 1996

The ISY is held every four years as an activity of the International Commission on Yeasts to foster interest in the science and technology of yeasts. This will be the first time that the ISY has been held in Australia. As an added attraction, it will be held concurrently with the 10th International Biotechnology Symposium (*IBS10*). The venue for both symposia is the Sydney Convention Centre, located in the heart of Sydney on the foreshore of its spectacular harbour. The scientific program has been designed to cover recent advances in all aspects of yeast biology and yeast technology. Advice on content and speakers has been obtained from the vast network of councillors with the International Commission on Yeasts. The program will consist of plenary presentations, contributions to specialised symposia, and poster presentations of which several will be selected for oral communication. With the exception of plenary presentations, the scientific program has been organised as two concurrent sessions, allowing some degree of flexibility and choice. It will include 16 symposium sessions of invited speakers and 6 proffered paper sessions. In addition,

opportunity will exist to attend sessions in the IBS program and there will be a substantial trade exhibition which is being held in conjunction with IBS. To encourage a strong submission of high quality posters, the International Commission on Yeasts is offering prizes of US\$500 each for the two best poster presentations. The posters will be judged by an international panel on the basis of scientific merit and quality of presentation. For one of the prizes, preference will be given to younger scientists. The social program has been designed to feature some of the unique attractions of Sydney (the Opera House, the harbour, the Power House Museum) and will be concluded with a dinner based on the theme *A taste of Australia*. The Australian Biotechnology Association is honoured to be hosting the 9th ISY and the 10th IBS. We anticipate symposia of excellence and excitement and look forward to seeing you in Australia in 1996.

For additional information, see the December issue of the Yeast Newsletter, or contact

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10th International Biotechnology Symposium, August 25-30, 1996, Sydney, Australia

Contact:

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**Eighth International Congress for Culture Collections (ICCC-8),
August 25-29 1996, Veldhoven, The Netherlands**

Organized by World Federation for Culture Collections (WFCC), Netherlands Culture Collections (NCC), and the Centraalbureau voor Schimmelcultures (CBS). The Netherlands will host the Eighth International Congress for Culture Collections. The conference is modular, enabling each participant to create an individual, tailor-made programme. The conference environment further enhances informal contacts in a comfortable, relaxing atmosphere. The conference centre "Koningshof" is surrounded by extensive woods. Comfortable hotel rooms and all other facilities are situated in one building, near Eindhoven Airport. This local airport

operates an international schedule connected to Schiphol International Airport. Shuttle buses will operate from and to Eindhoven Railway Station, also with excellent connections to major gateways.

Central theme: Culture Collections to improve the quality of life. Registration fee: Dfl. 1475 (including lodging single room for four nights and meals); Dfl. 1375 (including lodging double room for four nights and meals); Dfl. 900 (excluding lodging but with meals).

For information please contact

Secretariat ICCC-8
Centraalbureau voor Schimmelcultures
P.O.Box 273, 3740 AG Baarn
The Netherlands

Tel + 31-35-5481211
Fax + 31-35-5416142
E-Mail: iccc8@cbs.knaw.nl

Workshops offered by ATCC in 1996.

September 18-20 Downstream Processing, Recovery and Purification of Proteins
September 24-27 Fermentation Microbiology
October 2-4 Microscopy/Photomicrography

October 15-18 Freezing & Freeze-Drying of Microorganisms
November 11 -15 Advanced Recombinant DNA Techniques & Applications

For information on ATCC Workshops contact:

ATCC,
Workshop Coordinator,
12301 Parklawn Drive,
Rockville Md 20852
U.S.A.

Telephone: (301) 231 -5566
FAX: (301) 816-4364
<http://www.atcc.org/workshops/workshop.html>

**Ninth Meeting on "The Biology of *Kluyveromyces*",
Monday 23 and Tuesday 24 September, 1996, Centro Santa Elisabetta,
Univesità degli Studi di Parma, Viale delle Scienze, 43 100 Parma, Italy.**

Although the deadlines for registration and submission of abstracts have already passed, interested readers are encouraged to contact the organizers. The registration fee, payable at the meeting, is Italian Lire 160,000; it covers the costs of abstracts book, coffee

breaks, two lunches, and two dinners. Receipts will be issued on payment. **Contact**

Prof. Iliana Ferrero and Dr. Paola Goffrini
Istituto di Genetica
Universita degli Studi di Parma
Viale delle Scienze
43100 Parma, Italy

Tel.: +39 521905607/602
Fax: +39 521905604

**18th International Conference on Yeast Genetics and Molecular Biology,
University of Stellenbosch, Stellenbosch, South Africa, March 31 to April 5, 1997.**

The XVIII International Conference on Yeast Genetics and Molecular Biology will be held on the campus of the University of Stellenbosch, Stellenbosch, South Africa from 31 March to 5 April 1997. The breathtaking beauty of the Cape has inspired travellers for centuries. When Sir Francis Drake sailed around the southernmost tip of Africa in 1577, he called it, "The fairest Cape that I have seen in the whole circumference of the earth." It is to this magical region that I would like to invite you on behalf of the Organizing Committee. Stellenbosch, the second oldest town in the country, is situated in the heart of the winelands and surrounded by towering mountain ranges. The town was founded by Simon van der Stellenbosch in 1679 and is renowned for its impressive Cape Dutch buildings and oak-lined streets. It is also a prestigious centre of learning: the University, known as the Victoria College since 1887, was founded in 1918. We are looking forward to an exciting conference. The success of this meeting will, to a large extent, depend on its participants and we look forward to seeing you in

Deidre Cloete
Conferences *et al*
P.O. Box 452
7600 STELLENBOSCH
South Africa

Stellenbosch in 1997.

Local organizing committee. Hennie J.J. van Vuuren, Isak S. Pretorius, Willem H. van Zyl, Bernard A. Prior. **External advisors.** Terry G. Cooper, Rudi J. Planta. Scientific Programme. Leading scientists will be invited to present plenary lectures. In addition there will be workshops and poster sessions. Posters will be on display during the whole meeting. Further information will be provided in the second circular to be mailed around September 1996. **Accommodation.** For convenience, Stellenbosch is unrivalled. From Cape Town it is an easy 40 minute drive and from Cape Town International Airport a mere half hour. A shuttle service will be provided. Accommodation will be booked in University hostels, hotels and guest houses, all situated at a short distance from the conference venue.

Contact:

Tel.: 27-21-8864496
Fax: 27-21-88381 77
E-mail: <eikestad@iafrica.com>

**EFB Working Party Microbial Physiology Conference:
"Microbial Response to Stress: what's new and how can it be applied?"
Sesimbra, Portugal, 15-18 March 1997**

The conference, organized by the Working Party "Microbial Physiology" of the European Federation of Biotechnology and by Sociedade Portuguesa de Biotecnologia, will deal with: (1) Stress Response: sensing and signaling; (2) Stress-Induced Changes in Gene Expression; (3) Cellular Processes Affected by Stress; (4) Acquisition of Stress Tolerance, and (5) Biotechnological Applications, in Yeast and Bacteria. Many biotechnological industries can only compete with physical or chemical technologies if organisms can be found that can survive in extreme environments. Fundamental studies of how microbes respond to stress therefore provide the foundation on which to build new technologies or to optimize an old one. The aims of this intensive four day residential symposium will be to exchange information about most recent developments in our understanding of yeast and bacteria response to stress.

Prof. Isabel Sa-Correia
Secção de Biotecnologia,
Instituto Superior Tecnico,
Av. Rovisco Pais, 1096 Lisboa Codex,
Portugal

The conference will include lectures by invited speakers poster sessions and oral presentation of selected posters. We invite all biotechnologists to participate and especially encourage young scientists and PhD students to present their data and exchange ideas with leaders in their field.

Invited speakers: I.R. Booth (Aberdeen); A.M. Chakrabarty (Chicago); J.A. Cole (Birmingham); J.A.M. de Bont (Wageningen); A. Goffeau (Louvain-la-Neuve); R. Hengge-Aronis (Konstanz); R.C. Hockney (Zeneca Bioproducts); S. Hohmann (Göteborg); P.W. Piper (London); P.W. Postma (Amsterdam); U. Priefer (Aachen); H. Ruis (Wien); H. Santos (Oeiras); R. Serrano (Valencia); J. Thevelein (Leuven); D. Thiele (Michigan).

For further information and to receive the 1st announcement/call for abstracts please contact:

Telephone: 351-1-8417233/682
Fax: 351-1-8480072
E-mail: <qisc@beta.ist.utl.pt>
or <qviegas@beta.ist.utl.pt>

Yeast Protein Database

The Yeast Protein Database (YPD) is a public resource on the World Wide Web that provides many types of information about each yeast protein of known sequence. YPD stays up to date with new yeast sequences as they are released. Some information provided includes: Isoelectric point, molecular weight, codon bias, CAI. Chromosome number, presence of introns, viability of knockout mutation. Post-translational modifications (phos, N-glc, O-glc, prenylation, etc). Length of N-terminal precursor. Subcellular localization (nuclear, mitochondrial, nuclear pore, etc). Molecular environment (integral or peripheral membrane, DNA-associated, etc). Functional categories (transcription factor, protein kinase, GTPase, etc). Annotations (Extensive notes from the literature). References with titles (over 8000 total).

Of special interest: From the YPD Home page, one can access the new or renamed proteins for each week. Rather than just flagging new sequences, which are less frequent now, this feature flags proteins with new names, thereby pointing out those proteins that have been newly characterized and given functional names to replace their systematic (genome sequencing) names.

The Yeast Protein Database was initiated at the Cold Spring Harbor Laboratory with support from the National Center for Research Resources. It is now supported and maintained by Proteome Inc.

James I. Garrels, Ph.D.
PROTEOME INC.
181 Elliott St., Suite 909
Beverly, MA 01915

With the completion of the Yeast Genome Project, YPD will soon contain information on all yeast proteins. We want to enlist the help of the user community to be sure that YPD contains complete and accurate information. YPD is found at
<http://www.proteome.com/YPDhome.html>.

Ypd integrates the yeast literature and the sequence databases.

YPD contains information for all *S. cerevisiae* proteins of known sequence. It includes gene names and synonyms, protein names/descriptions, protein properties, annotations from the literature, and references.

The YPD entries now makes reference to more than 8300 papers in the yeast literature. YPD now contains about 20000 annotation lines describing protein functions, mutant phenotypes, protein domain structures, homologies, etc. We review most of the recent literature, and we continue to add information from the older literature.

We would appreciate your feedback.

You can help us by checking the entries for the proteins you work on. We would like to know about errors in the database, missing citations, and data that has not yet been included. We reply to all comments from users, and most corrections are entered within 24 hr.

Tel (508) 922-1643
FAX (508) 922-3971
Email: <jjg@proteome.com>
<http://www.proteome.com>
