ISSN 0513-5222

Y E A S T

A Newsletter for Persons Interested in Yeast

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

DECEMBER 1999 **Volume XLVIII, Number II**

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

Editorial

IXth IUMS International Congress of Mycology, Sydney, Australia

The organizers of the IXth International Congress of Mycology of the International Union of Microbiological Societies, held on August 16-20 1999 in Sydney, Australia, are to be congratulated for an excellent meeting. A small but vigorous representation of yeast researchers contributed to the symposia. The congress was an opportunity for researchers in various fields of microbiology, including bacteriology, mycology, and yeast biology, to mingle and exchange ideas and viewpoints. A significant number of members of the International Commission on Yeasts were in attendance and met officially. Minutes of the meeting are included in this issue of the Yeast Newsletter.

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

M.A. Lachance
Editor

I. Yeast Genetic Resource Center, American Type Culture Collection. 12301 Parklawn Drive, Rockville, Maryland 20852-1776, U.S.A. Communicated by T.K. Gu <kgu@atcc.org>.

ATCC to Distribute *Saccharomyces* Genome Deletion Mutants.

After fully sequencing the yeast genome, the yeast research community continues to collaborate to study functional genomics in the post-genome era. The *Saccharomyces* Genome Deletion Project (SGDP) is now underway. The goal of this project is to generate a complete set of yeast deletion mutants that will serve as the core set of strains for the functional analysis of each gene of the genome. These knockouts are being developed by a consortium of U.S. investigators funded by National Human Genome Research Institute of NIH, EUROFAN (EUROpean Functional Analysis Network), and Canadian colleagues. The SGDP deletion mutants will enable the analysis of each gene in the genome, continuing the study of function of each gene and whole genome that began after the full sequencing of the yeast genome. There are more than 6,000 open reading frames in the yeast genome. Each deletion mutant will be in **a** and **α** haploids and in homozygous and heterozygous diploids so that there will be 24,000 knockout mutants in the collection. These total 24,000 deletion strains are being generated by the consortium laboratories over a two-year period, and are made available to the research community after they arrive at ATCC. ATCC will serve as distributor and so far a total of 16,000 deletion mutants are already available for distribution. The on-line search engine of the deletion mutants is on the ATCC web site. (Click on Search Catalogs, then choose Yeast Genetic Stock Center and About the Collection).

Beside the deletion mutants, ATCC also curate the Yeast Genetic Stock Center collection from University of California-Berkeley. This collection is composed of approximately 1,200 genetically defined strains of the yeast *Saccharomyces cerevisiae*, and has served yeast geneticists and others in the scientific community by offering strains bearing mutations useful in the study of genetics, molecular biology, biotechnology, and medical research.

Now that two well-known yeast collections are available at ATCC, finding the right *Saccharomyces* mutant has never been easier. "These two collections represent a significant expansion of ATCC's yeast resources," said Dr. Raymond Cypess, President and CEO of ATCC. "As research in genomics continues to expand, the availability of these mutant strains and a comprehensive database will be of distinct value to scientists worldwide."

Please visit our new web site: (Genetic yeasts) http://www.atcc.org/ygsc/ and (Yeast deletion mutants) http://www-deletion.stanford.edu/cgi-bin/deletion/search3.pl.atcc

Requests and inquiries regarding the strains may be addressed to:

Dr. Kelong Tom Gu
Yeast Genetic Resource Center
American Type Culture Collection
10801 University Blvd.
Manassas, VA 20110-2209

<kgu@atcc.org>

Tel. 800-638-6597 (toll-free in North America)
or 703-365-2700
Fax. 703-365-2730

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

Recent publications.

1. Caridi A., Crucitti P., Ramondino D. 1999. Winemaking of musts at high osmotic strength by thermotolerant yeasts. Biotechnol. Lett. **21**:617-620.

Fermentation performance of 15 thermotolerant *Saccharomyces cerevisiae* in three musts from dried grapes at 25, 30, and 40°brix was studied. When osmotic strength increases, the volatile acidity and the SO_2 production in the wines also increases: in the must with 40°brix, yeasts produce from 1.63 to 3.65 g acetic acid l^{-1} and from 40.0 to 73.6 mg SO_2 l^{-1} due to osmotic stress. From 9.75 to 13.40 ethanol v/v production is observed in must at 30°brix, whereas at 40°brix there is a clear detrimental effect.

2. Scerra V., Caridi A., Foti F., Sinatra M.C., Caparra P. 1999. Changes in chemical composition during the colonisation of citrus pulps by a dairy *Penicillium roqueforti* strain. Biores. Technol. **72**:197-198.

Citrus pulps were examined during colonisation with *Penicillium roqueforti* Pr2. Fungal growth increased the crude protein content from 5.62 to 8.55 (% dry matter) in orange pulp and from 5.77 to 11.89 (% dry matter) in lemon pulp. The ether

extract increased respectively from 1.62 to 4.38 (% dry matter) and from 0.97 to 6.78 (% dry matter). The neutral detergent fiber content increased from 14.49 to 32.60 (% dry matter) and from 15.54 to 38.70 (% dry matter). The acid detergent fiber content increased from 11.15 to 24.79 (% dry matter) and from 10.96 to 30.73 (% dry matter).

3. Caridi A., Fuda S., Postorino S., Russo M., Sidari R. 1999. Selection of *Saccharomyces sensu stricto* for mead production. Food Technol. Biotechnol. **37**: 203-208.

The aim of this work was the selection of yeasts, of oenological origin, able to carry out the alcoholic fermentation of honey musts. We studied over one hundred strains of *Saccharomyces sensu stricto*. A preliminary screening with must prepared by mixing three parts of water and one of citrus honey was performed; the intensity of growth and the fermentation activity were evaluated. From the results of this screening, the strains with better characteristics were selected to test their fermentation performance using a must at higher sugar concentration. The majority of the yeasts did not show the ability to grow in the honey must during preliminary screening. Many of those strains that passed the preliminary screening manifested some defects when tested for fermentation performance. For some fermentation characteristics, such as fermentation vigour and ethanol production, the strains showed remarkable differences, which were particularly useful in the subsequent selection. Four strains exhibited good general performance. The research will go on to employ the best strains for the production of meads on an experimental scale, with or without the addition of nutrients, and using different varieties of honey; the meads thus produced will be examined for their chemical, physico-chemical and sensory profile.

4. Caridi A., Foti F., Sinatra M.C., Colacino T., Scerra V. 1999. Changes in nutrient content of sweet pepper waste after colonisation by dairy *Penicillium*. Ann. Microbiol. Enzymol. **49**: in press.

Sweet pepper waste was examined before and after colonisation with *Penicillium roqueforti* Pr1. Fungal growth increased crude protein content from 22.3 to 35.2 (% dry matter), gross energy from 4763 to 4899 (Kcal kg^{-1} DM), neutral detergent fiber content from 22.3 to 48.4 (% dry matter), and acid detergent fiber content from 17.5 to 38.6 (% dry matter). On the contrary, fungal growth decreased usable organic matter from 66.0 to 40.2 (% dry matter) and non-nitrogen usable organic matter from 35.7 to 5.0 (% dry matter).

III. Rosenstiel School of Marine and Atmospheric Science, University of Miami, 4600 Rickenbacker Causeway, Miami, Florida 33149, USA. Communicated by J.W. Fell <jfell@rsmas.miami.edu>.

Listed below are recent publications from our lab and in cooperation with other investigators. In addition, we released, to GenBank, the sequences for the D1/D2 LrDNA region for all described basidiomycetous yeasts. Identification of basidiomycetous yeasts should be possible through BLAST searches with D1/D2 sequences. The systematic placement of the yeasts in the GenBank data base is often curious as GenBank seems to have an ever fluctuating concept of yeast basidiomycete systematics. Our current ideas are presented in Fell et al. (2000).

1. Fell JW, Roeijmans H & Boekhout T. 1999 Cystofilobasidiales, a new order of basidiomycetous yeasts. Int. J. of Syst. Bacteriol. **49**:907-913.

2. Boekhout T, Fell JW, Kurtzman CP & Johnson EA. 1999. (1386) Proposal to reject the name *Rhodomyces dendrorhous* (Fungi, Basidiomycota). Taxon **48**:147-148.

3. Sato I, Kobayasi H, Hanya Y, Abe K, Murakami S, Scorzetti G & Fell JW. 1999. *Cryptococcus nodaensis* sp. nov., a yeast isolated from soil in Japan that produces salt-tolerant and thermostable glutaminase. J. Industr. Microbiol. Biotechnol. **22**:127-132.

4. Fonseca A, Fell JW, Kurtzman C P & Spencer-Martins I. *Candida tartarivorans* sp. nov., an anamorphic ascomycetous yeast with the capacity to degrade L(+)- and meso-tartaric acid. Int. J. Syst. Bacteriol. (accepted).

5. Scorzetti G, Petrescu I, Yarrow D & Fell JW. *Cryptococcus adeliensis* sp. nov., a xylanase producing basidiomycetous yeast from Antarctica. Antonie van Leeuwenhooek (in press).

6. Middelhoven WJ, Scorzetti G & Fell JW. *Trichosporon veenhuisii* sp. nov., an alkane-assimilating anamorphic basidiomycetous yeast. Int. J. Syst. Bacterol. (in press).

7. Middelhoven WJ, Scorzetti G & Fell JW. *Trichosporon guehoae*, sp. nov., an anamorphic basidiomycetous yeasts. Can. J. Microbiol. (in press).

8. Fell, JW & Blatt G. 1999. Separation of strains of the yeasts *Xanthophyllomyces dendrorohous* and *Phaffia rhodozyma* based on rDNA IGS and ITS sequence analysis. J. Industr. Microbiol. Biotechnol. **21**:677-681.

9. Diaz MR & Fell JW. 2000. Molecular analysis of ITS and IGS rDNA regions of the psychrophilic yeasts in the genus Mrakia. Antonie van Leeuwenhoek **77**:(in press).

10. Fonseca A, Scorzetti G, & Fell JW. 1999. Diversity in the yeasts *Cryptococcus albidus* and related species as revealed by ribosomal DNA sequence analysis. Can. J. Microbiol. **45**:1-21.

11. Sampaio JP, Fell JW, Gadanho M & Bauer R. 1999. *Kurtzmanomyces insolitus* sp. nov., a new anamorphic heterobasidiomycetous yeast species. Syst. Appl. Microbiol. **22**: (in press).

12. Fell, JW, Boekhout T, Fonseca A, Scorzetti G & Statzell-Tallman A. 2000. Biodiversity and systematics of basidiomycetous yeasts as determined by large subunit rD1/D2 domain sequence analysis. Int. J. Syst. Evol. Microbiol. (in press).

IV. Instituto de Investigaciones Biomédicas Alberto Sols, CSIC-UAM, 28029 Madrid, Spain. Communicated by C. Gancedo <cgancedo@iib.uam.es>.

The following are summaries of recent work from our laboratory.

1. C. Rodríguez & J. M. Gancedo. 1999. Glucose signalling in yeast is partially mimicked by galactose and does not require the Tps1 protein. Mol. Cel. Biol. Res. Commun. **1**:52-58

Glucose produces multiple effects in *Saccharomyces cerevisiae*, as it controls the expression of many genes and the activity of various enzymes. However, the elements involved in glucose signalling are not well characterized. In this work the capacity of galactose to bring about the same effects than glucose has been assessed. Galactose mimicks glucose only partially; it is suggested that it does not interact with a "sensor" in the plasma membrane and that it produces a weaker intracellular signal than glucose. To examine whether trehalose-6P synthase (Tps1) is required to transduce the glucose signal, we have constructed a *tps1hxk2*/*tps1HXK2* strain which, at difference of a *tps1* strain, grows on glucose, and, at difference of a *tps1hxk2* strain, still possess the Hxk2 protein, possibly involved in glucose repression. From the response of this strain to glucose, we conclude that Tps1 does not play a prominent role in glucose signalling.

2. M.J. Lafuente, C. Gancedo, J.C. Jauniaux & J.M. Gancedo. Mth1 receives the signal given by the glucose sensors Snf3 and Rgt2 in *Saccharomyces cerevisiae.* Mol. Microbiol. in press.

We have determined that the mutant genes *DGT1-1* and *BPC1-1* which impair glucose transport and catabolite repression in *Saccharomyces cerevisiae* are allelic forms of *MTH1*. Deletion of *MTH1* had only slight effects on the expression of *HXT1* or *SNF3* but increased expression of *HXT2* in the absence of glucose. A two-hybrid screen revealed that the Mth1 protein interacts with the cytoplasmic tails of the glucose sensors Snf3 and Rgt2. This interaction was affected by mutations in Mth1 and by the concentration of glucose in the medium. A double mutant *snf3 rgt2* recovered sensitivity to glucose when *MTH1* was deleted, thus showing that glucose signalling may occur independently of Snf3 and Rgt2. The following model is proposed : glucose will change the conformation of the cytoplasmic tails of Snf3 and Rgt2 thus influencing the binding of Mth1. This in turn will affect the transcription of several genes encoding glucose transporters.

3. O. Zaragoza, C. Rodríguez and C. Gancedo. Isolation of the gene *MIG1* from *Candida albicans* and effects of its disruption on catabolite repression. J. Bacteriol. (in press).

We have cloned a *Candida albicans* gene (*CaMIG1*) that encodes a protein homologous to the DNA binding protein Mig1 from *Saccharomyces cerevisiae*. The *C. albicans* Mig1 protein (*Ca*Mig1) differs from the *Sc*Mig1, among other things, in that it lacks a putative phosphorylation site for Snf1 and presents several long stretches rich in glutamine or in asparagine, serine and threonine and has the effector domain located at some distance (50 amino acids) from the carboxy terminus. Expression

of *CaMIG1* was low, and similar in glucose, sucrose or ethanol containing media. Disruption of the two *CaMIG1* genomic copies had no effect in filamentation or infectivity. Levels of a glucose repressible α-glucosidase, implicated in both, sucrose and produced by *mig1* or *tps1* mutations. In addition, *Ca*Mig1 formed specific complexes with the URS1 region of the *S. cerevisiae FBP1* gene. The existence of a possible functional analogue of maltose utilization, were similar in wild type or *mig1*/*mig1* cells. Disruption of *CaMIG1* had also no effect on the expression of the glucose repressed gene *CaGAL1*. *CaMIG1* was functional in *S. cerevisiae* as judged by its ability to suppress the phenotypes *CaMIG1* in *C. albicans* was suggested by the results of band shift experiments.

V. Food Research and Development Centre, Agriculture and Agri-Food Canada, Saint-Hyacinthe, Québec, Canada J2S 8E3. Communicated by P. Gélinas.

The following thesis was presented in August 1998.

1. Daigle, P. Production de composés aromatiques par *Geotrichum candidum* à partir de sous-produits de boulangerie. (In French). Laval University. Québec, Canada.

A scientific paper was prepared from this thesis.

2. Daigle, P., Gélinas, P., Leblanc, D. and Morin, A. 1999. Production of aroma compounds by *Geotrichum candidum* on waste bread crumb. Food Microbiol. **16**: (in press).

After selection from eight yeast commercial or type strains based on their aromatic potential to valorize bread by-products, *Geotrichum candidum* ATCC 62217 formed fruity aroma compounds (pineapple-like) on fermented waste bread (35% white bread crumb and 65% water). Fatty acids esters were identified, including ethyl esters of acetic acid, propionic acid, butyric acid and isobutyric acid. Their production corresponded to the stationary growth phase of the strain and, after 48 h, it was improved by agitation and, to a lesser extent, at 30°C compared to 25 or 20°C. Aromatic properties of the strain were linked to its ability to metabolize organic acids.

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

The following papers have been published since our last report.

1. S. Wang, K.C. Thomas, K. Sosulski, W.M. Ingledew, and F.W. Sosulski. 1999. Grain pearling and very high gravity (VHG) fermentation technologies for fuel alcohol production from rye and triticale. Proc. Biochem. **34**:421-428.

A SATAKE laboratory abrasive mill was used for rye and triticale grain processing. About 12% of dry grain mass was removed after three and five successive abrasions for triticale and rye, respectively. Starch contents in the pearled grain were increased by 8.0% for triticale, and by 7.1% for rye. The pearled rye and triticale were ground and fermented by active dry yeast for fuel alcohol production by very high gravity (VHG) fermentation at 20°C. VHG technology was applied to increase final ethanol concentrations in the fermentors from 9.5-10.0% (v/v) (normal gravity) to 12.9-15.1% (v/v). The grain pearling process coupled with VHG technology further raised the ethanol concentration to 15.7-16.1% (v/v). Partial removal of outer grain solids in an alcohol plant would improve plant efficiency and decrease energy requirements for mash heating, mash cooling, and ethanol distillation.

2. W.M. Ingledew, K.C. Thomas, S.H. Hynes and J.G. McLeod. 1999. Viscosity concerns with rye mashes used for ethanol production. Cereal Chem. **76**:459-464.

Rye cultivars leading to high viscosity extracts were easily mashed - even under very high gravity conditions - when enzymes were added to reduce viscosity caused by pentosans. The enzymes were so effective that for the fuel alcohol industry, the need for special genetic selections of rye with reduced extract viscosity was not indicated. High starch varieties of rye, however, were still most beneficial when alcohol was the end product of value. Both normal and very high gravity mashes fermented out within 48 hours under the conditions described. Yields (L/tonne starch) were, on average, 5.3% higher under VHG conditions than under normal gravity.

3. M.S. Whiting, S.L. Gares, W.M. Ingledew and B. Ziola. 1999. Brewing spoilage lactobacilli detected using monoclonal antibodies to bacterial surface antigens. Can. J. Microbiol. **45**:51-58.

A panel of thirteen monoclonal antibodies (Mabs) was assembled that reacts with surface antigens on eight of eleven *Lactobacillus* brewing spoilage organisms, including one or more of *L. brevis*, *L. buchneri*, *L. casei-alactosus*, *L. plantarum*, or unspeciated isolate(s). Immunoblotting was done to identify the antigens involved in Mab binding. Antigen stability in situ was tested by protease treatment and by surface antigen extraction of washed bacteria. Protease susceptibility of extracted surface antigens was also examined. In most cases, *Lactobacillus* surface antigens detected by the Mabs appear to be noncovalently bound proteins readily altered or removed from the bacterium by various environmental conditions. This research identifies brewing conditions that need to be tested to ascertain whether bacterial surface antigen-reactive Mabs can be used for the rapid, sensitive, and specific detection of *Lactobacillus* brewing spoilage organisms.

4. W.M. Ingledew. 1999. Yeast - Could you base a business on this bug? Biotechnology in the feed Industry. Proceedings of Alltech's Fifteenth Annual Symposium. Edited by: T.P. Lyons, and K.A. Jacques. Nottingham University Press. Nottingham UK.

5. M.S. Whiting, W.M. Ingledew, S.Y. Lee, and B. Ziola. 1999. Bacterial surface antigen-specific monoclonal antibodies used to detect beer spoilage pediococci. Can. J. Microbiol. 45: 670-677.

Fourteen monoclonal antibodies (Mabs) were isolated that react with surface antigens ofPediococcus beer spoilage organisms, including *P. damnosus*, *P. pentosaceous*, *P. acidilactici*, and unspeciated isolates. Immunoblotting, enzyme immunoassays (EIAs) of protease- and neuraminidase-treated surface antigen extracts, carbohydrate competition EIAs, and cardiolipin EIAs were used to characterize the bacterial antigens involved in Mab binding. Antigen stability in situ was tested by protease treatment or surface antigen extraction of washed bacteria. In most cases, the Mabs bind to *Pediococcus* surface antigens that appear to be covalently bound cell wall polymers resistant to alteration or removal from the bacterial surface. These bacterial surface antigen reactive Mabs show good potential for rapid, sensitive, and specific immunoassay detection of *Pediococcus* beer spoilage organisms.

6. B. Ziola, S. L. Gares, B. Lorrain, L. Gee, W. M. Ingledew, and S. Y. Lee. 1999. Epitope mapping of monoclonal antibodies specific for the directly cross-linked meso-diaminopimelic acid peptidoglycan found in the anaerobic beer spoilage bacterium *Pectinatus cerevisiiphilus*. Can. J. Microbiol. **45**:779-785.

Nineteen monoclonal antibodies (Mabs) were isolated based on reactivity with disrupted *Pectinatus cerevisiiphilus* cells. All of the Mabs reacted with cells from which the outer membrane had been stripped by incubation with sodium dodecyl sulphate, suggesting the peptidoglycan (PG) layer was involved in binding. Mab reactivity with purified PG confirmed this. Epitope mapping revealed the Mabs in total recognize four binding sites on the PG. Mabs specific for each of the four sites also bound strongly to disrupted *Pectinatus frisingensis*, *Selenomonas lacticifix*, *Zymophilus paucivorans*, and *Zymophilus raffinosivorans* cells, but weakly to disrupted *Megasphaera cerevisiae* cells. No antibody reactivity was seen with disrupted cells of 11 other species of Gram-negative bacteria. These results confirm that a common PG structure is used by several species of anaerobic Gram-negative beer spoilage bacteria. These results also indicate that PG-specific Mabs can be used to rapidly detect a range of anaerobic Gram-negative beer spoilage bacteria, provided the bacterial outer membrane is first removed to allow antibody binding.

VII. State Scientific-Research Institute for Genetics and Selection of Industrial Microorganisms, I-Dorozhnyi 1, Moscow 113545, Russia. Communicated by G.I. Naumov and E.S. Naumova.

We thank L. Tomaska, M. Kolarov, J. Nosek, L. Kovac (Comenius University, Bratislava), I. Masneuf, M. Aigle, D. Dubourdieu (Bordeaux 2 University, France), S. de Hoog and M. Smith (CBS, Barn/Delft) for fruitful collaboration in yeast research in 1997-1998. G.I.N. is grateful to C. Leão (Portugal), G. H. Fleet and J.I. Pitt (Australia) for the invitation to give lectures at the 19th ISSY, 30th August 1998, Braga, Portugal and IX ICM, 16th August 1999, Sydney, Australia. The following are our publications for 1997-1999.

1. G. Naumov. 1997. Genetic polymorphism of *Saccharomyces* yeasts in natural habitats. In: Yeast nutrition and natural habitats. 18th Int. Spec. Sym. on Yeasts. Bled, Slovenia, L4-05.

2. G. Naumov. 1998. Ecology of cultured and wild *Saccharomyces* sensu stricto yeasts and their adaptive fermentation peculiarities. In: Yeast in the production and spoilage of food and beverages. 19th Special Sym. on Yeasts, Braga, Portugal, 47.

3. G.I. Naumov, E.S. Naumova, I. Masneuf , M. Aigle & D. Dubourdieu. 1999. Genetical and molecular analysis of the phenomena of natural interspecific hybridization and introgression among *Saccharomyces bayanus* and *S. cerevisiae*: wine, cider and beer strains. In: Actualités Œnologiques. VIème Symp. Int. d'Œnologie, Bordeaux, France.

4. I. Masneuf, G.I. Naumov, M.L. Murat, N. Glumineau & D. Dubourdieu. 1999. Des hybrides *Saccharomyces cerevisiae, Saccharomyces bayanus* pour la vinification des vins de Sauvignon. In: Actualités Œnologiques. VIème Symp. Int. d'Œnologie, Bordeaux, France.

5. G.I. Naumov. 1999. Genetic diversity in yeasts: biological species, geographical and ecological populations, genetic genera. In: IX Int. Congr. Bacteriol. and Microbiol., IX Int. Congr. Mycol., Sydney, Australia, 180.

6. G.I. Naumov & D.G. Naumov. 1997. Genetic mapping of a new divergent family of a-galactosidase genes *MEL* in the yeast *Saccharomyces cerevisiae*. Russian Biotechnol. **1**:24-26.

7. G.I. Naumov, E.S. Naumova & A. Querol. 1997. Genetic study of natural introgression supports delimitation of biological species in the *Saccharomyces* sensu stricto complex. System. Appl. Microbiol. **20:**595-601.

8. G.I. Naumov, E.S. Naumova, G. Marinoni & J. Piškur. 1998. Genetic analysis of the yeast *Saccharomyces castelliii, S. exiguus* and *S. martiniae*. Russian Journal of Genetics. **34:** 457-460.

9. G.I. Naumov. 1999. Divergent Population of *Saccharomyces paradoxus* in the Hawaii Islands: an *in statu nascendi* yeast species. Dokl. Biol. Sciences. **364**: 51-53.

10. G.I. Naumov, V.I. Kondrat'eva, N.G. Dudkina, L. Tomashka & E.S. Naumova. 1999. Genetically isolated yeast species *Arthroascus fermentans* found in Taiwan. Dokl. Biol. Sciences. **367**: 382-385.

11. G.I. Naumov, M.T. Smith & G.S. de Hoog. 1999. Genetic interpretation of speciation and life cycle in *Galactomyces* fungi. Microbiology. **68**: 362-364 (Engl. Transl.).

12. G.I. Naumov & J. Piškur. 1999. Pheromone activity of collection strains of *Saccharomyces* sensu lato yeasts. Microbiology. **68**: 759-762 (Engl. Transl.).

VIII. Center for Process Biotechnology, Department of Biotechnology, Building 223, The Technical University of Denmark, DK-2800 Lyngby, Denmark. Communicated by L. Olsson <LO@ibt.dtu.dk>.

The research activities on yeast at the Center for Process Biotechnogy combines physiological studies with advanced analytical techniques and mathematical modelling with the objective of increasing our understanding of yeast. The following topics are studied: (1) Fermentation of complex substrates (metabolic engineering of the galactose and the xylose metabolism, mixed sugar utilisation and fermentation inhibitors). (2) Yeast physiology (pyruvate metabolism in *Saccharomyces klyveri,* modelling of the pyruvate node, transcriptome analysis of *Saccharomyces cerevisiae,* redox metabolism). (3) Metabolic network analysis (futile cycles, glucose repression, functional genomics). (4) Analytical biotechnology (measurement of intracellular metabolites, multiwave-length fluorescence, CE and combination sensors).

Recent publications:

1. Klein, C.J.L., Olsson, L., and J. Nielsen. 1998. Nitrogen-limited continuous cultivations as a tool to quantify glucose control in *Saccharomyces cerevisiae*. Enzyme Microb. Technol. **23**:91-100.

2. Smits, H.P., Cohen, A., Buttler, T., Nielsen J., and L. Olsson. 1998. Clean-up and analysis of sugar phosphates in biological extracts by using solid phase extraction and anion-exchange chromatography with pulsed amperometric detection. Anal. Biochem. **261**:36-42.

3. Dynesen, J., Smits, H.P., Olsson, L., J. Nielsen. 1998. Carbon catabolite repression of invertase during batch cultivations of *Saccharomyces cerevisiae*: The role of glucose, fructose and mannose. Appl. Microbiol. Biotechnol. **50**:579-582.

4. Klein, C.J.L., Rasmussen, J.J, Rønnow, B., Olsson L., and J. Nielsen. 1999. Investigation of the impact of *MIG1* and *MIG2* on the physiology of *Saccharomyces cerevisiae*. J. Biotechnol. **68**:197-212.

5. Rønnow, B., Olsson, L., Nielsen, J. and J. D. Mikkelsen. 1999. Derepression of galactose metabolism in melibiase producing bakers'and distillers' yeast. J. Biotechnol. **72**:213-228.

6. M. Anderlund, T. L. Nissen, J. Nielsen, J. Villadsen, J. Rydström, B. Hahn-Hägerdal, M.C. Kielland-Brandt. 1999. Expression of the *E. coli* pntA and pntB genes encoding nicotinamide nucleotide transhydrogenase in *Saccharomyces cerevisiae* and its effect on product formation during anaerobic glucose fermentation. Appl. Environ. Microbiol. **65**:2333-2340.

IX. Microbiology Group, Department of Genetics and Microbiology, Janus Pannonius University, H-7601 Pécs, P.O. Box 266, Hungary. Communicated by M. Pesti <micro@ttk.jpte.hu>.

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

1. M. Pesti, M. Sipiczki and Y. Pintér. 1999. Scanning electron microscopy characterisation of colonies of *Candida albicans* morphological mutants. J. Med. Microbiol. **48:**167-172.

The ultrastructure of colonies of two stable UV-induced morphological mutants and their parental strain of *Candida albicans* grown on glucose-containing solid medium, were investigated by scanning electron microscopy. The structures and ultrastructure of these three types of colonies were determined not only in terms of the proportions of blastospores, hyphae and pseudohyphae, but also with regard to the mode of budding of blastospores and the positions of these particular cell types within the colonies. Hyphae with an atypical appearance and branching characters were observed both in regular-wrinkled and in irregular-wrinkled mutant colonies. Smooth colonies of the parental strain and the mutants exhibited the same hyphal network within the agar, suggesting that microenvironmental factors in the agar overcame the effects of these mutations.

2. K. Czakó-Vér, M. Batic, P. Raspor, M. Sipiczki and M. Pesti. 1999. Hexavalent chromium uptake by sensitive and tolerant mutants of *Schizosaccharomyces pombe.* FEMS Microbiol. Lett. (accepted).

Lysine and leucine auxotrophic, heterothallic (h^+, h^-) strains of *Schizosaccharomyces pombe* were used to obtain chromium(VI)-sensitive and tolerant mutants by ultraviolet radiation-induced and nitrosoguanidine-induced mutagenesis. The minimal inhibitory concentrations of $K_2Cr_2O_7$ on YEA medium were 225 µM for the wild-type strain CW-6, 125 µM for the sensitive mutant CS-6.51 and 275 µM for the tolerant mutant CT-6.66. The mutants exhibited cross-sensitivity of various patterns to Cd^{2+}, Cu^{2+}, Ni^{2+}, Zn^{2+} and VO_4^{3-}. Cr(VI) was added to the actively-growing cultures and the total chromium (TOCr) content of the cells was determined. The sensitive mutant exhibited a high bioaccumulation ability, with a dry biomass of 810 µg/g after half an hour, while the tolerant mutant had a significantly lower such ability than the wild-type strain. In PIPES buffer, washed, lysine-starved biomasses were treated with 75 µM Cr(VI), and after 2 hours the TOCr and the organically bound chromium (OBCr) were determined. Under these conditions, the sensitive and tolerant mutants had the same TOCr content, 50% of which was OBCr. The wild-type strain exhibited a lower TOCr content than that of its mutants, and only 35% of this was OBCr. The Cr(VI) sensitivity was due to a significantly increased uptake of Cr(VI).

X. Angewandte Molekularbiologie, Universität des Saarlandes, FB 13.3 Gebäude 2, Postfach 15 11 50, D-66041 Saarbrücken, Germany. Communicated by M.J. Schmitt <mjs@microbiol.uni-sb.de>

Recent publications.

1. Schmitt, M.J. & K. Eisfeld. 1999. Killer viruses in *Saccharomyces cerevisiae* and their general importance in understanding eukaryotic cell biology. Rec. Res. Dev. Virol. (in press).

Recent studies on the virally encoded killer system in *S. cerevisiae* has shed light on essential aspects of yeast virology (such as host-cell interactions and viral RNA transcription, replication and packaging), and also provided important insights into basic and more general aspects of eukaryotic cell biology. The article focuses on the virally encoded killer system in *S. cerevisiae*, especially on the toxin-coding 'killer' viruses and the intracellular processing, maturation and toxicity of the virally encoded killer toxins.

2. Eisfeld, K., F. Riffer, J. Mentges & M.J. Schmitt. 1999. Endocytotic uptake and retrograde transport of a virally encoded killer toxin in yeast. J. Cell Biol. (in press).

We demonstrate that a virally encoded yeast 'killer' toxin uses endocytosis and retrograde transport in order to kill a eukaryotic target cell. The lethal K28 toxin is a secreted heterodimer that kills sensitive yeasts in a receptor-mediated fashion by blocking DNA synthesis. *In vivo* processing of the toxin precursor results in a protein whose -C-terminus carries the ER retention signal HDEL, which - as we show here - is essential for retrograde toxin transport. Yeast *end3/4* mutants as well as cells lacking the HDEL-receptor (D*erd2*) or mutants defective in Golgi-to-ER protein recycling (*erd1*) are toxin-resistant since the toxin can no longer enter and/or retrograde pass the cell. Site-directed mutagenesis further indicated that the toxin's -HDEL motif ensures retrograde transport, although in a toxin-secreting yeast the -C-terminus is initially masked by an R residue (-HDELR) until Kex1p cleavage uncovers the toxin's targeting signal in a late Golgi compartment. Prevention of Kex1p processing results in high level secretion of a biologically inactive protein uncapable of re-entering the secretory pathway. Finally, to our knowledge for the first time, we demonstrate that ER-to-cytosol toxin export is mediated by Kar2p and the major ER translocon Sec61p.

3. Theisen, S., E. Molkenau & M.J. Schmitt. 1999. Wicaltin, a unique broad-spectrum killer toxin of *Williopsis californica* and its antimycotic potential. J. Clin. Microbiol., in press.

The yeast *Williopsis californica* secretes a broad-spectrum killer toxin (Wicaltin) with antifungal activity against 14 yeast genera, including yeast-like and mycelia forms of the human pathogens *Candida* and *Sporothrix*. Agar diffusion bioassays indicated that its anti-*Candida* activity is more pronounced than the antifungal potential of frequently used antimycotics: 0.07 pmol Wicaltin showed the same toxicity as 0.2 pmol miconazol and 29 pmol clotrimazole. Since the toxin's primary target is likely to be the yeast cell wall, Wicaltin might be attractive in combatting clinically relevant yeast and fungal infections.

XI. Department of Genetics, University of Debrecen, POBox. 56,H-4010 Debrecen, Hungary. Communicated by M. Sipiczki.

List of recent publications and symposium abstracts.

1. Grallert, A., Grallert, B., Ribar, B., Sipiczki, M. 1998. Coordination of initiation of nuclear division and initiation of cell division in *Schizosaccharomyces pombe*: Genetic interactions of mutations. J. Bacteriol. **180**:892-900.

2. Sipiczki, M., Takeo, K., Yamaguchi, M., Yoshida, S., Miklos, I. 1998. Dimorphic cycle in a fission yeast. Microbiology UK **144**:1319-1330.

3. Sipiczki, M., Takeo, K. 1998. The effect of caffeine on cell cycle progression and polar growth in *Schizosaccharomyces pombe*. Biologia (Bratislava) **53**:295-300.

4. Sipiczki, M., Takeo, K., Grallert, A. 1998. Growth polarity transitions in a dimorphic fission yeast. Microbiology UK **144**:3475-3485.

5. Benko, Z., Sipiczki, M., Carr, A. M. 1998. Cloning of *caf1*$^+$, *caf2*$^+$ and *caf4*$^+$ of *Schizosaccharomyces pombe*: their involvement in multidrug resistance, UV- and pH-sensitivity. Mol. Gen. Genet. **260**:434-443.

6. Pesti, M., Sipiczki, M., Pinter, Y 1999. Scanning electron microscopy characterisation of colonies of *C. albicans* morphological mutants. J. Med. Microbiol. **48**:167-172,

7. Grallert, A., Grallert, B., Zilahi, E., Szilagyi, Z., Sipiczki, M. 1999. Eleven novel *sep* genes of *Schizosaccharomyces pombe* required for efficient cell separation and sexual differentiation. Yeast **15**:669-686.

8. Sipiczki, M., Grallert, A., Miklos, I., Zilahi. E., Bozsik, A., Szilagyi, Z. 1999. Genetics, physiology and cytology of yeast-mycelial dimorphism in fission yeasts. Acta Microbiol. Immunol. Hung. **46**:297-302.

9. Czako-Ver, K., Batie, M., Raspor, P., Sipiczki, M., Pesti, M. 1999. Hexavalent chromium uptake by sensitive and tolerant mutants of *Schizosaccharomyces pombe*. FEMS Microbiol. Lett. **178**:109-115.

10. Sipiczki, M. 1997. Protoplast fusion. Molecular genetics with the fission yeast *Schizosaccharomyces pombe.* EMBO Practical Course. Copenhagen, pp. 31-32.

11. Sipiczki, M. 1997. Phylogenesis of fission yeasts. Molecular Genetics with the Fission Yeast *Schizosaccharomyces pombe*. EMBO Practical Course. Copenhagen, p. 82.

12. Sipiczki, M., Miklos, I., Grallert, A. 1997. Polarity, spatial organisation of cytoskeleton and morphogenesis in fission yeasts. Proceedings of the International Symposium on Theoretical Biophisics and Biomathemathics. (eds. L. Luo, Q. Li and W. Lee) Inner Mongolia University Press, Hohhot, China, pp.129-132.

13. Pesti, M., Sipiczki, M., Pinter, Y. 1998. A scanning electronmicroscopy characterization of colonies of *Candida* morphological mutants. XXVII Annual Conference on Yeasts. Czechoslovak Society for Microbiology. Smolenice. Programme and Abstracts p. 35.

14. Paraggio, M., Romano, P., Miklos, I., Sipiczki, M. 1998. Characterization and improvement of *Saccharomyces cerevisiae* wine strains by genetic methods. 19th International Specialized Symposium on Yeasts. Yeast in the Production and Spoilage of Food and Beverages. Braga (Portugal). Book of Abstracts P8-8 p. 238.

15. Grallert, A., Sipiczki, M. 1999. Identification of novel *sep* genes of *Schizosaccharomyces pombe*. ISREC Conference "Cancer and the Cell Cycle". Lausanne (Switzerland). Abstract Book. PA-87.

16. Benko, Z., Sipiczki, M. 1999. Multidrug resistance caused by mutations in genes encoding thioredoxin reductase, AP1-like transcription factor and exportin. Curr. Genet. 35:377.

17. Miklos, I., Sipiczki, M. 1999. Analysis of a *Schizosaccharomyces pombe* cytokinesis mutant strain defective in the spl1 gene. Curr. Genet. 35:439.

18. Sipiczki, M., Paraggio, M., Miklos, I., Antunovics, Z., Romano, P. 1999. Isolation and characterisation of Tokay wine yeasts. IXth International Congress of Mycology. Sydney (Australia). Abstract Book p.251-252.

19. Bozsik, M., Sipiczki, M. 1999. Yeast-mycelial dimorphism in *Schizosaccharomyces*. IXth International Congress of Mycology. Sydney (Australia). Abstract Book p.266.

20. Benko, Z., Sipiczki, M. 1999. Caffeine resistance caused by mutations in genes encoding thioredoxin reductase, AP-1-like transcription factor and exportin. The First International Fission Yeast Meeting. Edinburgh (United Kingdom). Abstracts p. 18.

XII. Department of Food Science and Technology, Wiegand Hall, Oregon State University, Corvallis, OR 97331-6602, U.S.A. Communicated by A. Bakalinsky <bakalina@bcc.orst.edu>.

The following is an abstract of a paper recently accepted for publication in Current Genetics.

1. H. Park, N.I. Lopez, and A.T. Bakalinsky. 1999. Use of sulfite resistance in *Saccharomyces cerevisiae* as a dominant selectable marker.

Two *S. cerevisiae* genes were found to exhibit dominant phenotypes useful for selecting transformants of industrial and laboratory strains of *S. cerevisiae*. *FZF1-4*, which confers sulfite resistance, was originally isolated and identified as *RSU1-4*, but

the two genes were shown here to be allelic. Cysteine 57 in wild-type Fzflp was found to be replaced by tyrosine in Fzf1-4p. Multicopy *SSU1*, which also confers sulfite resistance, was found to be somewhat less efficient. In both cases, a period of outgrowth in non-selective medium following transformation was found to be necessary. The number of transformants obtained was found to be strain-dependent, and also to depend on the sulfite concentration used during selection. Undesirable background growth of non-transformants was not observed at cell densities as high as 2.5 x 10^7/plate. In two *ura3* laboratory strains where selection for *URA3* was applied independently of that for sulfite, the transformation efficiency for sulfite resistance was about 50% that for uracil prototrophy.

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

The following two contributions were recently published.

1. E. Minárik. Wine-related yeasts. In: Taxonomy and Biology, Forty Years of Activity in Czech and Slovak Yeast Research (l990-1999) pp.12-15. Veda-Publishing House of the Slovak Academy of Sciences, Bratislava 1999.
2. E. Minárik. Biotechnology of wine. In: Biotechnology. Forty Years of Act ivity in Czech and Slovak Yeast Research (1990-1999) pp. 75-78. Veda-Publishing House of the Slovak Academy of Sciences, Bratislava 1999.

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

Recent publication.

1. L.Colato[1], A. Cavazza & E. Poznanski. Biotechnologies for long leavened bread and sweet leavened products. (Produzione Di Pane a Lunga Fermentazione E Derivati Dolciari Mediante Biotecnologie). Proceedings of 4° CISETA (Congresso Italiano di Scienza e Tecnologie Alimentari), Cernobbio CO 16-17-9-1999.

   [1]SASIB Bakery M. V. & Specialities. Via del Garda, 34. Rovereto (TN).

Sourdough long fermentation, mainly due to yeast and lactic acid bacteria, leads to high quality baking products, but require long manual work. LAB-fermented liquid dough can be used as an ingredient in bread, pizza, and traditional cake making, and give rise to standardised handicraft-like industrial products. We set up a liquid dough (DY = 250), a sponge, inoculated with *Saccharomyces cerevisiae* and 3 bacterial strains: *Lactobacillus plantarum, Lactobacillus brevis, and Leuconostoc mesenteroides* subsp. *dextranicum*. The sponge requires 36 hours to be prepared, and can be used for 8 days, if kept refrigerated. It has high acidity, low pH, and a good shelf-life and has leavening activity. It can be used to prepare a wide range of different kinds of bread. No yeast is needed in the final dough, leading to a reduction of time and costs. Two plants are working with this technology, making 1 and 2.5 tons bread per hour, respectively.

XV. CREM – Centro de Recursos Microbiológicos, Biotechnology Unit, Faculty of Sciences & Technology, New University of Lisbon, 2825-114 Caparica, Portugal. Communicated by Á. Fonseca <amrf@mail.fct.unl.pt> & J.P. Sampaio <jss@mail.fct.unl.pt>.

Recent publications on yeast ecology and systematics.

1. Fonseca, A., Fell, J.W., Kurtzman, C.P., & Spencer-Martins, I. 2000. *Candida tartarivorans* sp. nov., an anamorphic ascomycetous yeast with the capacity to degrade L(+)- and *meso*-tartaric acid. Int. J. Syst. Evol. Microbiol. **50** (in press).

An undescribed anamorphic yeast species of ascomycetous affinity, for which the name *Candida tartarivorans* is proposed, was isolated from dried wine lees in Portugal using a selective medium with L(+)-tartaric acid as the sole source of carbon and energy. The single isolate (IGC 4854) showed the following characteristics: sympodial holoblastic conidiogenesis, absence of asci with ascospores, a negative colour reaction with Diazonium Blue B (DBB), production of elaborate pseudomycelium, and ability to grow with inositol as sole source of carbon. Analysis of the physiological data pointed to a close relationship with other inositol-assimilating taxa, namely the genera *Arxula*, *Stephanoascus*, *Sympodiomyces*, *Zygoascus* and selected *Candida* species. The comparative analysis of the D1/D2 variable domain of the 26S rRNA gene of all available sequences for ascomycetous yeasts showed that strain IGC 4854 did not match with any other species in the database. The closest relative was *Candida auringiensis* Santa Maria but the two species differed in 24 nucleotide positions. A description of the new species is given.

2. Fell, J.W., Scorzetti, G., Boekhout, T., Fonseca, A., Blatt, G., and Statzell-Tallman, A. 2000. Biodiversity and systematics of basidiomycetous yeasts as determined by D1/D2 large subunit rDNA sequence analysis. Int. J. Syst. Evol. Microbiol. (in press).

The molecular systematics of 337 strains of basidiomycetous yeasts and yeast-like fungi, representing 230 species in 18 anamorphic and 24 teleomorphic genera, was determined by sequence analysis of the D1/D2 region of the large subunit rDNA. The data was compared with published sequences of other basidiomycetous fungi. The results demonstrated that the yeast species and genera are phylogenetically distributed among the *Microbotryum*, *Sporidiobolus*, *Agaricostilbum* and *Erythrobasidium* major clades of the Urediniomycetes; the Tremellales, Trichosporonales ord. nov., Filobasidiales and Cystofilobasidiales clades of the Hymenomycetes; and the Ustilaginales, Tilletiales, Entylomatales, Doassansiales, Georgefisheriales, Microstromatales and Malasseziales clades of the Ustilaginomycetes. Genera such as *Bensingtonia*, *Cryptococcus*, *Rhodotorula* and *Sporobolomyces* are polyphyletic: they occur in 2 or more clades. In contrast, other genera e.g. *Cystofilobasidium*, *Fellomyces*, *Filobasidiella*, *Filobasidium*, *Kondoa*, *Kurtzmanomyces*, *Leucosporidium*, *Rhodosporidium Sporidiobolus* and *Udeniomyces* are monophyletic. The majority of the species can be identified using D1/D2 analyses, although the Internal Transcribed Spacer region is required to distinguish closely related species. The Intergenic Spacer region is recommended for additional differentiation of species and strains.

3. Fonseca, A., Scorzetti, G., & Fell, J.W. 1999. Diversity in the yeast *Cryptococcus albidus* and related species as revealed by ribosomal DNA sequence analysis. Can. J. Microbiol. **45** (in press).

Evidence accumulated from studies based on physiological, biochemical and molecular characteristics has pointed to the heterogeneity of the ubiquitous anamorphic basidiomycetous yeast species *Cryptococcus albidus* (Saito) Skinner, with its current varieties and synonyms. The taxonomic status of this species has not been re-appraised due to the lack of an integration of the different studies that involved, in most cases, limited numbers of strains. To assess species diversity within the clade that contains *Cryptococcus albidus* and other phylogenetically related *Cryptococcus* and *Filobasidium* species, we determined ribosomal DNA (rDNA) sequences from coding (5' end of the 26S gene, D1/D2 region) and, in some cases, the non-coding ITS2 region, of 69 strains. Analysis of the sequence data together with available physiological, biochemical and molecular characteristics, showed the segregation of *C. albidus* into at least 12 species, leading to the elevation of former varieties to the rank of species (*C. aerius*, *C. diffluens*), the reinstatement of synonyms (*C. liquefaciens*, *C. terricola*) and the proposal of new species (*C. arrabidensis*, *C. chernovii*, *C. cylindricus*, *C. oeirensis*, *C. phenolicus*, *C. saitoi*, *C. uzbekistanensis*, *C. wieringae*). The overall analyses of the results argues in favour of the use of rDNA sequence data to improve species delineation when integrated with other available physiological and molecular characteristics.

4. Fonseca, A., Sampaio, J.P., Inácio, J., & Fell, J.W. 2000. Emendation of the basidiomycetous yeast genus *Kondoa* and the description of *Kondoa aeria* sp. nov. Antonie van Leeuwenhoek (accepted).

The genus *Kondoa* Y. Yamada, Nakagawa & Banno was erected to accommodate a single taxon, *K. malvinella* (Fell & Hunter) Y. Yamada, Nakagawa & Banno, which was transferred from the teliospore-forming genus *Rhodosporidium* Banno based on pronounced differences in the 5S and 26S ribosomal RNA (rRNA) nucleotide sequences to *R. toruloides* Banno. In contrast with the original description, reinvestigation of *K. malvinella* revealed the formation of transversely septate (auricularioid) basidia that did not arise on teliospores, but formed directly on the dikaryotic mycelium. The four-celled basidia developed sterigmata on which forcibly discharged asymmetric basidiospores (ballistospores) were produced. Additionally, a new taxon emerged from the study of recent isolates, for which the name *K. aeria* sp. nov. is proposed. This new species produced two-celled auricularioid basidia on hyphae with incomplete clamp connections. Ballistospores arose on the basidia at the tip of sterigmata and, after ejection, germinated by budding. These observations led us to present an emended diagnosis for the genus *Kondoa*. Analysis of the sequence data from the D1/D2 region of the 26S rRNA gene showed a very close resemblance between *K. aeria* and *K. malvinella* in a cluster that also contained several *Bensingtonia* species. Taxa in this cluster share specific physiological traits and produce characteristic pinkish-cream to mauve colonies; in contrast, formation of ballistoconidia is only observed in the *Bensingtonia* species. Sequence data supported placement of *K. malvinella* and *K. aeria* in the '*Agaricostilbum* clade' of the Urediniomycetes.

5. Sampaio, J.P. 1999. Utilization of low molecular weight aromatic compounds by hetero-basidiomycetous yeasts: taxonomic implications. Can. J. Microbiol. **45**: 491-512.

The utilization of low molecular weight aromatic compounds implies the operation of complex metabolic pathways. In order to investigate the taxonomic relevance of this property among heterobasidiomycetous yeasts, both at the species level and at higher taxonomic ranks, the capacity to assimilate twenty such compounds was tested in a total of 332 strains representing

approximately 200 species. The substrates most frequently utilized were protocatechuic, caffeic and *p*-hydroxybenzoic acids, whereas cinnamic, sinapic and syringic acids and guaiacol were never assimilated. The assimilation of the majority of the aromatic compounds investigated correlated with the utilization of protocatechuic acid. Among the Urediniomycetes, the members of the Sporidiales and those of the *Naohidea - Rhodotorula minuta* clade showed a good ability to utilize aromatic compounds, whereas the members of the *Agaricostilbum-Kondoa* group were more heterogeneous, in agreement with the four sub-clades known. Among the Tremellomycetidae, the members of the *Cystofilobasidium* and *Tremella* clades showed a reduced or null ability to utilize aromatic compounds. In contrast, the members of the *Trichosporon* clade were able to utilize phenol and similar substrates and the representatives of the *Filobasidium* clade assimilated various aromatic compounds, including those requiring more complex catabolic routes. Assimilation tests using, as sole carbon and energy sources, low molecular weight aromatic compounds appear to be potentially useful in taxonomic studies of basidiomycetous yeasts. In those species in which a considerable number of strains was investigated, variable assimilation patterns were frequently observed. The possibility that such discrepant results indicate an incorrect species delimitation is discussed.

6. Sampaio, J.P., Bauer, R., Begerow, D. & Oberwinkler, F. 1999. *Occultifur externus* sp. nov., a new species of simple-pored auricularioid heterobasidiomycete from plant litter in Portugal. Mycologia **91**:1094-1104.

A new species of a dimorphic simple-pored auricularioid heterobasidiomycete, *Occultifur externus*, is described. This fungus was initially isolated in the unicellular stage and identified, on the basis of standard yeast identification tests, as the anamorphic pink yeast *Rhodotorula minuta*. Later, we found that this yeast was able to complete its life cycle on solid laboratory media. Two yeast cells conjugated, and gave origin to mycelium with clamp connections. The same generative hyphae produced clusters of four-celled basidia and characteristic swollen conidiogenic cells that released dikaryotic conidia. Haustoria-like structures were formed. Nuclear DNA-DNA hybridization experiments revealed that *O. externus* is not the teleomorphic stage of *R. minuta* and molecular sequence data from the large subunit of the rDNA supported the affiliation of *O. externus* to the *Naohidea-Sakaguchia* clade of the Urediniomycetes. Ultrastructural studies revealed the presence of simple septal pores. Cylindrical reticulate bodies were frequently found occluding them. These unique bodies could also be detected in *O. internus*, which supports the placement of both taxa in the same genus.

7. Sampaio, J.P., Fell, J.W., Gadanho, M. & Bauer, R. 1999. *Kurtzmanomyces insolitus* sp. nov., a new anamorphic heterobasidiomycetous yeast species. Syst. Appl. Microbiol. **22** (in press).

A new anamorphic heterobasidiomycetous yeast species, *Kurtzmanomyces insolitus*, is described using a polyphasic taxonomic approach. The new species has the salient characteristics of the genus *Kurtzmanomyces* and, additionally, the ability to produce ballistoconidia. Data derived from comparative micromorphological studies, physiological characterisation, ultrastructure and nucleic acid analyses led to assigning the new species to *Kurtzmanomyces* rather than to the currently accepted genera of ballistoconidia-forming fungi. An emendation of the genus *Kurtzmanomyces* is proposed to allow the inclusion of the new species.

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

The following article, whose abstract appeared in the previous issue of the Yeast Newsletter, has been published.

1. D. Dlauchy, J. Tornai-Lehoczki and G. Péter. 1999. Restriction enzyme analysis of PCR amplified rDNA as a taxonomic tool in yeast identification. Syst. Appl. Microbiol. **22**:445-453.

The following article has been accepted for publication.

2. G. Péter, J. Tornai-Lehoczki, D. Dlauchy & G. Vitányi. *Pichia sporocuriosa* sp. nov., a new yeast isolated from rambutan. Antonie van Leeuwenhoek. In press.

A strain of a hitherto undescribed yeast species with a unique ascospore morphology was isolated from rambutan (*Nephelium lappaceum*). A description of the new species, *Pichia sporocuriosa,* is given.

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

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

1. A. Cruz-Guerrero, E. Bárzana, M. García-Garibay and L. Gómez-Ruiz. 1999. Dissolved oxygen threshold for the repression of endo-polygalacturonase production by *Kluyveromyces marxianus*. Process Biochem. **34**:621-624.

A detailed study was conducted in terms of the influence of dissolved oxygen and growth temperature related to the endo-polygalacturonase synthesis by the yeast *Kluyveromyces marxianus* CDBB-L-278. It was found that 3.3 mg of D.O./litre was the threshold for the repression of enzyme production. Growth temperature had no effect on the synthesis of this enzyme, but had an indirect effect due to changes in oxygen solubility. Growth rate was influenced by temperature; maintaining the dissolved oxygen constant at 3.3 mg of D.O./litre the optimum growth temperature was 40°C. Pectin in the culture medium allowed higher endo-polygalacturonase production; even with 3.3 mg of D.O./litre, pectin de-repressed the enzyme production.

2. M. del C. Santillán-Valverde and M. García-Garibay. 1999. Congeners biosynthesis during alcoholic fermentations (In Spanish). Revista Latinoamericana de Microbiología. In press.

The flavor of alcoholic beverages is a consequence of a complex mixture of many compounds, including small concentrations of some volatile metabolites known as congeners, which are produced by the yeast during the fermentation. The more important compounds are those that can be found in all the alcoholic beverages in different concentrations, and they can be grouped on the following chemical species: higher alcohols, esters, and carbonyls. In the current paper the biochemical pathways that produce these compounds from the raw materials are reviewed. Research done in this field has led to a more complete knowledge concerning to organoleptic profiles of alcoholic beverages and to a better control for the production of the final product.

3. M. García-Garibay, L. Gómez-Ruiz and E. Bárzana. 1999. Single-Cell Protein. Yeasts and Bacteria. In Encyclopedia of Food Microbiology, edited by R.K. Robinson, C.A. Batt and P.D. Patel. Academic Press, London.

The single-cell protein (SCP) concept is applied to the massive growth of microorganisms for human or animal consumption. Single-cell protein is a generic term for crude or refined protein whose origin is bacteria, yeasts, moulds or algae. The production and utilization of the former two is discussed here. These two groups of microorganisms have been particularly important since yeasts and bacterial biomass has been consumed by human race since ancient times in fermented foods. The production of SCP has important advantages over other sources of proteins, such as its considerable shorter doubling time, the small land requirement and the fact that it is not affected by the weather conditions. Much attention was focused on the use of petroleum derivatives as substrates for the SCP production during the 1960's and 1970's when the price of this reserve was low; but currently, the production of SCP is based on renewable resources, and its interest is also kept as a means to confer value to waste materials. On the other hand, the organoleptic and functional properties of SCP are not always competitive, and its main drawback has been its high production costs.

XVIII. Japan Collection of Microorganisms, RIKEN, Wako, Saitama 351-0198, Japan. Communicated by M. Hamamoto <hamamoto@jcm.riken.go.jp> and T. Nakase <nakase@jcm.riken .go.jp>.

The following articles have been published recently.

1. Nagahama, T., Hamamoto, M., Nakase, T. & Horikoshi, K. 1999. Kluyveromyces nonfermentans sp. nov., a new yeast species isolated from the deep sea. Int. J. Syst. Bacteriol. **49**:1899-1905.

Eleven strains of a new species of the genus *Kluyveromyces*, characterized as having evanescent asci and Q-6 as the major ubiquinone, were isolated from sediments, a clam and a crab collected at depths of 1000-2000 m in Suruga Bay and Sagami Bay, Japan. A phylogenetic tree based on small-subunit (18S) rRNA gene sequences placed these isolates into a cluster of *Kluyveromyces*. DNA complementarity and phylogenetic trees of internal transcribed spacer (ITS) regions and 5.8S rRNA genes showed that the isolates are closely related to Kluyveromyces *aestuarii*, but that these two species are genetically distinct. The isolates are described as *Kluyveromyces nonfermentans* sp. nov. Because this species lacks the fermentative ability considered to be an important criterion for the genus *Kluyveromyces*, the definition of the genus has been emended. The type strain of *K. nonfermentans* is strain SY-33T (= JCM 10232T).

2. Bai, F.-Y., Takashima, M. & Nakase, T. 1999. Molecular phylogenetic studies on the *Bensingtonia* strain isolated from Yunnan, China. Mycosystema **18**:254-258.

A ballistospore-forming yeast strain CH 2.310 isolated from a stem sample of *Oryza sativa* L. collected in Yunnan Province, China, was identified as belonging to the genus *Bensingtonia* Ingold for its major ubiquinone was Q-9 and xylose was absent in its cell hydrolysate. The strain was similar to *B. yamatoana* (Nakase, M. Suzuki et M. Itoh) Nakase et Boekhout phenotypically, but differed from the original description of the species in some physiological properties. Molecular phylogenetic analysis based on SSU rDNA sequences showed that CH 2.310 was most closely related with the type strain of *B. yamatoana*. Further DNA-DNA reassociation study confirmed the conspecificity of CH 2.310 with the type and two other strains of *B. yamatoana* isolated from Japan.

3. Suzuki, M., Suh, S.-O., Sugita, T. & Nakase, T. A phylogenetic study on galactose-containing *Candida* species based on 18S ribosomal DNA sequences. J. Gen. Appl. Microbiol. (in press).

4. Suzuki, M. & Nakase, T. A phylogenetic study of ubiquinone Q-8 species of the genera Candida, Pichia and Citeromyces based on 18S ribosomal DNA sequence divergence. J. Gen. Appl. Microbiol. (in press).

XIX. Institut für Pflanzengenetik und Kulturpflanzenforschung, Corrensstr. 3, D-06466 Gatersleben, Germany. Communicated by G. Kunze <kunzeg@ipk-gatersleben.de>.

Recent publications.

1. C. Chan, M. Lehmann, K. Tag, M. Lung, G. Kunze, K. Riedel, B. Gruendig & R. Renneberg. 1999. Measurement of biodegradable substances using the salt tolerant yeast *Arxula adeninivorans* for a microbial sensor immobilized with poly(carbamoyl) sulfonate (PCS) part I: construction and characterization of the microbial sensor. Biosensors & Bioelectronics **14**:131-138.

A microbial biosensor based on the yeast *Arxula adeninivorans* has been developed for measurement of biodegradable substances. *Arxula* is immobilized in the hydrogel poly(carbamoyl) sulfonate (PCS). The immobilized yeast membrane is placed in front of an oxygen electrode with –600 mV versus Ag/AgCl. *Arxula* is salt tolerant; it can give a stable signal up to 2.5 M NaCl in sample (120 mM in increasing cell). The sensor´s measurements are highly correlated to BOD_5 measurements. It has a very high stability which can last for 40 days without any decrease signal. The linear range of the sensor is up to a corresponding BOD value of 550 mg/l.

2. M. Lehmann, C. Chan, A. Lo, M. Lung, K. Tag, G. Kunze, K. Riedel, B. Gruendig & R. Renneberg. 1999. Measurement of biodegradable substances using the salt tolerant yeast *Arxula adeninivorans* for a microbial sensor immobilized with poly(carbamoyl) sulfonate (PCS) part II: application of the novel biosensor to real samples from coastal and island regions. Biosensors & Bioelectronics **14**:295-302.

A microbial sensor for rapid measurement of the amount of biodegradable substances based on the salt-tolerant yeast *Arxula adeninivorans* LS3 has been developed especially for coastal and island regions. Our parameter, the so-called sensorBOD, that is available after only a few minutes, agrees with the 5-day value for the biochemical oxygen demand (BOD_5) very well. We have employed the *Arxula* sensor in the short-time estimation and supervision of the BOD of both domestic and industrial wastewater with high salinity. The novel sensor makes it possible to monitor the different types of wastewater rapidly without pretreatment, and it can be used for an active process control of sewage treatment works. Compared to a commercially available sensor, the novel sensor achieves better agreement between sensor BOD and BOD_5 measurements with salt containing samples.

XX. Laboratorium voor Microbiologie, Wageningen University, Hesselink van Suchtelenweg 4, 6703 CT Wageningen, The Netherlands. Communicated by W.J. Middelhoven <Wout.Middelhoven@Algemeen.MICR.WAU.NL>.

A seminar entitled "Phylogeny, physiology and metabolic diversity insome yeast genera" has been given on 27 Otober 1999 in the XXth Congress of Microbiology, organized by the Brazilian Society for Microbiology in Salvador de Bahia, Brazil. Phylogenetically closely related ascomycetous yeast species of the genera *Stephanoascus, Arxula, Blastobotrys* and *Sympodiomyces* (Kurtzman & Robnett, 1998) have many characters in common. With a few exceptions all of these 12 species, constituting one clade in the phylogenetic tree of the ascomycetous yeasts (Kurtzman & Robnett, 1998), assimilated uric acid, adenine, glycine, putrescine, leucine and isoleucine as sole sources of carbon and nitrogen, and n-hexadecane and isobutanol as sole carbon sources. Maybe these species have a chromosome or another long stretch of DNA in common, enabling them to carry out these different reactions. In this group of yeasts a strong correlation between phylogeny and physiology is evident. However, the phylogenetically unrelated *Candida blankii* has these growth responses in common with the species mentioned above,

except for the assimilation of adenine as sole source of carbon and nitrogen which appears to be shown only by the *Stephanoascus/Arxula/Blastobotrys* clade. Probably *Candida blankii*, which takes an isolated position in the phylogenetic tree (Kurtzman & Robnett, 1999), shares these DNA sequences with the other species mentioned. In the basidiomycetous genus *Trichosporon* assimilation of various carbon compounds not traditionally used in yeast taxonomy appeared to be scattered over the genus if the species are ordered according to the phylogenetic tree proposed by J.W. Fell and G. Scorzetti (unpublished, see Middelhoven et al., 1999). This was shown for the assimilation of uric acid and ethylamine as sole sources of carbon and nitrogen, and of polygalacturonate, xylan and some benzene compounds. These results confirm the monophyletic nature of the genus *Trichosporon*, earlier concluded by J.W. Fell and G. Scorzetti (personal communication). The growth responses to these compounds appeared to be species-specific and can be used for identification and distinction of the *Trichosporon* species.

Reference. Kurtzman, C.P. & Robnett, C. 1998. Identification and phylogeny of ascomycetous yeasts from analysis of nuclear large subunit (26S) ribosomal DNA partial sequences. Antonie van Leeuwenhoek **73**: 331-371.

The following paper has been published recently:

1. Middelhoven, W.J., Scorzetti, G. & Fell, J.W. (1999) *Trichosporon guehoae* sp.nov., an anamorphic basidiomycetous yeast. Can. J. Microbiol. **45**:686-690.

A morphological and physiological description of an anamorphic basidiomycetous yeast species, named *Trichosporon guehoae* (CBS 8521) is presented. The ability to assimilate several aliphatic and aromatic compounds as sole source of carbon and energy is reported. The phylogenetic position within the genus, based on nuclear base sequencing of the D1/D2 region of the large subunit of rDNA is discussed. A phylogenetic tree of the whole genus is provided.

Papers describing another novel *Trichosporon* species and a novel *Saccharomyces* species are in the press.

XXI. Universidade Federal do Rio de Janeiro, Instituto de Quimica - Departamento de Bioquimica, Lab 547, Cidade Universitaria - Ilha do Fundao, Rio de Janeiro - RJ - 21949-900 Brazil. Communicated by A.D. Panek.

Succesful defense of Master's dissertations.

1. A.P. Rodrigues Torres. Involvement of Ca^{2+}/calmodulin dependent protein kinase in response to environmental stresses in *Saccharomyces cerevisiae*.

2. M.D. Pereira. Protection factors involved in acquisition of resistance to oxidative stress in *Saccharomyces cerevisiae*.

Papers recently published or in press.

3. Diniz Mendes, L, Bernardes, E, de Araujo, PS, Panek, AD, and Paschoalin, VM. 1999. Preservation of frozen yeast cells by trehalose. Biotech. Bioeng. **65**:572-578.

4. Peixoto, DN, and Panek, AD. 1999. Involvement of hexokinases in trehalose synthesis Biochem. Mol. Biol. Int. **47**:873-880.

5. Ferreira, JC, de Araujo, PS, and Panek, AD. Inactivation of maltose permease and maltase in sporulating *Saccharomyces cerevisiae*. Can. J. Microbiol (in press).

XXII. Institut für Angewandte Mikrobiologie, Universität für Bodenkultur, Nuβdorfer Läende 11, A-1190 Vienna, Austria. Communicated by H. Prillinger <H.Prillinger@iam.boku.ac.at>.

The following publication has appeared recently.

1. Prillinger, H., Molnär, O., Eliskases-Lechner, F., Lopandic, K. 1999. Phenotypic and genotypic identification of yeasts from cheese. Antonie van Leeuwenhoek **75**:267-283.

Eighty-five yeast strains isolated from different cheeses of Austria, Denmark, France, Germany, and Italy were identified using physiological methods and genotypically using random amplified polymorphic DNA polymerase chain reaction (RAPD-PCR) analysis. Good congruence was found between the phenotypic and genotypic data for 39 of the isolates. However, 26

isolates of *Geotrichum* could only be identified to the species level using the genotypic methods and 7 isolates were correctly identified to the genus level only using phenotypic identification methods. The phenotypic identification did not agree with the genotypic data for 14 yeast isolates. Using ubiquinone analysis, yeast cell wall sugars and the diazonium blue B test 5 incorrectly identified isolates with phenotypic methods could be identified genotypically. In addition the 7 isolates identified only to the genus level by the phenotypic methods and the 26 *Geotrichum* strains were identified to the species level using the polyphasic molecular approach mentioned above. Eleven strains remained unidentified. The 76 identified yeast isolates were assigned to 39 species, the most frequent assignments were made to *Debaryomyces hansenii*, *Geotrichum candidum*, *Issatchenkia orientalis*, *Kluyveromyces lactis*, *K. marxianus*, *Saccharomyces cerevisiae*, *Yarrowia lipolytica*, and *Candida catenulata*. It is proposed that *Debaryomyces hansenii* (Zopf) Lodder et Kreger-van Rij and *Debaryomyces fabryi* Ota should be reinstated. The RAPD-PCR data reinforced the view that the species *Galactomyces geotrichum* is heterogeneous with all of the *Geotrichum* isolates from cheese products being assigned *G. geotrichum* group A *sensu* M. T. Smith. It is suggested that the name *Geotrichum candidum* be conserved for this rather common species.

XXIII. Planta Piloto de Procesos Industriales Microbiológicos (PROIMI), Avenida Belgrano y Pasaje Caseros, 4000 S.M. de Tucuman, Argentina. Communicated by J.F.T. Spencer <fspencer@proimi.edu.ar>.

Much of our work here has been on preparing a book, "Food Microbiology Protocols" (J.F.T Spencer and A.L. Ragout de Spencer, Eds.), It contains several chapters on methods for investigation of yeasts associated with foods, which we hope will be of some interest. The chapters dealing with yeasts are as follow.

1. Identification of yeasts and molds associated with poultry products. A.I. Gaitan.
2. Identification of yeasts present in sour fermented foods and fodders. W.J. Middelhoven.
3. Production of polyols by osmotolerant yeasts LIC de Figueroa and M.E. Lucca.
4. Carotenogenic microorganisms, a product-based biochemical characterization. J. Fontana
5. Genetic and chromosomal stability of wine yeasts. M. Sipiczki, I. Miklos, L. Levelski and Z. Antunovics.
6. Predictioon of prefermentation nutritional status of Grape juice - the FORMOL method. B.H. Gump, B-W- Zoecklein and K.C. Fugelswang.
7. Nutritional status of grape juice. B.W. Zoecklein, B.H. Gump and K.C. Fugelswang.
8. Selection of wine yeasts by means of oenological characteristics. Z.F. Vazquez, LIC de Figueroa, and Toro, M.E.
9. Genetic analysis of food spoilage yeasts S.A. James, M.D. Collins and I.N. Roberts.
10. Utilization of native cassava starch by yeasts. L.I.C. de Figueroa, L. Rubenstein and C. Gonzalez.
11. Molecular characterization of yeast strains by mitochondrial DNA restriction analysis M.T. Fernandez-Espinar, A.Querol and D. Ramon.

There will be a chapter on wine yeasts by Peter Raspor and also one by Tohoru Katsuragi, both of particular interest to workers with yeast. There are also chapters on food spoilage organisms, pathogens, fermented foods (Lactobacilli), methods and reviews, including one by I.G. Wilson on "Problems with PCR" and "Current Problems with acceptance of genetically modified foods", by J.-M. Bruno-Barcena. This is not a full description of the book, as there are approximately 30 chapters in all, but I have mentioned the chapters which are most likely to be interesting to readers of the Yeast Newsletter.

XXIV. School of Biological Sciences, University of East Anglia, Norwich NR47TJ, England, Communicated by J.A. Barnett <J.Barnett@ueaac.uk>.

In the press.

1. J.A. Barnett, R.W. Payne & D.Yarrow. New edition - Yeasts: Characteristics and Identification. To be published in May 2000 (Cambridge University Press - price about £200/$320. ISBN O 521 573963).

Features of this 3rd edition:
- by far the most up-to-date book on yeast systematics
- 678 species described, plus addendum summarizing 28 recently published species
- about 1400 photomicrographs (500 newly made for this edition)
- 9 identification keys
- plus all features of previous editions, updated and improved.

Yeast identification PC Program associated with the 3rd edition will also be available from J.A. Barnett.

XXV. Alkomohr Biotech Ltd, Viikinkaari 9, FIN-00710 Helsinki, Finland. Communicated by M. Korhola <matti.korhola@yeast.pp.fi>.

We are currently studying sour dough yeasts, originating especially from Finnish rye sour doughs, and the first paper has appeared.

1. Mäntynen, V.H., Korhola, M., Gudmundsson, H., Turakainen, H., Alfredsson, G.A., Salovaara, H. and Lindström, K. 1999. A polyphasic study on the taxonomic position of industrial sour dough yeasts. System. Appl. Microbiol.**22**:87-96.

The sour dough bread making process is extensively used to produce wholesome palatable rye bread. The process is traditionally done using a back-slopping procedure. Traditional sour doughs in Finland comprise of lactic acid bacteria and yeasts. The yeasts present in these doughs have been enriched in the doughs due to their metabolic activities, e.g. acid tolerance. We characterized the yeasts in five major sour bread bakeries in Finland. We found that most of the commercial sour doughs contained yeasts which were similar to *Candida milleri* on the basis of 18S rDNA and EF-3 PCR-RFLP patterns and metabolic activities. Some of the bakery yeasts exhibited extensive karyotype polymorphism. The minimum growth temperature was 8 ºC for *C. milleri* and for most of sour dough yeasts.

XXVI. SAB Brewing Research, South Africa. Communicated by B. Lodolo <betty.lodolo@sabreweries.com>.

I have recently completed my Ph.D through the University of Stellenbosh under Prof. Bernie Prior.

1. B. Lodolo. The effect of oxygen on the fermentation ability of *Saccharomyces cerevisiae* during high-gravity wort fermentations.

The advent of high-gravity brewing, due to its cost-effectiveness and increased efficiency, necessitated the optimisation of fermentations with regard to limiting nutritional factors. These factors were previously found to be usable nitrogen and substances synthesised in the presence of dissolved oxygen (DO). Oxygen has a central role in many critical biochemical processes in the yeast cell. These include sterol and unsaturated fatty acid synthesis and mitochondrial development. The aim of this investigation was to establish the various roles of DO in the industrial brewing yeast *Saccharomyces cerevisiae* AJL 2036 and then to optimise the DO requirement (amount and time) for this yeast strain.

Specific chemical inhibitors were used to elucidate the roles of DO in lipid metabolism and mitochondrial functionality. The lipid inhibitors showed that oxygen was able to stimulate yeast growth even in the absence of lipid biosynthesis. Furthermore, it was found that the capacity of yeast to synthesise critical lipids was related to the physiological condition of the yeast at inoculation. The mitochondrial inhibitors, bongkrekic acid (BA) and carbonyl cyanide-m-chlorophenylhydrazone (CCCP), demonstrated that the import of cytosolically-generated ATP was essential to maintain minimal fermentation performance. Blocking the intramitochondrial energy supply in *S. cerevisiae* AJL 2036 interfered with cellular metabolism more than blocking unsaturated lipid biosynthesis. This illustrated another role of DO in the brewing fermentation - to provide for mitochondrial development and functionality, and to ensure an adequate intramitochondrial energy supply.

The relative importance of mitochondrial versus cytosolic protein synthesis was demonstrated using the inhibitors chloramphenicol and cycloheximide. Cytosolic protein synthesis was much more critical, but blocking mitochondrial protein synthesis with chloramphenicol decreased yeast growth even in the presence of oxygen. Much of this effect may have been due to the lower lipid biosynthesis in chloramphenicol-treated cells. The blockage of mitochondrial protein synthesis did not alter the yeast performance as radically as inhibiting the energy supply to these organelles, but did inhibit the strain's ability to optimally ferment.

The DO addition optimisation studies showed that the optimised DO concentrations to add at the time of inoculation to 16°P worts were 16 mg/L DO for a maltose adjunct wort and 16 to 18 mg/L DO for a glucose adjunct wort. The initial vitality of the yeast affected this amount. The optimum time to provide DO was 4 hours post-inoculation with a greater flexibility in the DO supply time, up to 8 hours, in the maltose adjunct wort. Fermentations in the presence of greater glucose concentrations (glucose adjunct worts) had an absolute requirement for oxygenation at 4 hours post-inoculation. At the optimum time the DO demand was decreased. The maltose adjunct wort fermentations indicated that the best amount of DO to add at 4 hours post-inoculation was 8 mg/L DO. In all cases, when the DO was supplied at the optimum time in amounts ranging from 8 to 16 mg/L DO, the general performance was better than adding 16 mg/L DO at the time of inoculation. Practical application of this oxygenation regime could be achieved in a brewery with multiple fill operations where it is possible to omit oxygenating the first brew into which the entire yeast mass is pitched. Four hours later, an oxygenated brew of the same volume containing 16 mg/L DO can be added. This would result in the overall addition of 8 mg/L DO 4 hours post-inoculation as optimised for *S. cerevisiae* AJL 2036.

The latest publication that followed out of this work was a paper that I presented last year at the MBAA (Master Brewers Association of the Americas). For this I have recieved the MBAA presidential award for an outstanding refereed paper, as follows.

2. E. J. Lodolo, E.S.C. O'Connor-Cox and B.C. Axcell. 1999. Optimization of wort dissolved oxygen for an industrial brewer's yeast in high-gravity brewing. MBAA Techn. Quart. **36**(2):139-154.

Other publication.

3. E. J. Lodolo, E. S. C. O'Connor-Cox and B. C. Axcell. 1999. Evidence of antimycin-insensitive respiration in a commercial brewing yeast. J. Inst. Brew. **105**:35-43.

XXVII. Collection de Levures d'Intérêt Biotechnologique (CLIB), Laboratoire de Microbiologie et Génétique Moléculaire, INA-PG INRA, BP 01, F-78850 Thiverval-Grignon, France. Communicated by Nguyen H.V. <clib@platon.grignon.inra.fr>

The following have been recently published, accepted, or submitted.

1. H.V. Nguyen, G. Panon. 1998. The yeast *Metschnikowia pulcherrima* has an inhibitory effect against various yeast species. Sciences des Aliments **18**:515-526

An inhibitory effect has been shown in forty-three strains of *Metschnikowia pulcherrima* against *S. cerevisiae* or *S. uvarum* (syn *S. bayanus*). This effect found in *M. pulcherrima* is not a classical killer effect since some of the *Saccharomyces* tested are sensitive to *M. pulcherrima* but insensitive to *S. cerevisiae* killer toxin(s). Since the yeast *M. pulcherrima* produces pulcherrimic acid which complexes iron, the depletion of the medium of this element could prevent other yeasts from growing, although other toxic metabolite(s) produced by this yeast could not be excluded. Nevertheless, we showed that the inhibitory effect could be reversed by the addition of a high concentration of $FeCl_3$ in the test medium. We also found that the inhibitory effect produced by *M. pulcherrima* is nonspecific because many unrelated yeast species tested as : *S. servazzii, S. unisporus, D. hansenii, K. marxianus, P. anomala, P. jadinii* and *Candida albicans* were sensitive in various degrees to *M. pulcherrima*.

2. Nguyen, H.V., Lepingle, A., and Gaillardin, C. Molecular typing demonstrates homogeneity of *Saccharomyces uvarum* strains and reveals the existence of hybrids between *S. uvarum* and *S. cerevisiae,* including the *S. bayanus* type strain CBS 380. Systematic and Applied Microbiology (in press).

PCR/RFLP of the NTS2 sequence of rDNA was shown to be suitable for differentiating *Saccharomyces sensu stricto* species. We previously showed that, within the presently accepted *S. bayanus* taxon, strains formerly classified as *S. uvarum* represented a distinct subgroup (Nguyen & Gaillardin, Syst. Appl. Microbiol. 20, 286-294,1997). In this study, we reidentified 43 more strains isolated recently from wine, cider and various fermentation habitats, and confirmed by karyotyping, hybridization and mtDNA analysis the homogeneity of strains from the *S. uvarum* subspecies. Molecular typing of nuclear and mitochondrial genomes of strains conserved in collections, and often originating from beer like *S. pastorianus* NT, revealed the existence of hybrids between *S. uvarum* and *S. cerevisiae*. Surprisingly, *S. bayanus* T CBS 380 appeared itself to be a hybrid between *S. uvarum* and *S. cerevisiae*. This strain has a mitochondrial genome identical to that of *S. uvarum*, and a very similar karyotype with 13 isomorphic chromosomes, six of which at least hybridizing strongly with *S. uvarum* chromosomes or with a *S. uvarum* specific sequence. However, four of the chromosome bands of *S. bayanus* T beared Y' sequences indistinguishable from those of *S. cerevisiae*, a feature that is not observed among presently isolated *S. uvarum* strains. Because of the hybrid nature of *S. bayanus* T and of the scarcity of similar hybrids among present days isolates, we propose to reinstal *S. uvarum* as a proper species among the *Saccharomyces sensu stricto* complex.

3. S. Casaregola, H.V. Nguyen, G. Lapathitis,[1] A. Kotyk,[1] and C. Gaillardin. Analysis of the constitution of the brewing yeast genome by PCR and subtelomeric sequence hybridization. Yeast (submitted December 1999).

[1]Department of Membrane Transport, Institute of Physiology, Czech Academy of Sciences, Videnska 1083, 142 20, Prague 4, Czech Republic.

Lager brewing yeasts represented by *Saccharomyces carlsbergensis* isolated by Hansen in the Carlsberg breweries are allopolyploid, containing parts of two divergent genomes. *S. cerevisiae* contributed to the formation of these hybrids, however the identity of the other species is still unclear. By testing for the presence of alleles specific to *S. cerevisiae* and *S. carlsbergensis* by PCR/RFLP in brewing yeasts of various origins and in members of the *Saccharomyces sensu stricto* complex, we identified *S. cerevisiae*-type alleles of two genes, *HIS4* and *YCL008c*, in another brewing yeast, *S. monacensis*, also isolated by Hansen. This is consistent with the hybridization of *S. cerevisiae* subtelomeric sequences X and Y' to *S. monacensis* electrophoretic karyotype. *S. monacencis* is therefore a hybrid similar to *S. carlsbergenis*. Regions of the sequence of several genes (*HIS4, MET10, URA3*) were shown to be identical or very similar in *S. bayanus* and *S. carlsbergensis*, showing that these species have a common ancestor. A distinction between two subgroups within *S. bayanus* was made on the basis of sequence analysis of the same genes: the subgroup represented by *S. uvarum* had 7-8% sequence divergence from *S. bayanus/S. carlsbergensis*, indicating that *S. bayanus* and *S. uvarum* diverged recently. The detection of specific alleles by PCR/RFLP and hybridization with subtelomeric sequences X and Y' of *S. cerevisiae* to electrophoretic karyotypes of brewing yeasts and related species, confirmed our findings and revealed substantial heterogeneity in the genome constitution of brewing yeasts.

XXVIII. Departamento de Biologia, Universidade do Minho, 4709 Braga Codex, Portugal. Communicated by C.S.S. Pais <cpais@bio.uminho.pt>.

The following thesis was defended recently.

1. H. Gerós. 1999. PhD Thesis.

The aim of the present work was to contribute for the elucidation of the mechanisms underlying the transport and utilisation of sugars and weak carboxylic acids in yeasts. The studies were performed in whole cells and in plasma membrane vesicles of the yeasts *Dekkera anomala* IGC 5153 and *Candida utilis* IGC 3092. Cells of *D. anomala* grown in a medium with glucose, fructose, ethanol, glycerol or acetic acid, as only carbon and energy sources, were able to produce two transport systems for glucose which could be distinguished by their kinetic parameters, substrate specificity and regulation: a high affinity transport system, K_m 0.02 mM, constitutive, able to accept galactose and 2-deoxyglucose and subjected to glucose repression, and a lower affinity one, K_m 0.7 mM, shared by fructose and 2-deoxyglucose and present in cells grown in media with sugar or ethanol. The most probable mechanism involved in glucose transport by both carriers was facilitated diffusion. The carriers were negatively affected by ethanol and acetic acid, being the inhibitory effects of the acid more pronounced than those induced by ethanol. However, these compounds, at concentrations similar to those present in wine, did not inhibit significantly the glucose transport.

D. anomala behaved as a facultative anaerobe yeast of the fermentative type. In 2% glucose-grown cells, a yield coefficient of 0.28 g g^{-1} and a specific glucose transfer rate of 3.6 mmol g^{-1} h^{-1} were obtained and a high repression of the enzyme malate dehydrogenase was observed. On the other hand, 0.1% glucose-grown cells exhibited a higher activity of this enzyme, comparable to that measured in cells grown on respiratory substrates, such as ethanol and acetic acid. Additionally, an increase of the yield coefficient (0.4 g g^{-1}) and a decrease of the specific glucose transfer rate (1.7 mmol g^{-1} h^{-1}) were observed. In 2% glucose-grown cells the yeast appeared to be preferentially fermenting the sugar, while in cells grown in 0.1% glucose a higher contribution of respiration for the sugar catabolism seemed to be occurring. In both growth conditions, the values for the specific glucose transfer rate were very similar with those estimated for the V_{max} of glucose transport. The yeasts of the genera *Dekkera/Brettanomyces* are well known for their capacity to produce large amounts of acetic acid from growth on glucose. In growth conditions where large amounts of acetate are produced (glucose concentrations higher than 2%, w/v), the study of the activity of key enzymes involved in the glucose catabolism suggested that the production of acetate was associated to a strong repression of the enzyme acetyl-CoA synthetase.

The yeast *D. anomala* exhibited a limited ability to use carboxylic acids as the only carbon and energy sources. From the mono-, di-, and tricarboxylic acids tested only acetic acid was used in such a way. The cells were able to grow on acetic acid with concentrations from 0.1 to 3% (v/v) over a pH range of 3.5 - 5.5, and the specific growth rates decreased exponentially with the increase of the undissociated acetic acid concentration in the culture medium. Transport assays carried out at pH 5.0 in cells exhibiting higher specific growth rates (grown in media with 0.5% of acetic acid, pH 5.5) showed the presence of an electroneutral acetate-proton symport (K_m = 0.12 mM; V_{max} = 0.10 nmol s^{-1} mg^{-1} dry weight) associated to a simple diffusion component (k_d = 0.03 µl s^{-1} mg^{-1} dry weight). The acetate carrier was shared by propionic, formic and sorbic acids and was inducible and repressed by glucose as well as by concentrations of undissociated acetic acid in the culture medium above 0.3% (v/v).

Hybrid membrane vesicles were prepared by fusion of yeast plasma membranes with liposomes from *Escherichia coli* containing the enzyme cytochrome *c* oxidase as the proton-motive force (PMF) generating system. These hybrid vesicles were able to generate a PMF of about -140 mV, inside alkaline and negative, by the addition of an electron donor system. This *in vitro* system proved to be suitable for the functional reconstitution of the proton symports for mono-, di- and tricarboxylates of the yeast *C. utilis*. Indeed, in hybrid vesicles prepared with plasma membranes obtained from cells grown in a medium containing lactic, succinic or citric acid, the kinetics and energetics, as well as the specificity of the carriers, obeyed to the model observed in whole cells grown on the referred substrates.

Studies on the functional reconstitution in hybrid vesicles of the glucose transport systems of *D. anomala*, supported the involvement of facilitate diffusion mechanisms in the transport of the sugar by the high and low affinity components. In hybrid vesicles prepared with plasma membranes from cells grown on 2% glucose (only the low-affinity transport system present) or acetic acid (only the high-affinity transport system present), even in the absence of a PMF, the transport of D-glucose followed a Michaelis-Menten kinetics, the K_m value for the low- or the high-affinity systems and the substrate specificity pattern, being identical to those obtained in whole cells. Plasma membranes from 2% glucose-grown cells of *D. anomala* were solubilized with the detergent octylglucoside. Upon this procedure, a fraction of soluble protein was obtained which, after incorporation in a liposome bilayer was able to promote the glucose transport by the low affinity system. Biochemical studies were conductedin order to identify a putative lactate transporter protein in plasma membranes of the yeast *C. utilis*. Membranes were incubated with L-[^{14}C]lactic acid in the presence or absence of unlabelled carboxylic acids, after which proteins were separated by SDS-PAGE. In membrane preparations of lactic acid-grown cells, the labelled lactate was bound to a 43 kDa polypeptide that was probably a component of the lactate transporter of the yeast.

Recent publications.

1. Casal, M., Paiva, S., Andrade, R.P., Gancedo, C. and Leão, C. 1999. The lactate-proton symport of *Saccharomyces cerevisiae* is encoded by JEN1. J. Bacteriol. **181**:2620-2623.

A mutant of *Saccharomyces cerevisiae* deficient in the lactate-proton symport was isolated. Transformation of the mutant with a yeast genomic library allowed the isolation of the gene *JEN1* that restored lactate transport. Disruption of *JEN1* abolished uptake of lactate. The results indicate that, under the experimental conditions tested, no other monocarboxylate permease is able to efficiently transport lactate in *S. cerevisiae*.

2. Paiva, S., Althoff, S., Casal, M. and Leão, C. 1999. Transport of acetate in mutants of *Saccharomyces cerevisiae* defective in monocarboxylate permeasese. FEMS Microbiol. Lett. **170**:301-306.

The strain *Saccharomyces cerevisiae* W303-1a, able to grow in a medium containing acetic acid as the sole carbon and energy source, was subjected to mutagenesis in order to obtain mutants deficient in monocarboxylate permeases. Two mutant clones unable to grow in a medium with acetic acid as the sole carbon and energy source, although with normal growth rates in ethanol containing medium (mutants Ace12 and Ace8), were isolated. In both mutants the activity for the acetate carrier was strongly affected. The mutant Ace8 revealed not to be affected in the transport of lactate, while the mutant Ace12 did not display activity for that carrier. These results reinforced those previously found in the strain IGC 4072, where two distinct transport systems for monocarboxylates have been described, depending on the growth carbon source. The Ace8 mutant seems to be specifically affected in the structural gene coding for the acetate permease. In contrast, the absence of activity for both monocarboxylate permeases in mutant Ace12 could be attributed to a mutation in a gene coding for a regulatory protein not detected before.

3. Duarte, F., Pais, C., Spencer-Martins, I. and Leão, C. 1999. Distinctive electrophoretic isoenzyme profiles in the *Saccharomyces sensu strictu* complex. Int. J. Syst. Bacteriol. **49**:1907-1913.

Genetic variation among 35 strains representing the four currently recognised species of *Saccharomyces sensu stricto* (*Saccharomyces cerevisiae, S. bayanus, S. pastorianus/carlsbergensis* and *S. paradoxus*) was estimated by analysing the electrophoretic mobilities of nonspecific esterases, acid phosphatase, lactate dehydrogenase and glucose-6-phosphate dehydrogenase isoenzymes. Twenty-two electrophoretic types were identified, a result in agreement with the phenotypic and genetic polymorphisms reported for this group of yeasts. However, the four species were clearly distinguishable based on the patterns obtained using three of the enzymes assayed, the resolving power not being improved by the introduction of data correspondent to lactate dehydrogenase. The overall diversity was higher among *S. cerevisiae* isolates, in contrast with *S. paradoxus* which showed only two patterns, one of which was common to four of the five strains studied. Concordant results from the application of the method and DNA hybridisation experiments demonstrate its value for identification purposes.

4. Lages, F., Silva-Graça, M. and Lucas C. (1999). Glycerol active uptake is a mechanism underlying halotolerance in yeasts: a survey of 42 different species. *Microbiology*, **145**:2577-2585.

A comparison of 42 different yeast species in respect to growth in the presence of high NaCl concentration and characteristics of glycerol uptake is presented. The yeast species are classified into four classes on the basis of their ability to grow in the presence of 1, 2, 3 or 4 M NaCl. Considering that two different types of active transport systems for glycerol uptake have been described, Na^+/ and H^+/glycerol symports, glycerol transport was investigated searching proton uptake upon glycerol addition in cells incubated in the absence and in the presence of NaCl. Only strains belonging to the two classes of higher tolerance showed constitutive active glycerol uptake, and could accumulate internally glycerol against a concentration gradient. Five of these strains exhibited activity of a H^+/glycerol symport. All the other strains presented evidence of the activity of a salt-dependent glycerol uptake most similar to the one described in the literature for *D. hansenii*. The strains within the two classes of lower tolerance showed, to varying degree, glycerol active uptake only when glycerol was used as carbon and energy source, suggesting that this uptake system be involved in glycerol catabolism. The results within this work suggest that active glycerol uptake provides a basis for high halotolerance, helping to maintain a favourable glycerol intracellular concentration. The relation between the constitutive expression of such carriers and a higher level of salt-stress resistance suggests that this may be an evolutionary advantage for growth under such circumstances.

5. Nobre, A., Lucas, C. and Leão, C. 1999. Transport and utilization of hexoses and pentoses in the halotolerant yeast *Debaryomyces hansenii*. Appl. Environm. Microbiol. **65**:3594-3598.

Debaryomyces hansenii is a yeast species well known for its halotolerance. It has seldom been mentioned as a pentose consumer. In the present work, a strain of this species was investigated with respect to the utilization of pentoses and hexoses in mixtures and as single carbon sources. Growth parameters were calculated from batch aerobic cultures with pentoses, hexoses and mixtures of both sugars. Growth on pentoses was slower than on hexoses, but the values obtained for biomass yields were very similar in both types of sugars. Furthermore, in mixtures of two sugars, the preference for one carbon source did not inhibit the consumption of the other. Glucose and xylose were transported by cells grown on glucose, via a specific low- affinity facilitated diffusion system. Cells derepressed by growth on xylose exhibited two distinct high-affinity transport systems for glucose and xylose. The sensitivity of labeled glucose and xylose transport to the dissipation of transmembranar proton gradient by the protonophore

CCCP, allowed us to consider them as proton symports, although they displayed sugar associated proton uptake exclusively in the presence of NaCl or KCl. When the V_{max} of transport systems for glucose and xylose were compared with glucose and xylose specific consumption rates during growth on either sugar, transport appeared not to limit the growth rate.

6. Fernandes, L., Loureiro, L., V., Côrte-Real, M., and Leão, C. (1999). A peculiar behaviour for cell death induced by weak carboxylic acids in the wine spoilage yeast *Z. bailii* . Lett. Appl. Microbiol. **28**:345-349.

In glucose-grown cells of *Zygosaccharomyces bailii* ISA 1307 acetic acid and other carboxylic acids enhanced death. The effects were much lower than those described for *Saccharomyces cerevisiae* being only detectable at higher acid concentration. In *Z. bailii* acetic acid and other weak acids also induced intracellular acidification but the effect was less pronounced than the one of death, no relation being found with the death enhancement. The results suggested that in *Z. bailii*, unlike *S. cerevisiae*, the intracellular acidification induced by weak acids is less pronounced and appears not to have a significant role on death at the temperature range used.

7. Martins, M.A., Cardoso, H., Ramalho, M.T. and A.M.O. Campos. 1999. Biodegradation of Azo dyes by the yeast *Candida zelanoides* in batch aerated cultures. Chemosphere **18**:2455-2460.

A number of simple azo dyes was degraded in liquid aerated batch cultures by a strain of the yeast *Candida zeylanoides*. The standard decolorization medium contained glucose as a carbon and energy source, and its pH was either controlled to 5.0-5.2, or allowed to decrease to 3.2-2.8, in the course of microorganism growth. The extent of colour removal in the culture medium was assessed through the decrease in dye absorbance of the supernatants. The extend of colour removal ranged from 44 to 90%, after 7 days, for 5 out of 6 dyes studied in shaked cultures, without pH control, and from 46 to 67%, after 22 hours, for 6 out of 8 dyes in batch experiments, at controlled pH.

XXIX. Division of Industrial Microbiology, Department of Food Technology and Nutritional Sciences, Wageningen University , PO box 8129, 6700 EV Wageningen, The Netherlands. Communicated by J.C. Verdoes.

The following papers have recently been published.

1. J. C. Verdoes , N. Misawa and A.J.J. van Ooyen. 1999. Cloning and characterization of the astaxanthin biosynthetic gene encoding phytoene desaturase of *Xanthophyllomyces dendrorhous*. Biotechnol. Bioeng. **63**:750-755.

The first carotenoid biosynthetic gene from the basidiomycetous yeast *Xanthophyllomyces dendrorhous* was isolated by heterologous complementation in *Escherichia coli*. The isolated gene, denominated as *crtI,* was found to encode for phytoene desaturase. The coding region is interrupted by 11 introns. The deduced amino acid sequence showed significant homology with its bacterial and eukaryotic counterparts, especially those of fungal origin. A plasmid containing the geranylgeranyl diphosphate synthase and phytoene synthase encoding genes from *Erwinia uredovora* was introduced in *E. coli* together with the phytoene desaturase encoding cDNA from *X. dendrorhous*. As a result, lycopene accumulation was observed in these transformants. We conclude that in *X. dendrorhous* the four desaturase steps, by which phytoene is converted into lycopene, are carried out by a single gene product.

2. Jan C. Verdoes and Albert J.J. van Ooyen. 1999. Isolation of the isopentenyl diphosphate isomerase encoding gene of *Phaffia rhodozyma*; improved carotenoid production in *Escherichia coli*. Acta Bot. Gallica **146**:43-53.

The isolation of the isopentenyl diphosphate (IPP) isomerase (EC 5.3.3.2) encoding gene (*idi*) of *Phaffia rhodozyma* by a new and direct selection procedure in a carotenogenic *Escherichia coli* strain is described. Isopentenyl diphosphate (IPP) isomerase is a key enzyme in the isoprenoid biosynthetic pathway which catalyses the interconversion of the primary five-carbon diphosphate building blocks dimethylallyl diphosphate (DMAPP) and IPP. Our results imply that this method should be generally useful for the isolation of IPP isomerase encoding genes in both genomic and cDNA libraries from other organisms. The structural gene comprises 1294 nucleotides encoding a 28.7 kDa polypeptide of 251 amino acids. Analysis of the nucleotide and amino acid sequence indicates clear homology to IPP isomerases of non-carotenogenic yeasts, *Schizosaccharomyces pombe* (46%) and *Saccharomyces cerevisiae* (42%). By high level expression of the heterologous IPP isomerase gene in a recombinant *E. coli* a 3-fold increase in lycopene production was achieved. This indicates the potentials of this idi gene to improve carotenoid biosynthesis by metabolic pathway engineering in homologous and heterologous hosts.

3. A.L Botes, C.A.G.M. Weijers, P.J. Botes, M.S. van Dijk. Enantioselectivities of yeast epoxide hydrolases for 1,2-epoxides. 1999. Tetrahedron: *Asymmmetry* **10**:3327-3336.

Kinetic resolution of homologous ranges of unbranched 1,2-epoxyalkanes (C-4 to C-12), and 1,2-epoxyalkenes (C-4, C-6 and C-8), a 2,2-dialkylsubstituted epoxide (2-methyl-1,2-epoxyheptane) and a benzyloxy-substituted epoxide (benzyl glycidyl ether) was investigated using resting cells of 10 different yeast strains. Biocatalysts with excellent enantioselectivity (E>100) and high initial reaction rates (>300nmol/min.mg dry weight) were found for the 2-monosubstituted aliphatic epoxides C-6 to C-8. Yeast strains belonging to the genera *Rhodotorula*, *Rhodosporidium* and *Trichosporon*, all preferentially hydrolyzed (R)-1,2-epoxides with retention of configuration. The epoxide hydrolases of all the yeast strains are membrane-associated.

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

The following paper is in press.

1. H.J. Phaff, W.T. Starmer, and C.P. Kurtzman. *Pichia hawaiiensis* an. nov., occurring in decaying bark of *Charpentiera* trees in the Hawaiian archipelago. Int. J. Syst. Evol. Microbiol. (in press).

A description is given of Pichia hawaiiensis sp. nov., a nitrate-utilizing member of the genus *Pichia* E.C.Hansen emend. Kurtzman. Seven strains of the new species were isolated during the years 1972, 1973, and 1978 from rotting bark of the Hawaiian tree genera *Charpentiera*, *Pisonia*, and *Cheirodendron*. *P. hawaiiensis* is heterothallic but appears to occur in nature mainly in the diploid state. Asci are deliquescent and produce up to four hat-shaped spores per ascus. Phylogenetic analysis showed that *P. hawaiiensis* is most closely related to *Pichia populi* and *Willliopsis californica* (syn. *Hansenula californica*). The GenBank accession number for the sequence in this paper isAF15387S. The type strain of *P. hawaiiensis*, isolated on the Island of Hawaii from rotting bark of *Charpentiera* sp. with insect larvae, is strain UCD-FST 72-181 = CBS 8760 = NRRL Y-26270.

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

I thank Isabel Spencer-Martins and her colleagues at the Universidade Nova de Lisboa for their kindness during my visit in Portugal, and congratulate Gabriella Giménez-Jurado for her successful Ph.D. thesis defence. I welcome Gaelle Marinoni as a new doctoral students in my laboratory. Gaelle recently completed a Master's degree in Jure Piškur's laboratory, in Denmark, and is now working on the evolution of reproductive isolation in large-spored *Metschnikowia* species.

The following oral presentations were given recently.

1. Lachance, M.A. 1999. Understanding yeast biodiversity through the study of their adaptation to specific habitats. 19th Conference on Yeast Genetics and Molecular Biology, Rimini, Italy.

Bacterial ecology predicts that yeast biodiversity will be linked to extreme habitats. This approach, however, has led to the rediscovery of old species. Examples of extremes include growth in the presence of high ethanol (*Saccharomyces, Brettanomyces*), salt (*Debaryomyces*), and sugar (*Zygosaccharomyces*). More interestingly, through the study of specific but less extreme habitats, the true magnitude of yeast biodiversity has become apparent. Yeasts are nearly always found wherever a specific plant-insect interaction takes place. The best studied case is that of cactus necroses that serve as *Drosophila* habitats. The specific yeast community of cacti includes *Clavispora opuntiae, Sporopachydermia cereana*, several *Pichia* species (some that were reclassified as *Phaffiomyces* or *Starmera*), and nutritionally interesting *Candida* species. A whole genus (*Saccharomycopsis*) associated with tree beetles has ten species that all share two unusual traits, namely a requirement for organic sulfur and the ability to prey on other fungi by penetration. Ephemeral flowers of the Convolvulaceae and the Malvaceae harbour many unusual species of *Metschnikowia, Kodamaea, Wickerhamiella*, and *Starmerella*, some of which have unusual characteristics such as giant (250 µm) ascospores, or powerful extracellular lipases. Numerous other such communities await discovery.

2. Lachance, M.A. 1999. The ecological diversity of yeasts. 9th International Congress of Mycology (IUMS), Sydney, Autralia

The growth of our knowledge of yeast biodiversity is predicated on the discovery of new yeast habitats and the correct identification of isolates. Many new yeast species have been recognized in recent systematic studies of two habitats that also serve as insect habitats, namely necrotic plant tissue and ephemeral flowers. The advent of rapid genetic identification methods based on DNA sequencing has expanded our perception of yeast biodiversity and suggests that the global number of existing species

is greatly underestimated. Examples will be presented, with an emphasis on the "*Sporopachydermia cereana* species complex." The described species *S. cereana* is only one of several genetically separate taxa that are nearly indistinguishable from one another except by molecular characterization. Sequencing and PCR with group-specific primers in the ribosomal internal transcribed spacer DNA (ITS) revealed that the species complex actually consists of at least 10 species, one of which has three genetically distinct varieties. The known global distribution of the taxa follows a strong geographic pattern in an area bounded to the north by the Great Lakes region, to the southeast by the Atlantic coast of Brazil, and to the southwest by eastern Australia.

The following papers, whose abstracts was given in a previous issue of the YNL, have now appeared in print.

3. Rosa, C.A., R.P. Martins, E.M. Viana, Y. Antonini, and M.A. Lachance. 1999. *Candida batistae*, a new yeast species associated with solitary digger nesting bees in Brazil. Mycologia **91**:428-433.

4. Lachance, M.A., J.M. Bowles, W.T. Starmer, and J.S.F. Barker. 1999. *Kodamaea kakaduensis* and *Candida tolerans*, two new yeast species from Australian *Hibiscus* flowers. Can. J. Microbiol. **45**:172-177.

The following paper is in press.

5. Lachance, M.A., Starmer, W.T., Bowles, J.M., Phaff, H.J., and Rosa, C.A. In press. Ribosomal DNA, species structure, and biogeography of the cactophilic yeast *Clavispora opuntiae.* Can. J. Microbiol.

The ribosomal DNA of the cactophilic yeast species *Clavispora opuntiae* was studied in order to clarify the global distribution of the yeast. Over 500 strains, including isolates from several new localities worldwide, were characterized by rDNA restriction mapping. An unusual restriction pattern previously encountered only in one strain from Conception Island (Bahamas) was found in several Brazilian isolates. Sequences of the D1/D2 and D7/D8 divergent domains of the large subunit (LSU) and of the intergenic spacers (IGS) confirmed that these strains represent a genetically distinct variety of *Clavispora opuntiae*. This divergence had previously been hypothesized on the basis of reduced genetic recombination in inter-varietal crosses and the presence of a polymorphic *Apa*I restriction site located in the LSU. The exact position of the *Apa*I site in the D8 divergent domain and the nature of the variation that it reveals were determined. The complete sequences of 12 intergenic spacers clarified the significance of the species-wide variation uncovered by restriction mapping. Most of the polymorphic sites occur in the IGS1 and IGS2 regions, on either side of the 5S gene, and the variation is largely due to differences in the numbers and the sequences of internal repeats. Two other polymorphic sites are located in the external transcribed spacer (ETS) region. The reliability of various sites as indicators of overall spacer sequence divergence differed from one case to another. Variety-specific probes were devised and used to screen 120 strains for the presence of recombinant rDNA spacers. Three strains gave ambiguous results, but these did not constitute evidence that inter-varietal recombination has taken place in nature. The hypothesis that the global movement of *Clavispora opuntiae* has been influenced by the worldwide biological control of prickly pear with *Cactoblastis cactorum*, a moth of Argentinian origin, has received additional support from the demonstration that Argentinian strains have rDNAs similar to those found where the moth has been introduced. A dramatic founder effect was identified in a yeast population collected in cacti (island of Maui, Hawaii) in a site where the moth had been recently introduced.

Network: Yeasts in Food and Beverages

Dept. of Biologia, Difesa, Biotecnologie Agro-Forestali, University of Basilicata, Via Nazario Sauro 85, 85100 Potenza, Italy. Communicated by P. Romano <pot2930@iperbole.bologna.it>.

In this issue of the Yeast Newsletter we report events and recent publications on "Yeasts in Dairy Products".

Second Symposium on "Yeasts in the Dairy Industry"

The 2nd Symposium on "Yeasts in the Dairy Industry", promoted by the International Dairy Federation was held on 9-10 September 1999 at the Agricultural Faculty of Bologna University (Italy). The symposium, organized by Prof. Maria Elisabetta Guerzoni, Dept. Protezione, Valorizzazione Agroalimentare, was articulated in the following sections of oral presentations and posters.

Session I: Characterization and Identification of Yeasts from Dairy Products

1. Wyder, M.T., Meile L., Teuber M. Description of *Saccharomyces turicensis* sp. nov., a new species from Kefyr.

   Institute of Food Science, Laboratory of Food Microbiology, Swiss Federal Institute of Technology, ETH-Zentrum, Zurich, Switzerland.

   The new species *Saccharomyces turicensis* sp. nov. isolated from different kefyr grains is described. Although its morphological properties differ, its physiological characteristics come close to those of *Saccharomyces bayanus* Saccardo and *Saccharomyces pastorianus* Reess ex E.C. Hansen. However, electrophoretic karyotyping and restriction fragment length polymorphism of the internal transcribed spacer region yield clear differences. Sequences (270 nucleotides) of the D2 domain at the 5' - terminal end of the large subunit ribosomal RNA gene reveal 98.0 % identity with *Saccharomyces exiguus.* Since strains of a particular yeast species usually show less than 1% substitution in the D2 domain, the yeast in question is considered to be a new species. The name *Saccharomyces turicensis* is proposed indicating the place Zurich (Turicum in Latin) where the yeast had been isolated.

2. Sorensen, K., Jespersen, L., Jakobsen, M. DNA typing methods for differentiation of *Debaryomyces hansenii* at subspecies level.

   The Royal Veterinary and Agricultural University, Dept. Dairy and Food Science, Rolighedsvej 30, 1958 Frederiksberg, Copenhagen.

   Strains of *Debaryomyces hansenii* play an essential role in the production and maturation of surface ripened cheeses. One of the most important functions is the degradation of lactic acid increasing the pH of the cheese surface, which allows the growth of *Brevibacterium linens* and other bacteria present in the smear. Besides, yeast might produce aroma components and growth factors of importance for the bacteria. Traditionally yeasts are introduced in the production by process equipment and by reinoculation of the cheese surface with a slurry containing smear from previously produced cheeses. However, the use of well defined starter cultures of *D. hansenii* for surface ripening of cheese is paid increased attention. The *D. hansenii* starter cultures might be used alone or in combination with reinoculation. Little is known about the microbial succession between the *D. hansenii* starter culture and the strains of *D. hansenii* and other yeasts introduced from the environment and by reinoculation. In order to follow and control the starter culture during the production, fast and simple methods are required for identification of *D. hansenii* at subspecies level. In the present work DNA typing methods were evaluated for strain differentiation of *D. hansenii*. The methods used were Internal Transcribed Spacer-PCR Restriction Fragment Length Polymorphism (ITS-PCR RFLP) of ITS1/5.8S/ITS2 rDNA, mitochondrial DNA RFLP and chromosomal DNA RFLP. In total 20 isolates of both *Debaryomyces hansenii* var. *hansenii* and *Debaryomyces hansenii* var. *fabryii* obtained from Centraalbureau voor Schimmelcultures (CBS) were included together with 17 strains isolated from Danish surface ripened hard cheeses. Even though several restriction enzymes were used in the investigations, the discriminative power of the ITS-PCR RFLP method was found to be low for strains of *D. hansenii*. This observation was further confirmed by sequencing of the ITS1/5.8S/ITS2 region of the type strains of *D. hansenii* var. *hansenii* (CBS767) and *D. hansenii* var. *fabryii* (CBS789). By comparison of the two type strains it was found that their ITS regions only differed by one restriction site i.e. *TaqI*. By use of this restriction enzyme, the majority of the *D. hansenii* strains could be grouped into two clusters. Subspecies typing of *D. hansenii* was obtained by mtDNA RFLP and chromosomal DNA RFLP.

3. Romano P., Ricciardi A., Salzano G., Suzzi G. Yeasts from water Buffalo mozzarella, a traditional cheese of the Mediterranean area.

   Dept. Biologia, Difesa e Biotecnonologie Agro-forestali University of Basilicata, Potenza, Italy.

   Countries of the Mediterranean area are characterized by artisanal cheeses, obtained from goat, sheep, cow and buffalo raw milk. The numbers and species of yeasts in the different cheeses are variable, but some species are more frequently detected than other ones. *Kluyveromyces marxianus*, *K. lactis* with their anamorphous, *D. hansenii* and *Candida famata*, *C. colliculosa*, and *C. catenulata* are dominant species in several cheeses. However the non-lactose fermenting *Saccharomyces cerevisiae* is often detected in pasta filata cheeses, such as Water-Buffalo Mozzarella or Cacio Cavallo Podolico. Recently a comprehensive study of yeasts isolated from Mozzarella cheese produced in Basilicata (Southern Italy) has been carried out. The study was focused on lactose- and/or galactose fermenting species (*Saccharomyces* and *Kluyveromyces*), to evaluate their role on the functional and sensory properties of the product. Final products in milk were evaluated and the biodiversity (in terms of biotechnological properties) was studied. In particular *S. cerevisiae* strains from Water Buffalo Mozzarella cheese compared to same species strains isolated from different habitats such as wine, exhibited a considerable difference in the production of some compounds in function of the origin. The intraspecific diversity observed could be related to the particular microhabitat of *S. cerevisiae* present in whey and cheese of water -buffalo milk.

Session II: Yeasts as Probiotics and Microbial Interaction Involving Yeasts

4. Psomas[1] E., Andrighetto[2] C., Litopoulou – Tzanetaki[1] E., Lombardi[2] A., Tzanetakis[1] N. Some probiotic properties of yeast isolates from infant faeces and cheese.

   [1]Laboratory of Food Microbiology and Hygiene, Aristotele Univ.of Thessaloniki, Thessaloniki, Greece.

   [2]Veneto Agricoltura, Istituto per la Qualità e le Tecnologie Agroalimentari , Thiene (Vicenza), Italy.

   Twenty one yeast isolates from the faeces of new born infants were classified by phenotypic criteria, RAPD-PCR, and mt-DNA restriction analysis as *Saccharomyces cerevisiae* (7 strains) and *Candida albicans* (14 strains). Their enzyme profile was also studied. The isolates from infant faeces as well as 50 yeast strains previously isolated from the surface of Feta cheese were tested for their ability to withstand bile and low pH. The strains from faeces tolerated higher concentrations of bile and lower pH values compared to the strains from cheese. Selected strains might be used as components for fermented milk products.

5. Addis, E., G.H. Fleet, J.M. Cox. Evolution of yeasts and bacteria during the production of Australian Camembert and Blue-veined cheeses.

   Dept. Food Science and Technology, University of New South Wales, Sydney, New South Wales, Australia.

During the last 10 years, a significant industry has developed in Australia for the production of mould-ripened soft cheeses. Previous studies in our laboratory have demonstrated high but inconsistent populations of yeasts in retail samples of these cheeses suggesting that they are probably associated with the maturation process. The microbial ecology of Camembert and Blue-veined cheese production in Australia has not been previously studied. To better understand the contribution of microorganisms to cheese production, we have completed a detailed study of the occurrence and growth of yeasts, bacteria and moulds throughout the fermentation and maturation phases of locally produced Camembert and Blue-veined cheeses. *Debaryomyces hansenii* developed early in the maturation of the blue veined cheeses reaching maximum populations of 10^8-10^9cfu/g that remained at this level throughout the process. *Yarrowia lipolytica* and *Candida zeylanoides* were inconsistently found throughout the process. *Micrococcus* and *Staphylococcus* spp (10^7-10^8 cfu/g) were also significant in the maturation of these cheeses, especially at inner curd locations. *Debaryomyces hansenii* consistently grew during the production of Camembert cheeses giving final populations of 10^8-10^9 cfu/g or lower (10^4-10^5 cfu/g) depending on location within the cheese curd and place of manufacture. Low populations (10^2-10^4 cfu/g) of *Y. lipolytica* were inconsistently found in these cheeses. The evolution of Gram negative bacteria, especially *Acinetobacter* spp to 10^6-10^7 cfu/g, was significant during the maturation of these cheeses. Changes in the microbial ecology throughout production were correlated with changes in pH and salt concentration at both inner and outer locations of the curd, the proteolytic and lipolytic activities of the species and the interactive behaviour between the different species.

6. Neviani[1] E., Vannini[2] L., Suzzi[3] G., Gardini[2] F., Guerzoni[2] M.E. Contribution of Gal- lactic acid bacteria to *Saccharomyces cerevisiae* metabolic activity in milk.

   [1]Istituto Sperimentale Lattiero Caseario, Lodi, Italy; Dept. Protezione, Valorizzazione Agroalimentare, University of Bologna, Bologna, Italy.

   [2]Dept. Biologia Difesa, Biotecnologie Agroforestali, University of Basilicata, Potenza, Italy.

In the production and maturation of fermented foods, very complex biochemical processes leading to the formation of aromatic compounds are involved. Most of these chemical modifications originate from microbial activity by specific enzymatic pathways; moreover, microbially produced food or degradation of food is rarely the result of the activities of an individual but that of a group of micro-organism. Some of the processes utilising yeast and lactic acid bacteria (LAB) associations are among the oldest known food fermentations. In particular yeast and LAB are often encountered together during spontaneous fermentation of some food and beverages such as bread, wine, cider, and kefir which are media characterised by an acid pH. In the manufacture of Mozzarella cheese, yeasts are always present and their count can reach 10^7 cfu/ml. The dominant fermentative species are *Saccharomyces cerevisiae* and *Kluyveromyces marxianus* and *lactis* together with their anascosporogenous forms. In this work the metabolic interrelation between *S. cerevisiae* isolated from water Buffalo Mozzarella cheese and Gal$^-$ lactic acid bacteria was studied. *S. cerevisiae* proved to be able to grow in milk without using lactose or galactose; in particular the presence of peptides seems to be sufficient to ensure its growth. The growth of *S. cerevisiae* in presence of lactic acid bacteria is characterised by a stimulatory effect that involves both yeast and bacteria; however, the release of galactose by lactic acid bacteria does not seem to be the core metabolic event of these stimulatory effects on *S. cerevisiae*.

Session III: Yeasts as Potential Starter Cultures in Dairy Products

7. Rossi[1] J., Corsetti[1] A., Gobbetti[2] M., Smacchi[1] E. Interactions between yeast and bacteria in the smear surface-ripened cheeses.

   [1]Inst. of Industrie Agrarie (Microbiologia), University of Perugia, Italy

   [2]Inst. of Produzioni, Preparazioni Alimentari, University of Bari (Foggia), Foggia, Italy.

The term smear surface-ripened cheeses can loosely be applied to cheeses in which moulds, yeasts and bacteria are present in large numbers on the surface of the cheese and play a significant role in determining the final characteristics and attributed of the cheeses. Although the initial phase of ripening the surface microflora of bacterial smear surface-ripened cheeses such as Limburger, Brick, Munster and Saint Paulin and of surface mould-ripened cheeses such as Camembert and Brie may be similar, at the end of the ripening, bacteria such as *Brevibacterium* spp., *Arthrobacter* spp., *Micrococcus* spp and *Corynebacterium* spp. and moulds are, respectively, the dominant microorganisms. Yeasts such as *Candida* spp., *Cryptococcus* spp., *Debaryomyces* spp., *Geotrichum candidum* (*Galactomyces geotrichum*), *Pichia* spp., *Rhodotorula* spp., *Saccharomyces* spp. and *Yarrowia lipolytica* are often and variably isolated from the smear surface-ripened cheeses. Although not dominant within the microorganisms of the smear surface-ripened cheeses, yeast establish strict interactions with moulds and especially bacteria, including surface bacteria but also lactic acid bacteria. Yeast activities, such as lactate utilisation, ammonia production, proteolysis, lipolysis and liberation of bacteria growth factors are fundamental for the typical characteristics of the smear surface-ripened cheeses, which are determined by a very complex microbial community. A review of the main interactions between yeasts and bacteria in such type of cheeses, including experiences from our laboratory, is considered in this report.

8. Vannini L., Baldi D., Lanciotti R., Guerzoni M.E. Characterisation of *Yarrowia lipolytica* enzymes and their potential role as cheese ripening.

   Dept. Protezione, Valorizzazione Agroalimentare, University of Bologna, Bologna, Italy; Inst. Produzioni, Preparazioni Alimentari, University of Bari (Foggia), Foggia, Italy.

During recent years several investigations have shown the occurrence of different species of yeasts in dairy products. However, the significance of this presence has not been widely studied and yeasts are usually considered to be responsible for many defects, such as fruity, gassy, bitter, off-flavour or open texture, as well as to contribute positively to flavour development

during maturation. Although *Yarrowia lipolytica* is one of the most frequent species associated to milk products and its high extracellular protease and lipase are well known, relatively few are the study concerning the role of this microorganism in cheese ripening. The aim of this work was to evaluate the proteolytic activity of *Yarrowia lipolytica* strains, isolated from different habitats, as well as the potentiality of this species to be used as ripening accelerating agent. The results obtained indicate that *Yarrowia lipolytica* possess a remarkable proteolytic activity: in fact the differences observed in the electrophoretograms of a and b casein suggest that proteinase play a significant role in the breakdown of casein. Moreover, the comparison of the FTIR spectra of cheeses, obtained from milk inoculated with different strains of *Yarrowia lipolytica,* provides strong evidence for the importance of the enzymatic activities, as well as for the diversity in proteins hydrolysis pattern in relation to the ripening time course and to the strain used. This diversity within the species in relation to the isolation source could be exploited in order to obtain products having different flavours and textural characteristics.

9. Guerzoni[1] M.E., Lanciotti[2] R., Lanorte[3] M.T., Galgano[3] F., Lombardi[4] A., Suzzi[3] G. Biodiversity of *Yarrowia lipolytica* strains isolated from Caprino and Mozzarella cheeses.

   [1]Dept. Protezione, Valorizzazione Avgroalimentare, University of Bologna, Bologna.

   [2] Inst. Produzioni e Preparazioni Alimentari, University of Bari (Foggia), Foggia, Italy.

   [3]Dept. Biologia, Difesa, Biotecnologie Agroforestali, University of Basilicata, Potenza, Italy.

   [4]Veneto Agricoltura, Istituto per la Qualità e le Tecnologie Agroalimentari, Thiene (Vicenza), Italy.

   The contribution of fermentative yeast to flavour and aroma of cheeses is directly attributed to the ability of some species to ferment lactose and galactose and as a result producing ethanol, acetaldeyde, ethyl acetate and ethyl butyrate. Due to their proteolytic and lipolytic activity, oxidative yeasts indirectly play an important roleby the formation of precursor of the aroma. The aim of this work was to characterise starin of *Yarrowia lipolytica* both from a biochemical and genetic point of view. Five strains from goat cheese and three from water-buffalo Mozzarella cheese produced in Basilicata were studied. In order to confirm the identification obtained by conventional phenotipic methods, the starins were studied by means of RAPD-PCR with primers M13 and RF2. The presumtive identification as *Yarrowia lipolytica* was confirmed, except for a strain. In addition, RAPD-PCR with the two primers proved to be a rapid and reliable method to differentiate *Y. lipolytica* from other yeasts of dairy origin. Proteolytic activity was studied at two temperatures, 10°C and 25°C. At both temperatures the proteolytic activity was expressed at low levels during the first eight days of growth in skim milk, whereas from th 8th to the 14th day a great increase was observed. Low temperature affected all the strains. At 25°C a great variability of the character was observed, with strain producing more than 500 mg leucine/100 skim.

10. Larsen M.D., van den Tempel T., Hansen T.K., Jakobsen M. *Saccharomyces cerevisiae* as starter culture in Mycella.

    The Royal Veterinary and Agricultural University Department of Dairy and Food Science, Food Microbiology, Frederiksberg, Denmark.

    Yeasts are found in high numbers in blue veined cheeses, including the Danish types Danablu and Mycella, even though they are not added as starter cultures. The most commonly encountered yeast is *Debaryomyces hansenii/Candida famata*, but other species like *Yarrowia lipolytica* and *Saccharomyces cerevisiae* are found as well. Previous investigations have shown a positive interaction between *Penicillium roqueforti* and the *S. cerevisiae* strain FB7 in casein breakdown, the latter degrading ß-casein. The potential of *Sacch. cerevisiae* FB7 as a starter culture was investigated in two large scale productions of Mycella. For both reference and experimental cheeses the following examinations were carried out during the ripening period: chemical analysis (pH, a_w, NaCl), the evolution of the microflora, identification of the yeast flora, identification of aroma compounds by GC analysis and the level of proteolysis by CE analysis. Sensory analyses were made by a trained panel. Pictures of the interior of the cheeses were taken during the ripening period in order to follow the development of *P. roqueforti*.

Session IV: Yeasts recognised as Spoilage Organisms in Dairy products

11. Suzzi[1] G., Lombardi[2] A., Gardini[3] F., Galgano[1] F., Crudele[1] M.A., Andrighetto[2] C., Schirone[1] M. A survey of yeasts in traditional salami of Southern Italy: quali-quantitative aspects.

    [1]Dept. of Biologia, Difesa, Biotecnologie Agroforestali, University of Basilicata, Potenza, Italy.

    [2]Veneto Agricoltura, Istituto per la Qualità e le Tecnologie Agroalimentari , Thiene (Vicenza), Italy.

    [3]Dept. Protezione, Valorizzazione Avgroalimentare, University of Bologna, Bologna, Italy.

    In Italy, dry sausage production, especially on the small scale, is still based on the experience and the art of the local manufacturer. Most part of the commercially manufactured sausage are prepared without starter cultures and high quality products are produced. In this study the evolution of yeats population during manufacturing and ripening of "Salsiccia sotto sugna", a typical salami of Lucania region, were investigated. In particular, four different batches, produced in four farms located in Basilicata, were studied. In two batches the counts of yeasts started from 10^5-10^6 and in the other ones yeasts were not detected. However, after two weeks in all the sausage the number of yeasts reached values from 10^5 to 10^7. Each batch showed a particular yeast population, and only few yeast species, *Candida insectorum, C. famata* and *Rhodotorula mucilaginosa* were often present. *Debaryomyces hansenii* was found only in a sample and *Yarrowia lipolytica* in two samples. Only the strains of *Y. lipolytica* showed interesting biotechnological characteristics and consequently this study was further conducted on these strains, in particular on their lipolytic activity on fat pork. The greater lipolytic activity resulted to be exploited at pH 5.5 with oleic and palmitic acids as major free fatty acids produced. In addition, these strains resulted to belong to *Yarrowia lipolytica* species according to phenotypic characteristics, whereas from a genetic point of view they resulted to be two different species.

12. Davenport R. Selected yeasts problems associated with the dairy industry.

England.

Yeast problems in the dairy industry are not confined to products solely from milk origin. In this presentation case histories will be discussed to illustrate a range of yeast and their significance in a selection of dairy investigations. Also a "Test Case" cross section of a dairy will be described to indicate environmental relationships. As well as identity of yeasts and yeast like fungi. Finally some insight will be given to demonstrate workable forensic style protocols, developed as a "Bridge" between academic research and commercial needs.

13. Caggia C., Restuccia C., Pulvirenti A., Giudici P. Identification of yeasts isolated from spoiled yoghurt.

Inst. of Industrie Agrarie , University of Catania, Catania, Italy.

We have examined several packs of retailed yoghurt showing clear marks of swelling. The majority of the strains isolated belong to Pichia anomala and *Saccharomyces cerevisiae*. We found out that despite they are not able to ferment lactose they can spoil yoghurt due to the ability to ferment galactose. The presence of galactose, due to the lactose hydrolysis by lactic acid bacteria and by the activity of extracellular enzymes, is the main reason for the potential alteration of plain yogurt by galactose positive and non lactose fermenting yeasts.

14. Minervini F[1], Montagna MT[2], Erroi R[3], Monaci L[1], Spilotros G[2] and Visconti A[1]. On the presence of yeasts in cow and buffalo dairy products from the Southern Italy.

[1]Inst. Tossine, Micotossine da Parassiti Vegetali , CNR, Bari, Italy.

[2]Dept. of Med. Interna e Med. Pubblica, Sez. Igiene, University of Bari, Italy.

[3]Dept. of Biologia, Lab. Of Igiene, University of Lecce, Italy.

In recent years, economic losses due to spoilage by yeasts have increased in European companies because of the use of lower concentrations of preservatives, less severe preservation procedures, packaging in modified atmospheres, or new formulations that can allow the growth of yeasts. For this reason we studied the yeast population in cow and buffalo dairy products collected in Southern Italy. The study was carried out on 145 dairy products, of which 92 from cow (40 fresh, 36 middle and 16 long ripening) and 53 from buffalo (43 fresh, 2 middle and 8 long ripening). Yeast isolation and count were carried out on YGCA medium, with incubation at 30°C for 3-5 days. Yeasts were identified by API 20C Aux system. Yeasts were isolated from 73.9% and 56.6% of cow and buffalo products, respectively. About cow dairy products, *Torulopsis inconspicua* was predominant yeast (25.6%) among fresh samples, more frequently associated with other yeasts (*Candida* and *Torulopsis* spp). *Saccharomyces cerevisiae* was isolated in 11.6% of cases, principally in pure cultures. *C. albicans* was found in one stracciatella sample, associated with *T. inconspicua*. The range of mean total count (CFU/g) was between 2.2×10^3 and 1.1×10^5; the highest count was relevated among fresh cheeses. About middle ripening dairy products, *T.candida* was the most frequent yeast (15%), relevated in pure culture among scamorza samples, followed by *T.inconspicua* (15%) and *C.famata* (10.%). Moulds (*Penicillium roqueforti*, *P. expansum* and *P. aurantiogriseum*) were isolated from 10% of analyzed samples (especially in gorgonzola and caciotta samples) in mixed cultures with *Candida* spp. The range of mean count was included between 2.3×10^4 and $> 5 \times 10^5$. Also among long ripening dairy products, *T.candida* was the dominant yeast (31.2%). Moulds (*Alternaria alternata, Aspergillus sidowi, A.versicolor, G.penicillatum, P.commune, P.roqueforti, S.brevicaulis)* were found in 18.7% of analyzed samples, especially in provolone cheese, always in mixed cultures. Mean total count resulted between 5×10^2 and 3.5×10^4. About buffalo dairy products, *T.inconspicua* was isolated in pure culture in 46% of cases (in mozzarella particularly), followed by *C.kefyr* (11.4%) and *C.lusitaniae* (8.6%). Mean total count resulted between 3×10^2 and 2×10^5. Our study shows *T.inconspicua* is the predominant yeast among fresh samples. The presence of *T.candida* is demonstrated in middle and long ripening dairy products, frequently associated with other yeasts and moulds. Though they are enviromental contaminants, their occurence in dairy products, especially at high levels, could be a risk for human health, in particularly for immunocompromised patients. In addition, the isolation of *Candida albicans* from one stracciatella sample represents a significative result, because this yeast has been inclused among fecal indicators of human source.

POSTERS

1. Cappa F[1], Cocconcelli P.S.[1,2] . Identification of fungi from dairy products by means of 18 S rRNA analysis.

[1]Inst. of Microbiology, Università Cattolica del Sacro Cuore, Piacenza, Italy.

[2]Centro Ricerche Biotecnologiche, Università Cattolica del Sacro Cuore, Cremona, Italy.

Fungi are among the leading cause of spoilage of dairy products: yeasts carried by fruits are responsible of sweetened fruit yoghurt spoilage, inducing the alteration of the sensorial properties of this fermented product and the "doming" of the pot. Moreover, thread mould is a defect which occurs sporadically on surface of hard cheese, and packaged cheese, caused by the growth of fungi. The identification of fungi associated to spoilage of dairy products has been hampered by the lack of rapid system for the identification of these microorganisms. Over recent year the study of small subunits of ribosomal RNA has revolutionised the classification of microorganisms, both bacteria and fungi. These techniques based on the PCR amplification of genes coding for rRNAs and sequence comparison, offer a new tool for the identification and study of food associated fungi. A method for the rapid identification of food spoilage fungi has been developed during this work: colonies from agar plates or cells from food were mechanically disrupted and the DNA was purified by means of silica columns. The fungus-specific primers TR1 (5'-GTTTCTAGGACCGCCGTA-3') and TR2 (5'-CTCAAACTTCCATCGACTTG-3') were used to amplify a 581 bp fragment within the gene coding for the small ribosomal subunit (185 rRNA) of fungi. The resulting DNA fragment was sequenced using TR1 as sequencing primer, and the achieved sequences were compared with deposited sequences in the rRNA databases. This technique was applied to the study of fungi associated to spoilage of dairy products and hard packaged cheese and the most prevalent species identified were *Zygosaccharomyces microellipsoides,* in yoghurt, and *Cladosporium cladosporioides* and *Penicillium chrysogenum* in hard cheese.

2. Hansen, T.K., Jespersen, L. Jakobsen, M. Taxonomical and technological charateristics of *Saccharomycess* spp. associated with Blue veined cheese.

   The Royal Veterinary and Agricultural University, Dept. of Dairy and Food Science, Food Microbiology, Rolighedsvej., Frederiksberg C., Denmark.

Blue veined cheese often contains yeasts in high numbers. The dominant species in most cases is *Debaromyces hansenii*. *Saccharomyces* spp. occur less frequently but they are dominant in some blue mould cheeses in particular various Gorgonzola types. The taxonomy of the genus *Saccharomyces* has been changed significantly within the last years with more importance being placed on genotypic rather than phenotypic criteria. As a consequence the current taxonomic classification of *Saccharomyces* spp. associated with blue veined cheeses appears not to be known. The taxonomy of selected *Saccharomyces* yeast used either as commercial starter cultures or isolated from blue veined cheeses was elucidated by comparison to type strains of *Saccharomyces* spp. and *Saccharomyces* spp. isolated from other food sources. The isolates were classified by phenotypic characterisation, determination of chromosome length polymorphism by pulse field gel electrophoresis (PFGE) and by intergenic transcribed spacer (ITS)-PCR ribotyping. All strains originating from cheeses were found to belong to the *Saccharomyces sensu stricto* complex and could further be classified as *Saccharomyces cerevisiae*. Technological characteristics of five *Saccharomyces* isolates were studied. Lipolytic activity was investigated in agar diffusion systems and all the yeast strains isolated were capable of hydrolysing tributyrin. The degradation of casein by the *Saccharomyces* spp. were investigated by Capillary Electrophoresis. Only the *Saccharomyces cerevisiae* (FB7) were able to break down casein. A synergistic effect was found in the degradation of casein between *Penicillium roqueforti* and *Sacch. cerevisiae* (FB7) leading to a higher number and different grouping of the peptides. The positive interaction was also observed in cheese based model system. The strains of *Saccharomyces* spp. were screened for positive interaction with ten strains of *P. roqueforti*. Positive interactions were only demonstrated between the *Sacch. cerevisia*e (FB7) and the strains of *P. roqueforti*. The differences observed among the *Saccharomyces* spp. with regard to their technological characteristics and role in interactions indicates that not all the investigated strains were suitable as potential starter culture in blue veined cheese. To make use of yeast cultures in practice the cultures should be selected according to specific properties e.g. positive interaction with *Penicillium roqueforti*, lipolytic and proteolytic activity and assimilation or fermentation of lactic acid, lactate and residual lactose, galactose and glucose and be able to produce CO_2. The *Saccharomyces cerevisiae* FB7 seems to be the most suitable choice for a new starter culture and dairy trials have been carried out to verify *Sacch. cerevisiae* (FB7) as a potential starter culture in blue veined cheese.

3. Corbo M.R., Lanciotti R., Albenzio M., Sinigaglia M. Occurrence and characterization of yeasts isolated from milks and dairy products of Apulian region.

   Inst. of Produzione, Preparazioni Alimentari, University of Bari (Foggia), Foggia, Italy.

   One hundred and five strains of yeasts isolated from milk of different animal origin and from typical Apulian cheeses were studied to identify and characterize yeast strains for further selection as starter cultures for cheese production. The most prevalent isolates belonged to the species *Trichosporon cutaneum* (15,24%), *Candida catenulata* (10,48%) and *Yarrowia lipolytica* (8,57%). In order to evaluate the potential use as starter cultures, the occurrence of some selected properties, such as fermentation of glucose and lactose, assimilation of lactic acid, citric acid and lactose, growth at 4°C and production of lipolytic and proteolytic enzymes in the strains belonging to the most frequent species, was tested. *C. catenulata* and *C. zeylanoides* were positive for assimilation of lactic and citric acids and showed psychrotrophic aptitude. *T. cutaneum* was positive for all properties tested except for glucose and lactose fermentations. *Y. lipolitica* was endowed with remarkable lipolytic activity also at 4°C and was positive for assimilation of lactic acid, growth at 4°C and proteolytic activity.

4. Wojtatowicz M., Chrzanowska J., Juszczyk P., Skiba A., Gdula A. Identification and biochemical characteristics of yeast microflora of Rokpol cheese.

   Faculty of Food Technology Agricultural University of Wroclaw, Wroclaw, Poland.

Yeast microflora occuring on surface and inside of polish blue-veined cheese Rokpol, originated from three dairies located in south-west part of Poland, was evaluated. Yeasts were isolated and counted on OGY agar (Merck) supplemented with 0.2 % propionate. Representative strains were identified using API ID 32C tests and computer program APILAB Plus. Their proteolytic and lipolytic activities were investigated by plate diffussion tests. Further characteristics of their extra- and intracellular hydrolytic enzymes were done after cultivation of selected strains in model casein medium. Yeasts population on cheeses surface ranged from 10^6 to 10^8 cfu/g and inside cheeses it was lower by 1-2 logarithmic cycles. Among them the most frequently occured species: *Candida famata and C. spherica followed by C. lipolytica, C. intermedia and Saccharomyces kluyverii.* Majority of yeasts isolates revealed lipolytic acivity (ca. 90%) but only for one forth of them it was high. Their extracellular proteolytic activity was generally low and these proteases appeared to be more active against hemoglobin at acidic pH than against casein at pH 7.5. All yeast strains possesed higher intracellular proteolytic activity, as well as endo- and exo-peptidase activity. About 20% of strains were found to have especially high aminopeptidase activity. The highest level of this activity determined on Ala-pNA and Leu-pNA was observed for strains *C. lipolytica* KGIIW6a, *C. fam*ata WKIIIP6b and *C. spherica* WKIW3a.

5. Lourens Analie, Viljoen Bennie C. Growth and survival of dairy associated yeasts in yoghurt.

   Department of Microbiology and Biochemistry, University of the Orange Free State, Bloemfontein, South Africa.

The poor survival of probiotic bacteria is mainly due to the low pH of the yoghurt. Growth of yeasts in association with probiotic bacteria has been suggested with the intention to stimulate the growth of these organisms and to assure their survival. The probiotic yeast, *Saccharomyces boulardii* and four dairy associated yeasts, *Debaryomyces hansenii, Kluyveromyces*

marxianus, Yarrowia lipolytica and *Issatchenkia orientalis* were therefore incorporated into yoghurt and related dairy products. The survival and growth of the yeasts and probiotic bacteria were studied over a four-week period. Gas formation, pH, lactose utilisation and the production of organic acids were monitored at each interval. Based on the results obtained, the yeast species were able to progress in bio-yogurt reaching maximum counts exceeding 10^7 cfu/g. The number of yeast populations was substantially higher in the fruit based yogurt, mainly due to the presence of proportions of sucrose and fructose derived from the fruit. Despite the inability of some species to utilize lactose, the yeast species utilized available organic acids, galactose and glucose derived from bacterial metabolism of the milk lactose, as well as possible free fatty acids or free amino acids present in the dairy products. Excessive gas and alcohol production initiated by some yeast species proved, however, to be major constraints.

6. Fadda M. E., Cosentino S., Deplano M., Palmas F. Yeast populations in Sardinian Feta cheese.

   Dept. of Experimental Biology, Section of Hygiene University of Cagliari, Italy.

Feta cheese is the most popular traditional Greek cheese made from sheep's milk, but goats' milk may also be used or a mixture of both. In the last years this type of cheese is also being produced in Sardinia, one of the major ewe's milk producing regions in Italy. Many studies on yeasts occurrence in Greek feta cheese have been made but yeasts content in Sardinia feta cheese has not been reported yet. In this study the yeasts populations in feta cheese from two different Sardinian dairies was examined. 32 samples of feta of good quality and 10 samples of feta with slimy surface defect were examined from Dairy A. 23 samples of feta of good quality, 14 of feta with slimy surface and 6 of swelled samples were examined from Dairy *Kluyveromyces lactis* was the dominating species in feta from Dairy A (95.2% of samples) followed by *Debaromyces hansenii* (76,2%), *Dekkera anomala* (28,6%) amd *Dekkera bruxellensis* (19%). *Debaromyces hansenii* was dominant in samples from Dairy B (93%), followed by *Kluyveromyces lactis* (23,3%), *Geotrichum candidum* (23,3%) and *Dekkera anomala* (18,6%). No significant difference was observed between the occurrence of yeasts species in feta of good quality and in feta with slimy surface defect, thus confirming that slimy production is not associated with yeast contaminations. The swelling of sample observed in Dairy B seems to be caused by *Dekkera anomala*. In fact, this strong fermenting species was present in all swelled samples in numbers exceeding 10^6 CFU/g, while it was isolated in very low concentration in only 5,4% of good samples ($p<0.001$).

7. Cosentino S., Fadda M.E., Mulargia A.F., Palms F. Yeasts associated with Sardinian ewe's dairy products.

   Dept. of Experimental Biology, Section of Hygiene, University of Cagliari, Italy.

Yeasts play an important role both in production and spoilage of diary products. In fact they contribute to the taste and aroma of many cheese varieties but may also act as spoilage organisms, causing yeasty off-flavour, loss of texture quality, gas formation. In the present work the occurrence of yeasts in different types of typical Sardinian ewe's cheeses (32 samples of *pecorino*, 32 *caciotta*, 40 *feta*, 56 *ricotta*) was determined. On the strains isolated the following properties were studied: proteolytic and lipolytic activities, the ability to grow at different temperatures, different concentrations of salt, and to assimilate and/or ferment compounds like lactate, citrate lactose, glucose, galactose, lactic acid. Of 160 samples analysed 76.4% yielded growth of yeasts. Total yeast counts showed a certain variability among the sample. The highest levels were observed in caciotta and feta cheeses. A total of 281 strains belonging to 16 genera and 23 species were identified. In general *Debaryomyces hansenii* was the dominant species, being present in 28% of the total samples. Other frequently appearing species were *Geotrichum candidum*, *Kluyveromyces lactis* and *Kluyveromyces marxianus*. Other genera encountered were *Pichia*, *Candida*, *Dekkera*, *Yarrowia* and *Rhodotorula*. With regard to the biochemical and technological properties of the yeasts only *Kl. lactis*, *Kl. marxianus* and *Dekkera anomala* were able to assimilate and ferment lactose, whereas the majority of the species showed the ability to assimilate lactic acid. The assimilation of citrate was a characteristic of *Deb. hansenii, Rhodotorula rubra* and *Yarrowia lipolytica*. On the whole the yeasts were weakly proteolytic while lipolytic activity was present in several species. Only some stains of *Deb. hansenii* and *Kl. lactis* were able to grow at a 10% NaCl concentration. The results of our study confirmed that some of the yeast species isolated, e.g. *Deb. hansenii,* may play an active role in the ripening of sardinian cheeses whereas other species, such as *Yarrowia lipolytica* and *Rhodotorula rubra,* that are characterised by their strong enzymatic reactions, may cause spoilage of contaminated products when present in high numbers.

8. Viljoen, Bennie C. Interaction between yeasts and bacteria in dairy products.

   Dept Microbiology and Biochemistry, , University of the Free State, Bloemfontein, South Africa.

The general environment from which raw dairy products originate and the microbiological quality of the products in its processed state inevitably admit yeast growth and spoilage. Only part of the primary microflora survives under the selective pressures excerted by the intrinsic and extrinsic biotic factors present, processing procedures and preservatives. Yeasts that possess the proper physiological attributes to counteract the specific ecological determinants will be favored. Eventually, a particular yeast community will develop, and if the environmental factors permit, this characteristic yeast community will result in a specific association contributing positively or negatively to the final product. The association that develops between yeasts and bacteria is governed by specific key properties selecting for a few predominant yeasts. These yeasts may either stimulate or inhibit normal bacterial growth. The extent to which interaction between yeasts and bacteria contribute to the final product is investigated.

9. Kolak D., Leung T., Fleet G.H. Interactions between yeasts and bacterial species associated with cheese maturation.

   Dept. of Food Science and Technology, University of New South Wales, Sydney, New South Wales, Australia.

The maturation of mould-ripened soft cheeses involves a complex microbial ecology that comprises contributions from various species of yeasts, bacteria and filamentous fungi. The main yeasts include *Debaryomyces hansenii, Yarrowia lipolytica, Kluyveromyces marxianus, Saccharomyces cerevisiae* and

various *Candida* spp. The bacteria include various species of *Lactobacillus, Leuconostoc, Enterococcus, Staphylococcus, Micrococcus, Brevibacterium* and sometimes, Gram negative organisms. The ability to control the occurrence and growth of these species is important to management of product quality, but remains a challenge to cheese manufacturers because the factors which affect the presence of specific organisms are poorly understood. The potential of one microbial species or strain to affect the growth and survival of another species or strain is a significant factor in determining the microbiology of cheese maturation. Using the spot-on-lawn assay, we have examined the interactions which occur between various species of yeasts and bacteria that are associated with cheese maturation. Killer (antagonistic) interactions were found between various strains of *Debaryomyces hansenii* and between *D. hansenii* and *Pichia anomala*. These interactions were affected by the concentration of NaCl and pH. Some strains of *D. hansenii* enhanced the growth of *Yarrowia lipolytica, Kluyveromyces marxianus* and *Pichia membranaefaciens*. The growth of *Y. lipolytica* was also enhanced by strains of *Saccharomyces cerevisiae* and *P. anomala*. Some of these interactions were characterized by the development of yeast colonies with unusual appearances on agar media. Species of *Brevibacterium, Enterococcus, Micrococcus* and *Staphylococcus* did not show any antagonistic or stimulatory effects on the growth of a range of yeast species isolated from cheese. However, some strains of *D. hansenii, Y. lipolytica, K. marxianus* and *S. cerevisiae* stimulated the growth of *Staphylococcus* and *Enterococcus* spp isolated from cheese. The yeasts did not show any antagonistic effects on the bacteria examined.

10. Laubscher P.J., Viljoen B.C. The interaction between yeasts and bacteria during the manufacturing of mature cheddar cheeses.

    Dept. of Microbiology and Biochemistry, University of the Orange Free State, Bloemfontein, South Africa.

    The development of yeasts and bacteria in matured Cheddar cheese by using Bulk- and DVI-starters, from 6 separate days, was monitored during a 180 days ripening period. After 12 days, the numbers of yeasts reached their maximum (1.31×10^3 cfu/g), followed by a rapid decline. No yeasts were present after 3 months of maturation. Total bacterial counts as high as 5.18×10^8 cfu/g were observed after 48 hrs. The salting of the curd resulted in a decrease in total bacterial loads and an increase in yeast numbers. Coliforms as high as 8.6×10^2 cfu/g were present in the curd, but the numbers decreased to less than 10 cfu/g after 1 month. The pH of the cheese varied between 5.48 and 5.06 during the manufacturing of the curd and the ripening of the cheese. Moisture content remained between 41.45 % (curd) and 35.5 % (cheese after 6 months), whereas the salt concentration varied between 1.59 % and 2.50 % during the ripening period.

11. Loretan, T.[1], Mostert, J.F.[1], Viljoen, Bennie C.[2]. Interactions and fermentation characteristics of yeasts from traditional South African fermented milk.

    [1]ARC - Animal Nutrition and Animal Products Institute, Irene, South Africa.

    [2]Dept. of Microbiology, University of the Free State, Bloemfontein, South Africa.

    Fermentative and growth characteristics of three predominant dairy associated yeasts, *Kluyveromyces marxianus, Debaryomyces hansenii* and *Torulaspora delbrueckii*, originally isolated from naturally fermented milks in South Africa, were determined at different pH values, temperatures, lactose and lactate concentrations in UHT milk. Growth studies conducted at pH values ranging between 4.5 and 6.0 showed a general increase in viable cell numbers of all three species. At pH 4.0, however, only *T. delbrueckii* exhibited progressive growth. Although the optimum growth temperatures of the yeasts were established at 25°C, all three species progressed at temperatures of 7 and 35°C. With the exception of *T. delbrueckii* strains, the remaining two species showed marked increases in cell numbers in the presence of varying lactose and lactate concentrations at 25°C. The growth properties exhibited by these yeasts grown in milk, suggested a metabolically active role during, as well as at the end of lactic fermentation of milk. Differences in the growth patterns of the individual yeast species and lactic acid bacteria (different yeast:bacteria ratios) when grown simultaneously in non-fat milk were observed. The changes in pH, utilization of lactose, production of lactate, volatile flavour compounds, alcohol and CO_2 are also discussed.

12. Iklafeng, B., Viljoen, B.C. Interaction between yeasts and lactic acid bacteria during temperature abuse in yoghurt .

    Dept Microbiology and Biochemistry, University of the Free State, Bloemfontein, South Africa.

    Allergic reactions of consumers to foods and their contaminants are of increasing concern to health authorities and yeasts have been mentioned in this concern. The fermentative and spoilage activities of yeasts are well known in many food and beverage commodities while little attention has been given to the specific occurrence and significance of yeasts in dairy products. Since yeasts play a substantial role in the spoilage of commercial fruit yoghurts, especially when cold storage practices were neglected, the deterioration of yoghurt samples obtained from the manufacturers was evaluated at different temperatures for a period of 30 days. The highest number of yeast populations, up to 10^4 and 10^6 cfu/g, was found when yoghurts were exposed to abused temperatures in the range of 25°C, while insignificant counts were obtained from samples kept refrigerated at temperatures of 5°C. The most prevalent species isolated included strains of *Kluyveromyces marxianus*, *Saccharomyces cerevisiae,* C*andida famata, Candida krusei* and C*andida lusitaniae.*

13. Wyder M.T., Puhan Z. Role of selected yeasts in cheese ripening: evaluation in aseptic curd slurries.

    Laboratory of Dairy Science, Institute of Food Science, Swiss Federal Institute of Technology, ETH-Zentrum. Zürich Switzerland.

    Yeasts are encountered within the surface microflora of many cheese types, but no much is known about their direct contribution to cheese ripening. The objective of this study was to investigate, on a laboratory scale, the direct influence of yeasts to cheese ripening by means of aseptic cheese curd slurries. Isolates of all species found in three smear ripened cheese varieties were tested with respect to changes in pH, proteolysis and aroma development. Most yeasts could be assigned to two groups. One group was charecterised by the ability to ferment glucose, to utilise lactate, to increase pH and showing proteolytic

activity with mostly resulting alcoholic, acidic, fruity or fermented odour (*Clavispora lusitaniae, Pichia jadinii* and *Williopsis californica*). The second group was composed of non fermenting species which utilised lactate but did not affect pH (*Galactomyces geotrichum, Trichosporon ovoides* and *Yarrowia lipolitica*). They were proteolytic yielding a cheesy aroma. *Debaryomyces hansenii* B comprised characteristics of both groups.

14. Wyder[1] M.T., Bachmann[2] H.P., Puhan[1] Z. Role of selected yeasts in cheese ripening: an evalutation in foil wrapped Raclette cheese.

    [1]Laboratory of Dairy Science, Institute of Food Science, Swiss Federal Institute of Technology, ETH-Zentrum. Zürich, Switzerland.

    [2] Swiss Federal Dairy Research Station, FAM, Bern., Switzerland.

    The purpose of this study was to investigate selected yeasts (*Galactomyces geotrichum, Pichia jadinii, Yarrowia lipolytica, Debaryomyces hansenii*) for lactic acid utilisation, lipolysis, proteolysis and flavour development in foil ripened Raclette cheeses. An unreplicated 2^4 full factorial experimental design in two blocks of 8 vats was applied. In the mature cheeses, the lactic acid content was increased, probably as a result of increased lactic acid bacteria due tothe release of yeast metabolites. Yeasts seemed to show either esterase or lipase activity. Furthermore, yeasts revealed peptidolytic activity leading to an increase in smaller breakdown products and free amino acids. Except for *Galactomyces geotrichum,* they also enhanced the formation of biogenic amines. *Yarrowia lipolytica* was capable of improving the overall sensory characteristics of cheese, but all other species influenced the flavour rather negatively. Since hardly any yeasts were detectable in the mature cheese, the action by yeasts could be attributed to enzymes released after cell lysis.

15. Gdula[1] A., Vannini[2] L., Guerzoni[2] M.E., Chrzanowska[1] J. Chemico- physical features of cheeses obtained with *Yarrowia lipolytica* as co-starter.

    [1]Faculty of Food Technology - Agricultural University of Wroclaw, Wroclaw , Poland.

    [2] Dept. of Protezione , Valorizzazione Agroalimentare, University of Bologna, Bologna, Italy.

    The characteristics of each cheese type are determined by the influence of various factors, among which the type of starter culture plays one of the most important roles. Yeasts are frequently find in the microflora of a number of cheese varieties, especially soft-mould, smear and brine-ripened cheeses wherein they contribute to a flavour development in an uncontrolled way. *Yarrowia lipolytica* is one of the most predominant species isolated from dairy products, particularly associated with cheeses. It is known to have extracellular proteolytic and lipolytic activity which property has focused interest on *Yarrowia lipolytica* as a potential species in starter culture during cheese maturation. In this work the potential of this species as a cheese ripening agent has been evaluated in real system obtained from cow milk employing a traditional method. Microbiological analysis of microflora of cheeses obtained were conducted, Fourier transform infra-red spectroscopy (FTIR) as well as the chemical and physical characteristic of cheese were performed in order to evaluate all the changes induced by the enzymatic action of proteolytic enzymes of cultures starter of *Yarrowia lipolytica*. The results obtained suggest that *Yarrowia lipolytica* may be a good potential test organism for using yeast culture starter in cheese manufacturing.

We now list some recent publications and information on “Yeasts in Dairy Industry”.

Deptartment of Botany and Biological Engeneering, Instituto Superior de Agronomia, Lisboa Codex, Portugal - Communicated by P.V. Loureiro <Vloureiro@isa.utl.pt>.

---

1. Carreira, A., Paloma, L , Loureiro, V. 1998. Pigment producing yeasts in the brown surface discoloration of ewes' cheese. Int. J. Food Microb. **41**:223-230.

The appearance of a brown surface discoloration on Portuguese ewes' cheese has never previously been reported on. The regular occurrence of this defect over the past few years has caused serious financial losses to producers, which has led to growing interest in its study. This paper describes a preliminary approach to the problem, based on the hypothesis that pigment producing yeasts are involved. From a group of 51 yeast strains isolated from a number of brown rinds, it was possible to distinguish four pigment producing groups: group I (12 strains), produced an extracellular brown pigment from tyrosine and alkalised the tested media; group II (21 strains), produced a diffusible, reddish-brown pigment from resorcinol and alkalised the tested media; group III (three strains), alkalised the tested media without producing any pigments; group IV (15 strains), neither produced pigments nor alkalised the media. *Yarrowia lipolytica* and *Candida catenulata* type strains were also tested and their behaviour was similar to the strains in groups I and IV, respectively. The *Filobasidiella neoformans* type strain was distinct from all the other groups. The identification methods used for some strains in groups I, II and III suggest that *Yarrowia lipolytica* species is common to all strains in group I, and that *Debaryomyces hansenii* is present in both groups II and III. A study of several metal ions on the production of the brown pigment from tyrosine indicated Mn^{2+} to be a strong activator. Evidence is provided suggesting that the brown process may be related to tyrosine *Yarrowia lipolytica* metabolism.

2. Carreira, A., Loureiro, V. 1998. A differential medium to detect *Yarrowia lipolytica* within 24 hours. J. Food Mycol. **1**(1):3-12.

The ability of *Yarrowia lipolytica* to produce brown extracellular pigments from tyrosine was assessed. Although all strains tested were able to produce the pigments, the intensity of the final colour varied among the strains tested. Factors stimulating this process were sought in order to define a specific combination to maximise pigment production. Lactic acid, glycine, L-asparagine, peptone, Mn^{2+} showed a positive influence on the intensity of the brown colour produced. An inhibitory effect was observed for increased glucose concentrations. A medium containing peptone, Mn^{2+}, lactate, yeast extract and tyrosine induced the formation of strong brown colours within 24 h in all *Y. lipolytica* strains tested. From 44 yeast species also screened for the production of brown pigments from tyrosine, only the type strain of *Rhodotorula ingeniosa* produced a slight reddish colour within 24 h. As our results indicate that the ability to produce a rapid and intense brown pigmentation from tyrosine is, among yeasts, exclusive for *Y. lipolytica*, this differential medium is proposed for identification of this species. This medium is also proposed for use in quality assurance programmes for cheese, as *Y. lipolytica* has been associated with the occurrence of browning defects in cheese.

Dept. de Microbiologí, Facultad de Biologí Complutense de Madrid, Madrid, Spain - Communicated by Jose M. Peinado < Peinado@eucmax.sim.ucm.es>.

1. De Siloniz M. J., Gonzalo M. I., Peinado P. 1999. A differential medium for the isolation of *Kluyveromyces marxianus* and *Kluyveromyces lactis* from dairy products. J. Food Prot. **62**:189-193.

A selective and differential solid medium, called *Kluyveromyces* Differential medium (KDM), is described for the isolation *of Kluyveromyces marxianus* and *K. lactis* from dairy products. Its discriminative potential is based on the detection of the enzyme B-galactosidase, in the absence of lactose. Of the more than 95 strain tested, including yeasts, bacteria, and filamentous fungus, only the strains of *K. marxianus* and *K. lactis* produced blue colonies on the medium due to the presence of X-Gal /IPTG. The bacterial strains were not able to grow on KDM. On this basis, the medium was very satisfactory when testin naturally or experimentally contaminated dairy foods products. When quality assessment tests were performed, optimal values of productivity (growth and color) and selectivity were obtained for *K. marxianus* and *K. lactis*.

Institut fur Angewandte Mikrobiologie Univ.f.Bodenkultur Wien, Austria. Communicated by H. Prillinger <H.Prillinger@iam.boku.ac.at>.

1. Prillinger, H., Molná O., Eliskases-Lechner, F., Lopandic, K. 1999. Phenotypic and genotypic identification of yeasts from cheese. Antonie van Leeuwenhoek **75**:267-283.

Eighty-five yeast strains isolated from different cheeses of Austria, Denmark, France, Germany, and Italy were identified using physiological methods and genotypically using random amplified polymorphic DNA polymerase chain reaction (RAPD-PCR) analysis. Good congruence was found between the phenotypic and genotypic data for 39 of the isolates. However, 26 isolates of *Geotrichum* could only be identified to the species level using the genotypic methods and 7 isolates were correctly identified to the genus level only using phenotypic identification methods. The phenotypic identification did not agree with the genotypic data for 14 yeast isolates. Using ubiquinone analysis, yeast cell wall sugars and the diazonium blue B test 5 incorrectly identified isolates with phenotypic methods could be identified genotypically. In addition the 7 isolates identified only to the genus level by the phenotypic methods and the 26 *Geotrichum* strains were identified to the species level using the polyphasic molecular approach mentioned above. Eleven strains remained unidentified. The 76 identified yeast isolates were assigned to 39 species, the most frequent assignments were made to *Debaryomyces hansenii, Geotrichum candidum*, *Issatchenkia orientalis, Kluyveromyces lactis, K. marxianus, Saccharomyces cerevisiae, Yarrowia lipolytica*, and *Candida catenulata*. It is proposed that *Debaryomyces hansenii* (Zopf) Lodder et Kreger-van Rij and *Debaryomyces fabryi* Ota should be reinstated. The RAPD-PCR data reinforced the view that the species *Galactomyces geotrichum* is heterogeneous with all of the *Geotrichum* isolates from cheese products being assigned *G. geotrichum* group A *sensu* M. T. Smith. It is suggested that the name *Geotrichum candidum* be conserved for this rather common species.

Unilever Research / University of Amsterdam, Amsterdam, The Netherlands. Communicated by S. Brul <Stanley.Brul@unilever.com>.

1. Brul S., Klis 1999. Fungal Genetics & Biology **27**:199-208.

Fungal spoilage forms an increasing economic problem in the food industry. Chemical antifungals are becoming less attractive as food preservatives and hygiene agents due to the development of resistance and due to stricter legal regulations concerning the permitted concentrations. Finally, consumers tend to demand more 'naturally preserved' or preservative-free products. Here we review our understanding of the mechanisms of action and resistance to classical antifungals. Next, we evaluate the scientific basis underlying the application of novel, natural antifungals. Finally, we discuss the mathematical modelling of fungal growth and the development of preliminary predictive lag-time models. The eventual aim of the reviewed work is to generate mathematical lag-time models in real foods that predict the microbiological stability of the food, and are based on a mechanistic understanding of the chain of events that leads to cell death, or an extension of lag-time of the initiation of outgrowth.

2. Brul S., Coote 1999. Int. J. Food Micro. **50**:1-17.

Preservative agents are required to ensure that manufactured foods remain safe and unspoiled. In this review we will discuss the mode of action of both chemical and biological (nature-derived) preservatives and the stress response mechanisms induced by these compunds in micro-organisms of concern to the food industry. We will discuss the challenges that food manufacturers face with respect to the assurance of food safety and the prevention of spoilage. Following this, we will firstly discuss chemical preservatives, in particular, weak organic acids such as sorbic and benzoic acid which are widely used in preservation. Furthermore, we will discuss the mechanism of microbial inactivation with hydrogen peroxide mediated systems and chelators such as citric acid and EDTA and their potential use in preservation. Secondly, we will address the potential of naturally occurring 'preservatives'. Of the antimicrobial compounds present in nature we will first study the non-proteinaceous compounds often present in herbs and spices and speculate on the stress response(s) that micro-organisms may elicit to these natural compounds. Next we will address compounds that attack cell walls and membranes, for example, peptides, proteins and lytic enzymes. In discussing the resistance mechanisms against membrane and wall perturbation we will refer to the extensive knowledge of stress responses against osmotic stress and temperature stress. Finally, in the concluding paragraphs we will evaluate options for combination preservation systems.

International Commission on Yeasts

Minutes of the Meeting of Commissioners held at ISSY20, Smolenice, 24 May 1999

In Attendance: P. Biely (Slovak Rep.); M. Kopecka (Czech Rep.), R. Sentandreu (Spain); M. Breitenbach (Austria); P. Raspor (Slovenia); B. Prior (S. Africa); J. du Preez (S. Africa); H. Neujahr (Sweden); G. Fleet (Australia).

Apologies. Thanks to all those who registered apologies (too many to list).

Report of Chair - Graham H Fleet. The commissioner list has been culled and made current and workable in accordance with recommendations of previous meetings. Several new commissioners were proposed and accepted, these were: Professor S. Foda (Egypt); Dr. Jose Martinez Peinado (Spain); Dr. Merja Penttila (Finland), and Dr. Natalya Vesilyeva (Russia) to replace Dr N Elinov.

Symposium Reports

ISSY 20. Dr Biely summarised his experiences in coordinating this meeting and will prepare a detailed report for the December issue of the Yeast Newsletter.

ISY10 (Yeasts 2000) Papendal, The Netherlands, 27 August - 1 September 2000. Graham Fleet has had considerable correspondence with Lex Scheffers, Secretary of the Organising Committee for this major symposium.. Lex sent an outline of the program for discussion at ISSY20. Many keynote speakers and symposium sessions were now in place and the committee was very impressed with the planning to date. The first announcement is due for circulation around June 1999.

ISSY21. Biochemistry Genetics, Biotechnology and Ecology of non-Conventional Yeasts, Lviv, Ukraine, 19-23 August 2001. Andre Sibirny (Chair) has been in regular correspondence with Graham Fleet on progress.

ISSY22. Yeast and Fermentation, 2002, South Africa. James du Preez (Chair) reported that this will probably be held in a Wild Animal Game Park, in April. The commissioners discussed the theme of the symposium and suggested that it be made broader to include bioprocessing in the title - possibly "Yeast fermentations and other bioprocesses". James du Preez will finalise the name. This would have stronger appeal and attraction to participants. The Yeast Group of the International Dairy Federation (IDF) is also considering an international symposium in 2002 and there may be a possibility of organising this in South Africa the week before/after ISSY22, to help attract participants to the region. Graham Fleet will contact IDF to investigate this possibility.

ISSY23 (2003) and beyond. The following informal proposals have been received: Saprophytic Yeasts, T. Deak, Hungary. Morphogenesis, R. Sentandreu, Spain. Immobilised Yeasts, M. Becker, Latvia. Pathogenic Yeasts, R. Prasad, India.

Because 2003 is some years ahead, these proposals were only briefly discussed. There was a view that the theme of immobilised yeasts was too specialised and that this topic could be incorporated into ISSY 22. Professor Sentandreu proposed a good case for Morphogenesis. Graham Fleet is to seek more details from the various proponents for discussion at a future meeting of commissioners.

ISY11, 2004. Clete Kurtzman (USA) informally raised this topic at ISSY19 but further information needs to be submitted to commissioners for discussion.

Graham H Fleet
Chair, International Commission for Yeasts

Minutes of the Meeting of Commissioners held during IUMS, Sydney, 17 August 1999

In Attendance. S. Meyer (USA), C. Kurtzman (USA), A. Lachance (Canada), I. Russell (Canada), G. Stewart (UK), I. Spencer-Martins (Portugal), B. Hahn-Hagerdahl (Sweden), A. Gibriel (Egypt), G. Naumov (Russia), H. van Dijken (The Netherlands), M. Penntila (Finland), I. Pretorius (South Africa), K. Watson (Australia), G. Fleet (Australia).

Apologies. Thanks to the numerous apologies.

Report of Chair - Graham H Fleet. Graham welcomed Marj Penntila, Sakkie Pretorius and Ken Watson who were attending their first meeting of commissioners and thanked all for attendance, especially Clete Kurtzman, Andre Lachance, Isabel Spencer-Martins, Barbel Hahn-Hagerdahl, Gernadi Naumov and Ken Watson who presented papers at the IUMS Congress within the symposium, **Yeast Biodiversity**, organised by ICY.

The commissioner list is now in an up-to-date, active and operational format, listing 74 commissioners who represent 32 countries. However, there is a need for active commissioners from three key countries where yeast research is significant, Germany, France and Switzerland. The Committee agreed that Graham initiate correspondence to recruit commissioners from these countries, as well as others, especially in the S E Asia, Asia region.

Symposium Reports

ISY 10 (Yeasts 2000). Hans van Dijken reported good progress in the organisation of this meeting for next year at Papendal, and the first circular had been widely distributed. A second circular should be distributed late 1999 or early 2000.

ISSY 21. No further developments since the report at the meeting of commissioners at ISSY 20. Graham will keep in contact with Andrei Sibirny.

ISSY 22. No further developments since the report at the meeting of commissioners at ISSY 20. Graham has been in contact with the International Dairy Federation (September 1999) and it has been decided that the next (2002) symposium on **Yeasts In The Dairy Industry** will be held in Europe rather than South Africa. Consequently, there is no prospect of a "back to back" meeting with ISSY 22.

ISSY 23 and beyond. These proposals are outlined in the minutes of the meeting held at ISSY 20. The Committee considered these proposals to be at a very preliminary stage and should be scheduled for further discussion at our next meeting at ISY 2000. Graham will contact the various proponents beforehand so that more detailed information can be presented to the next meeting of commissioners.

General. There was general discussion about the lack of communication between the International Union of Microbiological Societies (IUMS) administration and its various Commissions or Committees which included the ICY. The problem lay with the IUMS organisation. The ICY is a Commission of the Mycology Division within IUMS. The new chair of the Mycology Division, Inge Russell, (one of our Canadian Commissioners) will endeavour to address this issue.

Isabel Spencer-Martins suggested that ICY establish a website on the internet. There was strong support for this proposal and Graham agreed to initiate this action.

Graham H Fleet
Chair, International Commission for Yeasts

Recent Meetings

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

The traditional annual conference on yeasts organized regularly by the Czech and Slovak Commission for Yeasts of the Czechoslovak Society for Microbiology and the Institute of Chemistry, Slovak Academy of Sciences, in Smolenice Castle, the popular Congress Center of the Slovak Academy of Sciences, was replaced this year by an international event. During May 23-27, 1999, Smolenice Castle hosted the 20th ISSY on "Yeast Cell Surfaces and Membrane Phenomena." The symposium was held under the auspices of the Federation of European Microbiological Societies (FEMS). FEMS provided the Young Scientists Grant to enable free participation to 13 scientists under 35 from various countries. The symposium was attended by 75 scientists from 20 countries and five continents. Its main theme was chosen to cover the main areas of yeast research in the Czech and Slovak

Republics. It was the fourth time that the ISSY was organized in Smolenice Castle. The 1st ISSY was held in Smolenice Castle in 1971, and so were the 9th ISSY in 1983 and the 14th ISSY in 1990.

The opening ceremony of the 20th ISSY took place on Sunday afternoon, May 23rd, and was moderated by Dr. Peter Biely, the chairman of the Organizing Committee and the chairman of the Czech and Slovak Yeast Commission. Speeches were given by the chairman of the International Commission for Yeasts (ICY) Prof. Graham H. Fleet from the University of South Wales in Sydney, Australia, Prof. Libor Ebringer, long time chairman of the Czechoslovak Society for Microbiology and a FEMS representative and delegate, Prof. Marie Kopecka, member of the ICY, and Dr. Jan Hirsch, Director of the Institute of Chemistry, the coorganizing institution.

The organizers were successful in attracting as plenary speakers leading scientists from all over the world and from the major areas of yeast research covered by the scientific program.

In the first section, devoted to **Cell Wall Structure and Biogenesis**, the following plenary lectures were presented:

G.H. Fleet (Australia): Diversity in the composition, structure and significance of yeast cell walls.

F.M. Klis, J.C. Kapteyn (The Netherlands): Dynamics of the molecular architecture of the cell wall of *Saccharomyces cerevisiae.*

R. Sentandreu, M. V. Elorza, S. Mormeneo, E. Valentin, H. Rico, F. Dubón (Spain): Fungal cell wall and dimorphism.

M. Breitenbach, E. Bogengruber, H. Farkas, R. Schricker, M. Rützler, T. Eichberger, P. Briza (Austria): Biochemistry and genetic regulation of the dityrosine pathway in *Saccharomyces cerevisiae.*

S. Shahinian, G.J.P. Dijkgraaf, T. Ketela, **H. Bussey** (Canada): Genetic studies on β-1,6-glucan synthesis in *S. cerevisiae* .

E. Cabib, J. Drgonová, T. Drgon, Dong-Hyun Roh (USA): The GTP-binding protein Rho1p controls cell wall synthesis and polarization in yeast.

M. Ecker, V. Mrsa, S. Strahl-Bolsinger, **W. Tanner** (Germany): Protein O-mannosylation and cell wall proteins.

A. Janik, M. Sosnowska, H. Krotkiewski, **G. Palamarczyk** (Poland): GDPMannose corrects defect in the synthesis of dolichol-linked saccharides in *S. cerevisiae.*

The second session, **Yeast Cytoskeleton**, featured the following plenary lectures:

J.R. Pringle, H. Harkins, N. Pagé, L.Schenkman, A. McKenzie, H. Bussey (USA): Roles of transmembrane, cell-wall-anchored glycoproteins in the bipolar budding pattern of *S. cerevisiae*.

D. Drubin (USA): The actions and interactions of the yeast actin cytoskeleton.

M. Osumi, M. Konomi, T. Takagi, S.H. Ishijima, B.M. Humbel, J. Ishiguro (Japan): Actin cytoskeleton is a conductor of cell wall formation of fission yeast.

J. Hasek, J. Palecek, L. Valasek, K. Malinska, P. Kovarik, J. Schneider, S.D. Kohlwein, H. Ruis (Czech Republic): Interaction of translation machinery with cytoskeleton in *Saccharomyces cerevisiae*.

V.J. Cid, **L. Adamikova**, R. Cenamor, M. Molina, M. Sanchez and C. Nombela (Slovakia): The role of septins in morphogenesis for septum development.

D.C. Amberg, A. Rodal, J. Tetreault, P. Lappalainen, D.G. Drubin (USA): Aip1p affects cofilin sorting and is an activator of cofilin mediated actin filament disassembly.

M. Kopecka, K. Takeo, M. Yamaguchi, M. Ohkusu, K. Hata, M. Gabriel, A. Svoboda (Czech Republic): Actin cytoskeleton in two human fungal pathogens *Aureobasidium pullulans* and *Cryptococcus neoformans.*

T. Toda, P. Radcliffe, L. Vardy, S. Katayama (United Kingdom): Cytoskeleton and cell polarity control in fission yeast.

A. Svoboda, I. Slaninová, M. Kopecká, M. Gabriel, O. Necas (Czech Republic): Restoration of the cytoskeleton during yeast protoplast regeneration.

The Last session of the meeting was devoted to **Membrane Transport and Membrane Phenomena**:

A. Goffeau (Belgium): Multiple drug-resistance genes in the yeast genome.

J. Subík (Slovakia): Antifungal antibiotic mucidin: From mode of action to multiple drug resistance.

K. Sigler, N. Stadler, B. Brodská, L. Váchová, M. Höfer (Czech Republic): Effect of oxidative stress on the yeast plasma membrane.

B.A. Prior, G. Kayingo, S. Hohmann (South Africa): Conservation of polyols in yeasts during osmotic stress.

M. Höfer, S. Heiland , H. Lichtenberg (Germany): Sugar transporters and ion channels of the *Schizosaccharomyces pombe* plasma membranes.

C.L. Slayman (USA): Active transport of potassium, and the structures of the Trk proteins in *Saccharomyces.*

C.W. Slayman (USA): Structure and function of the yeast PMA1 H+ ATPase.

R. De Philippis, A. Bastianini, L. Granchi, A. Messini, M. Vincenzini (Italy): Low-fermentation-temperature-induced changes in phospholipids and fatty acid composition of a cryotolerant *Saccharomyces cerevisiae* strain.

J.C. du Preez, S.H. de Kock, S.G. Kilian (South Africa): The relationship between nutritional requirements, membrane transport and continuous culture kinetics of *Saccharomyces cerevisiae* strains.

P. Raspor, M. Batic, K. Draslar, P. Jamnik, Dj. Josic, R. Milacic, M. Pas, M. Recek, V. Rezic-Derani, M. Skrt (Slovenia): The problem of chromium compounds bioaccumulation in yeasts.

The outstanding oral program went smoothly and without any changes. It was complemented by the 32 posters listed below.

J.O.M. Johansson, A.S. Byström (Sweden): Mutants linking tRNA biosynthesis/translation and cell wall formation?

K. Grabinska, T. Berges, F. Karst, G. Palamarczyk (Poland, France): Possible involvement of 26S proteasome in degradation of yeast farnesyl diphosphate synthase.

P. Capek, N. Kolarova (Slovakia): The capsular polysaccharides of *Cryptococcus laurentii.*

J. Sandula, G. Kogan, E. Machová, D. Chorvatovicová (Slovakia): Structure and biological activity of the yeast cell wall surface mannans.

G. Kogan, E. Machová, J. Sandula (Slovakia): Skeletal glucan

of *Saccharomyces cerevisiae* and its immunomodulating properties.

P. Bartek, N. Kolarova (Slovakia): Soluble microbial galactosyltransferase.

V. Puchart, M. Vrsanská, P. Biely (Slovakia): Inducible aryl b-glucosidase – an integral protein of the cell walls of *Cryptococcus albidus.*

S.K. Waghmare, Z. Lobo (India): Extragenic suppressor of *pdc2* mutant from yeast.

H. Martin, A. Dagkessamanskaia, J. François (France): Involvement of *KNR4* gene product in cell wall synthesis in *Saccharomyces cerevisiae.*

J.M. Rodriguez-Pena, V.J. Cid, M. Sánchez, C. Nombela, J. Arroyo (Spain): Crs1 and Crs2 code for cell wall-related proteins in *Saccharomyces cerevisiae.*

A. Holubárová, A. Svoboda (Czech Republic): Meiosis and sporulation in fission yeasts: the effect of cytoskeletal inhibitors.

J. Palecek, J. Hasek, H. Ruis (Czech Republic, Austria): Rpg1p/Tif32P, a subunit of the translation initiation factor, interacts with actin-associated protein End4P/SLA2P.

L. Adamíková, V.J. Cid, M. Molina, M. Sánchez and C. Nombela (Slovakia, Spain): Localization of wild type and mutant Cdc 10p-GFP fusions in *Saccharomyces cerevisiae.*

M. Havelková, E. Unger (Czech Republic, Germany): Calmodulin in cells and protoplasts of *Yarowia lipolytica.*

P. Mudry, P. Müller, E. Smejkalová, O. Necas (Czech Republic): Cold resistance of microtubules induced by UV irradiation in *Saccharomyces cerevisiae.*

K. Varecková, P Kempná, L. Sabová, P. Polcic, J. Kolarov (Slovakia): Targeting information within mitochondrial ADP/ATP carrier (AAC) protein investigated by fusion with green fluorescent protein (GFP) and b-galactosidase.

Y. Gbelská, M. Obernauerová, J. Subík (Slovakia): Structure of mitochondrial membranes and the requirement of mitochondrial genome integrity in yeast.

O. Kinclová, H. Sychrová (Czech Republic): Cloning of the *Zygosaccharomyces rouxii HOG1* gene.

D. Gásková, B. Brodská, K. Sigler: Interaction of yeast cells with killer toxin K1 studied by a fluorescence method for membrane potential assay.

N. Stadler, B. Brodská, M. Höfer, K. Sigler (Germany, Czech Republic): Sites of oxidative damage to the yeast *PMA1* H^+-ATP-ase.

S. Sesták, V. Farkas, K. Sigler, B. Brodská, D. Gásková (Slovakia, Czech Republic): Permeabilization of yeast cells induced by osmotic stress.

S. Sesták, V. Farkas (Slovakia): Effect of hyperosmotic shock on the activity of glucan synthase in *S. cerevisiae*.

P. Bafrncová, D. Smogrovicová, F. Malík, J. Pátková (Slovakia): Ethanolic fermentation at high sorbitol concentration.

J. Pátková, D. Smogrovicová, Z. Dömény, P. Bafrncová (Slovakia): Cell wall polysaccharides of free and immobilised yeast.

D. Smogrovicová, J. Pátková, P. Bafrncová, Z. Dömény (Slovakia): Trehalose and glycogen of stressed yeast cells.

E. Stratilová, E. Breierová, E. Machová: The influence of the stress on the production and the properties of cell wall bound polygalacturonases of yeasts.

E. Breierová, V. Sasinková, E. Stratilová (Slovakia):FT-IR spectroscopic study on the pectin utilized by the yeasts.

J. Sajbidor, E. Breierová, M. Lamacka (Slovakia): Effect of new ergosterol inhibitors on growth and lipid composition of some pathogen yeasts.

M. Pas, P. Jamnik, S. Prah, M. Batic, P. Raspor (Slovenia):Effect of chromium compounds on yeast viability.

P. Jamnik, M. Batic, P. Raspor (Slovenia):Model for measurement of yeast viability vs. mortality in the presence of chromium compounds.

E. Sláviková, B. Kosiková (Slovakia):The ability of yeast cells to modify surface-active lignin biopolymers.

A. Tomsiková (Czech Republic):*Candida dubliniensis* – a new emerging pathogen?

In addition to the program and abstract book, all participants of the symposium received a new publication of the Czech and Slovak Commission for Yeasts "Forty Years of Activity in Czech and Slovak Yeast Research - Period 1990-1999". The book is the fourth volume in a series that informs scientific community about the achievements of Czech and Slovak yeast researchers at approximately 10 year intervals. Based on the reaction of all participants, the 20th ISSY can be considered to have been a great success both scientifically and socially. The location and the size of the symposium contributed significantly to the friendly working atmosphere and high spirit of the meeting. The symposium was an occasion to hold the meeting of ICY commissioners. The minutes of this business meeting will be reported separately by Prof. Graham H. Fleet, Chair of ICY.

Peter Biely, Chair, Czech & Slovak Yeast Commission

Forthcoming Meetings

Yeast Genetics and Molecular Biology Meeting
University of Washington, Seattle Wa, July 25-30 2000

Y2K YCM Program Committee: Karen Amdt, Judith Berman, Jef Boeke, Martha Cyert, Beth DiDomenico, Stan Fields, Jim Haber, Mark Hochstrasser, Anita Hopper, Vickie Lundblad, Mark McCammon, Dave Kaback, Michael Lichten, Lorraine Pillus, Frank Rozennnreig, Mike Snyder, Reg Storms, Robin Wright, Mike Yaffe.

We welcome suggestions for this and future meetings. Please send your ideas via email to: Robin Wright <wrightr@u.washington.edu> or to any other committee member.

The facilities at the University of Washington include excellent meeting space for both poster and platform sessions, as well as dorm housing with views of Lake Washington and Mount Ranier. Downtown Seattle is a ten-minute bus ride from campus and provides interesting opportunities for dining, shopping, sightseeing, and other entertainment.

Deadlines: Abstract submission, April 3 2000; Registration at reduced rates and housing reservation, June 19 2000.

For further information see: http://genome-www.sfanford.edu/Saccharomyces/yeast2000
email the YGM meeting manager, Anne Marie Mahoney, at <amm60@aol.com>, or call the Genetics Society Administrative Office at 301-571-1825.

Tenth International Symposium on Yeasts - The Rising Power of Yeasts in Science and Industry
Sunday 27 August - Friday 1 September, 2000, Papendal, Arnhem, The Netherlands

The 10th International Symposium on Yeasts will bring together scientists from all disciplines involved in the study of yeasts and yeast-like organisms: Physiologists, geneticists, taxonomists, molecular biologists, biotechnologists, food microbiologists and medical mycologists. The Symposium will be structured for optimal interaction between scientists working in these fields, thus stimulating new developments in yeast research in the third millennium.

Organizing Committee: Hans van Dijken (Delft University of Technology), chairman. Lex Scheffers (Delft Univ. of Technology), general secretary. Pieter de Geus (Gist-brocades), treasurer. Wendel Iverson (Heineken Techn. Managem.), industrial liaisons. Ria Komen (Congress Office ASD), administrative affairs.

Scientific Committee: Hans van Dijken, Jack Pronk, Lex Scheffers

Symposium structure: Mornings: Plenary session (except on Friday): 3 Keynote speakers, introducing 3 afternoon sessions. Afternoons: 3 Parallel sessions (except on Friday). Convenors are keynote speakers of morning session. Programme proposed by convener + co-convener. Chairpersons: convener and co-convener. One lecture by co-convener. Short oral presentations selected from submitted abstracts. Evenings: Poster view sessions. Special sessions: continuation of afternoon sessions; workshops; other initiatives by conveners in consultation with organizing committee. Social events. Posters: Continuous display, up to 250. Session conveners together with Scientific Committee will, on basis of poster abstracts, select for short oral presentations in afternoon sessions. Registration and poster mounting: Sunday, 27 August 2000, 16.00-21.00, followed (19.00-21.30) by a Get-together party with food, drinks, and music.

Session themes and invited speakers (acceptation received): - Functional genome analysis - Oliver (UK); Medical yeasts - Calderone (USA), Sullivan (IRL); Food yeasts - Fleet (AUS), Raspor (SLO); Stress responses and signal transduction - Thevelein (B),Hohmann (S); Evolution - Van der Walt (Safr); Taxonomy, phylogenetics, evolution - Kurtzman (USA), Boekhout (NL); Regulation of carbohydrate metabolism - Entian (D), Grivell (NL); Metabolic engineering - Pretorius (Safr.), Pronk (NL); Beverages. Walsh (NL), Leão (P); Biodiversity and ecology - Lachance (CAN), Smith (NL); Heterologous protein production and secretion - De Geus (NL), Van Urk (UK); Alcohol from carbohydrates - Hahn-Hägerdal (S), Penttila (FIN); Cell cycle - Alberghina (I), Porro(I); Transport and energetics - Lagunas (E), Boles (D); Cell wall and flocculation - Klis (NL); Organelle biogenesis and function - Veenhuis (NL).

Important dates (tentative):

Final announcement/ registration form	1 May 2000
Deadline for abstracts	1 March 2000
Selection of abstracts	1 March-1 April 2000
Decision to authors	15 April 2000
Pre-registration deadline	15 March 2000

Accommodation at Papendal: Large hall, 400 seats. Medium halls. Small halls and rooms. Poster hall, 250 posts. Hotel rooms, tentative prices: Dfl. 50 - 120 pppd. Sports accomodations, incl. swimming pool. Meals.

Registration fee: Early Dfl. 1,175. Late Dfl. 1,350. Including meals, coffee, tea. Excluding hotel room. Financial support will be sought for (young) scientists. Symposium dinner: Dfl 150

Financial affairs: Auspices and guarantee of the Netherlands Foundation for Biotechnology. FEMS: support for young scientists.

Inquiries and correspondence:

Lex Scheffers
Department of Microbiology and Enzymology
Delft University of Technology
Julianalaan 67
NL-2628 BC Delft, The Netherlands

Tel. +31 15 278 2411
Fax: +31 15 278 2355
E-mail: lex.scheffers@stm.tudelft.nl

Yeasts of the Third Millenium - 21th International Specialized Symposium on Yeasts - 21 ISSY 2001 Biochemistry, Genetics, Biotechnology and Ecology of Non-conventional Yeasts (NCY). Lviv, Ukraine, 19-22 August, 2001

The Symposium will be held in the conference hall of the main building of Lviv State University. Lviv (also known as: Lvov, Lwow, Lemberg, Leopoli) is the largest scientific, cultural and economic city in the Western Ukraine with population near 1 mln located in the geographical center of Europe. The topics will include (preliminary list): Systematics of NCY. Ecology. Methods of NCY Molecular Genetics; Chromosome Structure and Genome Organization. Genome Sequencing in NCY. Regulation of Gene Expression. Metabolic Regulation. Organelles. *Saccharomyces* versus Non-*Saccharomyces*: Similarities and Differences. Membrane Structure and Functions. Stress Response. Heterologous Gene Expression. Biochemical Engineering. Industrial Applications. Medically Important Yeasts.

International Scientific Committee: Gerold Barth, Dresden Technical University, Germany (*Yarrowia lipolytica*). James M. Cregg, Oregon Graduate Institute, Portland, USA (heterologous gene expression, organelles). Graham H. Fleet, University of New South Wales, Sydney, Australia (ecology). Laura Frontali, Rome University "La Sapienza", Italy (*Kluyveromyces*). Sergei G. Inge-Vechtomov, St. Petersburg University, Russia (genetics). Cornelis P. Hollenberg, Duesseldorf University, Germany (heterologous gene expression). Cletus P. Kurtzman, Center of Agricultural Research, Peoria, USA (systematics). Jesus Pla, Madrid University, Spain (*Candida albicans*). Andrei A. Sibirny, Institute of Biochemistry, Lviv, Ukraine (metabolic regulation). Suresh Subramani, University of California at San Diego, La Jolla, USA (organelles). Masamichi Takagi, The University of Tokyo, Japan (*Candida maltosa*). Yuri A. Trotsenko, Institute of Biochemistry and Physiology of Microorganisms, Pushchino, Russia (biochemistry). Marten Veenhuis, Groningen University, The Netherlands (ultrastructure).

Local Organizing Committee: Andrei A. Sibirny, Inst. Biochem., Lviv, Chairman. Mykhailo V. Gonchar, Inst. Biochem., Lviv, Treasurer. Daria V. Fedorovych, Inst. Biochem., Lviv. Stepan P. Gudz, Lviv State University. Aleksandr R. Kulachkovsky, Lviv State University. Valentyn S. Pidgorsky, Inst. Microbiol. Virol., Kiev. Oleh V. Stasyk, Inst. Biochem., Lviv. Vira M. Ubyivovk, Inst. Biochem., Lviv.

Secretariat:

Lviv Division of Institute of Biochemistry
Drahomanov Street, 14/16
Lviv 290005
Ukraine

Phone: +380-322/740363
FAX: +380-322/721648
e-mail: ISSY2001@biochem.Lviv.ua

Brief News Items

Employment Opportunity - Yeast Physiologist - Lallemand Inc.

We have an opportunity in Montréal for a Ph.D. with 3-5 years of experience in yeast fermentation and interested by the industry. We are looking for an excellent yeast physiologist. **Contact:**

Dr. Richard Degré
<rdegre@lallemand.com>

Assistant Professor Position - Ecology - University of Western Ontario

ECOLOGIST. The Department of Plant Sciences at the University of Western Ontario invites applications for a tenure-track **ASSISTANT PROFESSOR** position in ecology, beginning **July 1, 2000**.

The successful applicant's research interests should be in the areas of ecology of plants and/or microorganisms at the population, community or ecosystem level. The applicant should have a broad based background in ecological methods, a good knowledge of plant diversity, and expertise in advanced quantitative data analysis and/or molecular approaches.

The preferred applicant will have a Ph.D. and appropriate postdoctoral training or equivalent experience and a proven research record including publications of high quality and will be expected to develop a vigorous and innovative research program, well supported by external funding. The applicant should also have the ability to work well with others and provide evidence of ability in teaching, conveying enthusiasm and ideas that will excite and inspire students, and to contribute to the department's commitment to excellence in both undergraduate and graduate teaching.

Further information about the position, the department and the University can be found at http://www.uwo.ca/plantsci.

Applications including a curriculum vitae and copies of recent significant papers should be forwarded to **Dr. I.F. Creed**, Chair, Ecologist Search Committee, Department of Plant Sciences, University of Western Ontario, London, Ontario, CANADA N6A 5B7. Please provide the names and addresses of three external referees who would be willing to assess your work and abilities. Applications will be accepted until January 31, 2000 or until a suitable candidate is found.

The University of Western Ontario is committed to the principle of equity of employment, welcomes diversity in the workplace, and encourages applications from all qualified individuals, including women, members of visible minorities, aboriginal persons, and persons with disabilities. In accordance with Canadian immigration requirements, priority will be given to Canadian citizens and permanent residents of Canada. All appointments are subject to budgetary authorization.

Kluyveromyces Genome Database

At the International Conference on *Kluyveromyces* which was held in Rimini (Italy) in May, Dr Hiroshi Fukuhara suggested that many of you may have unpublished fragments of *Kluyveromyces* DNA sequences "in your desk drawers". For example, during the cloning of a gene, you might have obtained a few sequencing runs from the neighboring gene. Dr Fukuhara showed many interesting examples from his own lab, in Rimini.

I am very interested in this sort of data because it can tell us a lot about the evolution of the *Kluyveromyces* genome and its relationship to the *S. cerevisiae* genome (see Keogh et al, Yeast **14**:443, 1998, and Ozier-Kalogeropoulos et al, NAR **26**:5511, 1998). It would also be useful to share this information because, for example, one lab might already have found accidentally a gene that another lab is trying to clone.

I would like to gather together all the sequence fragments of this type. I hope to do two things: (1) If I receive a good response, I will set up a web site containing the *Kluyveromyces* data (gene name, linkage information, name of the lab providing the data). If you are willing to make your sequences public, I will also put the sequences themselves on the web site and submit them to GenBank, but you can keep your sequence private if you prefer. A similar website is already successfully in operation for the *Candida albicans* community (http://alces.med.umn.edu/Candida.html -- see the "unpublished sequences" link). (2) If there are interesting evolutionary results, I hope to write a paper and will include as co-authors everybody who sends me data. It will thus be a joint enterprise by the *Kluyveromyces* community.

Any sequences will be useful, even 100 basepairs of low-quality single-strand sequence, whether or not they have homology to *S. cerevisiae*.

If you would like to participate in this project, please send me your sequences (by e-mail or regular mail) in any format. If you have lost the electronic copies of a sequence but still have a printout, just post me a copy of the printout and I will type it in.

Dr. Ken Wolfe
Department of Genetics
University of Dublin
Trinity College
Dublin 2, Ireland

Tel: +353-1-608-1253
Fax: +353-1-679-8558
<khwolfe@tcd.ie>

Change of Address: M. T. Wyder

I have moved from the Laboratory of Dairy Science, ETH Zentrum, Zurich, to the following address:

Dr. Marie-Therese Wyder
Swiss Federal Dairy Research Station FAM
Liebefeld
Schwarzenburgstr. 161
CH-3003 Bern, Switzerland

Tel: 031 323 82 23
Fax: 031 323 82 27
<marie-therese.wyder@fam.admin.ch>