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Y E A S T

A Newsletter for Persons Interested in Yeast

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Editorials

Dr. Nellie Margaretha Stelling-Dekker
28 May 1905 - 24 October 1998

We are saddened by the passing of Dr. Nellie Stelling-Dekker, a pioneer of yeast systematics. Her work paved the way to the enviable taxonomic system currently available for the classification and identification of yeast species. Dr. A. Scheffers kindly wrote on some of the highlights of Dr. Stelling-Dekker's contributions.

Network: Yeasts in Food and Beverages

In this issue appears the first communication from the network on yeasts in food and beverages, prepared by Dr. P. Romano. As evidenced by the number of entries, this field of yeast research is very active. It is hoped that this group will continue to share the progress of its work in this manner with our readers on a regular basis.

M.A. Lachance
Editor

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

The following papers have been published recently.

1. Golubev, W.I. 1998. New species of basidiomycetous yeasts, *Rhodotorula creatinovora* and *R. yakutica*, isolated from permafrost soils of Eastern-Siberian Arctic. Mykologiya i Phytopathologiya **32**:8-13.

Descriptions of two new species of the genus *Rhodotorula* are given to accomodate five yeast isolates. These yeasts are non-pigmented, sensitive to *R. glutinis* mycocin, glucuronate- and nitrate-positive. They use both creatine and cretinine as the only nitrogen source. Thenew species show the most close resemblance to *R. muscorum* but they differ from it in GC-content and sensitivity patterns to *R. mucilaginosa* mycocins.

2. Golubev, W.I. 1998. Killer activity of *Tilletiopsis albescens* Gokhale: Taxonomic and phylogenetic implication. System. Appl. Microbiol. **21**:429-432.

Killer activity expressed at pH values ranging from 3.5 to 8.0 was found in the *T. albescens* VKM Y-2822. Its killer phenotype was cureless. The toxin excreted with a molecular mass above 10 kDa is fungicidal, thermolabile, sensitive to proteinase K and was specified as a mycocin. The latter does not act against ascomycetous, sporidiobolaceous and tremellaceous yeasts. Its killing pattern includes the species of the genera *Exobasidium, Farysia, Pseudozyma, Sporisorium, Ustilago*, and also *Protomyces* and *Taphrina*. In contrast to all other *Rhodotorula* species, *Rh. acheniorum*, *Rh. bacarum*, *Rh. hinnulea* and *Rh. phylloplana* are sensitive to *T. albescens* mycocin. The host range of the mycocin studied is discussed from taxonomic and phylogenetic viewpoints.

3. Golubev, W.I. 1999. *Mastigobasidium*, a new teleomorphic genus for perfect state of ballistosporous yeast *Bensingtonia intermedia*. Int. J. System. Bacteriol. **49**(3): in press.

A new genus, *Mastigobasidium*, is proposed for teliospore-forming xylose-lacking ballistosporogenous glucuronate-positive yeasts. The distinguishing features of the genus are germination of teliospore by several long aseptate hyphe; curved phragmometabasidia developed on the apices of these hyphae; production of basidiospores on a peg in clusters. The type strain of heterothallic nitrate-negative species *M. intermedium* is VKM Y-2720 (*Bullera intermedia* type strain) and the allotype strain is VKM Y-2727 (*Sporobolomyces weijmanii* type strain).

II. State Institute for Genetics and Selection of Industrial Microorganisms, I Dorozhnyi 1, Moscow 113545, Russia. Communicated by G.I. Naumov.

Recent publications.

1. G. I. Naumov. 1999. Divergent Population of *Saccharomyces paradoxus* in the Hawaii Islands: an *in statu nascendi* yeast species. Doklady Biol. Sci. **364**(2): in press.

2. G.I. Naumov, E.S. Naumova & P.D. Sniegowski. 1998. *Saccharomyces paradoxus* and *Saccharomyces cerevisiae* are associated with exudates of North Arnerican oaks. Can. J. Microbiol. **44**:1045-1050.

Genetic hybridization and karyotypic analyses revealed the biological species *Saccharomyces paradoxus* and *Saccharomyces* by Phaff in 1961 and 1952 were reidentified as *S. paradoxus*. Each strain studied showed a unique profile of chromosomal hybridization *cerevisiae* in exudates from North American oaks for the first time. In addition, two strains collected from elm flux and from *Drosophila* with a probe for the retrotransposable element Tyl. The wild distribution of natural *Saccharomyces sensu stricto* yeasts is discussed.

III. Department of Biochemistry and Molecular Biology, University of Kansas Medical Center, Kansas City, Kansas, U.S.A. 66160-7421. Communicated by W.N. Arnold <warnold@kumc.edu>.

Recent publication.

1. Arnold, W.N., T. Binh, P. Nguyen, and L.C. Mann. 1998. Purification and characterization of a dextranase from *Sporothrix schenckii*. Arch. Microbiol. **170**:91-98.

A dextranase (EC 3.2.1.11) was purified and characterized from the IP-29 strain of *Sporothrix schenckii*, a dimorphic pathogenic fungus. Growing cells secrete the enzyme into a standard culture medium at 2 °C, that supports the mycelial phase. Soluble bacterial dextrans substitute for glucose as substrate with a small decrease in cellular yield, but a 10-fold increase in the production of dextranase. This enzyme is a monomeric protein with a molecular mass of 79 kDa, a pH optimum of 5.0, and an action pattern against a soluble 170 kDa

bacterial dextran which leads to a final mixture of glucose (38%), isomaltose (38%), and branched oligosaccharides (24%). In the presence of 200 mM sodium acetate buffer (pH 5.0), the K_m for soluble dextran is 0.067±0.003 % (w/v). Salts of Hg^{2+}, $(UO_2)^{2+}$, Pb^{2+}, Cu^{2+} cross-linked dextran chains in Sephadex G-50 to G-200, and the latter was a good substrate for cell growth at 20°C. Highly cross-linked grades, i.e. G-10 and G-25, were refractory to hydrolysis. Most strains of *S. schenckii* from Europe and North America tested positive for and Zn^{2+} inhibit by affecting both V_{max} and K_m. The enzyme is most stable between pH values of 4.50 and 4.75, where the half life at 55°C was 18 min, and the energy of activation for heat denaturation was 99 kcal/mol. *S. schenckii* dextranase catalyzed the degradation of dextranase when grown at 20°C. All of these isolates grew on glucose at 35°C, a condition which is typically associated with the yeast phase, but they did not express dextranase and were incapable of using dextran as a carbon source at the higher temperature.

IV. Fermentec s/c ltda and Esalq/USP, R. Treze de Maio, 768 salas 43/44, CEP 13400-900, Piracicaba, SP, Brazil. Communicated by H.V. Amorim <fermentec.ltda@merconet.com.br>.

The following paper was recently published.

1. Fernandes, E.A.N., Nepomuceno N., Trevizam A.B. & Amorim H.V. 1998. From potential to reality: Yeasts derived from ethanol production for animal nutrition. J. Radioanal. Nucl. Chem. **234**(1-2):113-118.

The high costs of cereals and vegetable protein supplements used for animal nutrition have directed much attention toward non-conventional alternative protein sources. Brazil has a significant potential to provide such material, since it is the world's largest producer of ethanol (13 billion liters per year) derived from fermentation by yeasts (sugar cane being the basic raw material). Distilleries are recovering surplus yeast to produce dry yeast for use in animal food formulations. With regard to the yeast biomass elemental composition, INAA analyses performed on a pool of samples from various different fermentations have shown the presence of various trace elements, *e.g.* As, Br, Ca, Ce, Co, Cr, Cs, Eu, Fe, Hf, K, La, Na, Rb, Sc, Sm, Th, and Zn. This reinforces the need for additional studies concerning the suitability of yeast in terms of maximum tolerable levels of these elements in formulations for domestic animals.

V. The American Type Culture Collection, 10801 University Boulevard, Manassas, VA 20110-2209, U.S.A. Communicated by K.T. Gu <http://www.atcc.org> <kgu@atcc.org>.

The world's largest yeast genetic resource center now resides at the American Type Culture Collection. Gu, Kelong Tom, Ph.D., Yeast Genetic Resource Center, Jong, Shung-Chang, Ph.D., Director of Microbiology Division.

The American Type Culture Collection (ATCC®) has been awarded a three-year grant by the NIH to combine the Yeast Genetic Stock Center (YGSC) and the ATCC yeast genetic collection into the world largest Yeast Genetics Resource Center. The eminent yeast geneticist, Dr. Robert K. Mortimer, established YGSC in 1960, at University of California, Berkeley. Over the last 40 years, YGSC has collected thousands of *Saccharomyces cerevisiae* strains from laboratories all over the world, and distributed tens of thousands of the strains to the yeast community. Dr. Mortimer and his co-workers used these genetically marked strains to construct the well-known genetic map of *S. cerevisiae*, which provided the basis for the complete DNA sequencing of the whole yeast genome (1).

Upon Dr. Mortimer's retirement, the 1,276 *S. cerevisiae* strains have been transferred to ATCC, where they will continue to exist as distinct collection, complementing ATCC's existing collection of 5,996 yeast strains, which include 1,360 *S. cerevisiae* strains bearing documented mutations. Today, ATCC maintains tens of thousands of yeast strains representing 20 species and 12 genera. With the determination of the yeast genome sequence, the goal of the Saccharomyces Genome Deletion Project (SGDP) 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 entire collection of deletion strains (24,000) are being generated by the consortium laboratories over the two-year period. The deletion mutants are made available to the research community after they arrive to ATCC. A total of 10,000 yeast genomic deletion strains have been transferred to the ATCC collection. In addition, ATCC also maintains plasmids useful for molecular biological research with yeasts, such as *S. cerevisiae/Escherichia coli* cloning and expression shuttle vectors, *S. pombe/E. coli* shuttle vectors, YACs and cosmids, genomic and cDNA libraries, and clones derived from yeast sources. The collection holds a set of clones of membrane protein genes as well as some *S. cerevisiae* genomic and cDNA libraries. ATCC also offers a set of clones constructed by Olson and co-workers [Olson et al. (1986); Link and Olson (1991); Riles et al. (1993)] for the physical mapping of *S. cerevisiae* genes, and some of the cosmids that were used for the sequencing of the yeast genome. The genetic strains carried by the YGSC include auxotrophic markers, temperature-sensitive lethals, markers conferring sensitivity to radiation, fermentation markers, mitochondrial markers, morphological markers, and other broad categories of mutants. The YGSC mutant alleles are very useful for functional studies of the corresponding gene. In particular, point mutations may help to reveal the molecular function of the corresponding protein. Most mutations are available in both mating types, some even in different genetic backgrounds. These deletion strains allow you to screen large numbers of yeast mutants individually or in genome-scale systematic analysis.

ATCC offers all the molecular biology and genetic tools necessary to study the yeast gene, as well as the expression of other foreign genes in yeast. Our molecular biology products can be used in the yeast two-hybrid system, yeast shuttle vector system, yeast expression system, and cloning-by-function system that uses yeast as a host to isolate the homologs of genes from other organisms. Yeast genetic strains can also be used to study other eukaryotic organisms or human disease due based on functional similarities. In addition, ATCC plans to add growth media and experimental reagents for customer convenience. ATCC will construct new yeast genetic strains for investigators upon special request. Easy access to yeast genetic resources will increase the use of yeast as a model organism.

Housing the YGSC at ATCC enhances it's security, utility, andavailability to the scientific community. The YGSC catalog will be accessible and searchable via ATCC's web site <www.atcc.org>. ATCC will support the YGSC users with information by phone, fax, mail, and e-mail. ATCC's web site also provides for electronic order placement, forms, and permits required for certain strains, and links to other databases or directories of information.

Requests for strains and plasmids should be sent to:

Yeast Genetic Resource Center
American Type Culture Collection
10801 University Blvd.
Manassas, VA 20110, USA

Phone: (800) 638-6597 (North America)
(703) 365-2700
Fax: (703) 365-2750
E-mail: <sales@atcc.org>

Reference:

Cherry, J.M., Ball, C., Weng, S., Juvik, G., Schmidt, R., Adler, C., Dunn, B., Dwight, S., Riles, L., Mortimer, R.K., and Botstein, D. (1997) Genetic and Physical Maps of *Saccharomyces cerevisiae*. Nature **387** (6632 Suppl.):67.

VI. Department of Plant Sciences, University of Western Ontario, London, Ontario N6A 5B7. Communicated by M.J. Butler.

The following paper was published recently.

1. M.J. Butler and A.W. Day. 1998. Fungal melanins: a review. Can. J. Microbiol. **44**:1115-1136.

The relationship of polyketide melanogenesis molecular biology to that of nonmelanin-producing pathways in a wide range of fungi and other organisms is discussed. Analytical methods and fundamental properties of melanins are discussed and fungal melanin properties are compared with those of animal and bacterial melanins. The enzymatic degradation of melanins by lignin peroxidases is described.

VII. Centraalbureau voor Schimmelcultures, P.O. Box 273, NL 3740 Ag Baarn, The Netherlands. Communicated by G.S. de Hoog <De.Hoog@cbs.knaw.nl>.

The following CBS publication has appeared recently.

1. CBS. 1999. Studies in Mycology **43**: Ecology and evolution of black yeasts and their relatives.

Contents.

VIII. 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. K.C. Thomas, S.H. Hynes and W.M. Ingledew. 1998. Initiation of anaerobic growth of *Saccharomyces cerevisiae* by amino acids or nucleic acid bases: ergosterol and unsaturated fatty acids cannot replace oxygen in minimal media. J. Indust. Microbiol. **21**:247-253.

Nine out of ten industrially important strains of *Saccharomyces cerevisiae* did not grow in minimal media under anaerobic conditions even when ergosterol and unsaturated fatty acids were provided. Anaerobiosis was maintained either by flushing the culture flasks with prepurified nitrogen or by incubating the flasks in an anaerobic chamber. Traces of oxygen present in "prepurified nitrogen gas" were sufficient to initiate yeast growth and on their removal by catalytic means the yeasts failed to grow. The yeast grew very well anaerobically if the medium was supplemented with a mixture of amino acids or with a mixture of purines and pyrimidines. The growth initiated by including a mixture of amino acids was further enhanced when the medium was supplemented with ergosterol and an unsaturated fatty acid. Since no oxygen requirement for the synthesis of amino acids or purines and pyrimidines has been demonstrated, growth promotion by these compounds under anaerobic conditions is most likely not by eliminating the need for oxygen for their synthesis. We suggest that the amino acids and the nucleic acid bases yielded, through some hitherto unknown reactions, small amounts of molecular or usable form of oxygen which allowed key reactions essential for "anaerobic" growth to proceed.

2. S. Wang, W.M. Ingledew, K.C. Thomas, K. Sosulski, and F.W. Sosulski. 1999. Optimization of fermentation temperature and mash specific gravity for fuel alcohol production. Cereal Chem. **76**:82-86.

The effects of fermentation temperature and dissolved solids concentration adjusted by changing mashing water-to-grain ratios on wheat fermentation efficiencies, fermentation times, final ethanol concentrations and ethanol production rates were studied by using Response Surface Methodology (RSM). Final ethanol concentrations in fermentors depended primarily on mash specific gravities. Predictably, increases in fermentation temperatures dramatically reduced fermentation times and thereby shortened fermentation cycles. Highest ethanol production rates were achieved with a high fermentation temperature of 30 °C and a low water-to-grain ratio of 2.0. At these settings, an ethanol concentration of 13.6% v/v, was attained with a fermentation time of 54 h, and an ethanol production rate of 2.45 ml ethanol/L/h. Optimization of operating conditions suggested in the current study will provide existing fuel alcohol plants with increased productivity without alteration of plant equipment or process flow.

3. C. A. Patterson and W. M. Ingledew. 1999. Utilization of peptides by a lager brewing yeast. J. Amer. Soc. Brew. Chem. **57**:1-8.

The nutritional quality of selected peptides [$(Ala)_2$, $(Asp)_2$ and $(Leu)_2$] and their constituent amino acids were compared by examining the growth response of the commercial lager brewing strain, *Saccharomyces cerevisiae* NCYC 1324 (formerly *Saccharomyces uvavum* NCYC 1324), to these nitrogen sources in a defined medium. Single amino acids were better sources of nitrogen than were single sources of each homodipeptides. Peptides [$(Ala)_2$ and $(Leu)_2$] and non-peptide nitrogen sources (ammonium sulphate, allantoin, arginine, leucine, proline and urea) were combined to increase the nutritional complexity of the defined medium. A synergistic effect on growth resulted in faster growth rates and shorter growth cycles. HPLC analysis of these culture supernatants demonstrated *Saccharomyces cerevisiae* NCYC 1324 utilized amino acids and peptides in a specific order. In binary mixtures of nitrogen sources, amino acids, ammonia, allantoin, and urea were preferred and appeared to inhibit the utilization of dipeptide from culture medium. In complex nitrogen mixtures (3 amino acids and 3 dipeptides), *Saccharomyces cerevisiae* NCYC 1324 simultaneously used both amino acids and peptides as sources of nitrogen. This research shows that these model dipeptides are definitely used as additional sources of nitrogen for continued yeast growth in a defined medium. Physiological conditions that influenced nitrogen repression of peptide utilization were also defined. It appears that the presence of ammonium ion in a defined culture medium inhibited peptide utilization inside the yeast cells, while leucine enhanced the ability of the yeast to utilize peptides.

4. C. A. Patterson and W. M. Ingledew. 1999. Effect of nitrogen source and concentration on the uptake of peptides by a lager yeast in continuous culture. J. Amer. Soc. Brew. Chem. **57**:9-17.

Continuous culture was used to demonstrate that both the type and the concentration of nitrogen source supplied in the medium influence the rate of peptide uptake by *Saccharomyces cerevisiae* NCYC 1324. Lower rates of peptide transport were observed in cells grown under ammonium- or allantoin-limitation or with excess ammonium or allantoin. Cells cultured in the chemostat with leucine (limiting or excess concentrations) exhibited the highest transport rates. Nitrogen starvation of ammonium-limited yeast cells removed the repressive effect of ammonium on peptide transport. The addition of leucine to ammonium- or allantoin-limited cells induced peptide transport. Physiological conditions that result in the repression, derepression and induction of peptide transport in the lager brewing yeast, *S. cerevisiae* NCYC 1324 were defined.

IX. Microbial Properties Research, National Center for Agricultural Utilization Research, Agricultural Research Service, United States Department of Agriculture, Peoria, Illinois 61604. Communicated by C.P. Kurtzman.

Recent publications.

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

Approximately 500 species of ascomycetous yeasts, including members of *Candida* and other anamorphic genera, were analyzed for extent of divergence in the variable D1/D2 domain of large subunit (26S) ribosomal DNA. Divergence in this domain is generally sufficient to resolve individual species, resulting in the prediction that 55 currently recognized taxa are synonyms of earlier described species. Phylogenetic relationships among the ascomycetous yeasts were analyzed from D1/D2 sequence divergence. For comparison, the phylogeny of selected members of the Saccharomyces clade was determined from 18S rDNA sequences. Species relationships were highly concordant between the D1/D2 and 18S trees when branches were statistically well supported.

2. C.P. Kurtzman and C.J. Robnett. 1998. Three new insect-associated species of the yeast genus *Candida*. Can. J. Microbiol. **44**:965-973.

Three new species of *Candida* are described that were determined to be genetically isolated from all other currently accepted ascomycetous yeasts based on their sequence divergence in the species-variable D1/D2 domain of large subunit (26S) ribosomal DNA. One of the species was isolated from an ambrosia beetle, whereas the other two were from the frass of wood-boring beetle larvae. The new species and their type strains are the following: *Candida ontarioensis* NRRL YB-1246 (CBS 8502), *Candida tammaniensis* NRRL Y-8257 (CBS 8504), and *Candida tripodendroni* NRRL Y-6488 (CBS 8505).

3. C.P. Kurtzman & B.S. Dien. 1998. *Candida arabinofermentans*, a new L-arabinose fermenting yeast. Antonie van Leeuwenhoek **74**:237-243

Candida arabinofermentans (type strain NRRL YB-2248, CBS 8468), a new yeast that ferments the pentose L-arabinose, is described. The three known strains of this new species were isolated from insect frass of pine and larch trees in the U.S. Phylogenetic analysis of nucleotide sequences from the DI/D2 domain of large subunit (26S) ribosomal DNA places *C. arabinofermentans* among the methanol-assimilating yeasts and most closely related to *Candida ovalis*. Strains of the new species produce 0.7-1.9 g/l ethanol from L-arabinose.

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

Recent publications.

1. Minárik, E. and Jungová, O. 1999. Yeast ghosts preparations and their application in winemaking (in Slovak). Vinohrad (Bratislava) **37**(1):16-17.

Yeast ghost preparations and microcrystalline cellulose stimulate the metabolism of wine yeasts *Saccharomyces cerevisiae* and related species of the genus *Saccharomyces*. The stimulation is manifested by an almost double intake of sugars in the lag-phase of yeasts, in more intensive alcohol formation and more sugar utilization of the grape juice. A more pronounced fermentation ability of yeasts caused by the prolongation of the stationary phase and by delay of the mortality of yeast cells as well as lower volatile acid formation induced by the modification of the fermentation glycero-pyruvate pathway could be observed. The preferential use of yeast ghost preparation in grape must fermentation under special conditions in winemaking is proposed.

2. Jungová, 0. and Minárik, E. 1999. FERMAID® - a complex preparation for the activation of alcoholic grape must fermentation (in Slovak). Vinohrad (Bratislava) **37**(3): in press.

The complex preparation FLRMAID® "E" shows favourable influence on the fermentation start and subsequent fermentation of grape must which is considerably accelerated even in unfavourable fermentation conditions, above all at low fermentation temperature, lack of nutrients or presence of inhibitory substances for yeasts.

3. Minárik, E. 1999. Reasons of sluggish or stuck grape must fermentation. (in Slovak) Kvasný průmysl (Prague) **45** (accepted for publication).

Retarded, sluggish or stuck grape must fermentations cause unfavourable consequences not only on wine quality but also on the economics of winemaking. A series of considerable and less serious factors may influence the course of fermentation, such as lack of thiamine, assimilable nitrogen, dissolved oxygen, inhibition of alcohol and C_8-C_{10} fatty acids may be considered as those negative factors decreasing yeast cell metabolism. Technological aspects are discussed in detail.

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

The following are abstracts of papers in press.

1. C. Groth, J. Hansen, and J. Piskur. A natural chimeric yeast containing genetic material from three species. Int. J. Syst. Bacteriol. (in press).

The *Saccharomyces* sp. CID1 isolate (CBS 8614) and several other *Saccharomyces sensu stricto* yeasts were analyzed for their mitochondrial and nuclear genes. Our data show that *Saccharomyces* sp. CID1, found so far only in one location in Europe, is a natural hybrid between three different *Saccharomyces* yeast species. Two of them, *S. cerevisiae*-like and *S. bayanus*-like are ubiquitous, and contributed parts of the nuclear genome, the third, *Saccharomyces* sp. IFO 1802-like, which has been found only in Japan, contributed the mitochondrial DNA molecule. These data suggest that the yeast cell is able to accommodate, express and propagate genetic material which origjnates from different species, and the resulting natural hybrids very existence indicates that such hybrids are well adapted to their habitats.

2. R.F. Petersen, T. Nilsson-Tillgren, and J. Piskur. Karyotypes of *Saccharomyces sensu lato* species. Int. J. Syst. Bacteriol. (in press).

An improved pulsed-field electrophoresis program was developed to study differently sized chromosomes within the genus *Saccharomyces*. The number of chromosomes in the type strains was shown to be 9 in *S. castellii* and *S. dairenensis*, 12 in *S. servazzii* and *S. unisporus*, 16 in *S. exiguus* and 7 in *S. kluyveri*. The size of individual chromosomes was resolved and the approximate genome sizes determined by the addition of individual chromosomes of thekaryotypes. Apparently, the genome of *S. exiguus*, which is the only *sensu lato* yeast to contain small chromosomes, is larger than that of *S. cerevisiae*. On the other hand other species exhibited sizes which were 10-25% smaller than *S. cerevisiae*. Well defined karyotypes represent the basis for future genome mapping and sequencing projects, as well as studies of the origin of the modern genomes.

3. R.F. Petersen, G. Marinoni, M.L. Nielsen, and J. Piskur. Molecular approaches for analyzing diversity and phylogeny among yeast species. In: Dimorphism in human pathogenic and apathogenic yeasts (J.F. Ernst and A. Schmidt, eds.)

1. Introduction
2. Diversity of yeast
3. Identification and classification
4. Principles of molecular phylogeny
5. Molecular phylogeny of the genus *Saccharomyces*
6. Genome structure of the genus *Saccharomyces*
7. Mitochondrial DNA molecule
8. Is the molecular approach always right?
9. Hybrids and mosaic genomes
10. Horizontal transfer of genetic material

XII. Department of Food Science and Technology, Dalhousie University, P.O. Box 1000 Halifax, NS B3J 2X4. Communicated by A. Speers <Alex.Speers@Dal.Ca>.

The following are recent publications.

1. Jin, Y-L. and Speers, R.A. 1999. Flocculation in *Saccharomyces cerevisiae*. Food Res. Int. **31**:421-440.

This paper reviews our current understanding of cell flocculation with particular emphasis of the process in brewing fermentations. While cell flocculation has been examined for over a century and has been the subject to a number of reviews in the early part of this decade, our view of the process is cloudy. Flocculation is affected by cell genetic behavior, cell age as well as the chemical and physical nature of the surrounding medium. Recently, a number of advances in our understanding of the genes governing the process have occurred. In conjunction with these genetic advances, new assay methods have also been developed. This review will discuss and update our current knowledge of cell flocculation and its use in brewing fermentations.

2. Jin, Y-L. and, Speers, R.A. 1999. Cell surface hydrophobicity of flocculent brewing yeast: Its relationship to cell flocculation and the effect of environmental conditions. Accepted for presentation at the CIFST Annual Meeting, June 6-9, Kelowna, BC.

The cell surface hydrophobicity (CSH) and ANS-hydrophobicity of two brewing yeast strains were measured by use of hydrophobic interaction charomatography and a fluorescent probe respectively. The CSH was determined via hydrophobic interaction chromatography using a Phenyl-Sepharose CL-4B column. As well, an anilino-naphthalene sulphonate (ANS) probe was employed to detect hydrophobic cavities on yeast cell surface. Presence of ethanol (0-10% v/v) in a pH 4.0 calcium containing sodium acetate buffer influenced CSH for both haploid and polyploid strains ($P<0.05$). In the pH range of 3.8-5.8 (similar to pH changes during fermentation) CSH showed different responses for the two strains. Temperature (5-25°C) had no effect on CSH of the polyploid strain ($P>0.05$) but resulted in an increased tendency of hydrophobic association for the haploid strain at low temperatures ($P<0.001$). Cell age, EDTA and α-methyl-D-mannopyranoside (a lectin blocking agent) all effected cell hydrophobicity. A correlationship ($r=0.684$) between CSH and flocculation of the two strains has been found over the conditions investigated above ($P<0.001$).

3. Jin, Y-L. and Speers, R.A. 1999. The effect of environmental conditions on the flocculation of *Saccharomyces cerevisiae*. Accepted for presentation at the ASBC Annual Meeting, June 19-23, Phoenix, AZ.

The flocculation behavior of two *S. cerevisiae* strains expressing either Flo1 or NewFlo phenotype were examined. The behavior of the two strains was examined after varying ethanol (0-10 %), pH (3.8-5.8), ionic strength (0.01-0.20 M) and temperature (5-25 °C). The flocculation behavior of Flo1 cells was insensitive to ethanol and pH 3.2 and 11.0 Kcal/mol for Flo1 and NewFlo respectively, indicating distinct sensitivities to temperature. Interestingly, flocculation inhibition by urea was overcome by washing with 100 mM acetate buffer (20 °C, pH 4, containing 1 mM Ca^{++}) presumably due to the reversible unfolding of zymolectin molecules. A semi-empirical model was developed that indicated that the flocculation behavior is affected change. Flocculation of NewFlo cells significantly increased with increases in ethanol concentration ($P<0.05$) and pH values ($P<0.01$). Increasing ionic strengths and decreasing temperatures significantly ($P<0.01$) retarded flocculation in both strains. The apparent activation energy of flocculation at pH 4 and 10^8 cells/ml was estimated to be by the cell volume fraction for both Flo1 ($R^2 = 0.98$) and NewFlo ($R^2 = 0.94$) strains. This semi-empirical model allows adjustment of Helms values due to variation in cell volume fraction thus partially explaining reported variations of Helms values with respect to fermentation time.

XIII. Culture Collection of Yeasts, Institute of Chemistry, Dúbravská cesta 9, 842 38 Bratislava, Slovakia. Communicated by E. Breierova <chememi@savba.sk>.

The following are abstracts of articles that were published recently.

1. Breierova, E., Kacurakova, M. and Stratilova, E. 1997. The effect of higher concentration of NaCl in the cultivation medium on changes in the composition of extracellular yeast glycoproteins. System. Appl. Microbiol. **20**:348-355.

Six yeast extracellular glycoproteins were isolated from different cultivation media containing higher concentration of NaCl (4%, 8%) or without NaCl. The glycoproteins produced under NaCl stress and physiological conditions differ mainly in the higher content of mannose in carbohydrate moiety and glutamic acid in the protein moiety. The correlation between osmotic stress and the composition and structure of the produced glycoproteins was observed. Chemical analysis and FT-IR spectroscopy was applied to investigation of these glycoproteins that are known to be associated with ability of binding water and protection of water-regime of cells.

2. Breierova E., Stratilova E., Sajbidor J. 1997. Salinity affects fatty acid and extracellular glycoprotein composition of *Dipodascus australiensis.* Food technol. Biotechnol. **35**:237-241.

Significant changes in the fatty acid composition of the cell lipids and extracellular glycoproteins of the yeast-like species *Dipodascus australiensis* grown under NaCl stress or in salt-free conditions were observed in the different growth phases. In the cells which were cultivated under hypertonic conditions, oleic acid content increased during the exponential growth phase while mean percentage of linoleic acid decreased. Significant changes in the fatty acid composition of the cell lipids occurred between the late exponential phase and early stationary phase of growth between ca. 3rd to 5th day for salt-free cultivation medium and between 6.5 and 8.5 day for hypertonic conditions. The resistance of yeast cells to osmotic-stress (caused with higher concentration of NaCl) can be correlated with the production of the extracellular glycoproteins. NaCl-stressed samples contained more mannose, galactose, and less glucose and glucosamine. The glycoproteins containing glutamic acid were produced under the influence of 8% NaCl predominantly during the early stationary phase of the growth.

3. Stratilova E., Breierova E., Vadkertiova R., Machova E. Malovikova A., Slavikova E. 1998. The adaptability of the methylotrophic yeast *Candida boidinii* on media containing pectic substances. Can. J. Microbiol. **44**:116-120.

The methylotrophic yeast *Candida boidinii* is able to utilize pectic substances as the only carbon source. The primary utilization of methanol released from pectin is followed by adaptation to pectate medium. The duration of activation of a secondary pathway was critical for survival of yeast in the absence of other carbon sources. The utilization of pectin-containing media is associated with the production of pectic enzymes. The main polygalactouronase activities were found within the upper layer of the cell wall as shown by the method of gradual ultrasonication.

4. Breierova E. 1998. Yeast exoglycoproteins produced under NaCl-stress conditions as efficient cryoprotective agents. Biological Procedures Online, www.science. unwaterloo.ca/~mwreimer/bpo.html.

Six extracellular yeast glycoproteins were prepared from three yeast species in osmotic equilibrium and unequilibrium environments and used as non-penetrating cryoadditives. Glycoproteins secreted by the strain *Dipodascus australiensis* into growth medium containing NaCl (8%w/v) were found to be the most effective cryoadditives. It was possible to use these glycoproteins alone (without DMSO as penetrating agent) for the cryoprotection of studied yeasts.

5. Breierova E., Stratilova E. 1998. Extracellular polysaccharides as protection of the yeasts to osmotic stress. Chem. Paper **52** (Focus Issue): 372-373.

6. Grabinska-Loniewska A., Pajor E., Slavikova E. 1997. Comparative analysis of yeast-like potentially useful to environmental biotechnology. Acta Mycologica **32**:99-106.

Five yeast-like species recommended for enhancing the bioremediation of wastewaters, soils and exhaust gases contaminated with recalcitrant compounds were analyzed with respect to their morphological characteristics and physiology.

7. Kosikova B., Slavikova E. 1997. Biochemical modification of lignin by yeast species. Biomass in energy, environment chemicals, fibers and materials (ed. R.P. Overend, E. Chornet) Vol. **2**:979-985. Pergamon, an imprint of Elsevier Science.

It has been reported that yeasts can utilize monomeric phenolic compounds. In spite of this, there is no information about the interaction of yeast with lignin macromolecules. The effect of beechwood prehydrolysis lignin on the growth of *Sporobolomyces roseus, Rhodotorula rubra, Bullera alba* and *Saccharomyces cerevisiae* species, isolated from natural microflora, was examined in the presence or absence of glucose. Production of biomass was observed in both cases IR and ^{13}C NMR spectroscopy was used to characterize the structure of lignin fractions isolated from culture media by extraction with organic solvents. Structural changes in prehydrolysis lignin caused by the yeasts indicate demethylation and degradation of aromatic rings, similar to that observed with lignin-degrading hyphal fungi. These results demonstrate a partial utilization of lignin by the yeasts as carbon source.

8. Slavikova E., Vadkertiova R. 1997. Seasonal occurrence of yeasts and yeast-like organisms in the river Danube. Antonie van Leeuwenhoek **72**:77-80.

One hundred and seventy yeast strains belonging to 14 genera and 29 species were isolated from 112 water samples of the river Danube in the area of Bratislava. The samples were collected through the year from April to March. *Saccharomyces cerevisiae, Candida maltosa, Aureobasidium pullulans, Cystofilobasidium capitatum, Rhodotorula glutinis, Geotrichum candidum,* and *Candida krusei* were the most frequent. The basidiomycetous yeasts and yeast-like organisms with oxidative metabolism were present in approximately equal numbers to those with fermentative metabolism. *Saccharomyces cerevisiae* was the dominant yeast and was isolated from 50% of all samples examined and represented approximately one quarter of the yeast community. Yeast densities ranged from 100 to 21,100 CFU per liter. The highest population density was observed in Oktober. *Cryptococcus albidus, Saccharomyces cerevisiae, Rhodotorula glutinis,* and *Aureobasidium pullulans* formed the main part of the yeast population in this month.

9. Slavikova E., Vadkertiova R. 1997. Yeasts and yeast-like organisms occurring in the river Morava. Food technol. Biotechnol. **35**:293-297.

One hundred and sixty seven yeast strains belonging to 27 species were isolated from 57 water samples of the river Morava of the Slovak Republic taken during one year. *Sporobolomyces roseus, Candida maltosa,* and *Aureobasidium pullulans* were found to be the most frequent species, from nearly 50% of all samples, and together with *Saccharomyces cerevisiae, Rhodotorula glutinis, Cystofilobasidium capitatum,* and *Cryptococcus laurentii* represented more than 80% of total yeast population. Yeast densities of the river water ranged from 100-37,800 CFU per liter. The highest yeast population density was observed in samples take in April, when *Sacch. cerevisiae, Cr. laurentii* and *Cys. capitatum* occurred the most frequently. The community was characterized by a broad assimilation activity, with the xylose, cellobiose, and trehalose, which are widespread in nature, being assimilated by nearly 90% of population.

10. Kosikova B., Slavikova E. 1998. Inhibition of the yeast growth by lignin biopolymers and related compounds. Drevarsky vyskum **43**:13-19.

Lignin model compounds as well as lignin biopolymers derived from prehydrolysis and neutralsulfite pulping of hardwood were investigated from the viewpoint of their influence on the growth of *Candida albicans, C. tropicalis,* and *Trichosporon cutaneum.* The results show that lignin preparation act as inhibitor of all three yeast strains. In agreement with behaviour of model compounds the oxidation of lignin decreased its inhibitory efficiency.

XIV. Instituto de Investigaciones Biomédicas del CSIC, Arturo Duperier 4. E-28029 Madrid. Spain. Communicated by C. Gancedo.

The following manuscripts have been submitted recently.

1. T. Petit & C. Gancedo. Molecular cloning and characterization of the gene *HXK1* encoding the hexokinase from *Yarrowia lipolytica*.

We have cloned the gene *HXK1* from the dimorphic yeast *Yarrowia lipolytica* that encodes the unique hexokinase of this yeast.

The gene has an intron located 39 base pairs after the A of the first ATG. The putative protein contains a sequence of 40 aminoacids which is absent from other known hexokinase sequences. Glucokinase allowed growth in glucose of *Y. lipolytica* strains devoided of hexokinase but the growth was slower than that of the wild type. The hexokinase from *Y. lipolytica* substituted effectively for the hexokinase II from *S. cerevisiae* in catabolite repression of invertase. The enzymes from *Schizosaccharomyces pombe* or *Kluyveromyces lactis* were much less effective in this role. The Km for glucose and fructose of hexokinase were respectively 0.38 mM and 3.56 mM. The Km of glucokinase for glucose was 0.17 mM. While the hexokinase was strongly inhibited by trehalose-6-phosphate (Ki= 3.6 mM) glucokinase was not affected by this compound.

2. C. Rodriguez & C.L. Flores. Mutations in *GAL2* or *GAL4* alleviate catabolite repression produced by galactose in *Saccharomyces cerevisiae*.

Galactose does not allow growth of pyruvate carboxylase mutants in media with ammonium as nitrogen source, and inhibits growth of strains defective in phosphoglyceromutase in ethanol-glycerol mixtures. Starting with *pyc1 pyc2* and *gpm1* strains we isolated mutants that eliminated those galactose effects. The mutations were recessive and were named dgr1-1 and dgr2-1. Strains bearing those mutations in an otherwise wild type background grew slower than the wild type in rich galactose media and made the growth dependent on respiration. Galactose repression of several enzymes was relieved in the mutants. Biochemical and genetic evidence showed that *dgr1-1* was allelic with *GAL2* and *dgr2-1* with *GAL4*. The results indicate that the rate of galactose consumption is critical to cause catabolite repression.

XV. Division of Industrial Microbiology, Department of Food Technology and Nutritional Sciences, Wageningen Agricultural University, PO Box 8129, 6700 EV Wageningen, The Netherlands. Communicated by J.C. Verdoes <Jan.Verdoes@imb.ftns.wau.nl>.

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

1. Van der Vlught-Bergmans, C.J.B. and van Ooyen A.J.J. 1999. Expression cloning in *Kluyveromyces lactis*. Biotechnol. Techniques **13:**87-92.

Kluyveromyces lactis was used as host for an *Aspergillus tubingensis* expression library. An new episomal vector was constructed to direct the expression of the *A. tubingensis* cDNAs and to alow subsequent analysis in *Escherichia coli*. Using three different plate assays, 18000 *K. lactis* recombinants were screened, yielding 60 galactanase-, 26 polygalacturonase- and 16 cellulase-secreting colonies. The galactanase-secreting recombinants were analysed in detail: they are transcripts of the same galactanase gene with similarity to an *A. aculeatus* beta-1,4- galatanase gene. The results of the *K. lactis* system compare favourably to those obtained by *Saccharomyces cerevisiae.*

2. Weijers, C.A.G.M. and de Bont, J.A.M. 1999. Review. Epoxide hydrolases from yeasts and other sources: versatile tools in biocatalysis. J. Mol. Catalysis B: Enzymatic **6**:199-214.

Major characteristics, substrate specificities and enantio-selectivities of epoxide hydrolases from various sources are described. Epoxide hydrolase activity in yeasts is discussed in more detail and is compared with activities in other microorganisms. Constitutively produced bacterial epoxide hydrolases are highly enantioselective in the hydrolysis of 2,2- and 2,3-disubstituted epoxides. A novel bacterial limonene-1,2-epoxide hydrolase, induced by growth on monoterpenes, showed high activities and selectivities in the hydrolysis of several substituted alicyclic epoxides. Constitutively produced epoxide hydrolases are found in eukaryotic microorganisms. Enzymes from filamentous fungi are useful biocatalysts in the resolution of aryl- and substituted alicyclic epoxides. Yeast epoxide hydrolase activity has been demonstrated for the enantioselective hydrolysis of various aryl-, alicyclic- and aliphatic epoxides by a strain of *Rhodotorula glutinis*. The yeast enzyme, moreover, is capable of asymmetric hydrolysis of meso epoxides and performs highly enantioselective resolution of unbranched aliphatic 1,2-epoxides. Screening for other yeast epoxide hydrolases shows that high enantioselectivity is restricted to a few basidiomycetes genera only. Resolution of very high substrate concentrations is possible by using selected basidiomycetes yeast strains.

3. Wery, J., Verdoes, J.C. and van Ooyen, A.J.J. 1999. Genetics of non-*Saccharomyces* industrial yeast. In: Manual of industrial microbiology and biotechnology, 2nd edition, Davies JE and Damain AL (Eds.), ASM Press, Washington, pp.447-459.

In this chapter a comparison is made between the available transformation systems for industrially important yeasts like *Kluyveromyces lactis, Pichia pastoris, Hansenula polymorpha* and *Yarrowia lipolitica*. The development of a transformation system for the unconventional basidiomycetous red yeast *Phaffia rhodozyma* is taken as a case study. Specific methods and problems in the various stages are discussed.

4. Kronenburg N.A.E., Mutter, M., Visser, H., de Bont, J.A.M. and Weijers, C.A.G.M. 1999. Purification of an epoxide hydrolase from *Rhodotorula glutinis.* Biotechnol. Lett. (in press.).

The epoxide hydrolase from *Rhodotorula glutinis* was isolated and initially characterized. The enzyme was membrane associated and could be solubilized by Triton X-100. Purification yielded an enzyme with sp. act. of 66 micromol 1,2-epoxyhexane hydrolyzed min^{-1} mg^{-1} protein. The enzyme was not completely purified to homogeneity but, nevertheless, a major protein was isolated by SDS-PAGE for subsequential amino acid determination of peptide fragments. From sequence alignments to related enzymes, a high homology towards the active site sequences of other microsomal epoxide hydrolases was found. Molecular mass determinations indicated that the native enzyme exists as a homodimer, with a subunit molecular mass of about 45 kDa. Based upon these, this epoxide hydrolase is structurally related to other microsomal epoxide hydrolases.

XVII. Department of Food Science and Technology, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061-0418, USA. Communicated by C.W. de Bordenave <bzoeckle@vt.edu>, H. McMahon <hmcmahon@vt.edu>, and B.W. Zoecklein <bzoeckle@vt.edu>.

The following are abstracts of some recently completed work.

1. H. McMahon, B.W. Zoecklein, G.W. Claus, K. Fugelsang, and Y. Jasinski. Quantification of glycosidase activities in selected yeasts and lactic acid bacteria.

The activities of α-L-arabinofuranosidase, β-glucosidase, and α-L-rhamnoyranosidase were determined in model systems for thirty-two strains of yeasts belonging to the following genera: *Aureobasidium, Candida, Cryptococcus, Hanseniaspora, Hansenula, Kloeckera, Metschnikowia, Pichia, Saccharomyces, Torulaspora,* and *Brettanomyces* (10 strains); and seven strains of bacteria (*Leuconostoc oenos)*. Only one *Saccharomyces* strain exhibited β-glucosidase activity, but several non-*Saccharomyces* yeast species had substantial production. *Aureobasidium pullulans* hydrolyzed α-L-arabinofuranoside, β-glucoside, and α-L-rhamnoyranoside. Eight *Brettanomyces* strains had β-glucosidase activity. Location of enzyme activity was determined for those species with enzymatic activity. The majority of β-glucosidase was located in the whole cell fraction, followed by the permeabilized fraction, and extracellular production. *Aureobasidium pullulans* was also capable of hydrolyzing grape glycosides.

2. C.W. de Bordenave, B.W. Zoecklein, K.C. Fugelsang[1], and L.S. Douglas. Effects of *Brettanomyces intermedius* strains on Pinot noir (*Vitis vinifera* L.) glycoconjugates, conjugate fractions, and wine sensory attributes.
[1]Viticulture and Enology Research Center, California State University, Fresno, CA 93740-8003

Glycoconjugates and conjugate fractions are, in part, aroma and flavor precursors. Their quantification may offer an objective means of determining the impact of vinification techniques on potential wine quality. Pinot noir (*Vitis vinifera* L.) wines were inoculated with one of six genetically different strains of *Brettanomyces intermedius* (Ave, M, 216, Vin 1, Vin 4, and Vin5). Wines stored *sur lie* and those racked immediately following the completion of secondary fermentation were analyzed to determine the total, red-free, and phenolic-free glycoside concentrations, estimated by the analysis of glycosyl-glucose. *Sur lie* wines inoculated with strain Vin 4 and racked wines inoculated with Vin 4 and Vin 5 had the lowest total glycoside concentration. Hydrolysis of red-free glycosides appeared greater in *sur lie* wines inoculated with Vin 4 and racked wines inoculated with Vin 4 and Vin 5. Wines stored *sur lie* that were inoculated with M and Vin 1 and racked wines inoculated with Vin 1, Vin 4, and Vin 5 had the lowest concentration of phenolic-free glycosides. Duo-trio significance testing resulted in differences between the control and wines inoculated with strains Ave, M, Vin 1, Vin 4, and Vin 5. Wines inoculated with strains Ave and Vin 5 differed, as did wines with strains M and Vin 4.

XVIII. School of Biological Sciences, University of East Anglia, Norwich NR4 7TJ, England, Communicated by J.A. Barnett.

The following has been published.

1. Barnett, J.A. 1998. A history of research on yeasts. i. work by chemists and biologists 1789-1850. Yeast **14**:1439-1451.

XIX. The Australian Wine Research Institute, PO Box 197, Glen Osmond, SA 5064, Australia. Communicated by M. de Barros Lopes <mlopes@waite.adelaide.edu.au>.

Recent publication.

1. M. de Barros Lopes, S. Rainieri, P.A. Henschke, P. Langridge. 1999. AFLP fingerprinting for analysis of yeast genetic variation. Int. J. Syst. Bacteriol. **49**:915-924.

Amplified fragment length polymorphism (AFLP) was used to investigate genetic variation in commercial strains, type strains and winery isolates from a number of yeast species. AFLP was shown to be effective in discriminating closely related strains. Furthermore,

sufficient similarity in the fingerprints produced by yeasts of a given species allowed classification of unknown isolates. The applicability of the method for determining genome similarities between yeasts was investigated by performing cluster analysis on the AFLP data. Results from two species, *Saccharomyces* cerevisiae and *Dekkera bruxellensis*, illustrate that AFLP is useful for the study of intraspecific genetic relatedness. The value of the technique in strain differentiation, species identification and the analysis of genetic similarity demonstrates the potential of AFLP in yeast ecology and evolutionary studies.

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

Recent publications.

1. Buck, J. W. and Andrews, J. H. 1999. Attachment of the yeast *Rhodosporidium toruloides* is mediated by adhesives localized at sites of bud cell development. Appl. Environ. Microbiol. **65**:465-471.

2. Buck, J. W. and Andrews, J. H. 1999. Localized, positive charge mediates adhesion of *Rhodosporidium toruloides* to barley leaves and polystyrene. Appl. Environ. Microbiol. **65**:2179-2183.

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

The following are abstracts that were presented at the 5th *Candida* and Candidiasis meeting in Charleston, SC, March 1-4, 1999.

1. Y. Zhang and M.J. Leibowitz. Impact of Mg^{++} on pentamidine inhibition of *Candida* ribozyme activity.

Many *Candida albicans* isolates harbor the Ca.LSU self-splicing group I intron ribozyme in their 25S rRNA genes. Our laboratory has found that processing of this intron from the large rRNA gene transcript is sensitive to pentamidine *in vitro* and *in vivo*. We have found that *C. albicans* strains harboring this intron are more sensitive to pentamidine growth inhibition than are intronless strains, with differential growth inhibition seen under conditions in which splicing is inhibited *in vivo* (K.E. Miletti and M.J. Leibowitz, this meeting). Mg^{++} is required for the *in vitro* splicing of group I introns. In such ribozymes, this ion has been proven to play a role in the folding of ribozyme RNA to its tertiary structure and to directly participate in ribozyme catalysis. In this work, we used pentamidine inhibition of group I intron ribozyme splicing to study the interactions of Mg^{++} with the Ca.LSU ribozyme. Three distinct interactions of Mg^{++} with the ribozyme were observed. (1) *In vitro* splicing of this intron was inhibited by pentamidine, but pentamidine was less inhihitorv with increasing concentration of Mg^{++} in the reaction (200 μM pentamidine pentamidine completely inhibited at 1.25 mM Mg^{++}, 300 μM pentamidine was required at 2.5 mM Mg^{++}, no inhibition up to 500 μM pentamidine at 20 mM Mg^{++}). This result indicates that mM Mg^{++} competes with pentamidine for interaction with the ribozyme. (2) Pre-incubation of the Mg^{++} resulted in greater inhibition upon subsequent reaction than did addition of pentamidine to the complete reaction. However, inclusion of Mg^{++} in the preincubation eliminated this additional inhibitory effect of preincubation with pentamidine. This further supports Mg^{++}/pentamidine competition. (3) Pre-incubation of the Mg^{++} or Ca^{++} resulted in a dose-dependent reduction in sensitivity to pentamidine added in a subsequent reaction, suggesting that Mg^{++} or Ca^{++} alters the conformation of the RNA to generate a structure more resistant to pentamidine. Comparable concentrations of Na^{+} or NH_4^{+} had no such protective effect. These results support the role of divalent cations in the folding of group I intron RNA. Ribozyme inhibitors can be useful to study factors influencing ribozyme conformation. Further work will be needed to define the structural basis of the protective effect of Mg^{++} against pentamidine inhibition of the ribozyme.

2. K.E. Miletti & M.J. Leibowitz. The *Candida* ribozyme: a new antimicrobial target.

In HIV-infected and AIDS patients, opportunistic fungal infections are a major cause of morbidity and mortality. Despite improvements in treatment, toxicity and intolerance, as well as emergence of microbial resistance, are major issues yet to be resolved. *Candida albicans* is responsible for the most common fungal infection in HIV-infected patients. In about half of the isolates tested, a group I intron ribozyme (Ca.LSU) is present in the 25S rRNA gene. Previous work from this lab has shown that a similar ribozyme from *Pneumocysitis* carinii is sensitive to inhibition by pentamidine in vitro. Therefore, we tested whether pentamidine inhibited Ca.LSU enzymatic activity *in vitro* and *in vivo*, in order to determine if such inhibition results in antifungal activity. Splicing reactions of *in vitro* transcribed precursor RNA containing Ca.LSU were performed in the presence or absence of pentamidine. Two phylogenetically closely related intron-containing (4-1) and intronless (62-1) *Candida* strains were compared for their susceptibilities to pentamidine, and the effect of pentamidine on RNA splicing in vive was determined by reverse transcription-PCR (RT-PCR). The *in vitro* splicing of Ca.LSU was completely inhibited by pentamidine at 200 μM. Consistent with this result, intron-containing strain 4-1 was at least 16-fold more susceptible to growth inhibition by pentamidine than was strain 62-1, as shown by a YPD (dextrose) broth microdilution assay with growth measured after 24 h treatment (MIC_{80}). In YPD, only the intron-containing strain was inhibited by I CIM pentamidine. No significant differences were observed in YPG (glycerol), suggesting that a nuclear target rather than a mitochondrial target is responsible for the differential susceptibility. This is important since in *Saccharomyces*, which only has group I introns in its mitochondria, the most sensitive drug target appears to be mitochondrial. RT-PCR data showed a ≥10-fold accumulation of the unspliced precursor rRNA within 1 min after adding 1 μM pentamidine to strain 4-1. Precursor rRNA containing internal transcribed spacers also increased at this time. No effect on

rRNA processing was observed by similar RT-PCR analyses of intronless strain 62-1. Furthermore, when 12 *Candida* strains were tested for growth inhibition by pentamidine, the seven intron-containing strains were generally more sensitive to pentamidine than the five intronless strains. Thus, physiological and molecular data strongly suggest that the growth inhibition of strain 4-1 (and presumably all other intron-containing strains tested) is due to the *in vivo* inhibition of Ca.LSU splicing by pentamidine. These results indicate that nuclear group I intron ribozymes are potential targets for antimicrobial action. We thank G. Lemay and G. St.Germain for providing *Candida* strains.

XXII. Institut für Angewandte Mikrobiologie, Universität für Bodenkultur, Nuβdorfer Läende 11, A-1190 Vienna, Austria. Communicated by H. Prillinger.

Dr. W. Scheigkofler moved from Vienna to "Land- und Forstwirtschaftliches Versuchszentrum Laimburg" I-39040 Auer, Italy. The following paper is in press.

1. W. Schweigkofler, H. Prillinger. 1999. Molecular identification and phylogenetic analyses of endophytic and latent pathogenic fungi from grapevine. Mitteilungen Klosterneuburg (in press, in German).

In the present work we used molecular methods of DNA sequence analysis to investigate two points: first to identify fungal isolates from grapevine and second to determine the phylogenetic positions of these microorganisms. Using partial sequences of the 18S rRNA gene and the 26S rRNA gene yeast isolates have been identified unambigously, with filamentous fungi on the other hand, lack of sequences in the GenBank may lead to unclear results. The phylogenetic tree based on complete 18S rDNA sequences of the division Ascomycota comprises three distinct classes, the Hemiascomycetes, the Protomycetes, and the Euascomycetes. Similarly the phylogenetic tree of the division Basidiomycota is tripartite and includes the Urediniomycetes, the Ustilaginomycetes, and the Hymenomycetes. The phylogenetic tree clusters all endophytic filamentous fungi from grapevine analyzed so far in different orders of the Euascomycetes. All endophytic yeast isolates belong either to the Urediniomycetes (*Rhodotorula glutinis*, *Sporobolomyces* spec. nov.) or to the Hymenomycetes (*Cystofilobasidium macerans*, syn. *Cryptococcus macerans*). The phylogenetic trees of the Ascomycota and Basidiomycota give an overview about presently accepted natural orders of higher fungi. In contrast to endophytes of grasses the fungi colonizing grapevine wood show a very remarkable biodiversity of phylogenetically different species.

The following is the abstract of an invited lecture in the meeting "25 Jahre Mykologie in Tuebingen" June 3-6.

2. H. Prillinger, W. Schweigkofler, K Lopandic, R.,[1] U.G. Mueller[2] Evolution of Asco- and Basidiomycota based on cell wall sugars, 18S ribosomal DNA sequences and coevolution with animals and plants.
[1]Universität Tübingen, Spezielle Botanik Mykologie, Auf der Morgenstelle 1, D-72076 Tübingen.
[2]University of Maryland, Dept. Biology, College Park, MD 20742, USA.

In the Basidiomycota the qualitative and quantitative monosaccharide pattern of purified cell walls correlates well with complete sequences of the 18S ribosomal DNA to separate the Basidiomycota in three distinct classes. The *Microbotryum*-type (dominant amounts of mannose, glucose, galactose, fucose, sporadically rhamnose) correlates with the Urediniomycetes, the *Ustilago*-type (dominant amounts of glucose, mannose, galactose) with the Ustilaginomycetes and the *Tremella*-type (glucose, mannose, xylose, galactose may be present) with the Hymenomycetes (Prillinger et al., J. Gen. Appl. Microbiol. 39: 1-34, 1993). Xylose, however, may disappear by reduction in some derived genera of the Hymenomycetes (*Schizophyllum commune*, yeast stages from agarics of fungus-growing ants; Mueller et al., Science 281: 2034-2038, 1998). *Cryptococcus yarrowii* is so far the only basidiomycetous fungus which has xylose in its cell walls, but dominant amounts of mannose, however, suggest a Urediniomycetes affinity. Based on complete sequences of the 18S ribosomal DNA the yeasts isolates of the agarics *Asterophora lycoperdoides* and *A. parasitica* were assigned together with *Cryptococcus humicolus* to the new genus *Asterotremella*. They were regarded as sexual symbionts and missing links in the evolution from mycoparasitism to heterothallism (Prillinger, H., In: Evolutionary biology of the fungi; Rayner & al. eds., Cambridge Uiv. Press pp. 355-377, 1987). In the Ascomycota presently only 18S ribosomal DNA sequences are useful to separate the Ascomycota into three classes, the Hemiascomycetes, the Protomycetes, and the Euascomycetes. Based on cell wall sugars (glucose, mannose, galactose or glucose, mannose, galactose, rhamnose), urease activity, the ultrastructure of septal pores and complete 18S ribosomal DNA sequences the Protomycetes appear as a sister group of the Euascomycetes. Morphological and ultrastructural data of *Mixia osmundae*, cell wall sugars of *Taphrina vestergrenii* (Prillinger et al., Z. Mykol. 56: 219-250, 1990) and 5S ribosomal DNA data from Gottschalk & Blanz (Z. Mykol. 51: 205-243, 1985) and Walker (System. Appl. Microbiol. 6: 48-53, 1985) suggest the Protomycetes to be ancestral to the Euascomycetes and Urediniomycetes. Based on coevolution the Hemiascomycetes may be traced back by parasitic *Metschnikowia* species with their Crustacean host at least to the Cambrian (500 million years ago; Prillinger et al., Yeast 13: 945-960, 1997). Within the Basidiomycota the Urediniomycetes already have existed in the Carboniferous (300 million years ago) as indicated by coevolution from *Mixia osmundae* with its host plant *Osmunda regalis* (Prillinger et al., Z. Mykol. 56: 219-250, 1990).

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

The following papers are in press.

1. M.T. Wyder and Z. Puhan. 1999. Investigation of the yeast flora in smear ripened cheeses. Milchwissenschaft. In press.

In a large number of cheese varieties, yeasts belong to the specific surface microflora composed of moulds, yeasts, micrococci and coryneform bacteria. They are known for their high tolerance towards low pH, moisture and temperature, and contribute mainly to the de-acidification process. The aim of the project was to investigate the occurrence of yeasts in two smear ripened soft cheeses made with pasteurised milk (Limburger and Münster) and in one smear ripened semi-hard cheese made with raw milk (Tête de Moine). A non-traditional technique which is the amplification of the ITS region with subsequent restriction analysis, was used to characterise and group all isolates from cheese. The method proved to be easy, rapid and accurate. A total of 12 species were isolated from the surface and the interior of Limburger, Münster and Tête de Moine. The predominant species in all investigated cheese varieties was *Debaryomyces hansenii* regardless of whether it had been added as a starter to the smear or of whether it originated from spontaneous contamination. The second most frequent species was *Galactomyces geotrichum* followed by *Yarrowia lipolytica*. The surface of Tête de Moine, and both the interior and the surface of the soft cheeses revealed a homogeneous yeast flora with a maximum of 3 – 5 species. However, the yeast flora in the interior of Tête de Moine was quite heterogeneous with a spectrum of seven species. The counts of 100 – 1000 CFU/g inside the cheeses were always much lower than on the surface. It has been concluded that even though the yeast flora composition inside the cheese depends strongly on the applied technology e.g. whether pasteurised or raw milk is used, the dominating species on the surface always is *D. hansenii*.

2. M.T. Wyder and Z. Puhan. 1999. Role of selected yeasts in cheese ripening: an evaluation in aseptic cheese curd slurries. Int. Dairy J. In press.

Yeasts are found within the surface microflora of many cheese types, but not much is known about their direct contribution to cheese ripening. The aim of this study was to investigate, on a laboratory scale, the direct influence of yeasts on 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 characterised by the ability to ferment glucose, to utilise lactate and to increase pH values and by the fact that proteolytic activity was not shown. This resulted in alcoholic, acidic, fruity or fermented odours (*Clavispora lusitaniae*, *Pichia jadinii* and *Williopsis californica*). The second group was composed of non fermenting species which utilised lactate, but the pH was not affected (*Galactomyces geotrichum*, *Trichosporon ovoides* and *Yarrowia lipolytica*). These yeasts were proteolytic yielding a cheesy aroma. *Debaryomyces hansenii* B comprised characteristics of both groups.

3. M.T. Wyder, H.P. Bachmann[1] and Z. Puhan. 1999. Role of selected yeasts in cheese ripening: an evaluation in foil wrapped raclette cheese. Lebensmittel Wissenschaft und Technologie. In press.
[1]Swiss Federal Dairy Research Station, FAM, Liebefeld, 3003 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. Foil ripening was chosen in order to exclude the influence of the surface flora. An unreplicated 2^4 full factorial experimental design in two blocks of 8 vats was used. In the mature cheeses, the lactic acid content was increased, probably as a result of increased lactic acid bacteria due to the release of yeast metabolites. Yeasts seemed to show either esterase or lipase activity. Furthermore, yeasts revealed peptidolytic activity leading to an increase in smaller peptides and free amino acids. Except for *Gal. geotrichum*, they also enhanced the formation of biogenic amines. *Y. lipolytica* was capable of improving the overall sensory characteristics of cheese, but all other species influenced the flavour rather negatively. Since hardly any viable yeasts were detectable in the mature cheeses, the action by yeasts could be attributed to enzymes released after cell lysis.

XXIV. Department of Biology, Queen's University, Kingston, Ontario, K7L 3N6 Canada. Communicated by J. Karagiannis <karagiaj@biology.queensu.ca>.

Recently published papers.

1. J. Karagiannis, R. Saleki & P.G. Young. The *pub1* E3 ubiquitin ligase negatively regulates leucine uptake in response to NH_4^+ in fission yeast.

Fission yeast strains auxotrophic for leucine are unable to proliferate in normally supplemented minimal media adjusted to ph 6.4 or above. High ph sensitivity can be suppressed by the loss of pub1, an e3 ubiquitin ligase, or by the replacement of NH_4^+ with a non-repressing source of nitrogen such as l-proline. In this report we show *pub1* to be required for the rapid down-regulation of leucine uptake observed in response to the addition of NH_4^+ to the growth media. Furthermore, we corroborate earlier results demonstrating the transport of leucine to be negatively influenced by high extracellular ph. *Pub1* is homologous to the budding yeast nitrogen permease inactivator, *npi1/rsp5,* which mediates the ubiquitination and subsequent destruction of NH_4^+-sensitive permeases. The high ph sensitivity of cells auxotrophic for leucine thus seems to reflect an inability of NH_4^+-insensitive permeases to transport sufficient leucine under conditions where the proton gradient driving nutrient transport is low, and NH_4^+-sensitive permeases have been destroyed. Intriguingly, the partial suppression of both high ph sensitivity, and the inactivating effect of NH_4^+ on leucine transport, seen in *pub1-1* point mutants, becomes as complete as seen in *pub1* backgrounds when cells have concomitantly lost the function of the *spc1* stress activated mapk.

2. I. Rupeš, Z. Jia & P.G. Young. *Ssp1* promotes actin depolymerization and is involved in stress response and NETO control in fission yeast.

The *ssp1* gene encodes a protein kinase involved in alteration of cell polarity in *S. pombe*. *ssp1* deletion causes stress sensitivity, reminiscent of defects in the stress-activated MAP kinase, Spc1. However, the two protein kinases do not act through the same pathway. Ssp1 is localized mainly in the cytoplasm, but following a rise in external osmolarity it is rapidly recruited to the plasma membrane, preferentially to active growth zones and septa. Loss of Ssp1 function inhibits actin relocalization during osmotic stress, in *cdc3* and *cdc8* mutant backgrounds and in the presence of latrunculin A, implicating Ssp1 in promotion of actin depolymerization. We propose a model in which Ssp1 can be activated independently of Spc1 and can partially compensate for its loss. The *ssp1* deletion mutant exhibited monopolar actin distribution, but NETO (new end take-off) could be induced in these cells by exposure to KCl or to latrunculin A pulse treatment. This treatment induced NETO in *cdc10* cells arrested in G1 but not in *tea1* cells. This suggests that cells that contain intact cell end markers are competent to undergo NETO throughout interphase and Ssp1 is involved in generating the NETO stimulus by enlarging the actin monomer pool.

XIV. Japan Collection of Microorganisms, The Institute of Physical and Chemical Research (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. Golubev, W. I. & Nakase, T. 1998. Mycocinogeny in the genus *Bullera*: killer activity of *Bullera unica* and intrageneric killer-sensitive relationships. Mikrobiologiya **67**:225-230 (English translation: 184-188). See abstract under Dr. W. I. Golubev's communication (the Fall issue of the Yeast Newsletter, 1998).

2. Suzuki, M. & Nakase, T. 1998. Cellular neutral sugar compositions and ubiquinone systems of the genus Candida. Microbiol. Cult. Coll. **14**:49-62.

Cellular neutral sugar compositions and ubiquinone systems of the type strains of 196 species and 9 varieties of the genus *Candida* were examined. One hundred sixty-two species and 7 varieties contained glucose and mannose in the whole cells, while 34 species and 2 varieties contained glucose, mannose, and galactose. As the major ubiquinone, the genus *Candida* was clearly demonstrated to have four ubiquinone types of Q-6, Q-7, Q-8, and Q-9, i.e., 8 species had Q-6, 50 species had Q-7, 20 species had Q-8, and 118 species and 9 varieties had Q-9. On the basis of cellular neutral sugar compositions and ubiquinone systems, *Candida* species were divided into six groups. Group Ia (8 species) has glucose, mannose, and Q-6 type of ubiquinone. Group Ib (50 species) has glucose, mannose, and Q-7 type of ubiquinone. Group Ic (17 species) has glucose, mannose, and Q-8 type of ubiquinone. Group Id (87 species and 7 varieties) has glucose, mannose, and Q-9 type of ubiquinone. Group IIa (3 species) has glucose, mannose, galactose, and Q-8 type of ubiquinone. Group IIb (31 species and 2 varieties) has glucose, mannose, galactose, and Q-9 type of ubiquinone. Each of the six groups is assumed to be still an assembly of phylogenetically different species because each group contains several teleomorphic genera and is heterogeneous in cellular fatty acid compositions.

3. Tsuruta, S., Takaya, N., Zhang, L., Shoun, H., Kimura, K., Hamamoto, M. and Nakase, T. 1998. Denitrification by yeasts and occurrence of cytochrome P450nor in Trichosporon cutaneum. FEMS Microbiol. Lett. **168**:105-110.

Yeasts of various genera were screened for denitrifying activity, and several yeast strains such as *Trichosporon cutaneum*, *Fellomyces fuzhouensis*, and *Candida* sp. were found to exhibit distinct activities to convert nitrite to nitrous oxide. Dissimilatory nitrite reductase (Nir) or nitric oxide reductase (Nor) activities were detected in the cell-free extracts of these yeasts. Spectrophotometric as well as Western blot analyses showed that *T. cutaneum* contains Nor of the cytochrome P450 type. This is the first report that shows that denitrification is also distributed among yeasts although their systems are incomplete, only capable of reducing nitrite to nitrous oxide.

4. Sugita, T. & Nakase, T. 1999. Non-universal usage of the leucine CUG codon and the molecular phylogeny of the genus *Candida*. System. Appl. Microbiol. **22**:79-86.

CUG, a universal leucine codon, was reported to be read as serine in 10 species of the genus *Candida*. We used an in vitro cell-free translation system to identify the amino acid assignment of codon CUG in 78 species and 7 varieties of galactose-lacking *Candida* species equipped with Q9 as the major ubiquinone. Of these, only 11 species used codon CUG as a leucine codon. The remaining species decoded CUG as serine. Their small subunit ribosomal DNA sequences were also determined and anlyzed using both Neighbor-Joining and Maximum Likelihood methods. The species decoding CUG as serine and leucine formed distinct clusters on both molecular phylogenetic trees. Our result suggests that non-universal decoding is not a rare event, and that it is widely distributed in the genus *Candida*.

XV. Department of Soil Biology, Lomonosov Moscow State University, Vorobjevy Gory, Moscow 119899, Russia. Communicated by I.P.Bab'eva <boris.byzov@lvt.ss.msu.ru>.

Current research.

1. Bab'eva I.P., Chernov I.Yu., Karelina M.E., Terenina E.E. 1999. Yeasts in Arctic deserts of Bolshevik Island, archipelago Severnaja. Zemlja (Northern Land).

Research of yeasts in different geographical zones has been conducted in our laboratory for a long time. But yeasts never were investigated in Arctic deserts located in Russian islands in the Arctic Ocean. Recently we have had the opportunity to investigate yeast communities in several dozens of samples collected in Bolshevik island (78°13'N,103°15'E). Various substrates including flowers, live and dead leaves of plants, plant debris, roots, mosses, lichens, and different soil horizons were investigated using diluted plate method with malt extract. All yeasts in polar desert was found to be associated with plants and plant debris and thoroughly absent in mineral soil layers. The following species of yeasts were found to be dominant: *Mrakia frigida*, *Cryptococcus laurentii*, *Cr.gilvescens*, *Cr.albidus*, *Cr. hungaricus*. Unidentified yeasts probably belonging to new species were very frequent too. They were similar to the genus *Mrakia*. Arctic deserts differ from souther zones not only by specific vegetation, but by character of yeast communities also. The yeast communities in Arctic deserts are found to be quite similar to those found in the more southern tundra zone. But the number of yeast species found here is significantly less and represented by two ecological types: cosmopolitan species (e.g. *Cr. laurentii*) and species distributed exclusively in high latitudes (*Cr. gilvescens*, *M. frigida*).

A Masters thesis has been defended on the basis of these results by M.E. Karelina.

The following papers have been prepared recently.

2. Polyakova A.V., Chernov I.Yu. 1999. Yeasts in fruit bodies of mushrooms. Mikologia i phytopathologia (Mycology and Phytopathology, in Russian, in press).

3. Bab'eva I.P., Kartintseva A.A., Maximova I.A., Chernov I.Yu. 1999. Yeasts in coniferous forest of Central Forest Reserve (Middle Russia). Vestnik MGU, (Proceedings of Moscow State University, in Russian, in press).

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

The following article was recently published.

1. Avram, D., Leid, M., and Bakalinsky, A.T. 1999. *Fzf1p* of *S. cerevisiae* is a positive regulator of *SSU1* transcription and its first zinc finger region is required for DNA binding. Yeast **15**:473-480.

The *FZF1* gene of *Saccharomyces cerevisiae* encodes a five zinc finger transcription factor involved in sulfite tolerance. Previous work based on multicopy suppression analysis placed *FZF1* upstream of *SSU1*, which encodes a plasma membrane protein and putative transporter also implicated in sulfite detoxification. Consistent with this analysis, Fzf1p was found to be a positive regulator of *SSU1* transcription. The *SSU1* promoter region involved in activation by *FZF1* was defined, and the Fzf1 protein was shown to bind to it directly *in vitro*. Deletion of a single, amino terminal zinc finger of *Fzf1p* resulted in loss of DNA binding, while the fourth and fifth zinc fingers were found to be dispensable.

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

Recent publications.

1. V. Scerra, A. Caridi, F. Foti, M.C. Sinatra. 1999. Influence of dairy *Penicillium spp.* on nutrient content of citrus fruit peel. Animal Feed Science and Technology **78**:169-176.

The aim of this study is to improve the nutritional value of bergamot fruit peel by solid-state fermentation with three strains of *Penicillium camemberti* and seven strains of *Penicillium roqueforti*. non-proteic nitrogen, dry matter, ether extract, ash, neutral detergent fiber, acid detergent fiber, hemicellulose, cellulose, organic matter, To assess the effect of their growth on the chemical composition of the substrate, the following analytical determinations were carried out before and after microbial colonization: crude protein, pure protein, and gross energy. The colonization with the moulds produces substantial changes in the chemical composition of the bergamot fruit

peel, notably significant increases in crude protein, in ether extract, in gross energy, and in structural carbohydrates. The solid-state fermentation activity of these moulds could prove a cost-effective way of recycling citrus fruit peel in animal feed, thus increasing its nutritional value by single cell protein. Nevertheless further research on the matter must be carried out, especially on the estimation of the biomass digestibility – *in vitro*, *in sacco* or *in vivo* – and on the check for eventual mycotoxins (roquefortin, cyclopiazoic acid) produced by the moulds in the cellulose waste.

2. A. Caridi, P. Crucitti, D. Ramondino, E. Santagati, P. Audino. 1999. Isolation and initial characterisation of thermotolerant yeasts for oenological use. (in Italian). Industrie delle Bevande **28**(3): in press.

The aim of this research is the selection of thermotolerant yeasts to control the winemaking of musts from dried grapes. One hundred and forty saccharose fermenting elliptic yeasts, able to grow even at 40°C, were isolated and identified. Seventeen strains of *Saccharomyces cerevisiae* were selected by determining their fermentative activity and resistance to SO_2 after two and after seven days compared to the control strain 220. Winemakings were performed at 25 and 40°C. At the end of fermentation, wines were analysed for alcoholic content, residual sugar, and total SO_2. The most interesting strains are *Saccharomyces cerevisiae* p.r. *cerevisiae* TT141 and TT254. Both show high fermentative activity and good resistance to SO_2 also at 40°C.

3. A. Caridi. 1999. Influence of temperature and sugar concentration on growth kinetic and fermentation performance of *Schizosaccharomyces* strains. (in Italian). Industrie delle Bevande **28**(4): in press.

The aim of this research was to study the growth kinetic and the fermentation performance of eight strains of Calabrian *Schizosaccharomyces*. Fermentations were carried out at 18°C and at 35°C, using four samples of must with increasing sugar content. Fermentation vigour after two and after seven days and, at the end of winemaking, titratable acidity, volatile acidity and colour, by optical density at 420 nm, were determined. The growth kinetic of these yeasts suggest that, to inhibit their growth, it would be useful to control the rise in temperature of musts during fermentation. The fermentation performance of these strains produces a notable deacidification of musts both at 18°C and at 35°C, a remarkable biodiversity for the production of volatile acidity and a positive effect on the colour of wines. On the whole, the results explain the high frequency of *Schizosaccharomyces* in spontaneous fermentation of musts from dried grapes.

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

Recent publications.

1. R. Stoltenburg, O. Lösche, G. Klappach & G. Kunze. 1999. Molecular cloning and expression of the *ARFC1* gene, a component of the replication factor C from the salt-tolerant, dimorphic yeast *Arxula adeninivorans* LS3. Curr. Genet. **35**:8-13.

The yeast replication factor C (RF-C) is a multipolypeptide complex with five sub-units involved in chromosomal DNA replication. This factor, encoded by five genes, is well characterized for the yeast *Saccharomyces cerevisiae*. However, RF-C components from other yeast species were not analysed yet. Here we report cloning and characterization of the gene *ARFC3* from the dimorphic and osmo-tolerant yeast *Arxula adeninivorans*. This gene encodes one subunit of the RF-C complex. It is localized on chromosome 1 of the four *Arxula* chromosomes and comprises a coding region of 1,014 bp, which corresponds to 338 amino acids. Two introns are contained within this gene. The *ARFC3* transcript level is influenced by both salt and temperature. The latter influences also the morphological state (budding cells-mycelium). High salt concentration and high temperature result in a rapid decrease of the *ARFC3* mRNA.

2. I. Kunze, G. Hensel, K. Adler, J. Bernard, B. Neubohn, C. Nilsson, R. Stoltenburg, S.D. Kohlwein & G. Kunze. 1999. The green fluorescent protein targets secretory proteins to the yeast vacuole. Biochim. Biophys. Acta **1410**:287-298.

The green fluorescent protein (GFP) was used as a marker to study the intracellular transport of vacuolar and secretory proteins in yeast. Therefore, the following gene constructs were expressed in *Saccharomyces cerevisiae* under control of the *GAL*1 promoter: GFP N-terminally fused to the yeast secretory invertase (INV-GFP), the plant vacuolar chitinase (CHN-GFP) and its secretory derivative (CHNΔVTP-GFP), which did not contain the vacuolar targeting peptide (VTP), both chitinase forms (CHN and CHNΔVTP), GFP without any targeting information (GFP) and two secretory GFP variants with and without the VTP of chitinase (N-GFP-V and N-GFP). Whereas chitinase without VTP is accumulated in the culture medium the other gene products are retained inside the cell up to 48 hours of induction. Independently of a known VTP they are transported to the vacuole, so far as they contain a signal peptide for entering the endoplasmic reticulum. This was demonstrated by confocal laser scanning microscopy, immunocytochemical analysis and subcellular fractionation experiments as well. The transport of the GFP fusion proteins is temporary delayed by a transient accumulation in electron-dense structures very likely derived from the ER, because they also contain the ER chaperone Kar2p/Bip. Our results demonstrate that GFP directs secretory proteins without VTP to the yeast vacuole, possibly by the recognition of an unknown vacuolar signal and demonstrates, therefore, a first limitation for the application of GFP as a marker for the secretory pathway in yeast.

XIX. Departamento de Microbiologia, Instituto de Ciências Biológicas, C.P. 486, Universidade Federal de Minas Gerais, Belo Horizonte, MG, 31270-901, Brazil. Communicated by C.A. Rosa <carlrosa@mono.icb.ufmg.br>.

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

1. Pataro, C., A. Santos, S. R. Correa, P.B. Morais, V.R. Linardi, and C.A. Rosa. 1998. Physiological characterization of yeasts isolated from artisanal fermentation in an aguardente distillery. Rev. Microbiol. **29**:104-108.

2. Braga, A.A., P.B. Morais, and V.R. Linardi. 1998. Screening of yeasts from Brazilian Amazon Rain Forest for extracellular proteinases production. System. Appl. Microbiol. **21**:353-359.

3. Ribeiro, M.J.S., L.S.C. Leão, P.B. Morais, C.A. Rosa, and A.D. Panek. 1999. Trehalose accumulation by tropical yeast strains submitted to stress conditions. Antonie van Leeuwenhoek. In press.

Trehalose, a non-reducing disaccharide, that accumulates in *Saccharomyces cerevisiae* has been implicated in survival under various stress conditions by acting as membrane protectant, as a supplementary compatible solute or as a reserve carbohydrate which may be mobilized during stress. However, most of these studies have been done with strains isolated from European or Asian habitats, of temperate climate. In this study yeasts living in tropical environments, isolated from different microhabitats in Southeastern Brazil, were used to evaluate whether trehalose contributes to survival under osmotic, ethanol and heat stress. The survival under severe stress was compared to a well-characterized laboratorial wild-type strain (D273-10B). Most of the *Saccharomyces cerevisiae* strains isolated from *Drosophila* in Tropical Rain Forest were able to accumulate trehalose after a preconditioning treatment at 40°C for 1h. The amount of intracellular trehalose levels was better correlated with survival during a challenging heat shock at 50.5°C for 8 min. *Saccharomyces cerevisiae* and *Candida guilliermondii* were observed to be thermotolerant as well as osmotolerant. No clear correlation between intracellular trehalose levels and survival could be derived during ethanol stress. In some cases, the amount of trehalose accumulated before the ethanol stress seemed to play an important role for the survival of these strains.

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

The following paper has been accepted for publication in Systematic and Applied Microbiology:

1. D. Dlauchy, J. Tornai-Lehoczki and G. Péter. 1999. Restriction enzyme analysis of PCR amplified rDNA as a taxonomic tool in yeast identification.

A method has been developed to simplify the identification of yeast strains. We used the restriction fragment patterns of PCR-amplified 18S rRNA-coding DNA with the neighbouring ITS1 region for differentiation and identification of 169 yeast strains representing 128 species associated mainly with food, wine, beer, and soft drinks The amplicons were digested with four different four-base-cutting restriction enzymes. To construct a database of restriction fragment patterns, the gels have been scanned and analyzed using the Molecular Analyst Fingerprint 2.0 software. The use of four enzymes proved to be sufficient for strain identification.

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

Dr. Vincent Robert <Robert@STM.TUDELFT.NL> started at the CBS in May and replaces Dr. David Yarrow as curator of the Yeast Collection. The following articles have appeared or are in press.

1. D.Bergerow, R.Bauer & T.Boekhout. Phylogenetic placements of ustilaginomycetous anamorphs as deduced from nuclear LSU rDNA sequences. Mycological research (in press).

2. T.Boekhout, J.W.Fell, C.P.Kurtzman, & E.A.Johnson. 1999. Proposal to reject the name *Rhodomyces dendrorhous* (Fungi, Basidiomycota). Taxon **48**:147-148.

3. T.Boekhout, M.Kamp, & E. Guého. 1999. Molecular typing of *Malassezia* species with PFGE and RAPD. Med. Mycol. **36**:365-372.

The currently recognized seven species of *Malassezia* all have different karyotypes which do not vary intraspecifically, except in *M. furfur*, which displayed two different karyotypes. In contrast, random amplified polymorphic DNA (RAPD) typing showed the presence of genetic variation in all species. It is concluded that karyotype analysis is useful for species identification, and RAPD typing can be used in epidemiological investigations.

4. J.W.Fell, R.Roeijmans & T.Boekhout. Cystofilobasidiales, a new order of basidiomycetous yeasts. 1999. Int.J.System.Bacteriol. **49**:907-913.

The order Cystofilobasidiales is described for teleomorphic basidiomycetous yeasts with holobasisia and teliospores. Their septa have dolipores, but lack parenthesomes, D-Glucuronate, nitrate and nitrite are assimilated and *myo*-inositol is usually assimilated. Coenzyme Q has 8 or 10 isopenologues. 25S and 18S rDNA sequence analysis indicates a momopheletic branch within the Tremellomycetidae of the Hymenomycetes. *Cystofilobasidium* is the type genus.

5. M.L.Kerkmann, G.Schönian, K.D.Paul, K.Ulbrich, M.T.Smith & M.Schuppler. 1999. Red-pigmented *Candida albicans* in patients with cystic fibrosis. J.Clinical Microbiol. **37**:278

6. W.H.Müller, B.M.Humbel, A.C.van Aelst, T.P.van der Krift & T.Boekhout. The perforate septal pore cap of basidiomycetes. In: Plasmodesmata. (Ed. A.van Bel), in press.

Recent acquisitions.

For recent acquisitions, please consult the catalogue on the www at http://www.cbs.knaw.nl/www/cbshome.html

XXII. VTT Biotechnology & Food Research, P.O.Box 1501, FIN-02044 VTT, Finland. Communicated by J. Londesborough <john.londesborough@vtt.fi>.

Recent publications include.

1. Aristidou, A., Londesborough, J., Penttilä, M., Richard, P., Ruohonen, L., Söderlund, H., Teleman, A., and Toivari, M. 1998. Transformed organisms with improved properties. Pat. Appl. 980551

2. Jäntti, J., Lahdenranta, J., Olkkonen, V.M., Söderlund, H. and Keränen, S. 1999. *SEM1*, a homologue of the Split hand/split foot malformation candidate gene, *Dss1*, regulates exocytosis and pseudohyphal differentiation in yeast. Proc.Natl. Acad. Sci. USA **96**:909-914.

3. Kesti, T., Flick, K., Keränen, S., Syväoja, J.E. and Wittenberg, C. 1999. DNA polymerase e catalytic domains are dispensable for replication, repair and cell viability. Molecular Cell (in press).

4. Serón, K., Tieaho, V., Prescianotto-Baschong, C., Aust, T., Blondel, M-O., Guillaud, P., Deviellers, G., Rossanese, O.W., Glick, B.S., Riezman, H., Keränen, S. and Hagenauer-Tsapis, R. 1998. A yeast t-SNARE involved in endocytosis. Mol. Biol. Cell **9**:2873-2889.

5. Teleman, A., Richard, P., Toivari, M. and Penttilä, M. 1999. Identification and quantification of phosphorus metabolites in yeast neutral pH extracts by NMR spectroscopy. Anal. Biochem., in press.

6. Virkjärvi, I., Lindborg, N., Kronlöf, J. and Pajunen, E. (1999) Effects of aeration on flavour compounds in immobilized primary fermentation. Monatsschrift für Brauwissenschaft, **52**:9-28.

XXIII. Microbiology Department, Miami University, Oxford, Ohio 45056, U.S.A. Communicated by J.K. Bhattacharjee.

Recent publications.

1. K. Suvarna, L. Seah, V. Bhattacherjee, and J.K. Bhattacharjee. 1998. Molecular analysis of the *Lys2* gene of *Candida albicans*: homology to peptide antibiotic synthetases and the regulation of the α-aminoadipate reductase. Curr. Genet. **33**:268-275.

2. S.D. Irvin and J.K. Bhattacharjee. 1998. A unique fungal lysine biosynthesis enzyme shares a common ancestor with tricarboxylic acid cycle and leucine biosynthetic enzymes found in diverse organisms. J. Mol. Evol. **46**:401-408.

3. V. Bhattacherjee and J.K. Bhattacharjee. 1998. Nucleotide sequence of the *Schizosaccharomyces pombe lysl*$^{+}$ gene and similarities of the lysl^{+} protein to peptide antibiotic synthetases. Yeast **14**:479-484.

4. V. Bhattacherjee and J.K. Bhattacharjee. Characterization ofa double gene disruption in the *LYS2* locus of the pathogenic yeast *Candida albicans*. J. Med. Mycol. (in press).

Patent Applications.

5. J.K. Bhattacharjee, R.C. Garrad, P.L. Skatrud, and R.B. Peery. Methods and reagents for detecting fungal pathogens in biological sample. U.S. serial no. 08/360,606; case no. (Allegretti and Witcoff, Ltd.) 94,319.

6. J.K. Bhattacharjee and V. Bhattachejee. Methods and reagents for detecting fungal pathogens in a biological sample. U.S. serial no. 08/650,809, case no. (McDonnell, Bochner Hulbert and Berghoff) 96,247.

7. J.K. Bhattacharjee, K. Suvarna, and V. Bhattachejee. Methods and reagents for detecting fungal pathogens in a biological sample", case no. (McDonnell, Boehner Hulbert and Berghoff) 96,247-A.

Network: Yeasts in Food and Beverages

Recent publications regarding in particular the topic: Methods for isolation, enumeration, identification and characterization of yeasts in food and beverages. Communicated by P. Romano <pot2930@iperbole.bologna.it>.

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

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

85 yeast strains isolated from different cheeses of Austria, Denmark, France, Germany, and Italy identified phenotypically by traditional physiological methods were investigated genotypically using type strains and random amplified polymorphic DNA polymerase chain reaction (RAPD-PCR) analysis. In 39 yeast strains the results obtained phenotypically agreed well with the genotypic investigation. 26 isolates of Geotrichum could be identified at the species level with genotypic methods only. 7 yeast isolates were correctly identified at the genus level only using phenotypic identification methods. For 14 yeast isolates the phenotypic identification does not agree with the genotypic results. Using the diazonium blue B test and a polyphasic molecular approach including ubiquinone analysis, yeast cell wall sugars, and RAPD-PCR 5 isolates which were wrongly identified phenotypically, the 7 isolates which were identified correctly at the genus level as well as the 26 *Geotrichum* strains could be identified genotypically. 9 yeast strains remained unidentified. The 76 yeast isolates were assigned to 39 species. *Debaryomyces hansenii, Geotrichum candidum, Issatchenkia orientalis, Kluyveromyces lactis, K. marxianus, Saccharomyces cerevisiae, Yarrowia lipolytica,* and *Candida catenulata* were the most common species. The species *Debaryomyces hansenii* (Zopf) Lodder et Kreger-van Rij and *Debaryomyces fabryi* Ota were reinstated. Our RAPD-PCR data reinforced that the species *Galactomyces geotrichum* is heterogeneous. All the *Geotrichum* isolates from cheese products belong to *Ga. geotrichum* group A *sensu* M. T. Smith. It was suggested to conserve the name *Geotrichum candidum* for this rather common species.

France: Laboratoire de Génie Chimique, CNRS UMR 5503, ENSIGC , 18 chemin de la loge, F-31078 Toulouse Cedex 04. Communicated by M.L. Delia <MarieLine.Delia@ensigct.fr >.

1. Ramon-Portugal, F., Delia-Dupuy, M.L., Pingaud, H., Riba, J.P. 1997. Kinetic study and mathematical modelling of the *Saccharomyces cerevisiae* 522D sensitive strain growth in presence of K2 killer toxin. J. Chem. Technol. Biotechnol. **68**(2):195-201.

The killer phenomenon is quantified through the action of a toxic medium on a sensitive strain culture. Indeed, the behaviour of a sensitive *Saccharomyces cerevisiae* strain (522D) growing in a culture medium containing K2 killer protein was studied in batch conditions. The kinetics of reaction were analysed. The inhibition due to the killer toxin was measured by the reduction of the viable biomass, ethanol production and glucose consumption compared with a reference fermentation. The reference culture was run under the same conditions, but using a heat-denatured solution of killer protein (this method was developed in our group). A decrease of the viable population of 68%

was observed after 15h incubation. The fermentation time for the total consumption of the glucose was significantly affected by the presence of the killer toxin. The specific rate of alcohol production was also affected during the fermentation. However the yields of ethanol and biomass were very similar for both fermentations. A kinetic mathematical model was proposed to explain the dynamics of the *S. cerevisiae* growth in the presence of the K2 killer protein. It takes into account the biomass evolution, the microbial death and the toxin consumption. The results of the suggested simulation were in agreement with the experimental data.

2. Furlan, S., Delia-Dupuy, M.L., Strehaiano, P. 1997. Xylitol production in repeated fed-batch cultivation. World J. Microbiol. Biotechnol. **13**:591-592.

The ability of *Candida parapsilosis* to produce xylitol was tested using successive substrate supplies, its pointed out the importance of the amount of viable cells in enhancing the xylose-xylitol conversion rate. Using repeated fed-batch cultivation, with pure xylose or mixtures of xylose and glucose as substrate, the productivity was increased by about 40% compared with simple batch culture without loss of yield of product on substrate. The presence of a low level of glucose in the culture medium stimulates the specific growth rate, and has no influence on other fermentative parameters.

3. Ramon-Portugal, F., Delia-Dupuy, M.L., Pingaud, H., Carrillo-Leroux, G.A., Riba, J.P. 1997. Kinetics study and mathematical modelling of killer and sensitive *Saccharomyces cerevisiae* strains growing in mixed culture. Bioprocess Engin. **17**:375-381.

The kinetics of growth of two yeasts growing in pure and mixed batch cultures were studied. Two winemaking strains were concerned: *S. cerevisiae* K1 possessing the K2 killer character and *S. cerevisiae* 522D sensitive to this toxin. First of all, pure cultures were analysed. In this case, the kinetic profiles of biomass production have shown that the growth rate of the K1 strain is slightly higher than the 522D strain one. During the fermentation, the viability for both populations was higher than 90%. After, fermentations in mixed culture with an initial percentage in killer strain of 5 and 10% with respect to the total inoculum showed a more important decrease in the percentage of total viable yeasts whent the initial concentration of killer strain increased. However the kinetic profiles of total biomass (killer+sensitive) were very similar in both cases. The microbial growth of the killer and sensitive strains in pure and mixed cultures were simulated. The mathematical model is based on three main reactions the evolution of the killer toxin in the culture medium, the duplication and the mortality rates for each microbial population. The model is adequate to represent the experiments.

4. Ramon-Portugal, F., Delia-Dupuy, M.L., Strehaiano, P., Riba, J.P. 1998. Mixed culture of killer and sensitive *Saccharomyces cerevisiae* strains in batch and continuous fermentations. World J. Microbiol. Biotechnol. **14**:83-87.

In winemaking, the killer mechanism plays an important role in the balance of the wild population. The possession of the killer characteristic is considered as a supplementary guarantee for the implantation of an inoculated killer strain. The balance between two strains of *S. cerevisiae* (one killer *S. cerevisiae* K1 and one sensitive *S. cerevisiae* 522D) was studied in mixed cultures in batch and continuous fermentations. Batch fermentations were run with initial killer strain concentrations of 5 and 10% of the total population. The influence of the killer strain was measured in comparison with a reference fermentation (sensitive strain alone). An initial concentration of 10% of killer strain affects the microbial population balance and the rate of ethanol production. However the fermentation was only slightly disturbed when the proportion of killer to sensitive yeast at the beginning of the mixed culture was 5%. To achieve total displacement by the killer yeast at low concentrations, the mixed cultures were carried out in a continuous system.In these conditions, with the same strains, a level of contamination as low as 0,8% of killer strain is sufficient to completely displace the original sensitive population after 150h incubation.

The following posters have been presented at the 19th ISSY " Yeast in the production and spoilage of food and beverages " 30 August-3 September 1998, Braga, Portugal.

5. Ramon-Portugal, F., Seiller, I., Taillandier, P., Favarel, J.L., Nepveu, F., Strehaiano, P. Consumption and production of organic acids by acidifying wine yeasts during alcoholic fermentation.

6. Ramon-Portugal, F., Pingaud, H., Strehaiano, P. Kinetic study of the transition step ethanol consumption/sugar consumption by *Saccharomyces cerevisiae*.

7. Aranda-Barradas, J., Taillandier, P., Riba, J.P. Fed-batch cultures of yeast: relationship between high cellular trehalose concentration and fermentative activity.

8. Alfenore, S., Delia, M.L., Strehaiano, P. Quantification of killer effect: experimental aspects.

9. Delia, M.L., Aguilar-Uscanga, M.G., Strehaiano, P. Influence of oxygen supply on the metabolism of *Brettanomyces bruxellensis*.

Germany: FML-Mikrobiologie,Weihenstephaner Berg 3,D-85350 Freising. Communicated by H. Seiler <seiler@lrz.tu.muenchen.de>.

1. Kosse, D., Seiler, H.,. Amann, R, Ludwig, W., Scherer, S. 1997. Identification of yoghurt-spoiling yeasts with 18S rRNA-targeted oligonucleotide probes. System. Appl. Microbiol. **20**:468-480.

2. Seiler, H., Kümmerle, M. 1997. Die Hefenflora von Handelskefir (The yeasts of commercial kefirs). Deutsche Milchwirtschaft **48**:438-440.

3. Kümmerle, M., Scherer, S., Seiler, H. 1998. Rapid and reliable identification of food-borne yeasts by Fourier-transform infrared spectroscopy. Appl. Envir. Microbiol. **64**:2207-2214.

4. Michel, D. 1998. Identifizierung und Characterisierung von lebensmittelrelevanten Hefen mit Oligonucleotidsonden und RAPD-Analyse (Identification and characterization of food-borne yeasts with oligonucleotide probes and RAPD analysis). Thesis, TU Munich.

5. Seiler, H., Kümmerle, M. 1998. Yeast species in the enviroment of dairy production lines. In: Yeast in the dairy industry. International dairy federation.

6. Kosse, D., Ostenrieder, I., Seiler, H, Scherer, S. 1998. Rapid detection and identification of yeasts in yoghurt using fluorescently labelled oligonucleotide probes. In: Yeast in the dairy industry. International dairy federation.

7. Kümmerle, M., Scherer, S., H. Seiler, H. 1998. Food spoilage yeasts identified by Fourier-transform infrared spectroscopy. In: Yeast in the dairy industry. International dairy federation.

Hungary: University of Horticulture & Food Science, Somloi UT 14/16 M-11187, Budapest. Communicated by T. Deak <tdeak@cfsqe.griffin.peachnet.edu>.

1. Mani, K., Peter, G., Deak, T. 1997. Identification of industrial yeast strains of Indian origin. Acta Alimentaria **26**:393-401.

Nine yeast strains were isolated from red wine, beer and baker's yeast, originating from India, and were identified as members of *Saccharomyces sensu stricto* group (7), *Pichia fabiani* (1) and *Issatchenkia orientalis* (1). All *Saccharomyces* isolates were found to be *S. cerevisiae*, although they differed in fermentative characteristics.

2. Peter, G., Tornai-Lehoczki, J., Deak, T. 1997. *Candida novakii*, sp. nov, a new anamorphic yeast species of ascomycetous affinity. Antonie van Leeuwenhoek **71**:375-378.

Two strains of an undescribed species of the genus *Candida* were isolated from decaying wood of *Quercus* sp. A description of the new species *C. novakii* is given.

3. Mozina, S.S., Dlauchy, D., Deak, T., Raspor, P. 1997. Identification of *Saccharomyces sensu stricto* and *Torulaspora* yeasts by PCR ribotyping. Lett. Appl. Microbiol. **24**:311-315.

18S rDNA + ITS1 and 25S rDNA PCR products covering more than 95% of the rDNA repeat unit of 28 *Saccharomyces sensu stricto* and *Torulaspora* yeasts and their anamorphs were digested with HaeIII, MspI and CfoI. Using combinations of two restriction enzymes, specific ribotyping patterns of six species were found offering a convenient tool for rapid identification of yeast isolates. However, for practical application, a large database should be developed including a large number of strains to cover intraspecies variations.

4. Deak, T., Beuchat, L.R., Guerzoni, M.E. 1998. A collaborative study on media for the enumeration of yeasts in foods. Int. J. Food Microbiol. **43**:91-95.

A collaborative study was made to evaluate the effectivity of a general purpose medium, tryptone yeast extract (TGY) agar on the detection and enumeration of yeasts from food. Nine laboratories participated in the study and compared four kinds of TGY with different concentrations of glucose, and, for comparison, DRBC agar. TGY at any concentration of glucose showed a reliable performance to recover yeasts from food, and did not differ from that of DRBC. However, DRBC incubated in light resulted lower counts compared to that incubated in the dark.

5. Deak, T. 1998. Biodiversity of yeasts in man-made environments. Food Technol. Biotechnol. **36**:279-283.

Agroecosystems and foods are based on natural habitats which have become partially or largely artificial by human interventions. Nevertheless, foods can be treated in terms of microbial ecology, and wineyards and wineries provide examples of man-made ecosystems in which the biodiversity of yeasts can be studied. Molecular techniques have made it possible to characterize the intraspecies population of wine yeast, *Saccharomyces cerevisiae* at the genetic level, to follow its distribution, variation and dynamics from the grapes through spontaneous and induced fermentation, as well as to study the speciation and domestication of *Saccharomyces sensu stricto* yeasts.

6. Mani, K., Dlauchy, D., Deak, T. 1998. A comparative study of karyotyping, RAPD-PCR and nDNA/nDNA homology methods for identification of yeasts isolated from Indian beverages. Acta Alimentaria **27**:43-52.

A survey of three different methods in yeast strain identification (karyotyping, RAPD-PCR and nDNA/nDNA reassociation) has been carried out in porder to differentiate, with industrial purposes, the strains present in red wine, beer and baker's yeast originating from India. All thre methods were able to successfully identify the strains. However, RAPD-PCR was more rapid than the other two.

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1. Suzzi, G., Lombardi, A. 1997. Understanding the role of Yeasts in cheese. Proc. "Mastering the Influence of Microorganism on the Final Properties of Raw milk cheese". Povoa de Varzim (Portugal, 26-27 May), pp. 129-135.

The main characteristics of cheese yeasts are lactose fermentation or assimilation, proteolytic and lypolitic activities, lactic acid and citric acid assimilation, tolerance at low water activities and grow at low temperatures. Among the cheese yeasts, *Yarrowia ipolytica* is one of the species most frequently associated with milk products. Due to enzymatic activities, it can be regarded as ripening agent for milk products. The *Yarrowia* inoculum in cheese can modify the microbial ecosystem and pH in the different parts of cheese. In addition can inhibit total and faecal coliform and mesophiles and can have growth stimulant effects on lactic acid bacteria. Also non-lactose-fermenting yeasts, in particular *Saccharomyces cerevisiae*, can contribute to the organoleptic quality of cheese. The main characteristics of *S.cerevisiae* from cheese are the production of ethanol, ethyl acetate and acetaldehyde.

2. Romano, P., Paraggio, M., Turbanti, L. 1998. Stability in by-product formation as a strain selection tool of *Saccharomyces cerevisiae* wine yeasts. J. Appl. Microbiol. **84**:336-341.

In this work we used a strain of *Saccharomyces cerevisiae* homozygous for different physiological and metabolic characters. The strain was inoculated in two grape musts and the stability of the characters was tested by isolating clones at different fermentation stages. A total of 60 cell-clones were collected and asci dissected from each, yielding a total of 1200 single spore cultures, which were then tested for the segregation of several genetically controlled traits. From the parental strain 10 asci were dissected and the 40 single spore cultures obtained were used as controls. Microfermentations were performed with the 200 single spore cultures obtained from clones, isolated at the end of Trebbiano and Aglianico must fermentations. The majority of these spore cultures corresponded with the parental strain in the production of all the by-products determined. The progeny of three clones from the Trebbiano fermentation exhibited a significant increase in the production of isoamyl alcohol, whereas the progeny of one clone from the Aglianico fermentation differed in the production of acetoin and arnyl alcohols. The variability found in the levels of by-products can also affect the organoleptic properties of the final product. The introduction of the "metabolic characteristic stability" as a selective index for industrial strains is advised.

3. Romano, P., Brandolini, V., Ansaloni, C., Menziani, E. 1998. Characterization of wine yeasts for 2,3-butanediol production. World J. Microbiol. Biotechnol. **14**:649-653.

The capacity to produce 2,3-butanediol by different species of wine yeasts (*Kloeckera apiculata, Saccharomyces cerevisiae, Saccharomycodes ludwigii, Zygosaccharomyces bailii*) was tested in grape must by AMD technique. The total amount varied from 23 mg l^{-1} to 857.7 mg l^{-1} and on the basis of 2,3-butanediol variation the yeast species showed a different behaviour. *Saccharomyces cerevisiae* and *Zgosaccharomyces bailii* behaved similarly, producing elevated amounts of the compound, resulting as high producers. *Kloeckera apiculata* and *Saccharomycodes ludwigii* produced low arnounts, resulting as low producers. When considerable amounts of 2,3-butanediol were found, little acetoin was present, the amount of butanediol and acetoin being in relation to the individual species.

4. Romano, P. 1998. Metabolic characteristics of wine strains during spontaneous and inoculated fermentation. Food Technol. Biotechnol. **35**(4):255-260.

The spontaneous alcoholic fermentation is characterized by the contribution of different S*accharomyces cerevisiae* strains, which grow in succession or in combination throughout the fermentation process and exhibit different metabolic patterns. The formation of secondary compounds is a strain specific characteristic and the strains are distinguishable in phenotypes through the production of different

amounts of by-products. Natural fermentation represents a source of indigenous *S.cerevisiae* strains, which seem more suitable to be used as starter cultures for that particular wine because they were isolated from the original region and, consequently, better adapted to the particular vinification conditions of that enological area. Among the indigenous strains, the cultures for must fermentation should be chosen on the basis of aroma and flavour-determinants typical of the wine under study. Successively, the selected cultures should be tested for the genetic segregation of traits under consideration in order to identify strains completely homozygous for the metabolic characteristics. Only a small proportion of natural wine strains is completely homozygous, the majority being heterozygous for one or more traits. In addition, a significant proportion of natural wine strains can sporulate on rich media, such as grape must, and, as a consequence, the progeny of such strains can exhibit differences in the levels of by-products, thereby affecting the organoleptic properties of the ultimate product. Determination of the degree of strain stability overcomes this problem and allows the choice of the most suitable selected culture to use in inoculated fermentation. The character "stability of metabolic phenotype in industrial strains" represents a selective index, which ensures that the final product is always consistent with the own properties of each wine.

5. Romano, P., Monteleone, E., Paraggio, M., Marchese, R., Caporale, G. 1998. A methodological approach for the selection of *Saccharomyces cerevisiae* wine strains. Food Technol. Biotech. **36**(1):69-74.

Yeast strains, producing different amounts of secondary compounds, exert a definite influence on the flavour and aroma of the wines and impart their imprinting. This suggests that the use of a single strain for different types of wines is not be appropriate, due to a potential uniformity of aromatic characteristics in the final products. In order to typify each product for the varietal and geographic characteristics, it becomes necessary to isolate natural autochtonous strains, which, in addition to the desirable technological characteristics, exhibit a metabolic profile corresponding to each wine. Thirty strains of *Saccharomyces cerevisiae*, isolated from different Aglianico grape cultivars, were tested for fermentation power, SO_2-resistance, Cu-resistance and the production of secondary compounds. The results for each strain were transformed into individual functions of desirability (d_i), i.e. dimensionless values between 0 and 1, and then combined to obtain a response of total desirability (D_{tot}). The form of the transformation was subjectively selected according to the level of knowledge of the desired optimal response. The strains were tested in Aglianico fermentations and only three showed a D_{tot} value higher than 0.7. By comparing D_{tot} values of selected strains with D_{tot} values of experimental wines, an evident correspondence was found. This demonstrates the goodness of the selection method utilised.

6. Palla, G., Brandolini, V., Caligiani, A., Marchese, R., Romano, P. 1998. Chiral resolution of acetoin and 2,3-butanediols by GC/MS as a tool for the characterization of fermenting yeasts. XX Int. Symp. Capillary Chromat., 26-29 May, Riva del Garda (Italy), p.32.

Acetyl methyl carbinol (acetoin) and 2,3-butanediol isomers formed during fermentation of Aglianico grape must by *Saccharomyces cerevisiae, Saccharomycodes ludwigii* and *Kloeckera apiculata* strains, have been resolved and quantified by GC/MS, using chiral capillary colums. Wines were alkalinized with sodium hydroxide, additioned of 1,4-butanediol as internal standard, extracted with ethyl acetate and the organic phase was injected in the GC/MS system, operating both splitless and SIM mode. The content of D,L-acetoin and (2R,3R)-(-)-2,3-butanediol is higher in wines obtained by *S.cerevisiae* than in those by *S'codes ludwigii* and *K. apiculata*, that show more racemates and meso forms. The total amounts of acetoin and 2,3-butanediols, together with D/L values, allow to discriminate between wines coming from different yeast fermentations.

7. Reyes, A., Paraggio, M, Pesole, G., Romano, P. 1998. Genetic characterization of dsRNA plasmids in *Saccharomyces cerevisiae* wine yeast. 19th ISSY, 30 Aug.-3 Sept., Braga, Portugal, P1.20, p.67.

The killing ability of *Saccharomyces cerevisiae* wine strains is of great interest in winemaking and problems associated with slow or stuck fermentations have been reported to involve a relevant incidence in grape must of wild killer yeasts, which can inactivate the inoculated strains of *S.cerevisiae*. Killer activity has been found in wine yeasts from many different geographical origins, being a relatively wide spread phenomenon. The existence of dsRNA plasmids in the cytoplasm of yeast was described in the 70s and soon their presence was correlated with the production of a killer toxin lethal to other strains of the same species. We have analyzed a total of 59 strains of *S. cerevisiae* from two different geographical areas of Italy: 30 of them came from the Basilicata region (South) while the remaining 29 strains were from Northern regions. These strains were assayed for the presence of dsRNA plasmids in their cytoplasm. The same strains were also tested against a sensitive strain for the production of the killer toxin. Correlation between the presence of a particular dsRNA plasmid and the killer activity were done. Furthermore, the frequencies of the different dsRNA plasmids in both Italian regions were compared.

8. Paraggio, M., Capece, A., Lipani, G., Romano, P. 1998. Fermentation characteristics of *Saccharomyces cerevisiae* isolates from Aglianico of Vulture in the Basilicata region of Southern Italy. Alcologia.

One hundred and fifteen strains of *Saccharomyces cerevisiae*, isolated from Aglianico must**,** were characterized for some traits of technological interest in winemaking, namely fermentation efficiency, resistance to sulphur dioxide and copper and ethanol tolerance. All strains exhibited a general high fermentation activity, reaching the maximal weight loss after ten days. The most of the strains were able to tolerate a considerable ethanol concentration and exhibited also a high SO_2 resistance, being at least resistant to 175 ppm of SO_2. The majority of the strains, 48% and 27%, tolerated SO_2 concentrations of 275 ppm and 300 ppm, respectively. Conversely, the majority of

the strains did not tolerate concentrations of Cu^{++} exceeding 40 mmol/L, being considered low resistant to this compound, while only 10% of strains possessed a high tolerance to copper ion, surviving at concentrations of 120 mmol/L. Three strains, exhibiting different responses to the considered parameters, were tested for genetic analysis. The progeny showed the same characteristics than the parental strains and was also homozygous for the characters considered. The phenotypic variation of the Aglianico strains for the traits under consideration confirms the environmental role of natural yeasts and the importance of individuating the main and desirable traits, that the selected culture must possess to preserve the individual characteristics of that specific product. In view of an increasing of biological production one of the desirable properties of the starter yeast becomes a high resistance to copper.

9. Romano, P., Marchese, R. 1998. Characterization of *Kloeckera apiculata* strains from star fruit fermentation. Antonie van Leeuwenhoek **73**:321-325.

A total of 37 strains of *Kloeckera apiculata* was isolated during the spontaneous fermentation of star fruit must. Each strain was differentiated from the others on the basis of its capacity to produce acetaldehyde, ethyl acetate, higher alcohols, acetoin and acetic acid. All the strains were characterized by the low production of higher alcohols and the high production of ethyl acetate, whereas consistent differences in the production of acetaldehyde, acetoin and acetic acid served to differentiate star fruit apiculate strains into six different phenotypes, present at different stages of the fermentation process. The metabolic strain diversity found can be interpreted as a natural consequence of environmental conditions, which influenced the frequency and selection of specific apiculate strains. From the biotechnological point of view the different metabolic biotypes represent an important source of strains for potential use as starter cultures for star fruit fermentation.

10. Torriani, S., Zapparoli, G., Suzzi, G. 1999. Genetic and phenotipic diversity of *Saccharomyces sensu stricto* isolated from Amarone wine. Antonie van Leeuwenhoek, in press.

Yeast strains. isolated from Amarone wine produced in four cellars in the Valpolicella area (Italy), were studied for genetical and biochemical characteristics. Individual strains belonging to the *Saccharomyces sensu stricto* complex were identified by traditional physiological tests and by RAPD-PCR and mtDNA restriction assays. Fourteen out of 20 strains were classified as *S.cerevisiae* and the remaining as *S.bayanus*. RAPD-PCR assay proved to be fast and reliable for identification of *Saccharomyces sensu stricto* strains and gave also intraspecific discrimination. RFLP of mtDNA gave a good discrimination between *S.cerevisiae* and *S.bayanus* with a better discriminatory power for *S.cerevisiae* species. With regard to secondary fermentation products most of the strains produced low amounts of isobutanol, and all *S.bayanus* strains produced low amounts of amylic alcohols. Each winery possessed specific strains with different genetical and biochemical characteristics that could be selected during Amarone winemaking at low temperature in presence of high sugar content.

11. Romano, P., Marchese, R., Laurita, C., Turbanti, L. 1999. Biotechnological suitability of *Saccharomycodes ludwigii* for fermented beverages. World J. Microbiol. Biotechnol., in press.

Nineteen *Saccharomycodes ludwigii* strains were tested for the production of secondary products in grape must fermentation. A predominant metabolic pattern was obtained characterized by high production of isobutyl alcohol, acetoin and ethyl acetate. The occurrence of some strains producing enhanced amounts of these compounds suggest a potential utilization of this species for industrial applications. Feijoa juice was inoculated with a selected *S'codes ludwigii* strain in comparison to a control strain of *S.cerevisiae* and the evaluation of the fermented products, was performed by 30 consumers on the odour, flavour and taste. The sample fermented by *S'codes ludwigii* was characterized by a fresh odour with a fruity flavour, identified as flavour of apple and kiwi. This product was compared to apple juice, with a more acid taste. Despite the high concentrations of acetic acid, this beverage might be considered a potential summer refreshing drink, addressed to a target of consumers, who prefer fruit drinks leaving a slightly acid and little sugary taste in the mouth.

12. Suzzi, G., Lombardi, A., Lanorte, M.T., Caruso, M., Andrighetto, C., Gardini, F. Phenotypic and genotypic diversity of yeasts isolated from water-buffalo mozzarella cheese. J. Appl. Microbiol., in press.

Water-Buffalo Mozzarella cheese (WBM) is one of the several "pasta filata" or stretched curd cheeses that originated in Southern Italy, traditionally manufactured from raw milk employing matural whey starter cultures. Lactose and galactose fermenting yeasts isolated in WBM were studied with the aim to evaluate their role in the ripening oh this cheese. The kinetic parameters of the growth of the yeasts as well as their principal metabolic end-products showed a great variability depending on the species. Moreover, the genetic polymorphism of the yeasts was studied for their differentiation at species level by means of the PCR fingerprinting and mtDNA restriction analysis. While the differentiation based on physiological traits was not able to discriminate *Kluyveromyces marxianus, Candida kefir* and *Candida sphaerica*, the PCR analysis with primer M13 and RF2 resulted a reliable and rapid method for differentiating at species level among *Saccharomyces cerevisiae, K.marxianus, K. lactis* and their anamorphic species. Furthermore, mtDNA analysis proved to be more discriminating at strain level.

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1. Granchi, L., Bosco, M., Messini, A., Vincenzini, M. 1998. Identificazione rapida di lieviti di interesse enologico mediante PCR-RFLP dello spaziatore ribosomale interno trascritto (ITS). Proc. Congresso Nazionale Biotecnologie. 1-3 luglio, Parma, Italy, p.133.

2. Granchi, L., Bosco, M., Messini, A., Vincenzini, M. 1998. PCR-RFLP analysis of ribosomal ITS region to rapidly identify yeast species occurring in spontaneous wine fermentations. Proc. 19th. ISSY, 30 Aug.-3 Sept., Braga, Portugal, p. 212.

3. Granchi, L., Bosco, M., Messini, A., Vincenzini, M. 1999. Rapid detection and quantification of yeast species during spontaneous wine fermentation by PCR-RFLP analysis of the rDNA ITS region. (Submitted)

PCR-RFLP analysis of the rDNA-ITS region with five endonucleases was applied to 174 yeast strains belonging to 30 species of oenological significance and including 27 type strains in order to develop a rapid protocol for yeast identification. PCR-RFLP patterns resulted species-specific with the exception of teleomorphic and anamorphic forms. According to these findings, a protocol taking about 30 hours was set up and applied for the detection and quantification of yeast species occurring in the course of a spontaneous wine fermentation at industrial level. This protocol consisted of the following steps: 1) plating of must or wine sample; 2) 24 hours of plate incubation; 3) sampling of all microcolonies (90 or more/plate); 4) cell lysis by heating at 80°C for 10 min; 5) amplification of rITS and direct restriction analysis 6) separation of the restriction fragments on 3.5% agarose gel electrophoresis using chilled buffer; 7) digital acquisition of restriction patterns images and comparison with our database of yeast rITS PCR-RFLP patterns. A representative sample of colonies from WL agar plates were identified by traditional methods, too. Both procedures gave identical responses. However, PCR-RFLP analysis furnished more precise quantitative data, proving to be a reliable and simple method for monitoring the development of the yeast community throughout wine fermentation and its storage. On the other hand, the timely knowledge on type and level of yeast populations which are transforming grape must into wine is believed to be absolutely necessary in order to give a rationale for any technological operation feasible in the cellar.

Italy: Università di Perugia, Dipartimento di Biologia Vegetale-Microbiologia, Via Borgo XX Giugno 74, 06121 Perugia. Communicated by M. Ciani <mciani@egeo.unipg.it>.

1. Ciani, M., Ferraro, L. 1997. Role of oxygen on acetic acid production by *Brettanomyces*/*Dekkera* in winemaking. J. Sci. Food Agric. **75**:489-495.

Since a large occurrence of *Brettanomyces* yeasts in strict anaerobiosis environment (sparkling wines) has been found without an increase in acetic acid content, we evaluated the influence of the oxygen concentration on the acetic acid production. Results showed that the oxygen concentration exerted a strong influence on both growth and acetic acid production by *Brettanomyces* yeasts in winemaking. Full aerobiosis determined a large production of acetic acid causing a stuck of metabolic activity. Semi-aerobiosis resulted in the best condition for alcoholic fermentation (Custers effect) combined with acetic acid production. In anaerobic condition *Brettanomyces* yeasts did not result in high acetic acid producers and a pure, even if slow, alcoholic fermentation occurred. The absence of an increase in acetic acid in wines, does not exclude the active presence of *Brettanomyces* yeast since the characteristic "high acetic producer" in *Brettanomyces* yeast is linked to the presence of oxygen.

2. Ciani, M. 1997. Role, enological properties and potential biotechnological use of non-*Saccharomyces* wine yeasts. Eds. S.G. Pandalai, in "Recent Res. Devel. in Microbiology"1, pp.317-331. Research Signpost, Trivandrum, India.

Recent quantitative studies showed that non-*Saccharomyces* wine yeasts suvived during fermentation for longer perids than previously thought even in inoculated fermentations with *Saccharo-Saccharomyces* wine yeasts revealed some interesting characters useful in winemaking. However, for the use of these yeasts in winemaking more specific information on the metabolic interactions with *myces cerevisiae* selected cultures. On the basis of these experimental evidences the role of non-*Saccharomyces* wine yeasts on analytical profile is revaluated. The analyses of enological properties of non-*Saccharomyces* yeasts and the develop of appropriate control strategies are required. Two biotechnological processes, using *Schizo-saccharomyces pombe* and *Candida stellata*, are analyzed.

3. Ciani, M., Maccarelli, F. 1998. Oenological properties of non-*Saccharomyces* yeasts associated with wine-making. Word J. Microbiol. Biotechnol. **14**:199-203.

Several yeast cultures belonging to five non-*Saccharomyces* species associated with wine-making were evaluated for their oenological properties. Results showed that *Candida stellata* and *Torulaspora delbrueckii* could positively affect the taste and flavour of alcoholic beverages. Apiculate yeasts exhibited large amounts of negative by-products particularly ethyl acetate. Nevertheless, *Kloeckera apiculata* showed a significantly negative correlation between either acetic acid and ethyl acetate formation and ethanol production. Selected non-*Saccharomyces* yeast cultures could profitably be applied in wine-making for optimization of wine bouquet using new fermentation technologies.

4. Ciani, M. 1998. Wine vinegar production using wines made with different yeast species. J. Sci. Food Agric. (in press).

The influence of base wines obtained by the fermentation of different yeast species on acetic acid bacteria growth and on the analytical profile of vinegars was investigated. Results show that the substrates for wine vinegar production exerted a strong influence on both acetic acid bacteria growth and analytical profile of vinegars. The base wine obtained from the alcoholic fermentation of *Saccharomyces cerevisiae* was not always the best substrate. The fermentate made with *Candida stellata* positively influenced the acetic acid bacteria growth and the quality of winegar while the wine obtained from the fermentation of *Kloeckera apiculata* was a good substrate for acetic acid bacteria growth and acetic acid production and could be used for "ordinary" vinegar production.

5. Ciani, M., Ferraro, L. 1998. Combined use of immobilized *Candida stellata* cells and *Saccharomyces cerevisiae* to improve the quality of wines. J. Appl. Microbiol. (in press).

Grape must fermentation by the combination of immobilized *Candida stellata* cells and *Saccharomyces cerevisiae* was carried out in order to enhance the analytical profiles of wine. Batch and continuous pretreating of must with immobilized *C. stellata* cells followed by an inoculum of *S. cerevisiae* enhanced the analytical profiles of fermentates. The metabolic interactions between the two yeast species showed a positive influence on reducing sugars, acetaldehyde and acetoin metabolism. Sequential fermentation was the best combination to improve the analytical profiles of wine but caused a loss of viability and metabolic activity of beads by limiting their successive use. Continuous pretreating of must on the beads of *C. stellata* could be a more interesting modality to improve the quality of wines. This biotechnological process could be profitably used to produce specific and special wines.

Slovakia: Research Institute for Viticulture and Enology, 833 11, Matúškova 25, Bratislava; communicated by E. Minárik.

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1. Einflub von Hefezellwand-Präparaten auf die durch *Leuconostoc oenos* eingeleitete Âpfelsäure-Milchsäure-Gärung in Wein.

Influence of yeast ghost preparation on the L-malic acid decomposition in wine by *Leconostoc oenos*. Yeast ghost preparations stimulate the L-malic acid decomposition induced by *Leuconoctoc oenos* cultures only insignificantly,. Important preconditions for an effective course of malolactic fermentation by *Leuconostoc oenos* cultures are described.

2. Jungová, O. Autochtónna mikroflóra vinohradníckych oblastí Slovenska I.

The qualitative and quantitative representation of yeasts and yeast-like microorganisms in the wine growing regions of South and Middle Slovakia was investigated. 255 *Saccharomyces cerevisiae* strains were tested in order to acquire efficient autochthonous pure yeast cultures ensuring the authenticity of varietal wines with controlled appellation of origin. The highest alcohol production was attained by isolates originating from the locality Strekovithe, the lowest from the locality Muzia.

3. Jungová, O., Minárik, E. Genofond vínnych kvasiniek a jeho význam pre vinársku prax.

The yeast collection of the Research Institute for Viticulture and Enology in Bratislkava (Rive 28) represents an important yeast gene bank utilized in the wine industry. Results of examination of basic technological properties of 471 yeast strains of the species *Saccharomyces cerevisiae* had been confirmed: 65% of strains fermented grape must up to 13 - 15 vol. % alcohol, 12% examined strains showed better results in practice than the verified strain Bratislava 1.

4. Jungová, O., Minárik, E. Problémy so *Zygosaccharomyces bailii* pretrvávajú.

Microbiological investigations of wine and wine production units confirmed that the occurrence of resistant yeasts *Zygosaccharomyces bailii* is still of immediate interest. As source of contamination of bottled wine may be pointed out concentrated grape must used for adjustment of residual sugar or the equipment of the wine. The occurrence of *Z. bailii* and haze formation may be avoided by total decontamination by membrane filtration of the wine and by prevention of recontamination during bottling.

Spain: Universidad de Valencia, Departamento de Bioquimica y Biologia Molecular, Instituto de Agroquimica y Tecnologia de Alimentos, CSIC Apartado de Correos 73, 46100 Burjassot, Valencia. Communicated by E. Matallana <emilia.matallana@uv.es>.

1. Ivorra, C., Pérez-Ortín, J.E., del Olmo, M. An inverse correlation between stress resistance and stuck fermentations in wine yeasts. A molecular study. Biotechnology and Bioengineering (in press).

During alcoholic fermentations yeast cells are subjected to several stress conditions and, therefore, yeasts have developed molecular mechanisms in order to resist this adverse situation. The mechanisms involved in stress response have been studied in *Saccharomyces cerevisiae* laboratory strains. However a better understanding of these mechanisms in wine yeasts could open the possibility to improve the fermentation process. In this work an analysis of the stress response in three wine yeasts has been carried out by studying the expression of several representative genes under several stress conditions which occur during fermentation. We propose a simplified method to study how these stress conditions affect the viability of yeast cells. Using this approach an inverse correlation between stress-resistance and stuck fermentations has been found. We also have preliminary data about the use of the HSP12 gene as a molecular marker for stress-resistance in wine yeasts.

2. Gimeno-Alcañiz, J.V., Pérez-Ortín, J.E., Matallana, E. Differential pattern of trehalose acculumation in wine yeast strains during the microvinification process. Biotechology Letters (in press).

Trehalose accumulation in wine yeast strains growing under microvinification conditions was determined and compared to that obtained under laboratory conditions. Industrial strains accumulate 10-fold more trehalose than laboratory strains. Contrary to batch-culture growth, under microvinification conditions trehalose accumulation is not consequence of glucose exhaustion. Physiological relevance of trehalose during the process of wine making and their use for potential improvements of alcoholic fermentation are discussed.

Spain: Universidad de Valencia, Departamento de Biotecnologia-Jata (CSIC), Instituto de Agroquimica y Tecnologia de Alimentos, P.O.Box 73, 46100 Burjassot, Valencia. Communicated by A. Querol <aquerol@iata.csic.es>.

1. Puig, S., Querol, A., Ramón, D., Pérez-Ortín, J.E. 1996. Evaluation of the use of phase specific gene promoters for the expression of enological enzymes in an industrial wine yeast strain. Biotechnol. Lett. **18**:887-892.

Genes as *POT1*, *HSP104* and *SSA3*, which are late expressed in laboratory culture conditions are expressed only during the first few days in microvinifications in wine yeast cells. This effect is probably due to the different growth conditions and leads to useless levels of enzyme activity for a reporter gene. However the *ACT1* promoter, which is constitutively expressed in laboratory conditions, produces sufficient amounts of enzyme activity in late fermentation phases.

2. Puig, S., Ramón, D., Pérez-Ortín, J.E. An optimized method to obtain stable food-safe recombinant wine yeast strains. J. Agric. Food Chem. **46**:1689-1693.

Pure *Saccharomyces cerevisiae* cultures are commonly used to inoculate fresh musts. The added active dry yeasts dominate the fermentation and produce wine of more reproducible quality. This microbiological simplification of the vinification process opens the way for the genetic modification of active dry yeasts. We have successfully transformed industrial yeast strains selecting for G418 resistance. The use of this method for integrative transformation facilitates the construction of food-safe recombinant yeast strains with high efficiency. To demonstrate the potential of the method we have disrupted the two *URA3* alleles of the wine yeast T_{73} strain in two consecutive steps obtaining a stable recombinant strain which retains all its useful wine making properties.

3. Belloch, C., Barrio, E., M., García, M., Querol, A. 1998. Inter- and intraspecific chromosome pattern variation in the yeast genus *Kluyveromyces*. Yeast **14**:1341-1354.

The analysis of the electrophoretic chromosome patterns of the species of the genus *Kluyveromyces*, reveals a high polymorphism in size, number and intensity of bands. Different sets of electrophoresis running conditions were used to establish species-specific patterns and also to detect intraspecific variation. According to their karyotypes, the species of this genus can be divided into two major groups. The first group includes the species *K. africanus*, *K. bacillisporus*, *K.delphensis, K. lodderae*, *K. phaffi*, *K. polysporus* and *K. yarrowii*, composing the so-called "*Saccharomyces cerevisiae*-like", because their karyotypes resemble that of the species *S. cerevisiae*. The second group comprises the species *K. aestuarii*, *K. blattae*, *K. dobzhanskii*, *K. lactis*, *K. marxianus, K. thermotolerans*, *K. waltii*, and *K. wickerhamii*, whose chromosomal patterns exhibit common characteristics very different to those of the species included in the "*S. cerevisiae*-like" group. This division is concordant with the position of these species in previous phylogenetic reconstructions. Additionally, the intraspecific analysis of the chromosome patterns show a rich polymorphism in the heterogeneous species *K. dobzhanskii*, *K. lactis*, and *K. marxianus*, which is in concordance with the variability observed with other phenotypic or genetic markers. On the contrary, *K. thermotolerans* exhibits a homogeneous karyotype indicative of a very low level of chromosomal polymorphism, which is congruent with the reduced variability found in this species with other molecular markers.

4. Manzanares, P., Ramón, D., Querol A. 1999. Screening of non-*Saccharomyces* wine yeasts for the production of β-D-xylosidase activity. Int. J. Food Microbiol. **46**:105-112.

Fifty-four yeast strains belonging to the genera *Candida, Dekkera, Hanseniaspora, Metschnikowia, Pichia, Rhodotorula, Schizosaccharomyces* and *Zygosaccharomyces,* mainly isolated from grapes and wines, were screened for the production of β-D-xylosidase activity was only detected in eight yeast strains belonging to the genera Hanseniaspora (*H'spora osmophila* and *H'spora uvarum*) and *Pichia* (*P.anomala*). D-xylosidase preparations active againstnitrophenyl-β-D-xyloside were characterised with respect to their optimal pH and temperature conditions. *H'spora uvarum* 11105 and 11107 and *P. anomala* 10320 β-D-xylosidase preparations were active at pH and temperature ranges and at concentrations of glucose and ethanol usually found during winemaking processes.

5. Puig, S., Pérez-Ortín, J.E. Stress response and expression patterns in wine fermentations of yeast genes induced at the diauxic shift. Yeast, submited.

During wine fermentation yeasts quickly reach a stationary phase, where cells are metabolically active by consuming sugars present in grape must. It is, consequently, of great interest at this stage to identify suitable gene promoters that may be used to induce the expression of genes with enological applications. With this aim, we have studied a group of genes showing an induction peak at the diauxic shift, and possessing stress response elements (STRE) at their promoters. We have determined their induction levels under individualised stress conditions, such as carbon source starvation or high salt concentrations. In all the cases studied the activation and/or basal transcription are dependent on the transcriptional factors Msn2p and Msn4p. We have analysed the expression patterns and mRNA levels during wine fermentation, and have found that they are all activated at the stationary phase. Finally, we have identified *SPI1*, a new highly expressed yeast gene which is specifically induced at the stationary phase of both microvinification and laboratory growth conditions.

Obituary

Dr. Nellie Margaretha Stelling-Dekker
28 May 1905 - 24 October 1998

Dr. Nellie Stelling-Dekker passed away at the age of 93 years. She had made important contributions to the systematics of yeasts.

In 1931, she presented her Thesis for Dr.Sci. at the State University of Utrecht. The title of the dissertation was: "Die Hefesammlung des 'Centraalbureau voor Schimmelcultures' Beiträge zu einer Monographie der Hefearten. I. Tell. Die Sporogenen Hefen". Her promoter on 8 July 1931 was Prof. Johanna Westerdijk. Prof. Albert Jan Kluyver was a member of the promotion commission. He had directed and supervised the work in the CBS Yeast Division at the Laboratory of Microbiology of the Technical University, on the Nieuwelaan in Delft. The thesis (547 pp.) includes a critical review of existing methods and yeast systematics; a systematic treatment of the sporogenous yeast genera, and a proposal for a rational system for the sporogenous yeasts.

The descriptions of genera and species, including many morphological drawings, have set the example for later works by Lodder, Diddens, Kreger-van Rij, up to the present work edited by Kurtzman and Fell.

In a preface to the thesis, Kluyver wrote (in German):

"The hope may be expressed that many yeast scientists will accept the results of this work as a scheme for further elaboration of the systematics of sporogenous yeasts. In that case, on the one hand the discovery of many, still unknown yeast species may be greatly promoted. On the other hand - perhaps even more important for a viable yeast systematics - the establishment of many putative new species may be prevented."

The genus *Dekkeromyces* did not stand the tooth of nomenclatural development. But the genus *Dekkera* still stands as a homage and memory to a pioneer in yeast taxonomy.

W.A. Scheffers, Delft

Forthcoming meetings

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

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

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Yeasts in the Dairy Industry
Second symposium organised by the International Dairy Federation
Bologna, Italy 9-10 September, 1999.

Objectives of Symposium: *Systematic description of the use of yeasts as starter cultures and in development of milk based products relying upon technological properties of yeasts like aroma formation, lipolysis and proteolysis. *Development and evaluation of new methods including molecular techniques for characterisation of yeasts with potential use as starter cultures. *Detection and enumeration of specific yeasts, including spoilage organisms, in dairy products. *Information on yeasts as spoilage organisms in dairy products. *Information on yeasts as probiotics in dairy products. *Discussion of the subject of adding value to waste (e.g. whey) using yeasts. Contact:

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Yeast Genetics and Molecular Biology, a Symposium to honor Fred Sherman, University of Rochester, New York. October 8-10 1999.

We invite you to attend a symposium entitled "Yeast Genetics and Molecular Biology" that is being held in honor of Fred Sherman on Oct. 8-10 1999. As you may know, as of Jan. 1, 1999, Fred stepped down as Chairman of the Department of Biochemistry and Biophysics of the University of Rochester. The symposium is a chance to celebrate his many contributions over the years to the field of yeast genetics and to the University of Rochester. We are inviting all former members of the Sherman laboratory, as well as past and present collaborators and members of the yeast community, to come to Rochester for this event. The symposium will include oral presentations from the following confirmed speakers: Joachim Ernst, Beverly Errede, Gerald Fink, Sue Liebman, Michael Hampsey, Ira Herskowitz, Chris Lawrence,

Rodney Rothstein, and Ken Zaret. In addition, all attendees are encouraged to present their work, either as an additional oral presentation or by participation in a poster session. We hope that the talks and posters will stimulate lively and wide-ranging discussions of the variety of scientific areas to which Fred has contributed.

The symposium will begin with an informal get-together on the evening of Friday, Oct. 8, and will extend into early afternoon on Sunday Oct. 10. It will include luncheons on Saturday and Sunday and a dinner on the night of Saturday, Oct. 9. This will be one of the first events held in the new research building of the Rochester Institutes of Biomedical Sciences that is part of the University of Rochester Medical Center.

The meeting is being organized by Mark Dumont, Elizabeth Grayhack, and Eric Phizicky of the Department of Biochemistry and Biophysics of the University of Rochester with administrative assistance from Linda Altpeter.

To reserve a place, reply by July 1, 1999 to Linda, by calling her at 716-275-3723, e-mailing her at Linda Altpeter@urmc.rochester.edu, or faxing her at 716-271-6007. Please include your e-mail address. Abstract forms will be sent to registrants over the summer. The deadline for abstract submission will be Sept. 1.

To cover the costs of the meeting and the included meals, there will be a registration charge of $65.00 per person. In addition, accommodations at the Strathallan Hotel are available at a reduced rate. To contact the Strathallan, call 800-678-7284 or 716-461-5010 and refer to the group code U.OF.R.108. The hotel is located at 550 East Avenue, Rochester, NY 14607 (Fax: 716-461-3387, website: www.strathallan.com).

We hope that you will be able to join Fred at this symposium and welcome any suggestions or comments you may have regarding the plans for this event.

Mark E. Dumont, Elizabeth Grayhack, Eric M. Phizicky, and Linda L. Altpeter.

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):

First announcement	June 1999
Second announcement/ registration form	15 October 1999
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.

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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.

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Brief News Item

Change of address and employment: J. W. Buck

I shall be joining the faculty at the University of Georgia on August 1st 1999 as Assistant Professor - Diseases of Ornamentals. My new address will be

Dr. James W. Buck
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