ISSN 0513-5222

# Y E A S T

## A Newsletter for Persons Interested in Yeast

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

**DECEMBER 1997** **Volume XLVI, Number II**

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

# Editorials

## Prof. Dr. Dr. h.c. Helmut Holzer 1921-1997

With sadness, I announce the passing of Prof. Helmut Holzer of the University of Freiburg, Germany. Prof. Holzer was for many years a regular contributor to the Yeast Newsletter. His most recent work was in the area of the biochemistry and genetics of trehalose metabolism, function, and regulation in yeast. As I did not know Prof. Holzer personally, I invite any readers who are more familiar with his life and accomplishments to share with us any information they deem relevant.

## New payment option for readers in the United Kingdom

We are now offering to subscribers in the British Isles the opportunity to pay their subscription fees by cheque in Pounds Sterling (£5 surface mail or £7.50 for airmail) to Prof. G.G. Stewart, in Edinborough. If this experiment is successful, we hope to offer a similar, more convenient mode of payment to subscribers across Europe. We are aware that astronomical bank fees are levied by some European financial institutions to issue international cheques or money orders, and we cannot accept any cheques (including Eurocheques) that are not cashable directly at a U.S. or Canadian bank. Some readers have found it most convenient to purchase U.S. bank notes. Despite several requests, we cannot accept direct bank transfers.

## Network: yeasts in food and beverages

In collaboration with the International Yeast Commission, Dr. Pratrizia Romano will be coordinating a new network for persons interested in yeasts of foods and beverages. The activities of the group will be publicized in the Yeast Newsletter. Interested readers are urged to read the details in the ICY section of this issue of the Yeast Newsletter.

## Short reviews, essays, and images in the Yeast Newsletter

We are delighted to see that some readers have communicated, for this issue, a review and two essays on topics that ought to be of interest to many readers. As stated on the reminder cards sent in April and October, readers are welcome to communicate materials in many formats. As the content of the Yeast Newsletter is not subject to formal peer review, it is entirely appropriate that readers should send any comments they may have on material presented in review or essay format.

Recent developments in desktop publishing make it easier to incorporate illustrations when appropriate, subject of course to copyright law. We have included images in the "Forthcoming meetings" section, and hope that this will enhance the YNL visually. Readers are encouraged to send images of general interest as part of their submissions.

---

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

M.A. Lachance
Editor

**I. Virology Group, International Centre for Genetic Engineering & Biotechnology, Aruna Asaf Ali Marg, New Delhi 110067, India. Communicated by S.K. Lal <sunillal@icgebnd.ernet.in>.**

The following is a short review article. Readers interested in additional details are welcome to contact Dr. Lal.

## The *Pichia pastoris* Gene Expression System: A Review.

Baker's yeast, *Saccharomyces cerevisiae*, was the initial choice as a yeast host due to the accumulation of information concerning molecular and genetic aspects of the organism. Since the first report of heterologous gene expression in *S. cerevisiae* in 1981,[1] a number of groups have reported success in producing small quantities of foreign proteins on a laboratory scale. However, several problems have been encountered in attempts to adapt laboratory-scale processes to an industrial scale. First, most of the promoters selected to express the heterologous genes must be present on multi-copy plasmids for high levels of expression. In the high cell-density environment of a fermentor, selection for plasmid maintenance is often lost, and plasmid distribution, copy number, and stability become significant problems. Second, some promoters, such as those from the glycolytic genes are constitutive, and therefore, strains which express the product can be at a selective disadvantage relative to non-expressing strains. The increased number of generations required to produce the cell mass of an industrial-scale process may allow sufficient time for the succession of non-expressing strains over the desired expressing population. Third, laboratory culture media are often complex and expensive, and when switching to a medium which is acceptable in large-scale fermentations, significant decreases in product yield are often noted. Another weakness occasionally demonstrated by S. *cerevisiae* is the inability to perform "correct" glycosylation. In *S. cerevisiae*, N-linked glycosylation can consist of a polymer ranging from twenty to one hundred fifty mannose residues in length. Such glycosylation may be detrimental to the efficient secretion of proteins into the growth medium, as well as to the biological activity of the secreted heterologous protein.

In the light of these problems, in recent years, several industrial yeasts owing to their robust growth and certain other unique characteristics, have been developed as recombinant host systems for commercial production of heterologous proteins.[2] One such yeast, *Pichia pastoris,* has proved to be an excellent host for production of both secreted and intracellular proteins.[3]

Metabolism of methanol by *P. pastoris* proceeds by a well defined pathway. Alcohol oxidase, the first enzyme in the methanol utilization pathway of *Pichia pastoris*, can constitute as much as 30% of the soluble protein of the cell during growth on methanol. In contrast, when this yeast is grown in excess amounts of repressible carbon sources, such as glucose or glycerol, no alcohol oxidase is present. Alcohol oxidase converts methanol to formaldehyde within the protective confines of yeast peroxisomes.[4] One of these highly expressed and tightly methanol regulated promoters from *P. pastoris* controlling the alcohol oxidase 1gene (*AOX1*), has been used with a foreign gene. This gene is thus regulated by the *AOX1* promoter and would be expressed at a high level, even in the cells that contained only a single copy of an *AOX1* promoter-foreign gene construct (expression cassette). This expression cassette can be inserted into the host genome, thereby avoiding potential plasmid instability problems. Most expression vectors designed to date are single copy integrants and have excellent mitotic stability. The expression vectors constructed to date are for both intracellular expression and secretion. These vectors utilize the protein of *E. coli* plasmid pBR322 encoding ampicillin resistance and containing the origin of replication. The *P. pastoris HIS4* gene is generally used for selection in the histidine auxotrophic strain GS115. The alcohol oxidase 5' and 3' regions are utilized in each vector for control of gene expression and proper termination. Several genes of the methanol-utilization pathway have been cloned and characterized. The methanol-inducible promoter regions have been sequenced and have been used to construct various efficient expression vectors.

Regulation of gene expression by methanol in such yeast strains is simple and cost effective for industrial fermentations. *P. pastoris* has been fermentor optimized and is capable of high cell density growth (150 g/l dry weight) in continuous fermentation. High-level expression simplifies product recovery and purification. The growth medium consists of a simple, well defined, inexpensive formulation, thus media and purification costs are lower. *P. pastoris* is easily scaled up from shake flask to fermentor. Economics of scale-up impact the rate of return and payback period. High cell density is achieved while maintaining optimal production of the foreign protein. For example, the yield of HBsAg particles increased linearly with total protein through a 400 liter fermentation (240 litre working volume) at 59 grams per liter (dry weight). Tightly controlled gene expression helps to minimize any possible toxic effects of a foreign protein on the yeast. By varying the carbon source, the level of gene expression can be manipulated to reduce possible toxic effects of foreign protein on the host organism. High productivity under a variety of fermentation conditions (batch or continuous) offers the flexibility needed in the production of proteins with various characteristics, for example, in particle assembly of viral antigens. Few proteins have exhibited toxic effects or have shown non-linear scale-up kinetics, thus greatly shortening product development time.

The methylotrophic yeast *P. pastoris* has become of interest to both academics and industry and has been used to successfully express a wide variety of proteins ranging in size from 6kD to 135kD. Initially industry was interested in *P. pastoris* for single-cell protein production.[5] Various examples of the potential of the *P. pastoris* expression system have been cited to date with genes from the hepatitis B virus HBsAg[6] and X,[7] tetanus toxin fragment C,[8] hepatitis E virus ORF3,[9] tumor necrosis factor, superoxide dismutase, human interleukin-2, *Bordetella pertussis* pertactin (P69), tetanus toxin fragment C (ref. 10, for review), streptokinase,[11] β-galactosidase, salmon growth hormone, mature IL-2, gamma interferon, human serum albumin, HIV antigens (ref. 12 for review), spinach glycolate oxidase,[13] spiny dogfish shark cytochrome P450c17,[14] N-terminal half transferrin,[15] human mutant monocyte chemotactic protein 3,[16] trimeric CD40L.[17]

The *P. pastoris* system has been shown to secrete extremely

high levels of various proteins into the culture media where purification is further facilitated by naturally low levels of native secreted proteins. Some of the secretion levels that have been reported include human serum albumin (4g/l), invertase (2.5g/l), bovine lysozyme (550 mg/l), mouse epidermal growth factor (ref. 10, for review), aprotinin (930 mg/l),[18] bovine enterokinase catalytic subunit (6.3mg/l),[19] alpha amylase (2.5g/l).[20] Although the initial application of the methylotrophic yeast was during the early 1970s when there was considerable interest in using them to produce single cell protein. Alcohol oxidase continues to enjoy a small market as a diagnostic enzyme. However, potentially large volume applications, such as to produce aldehydes or hydrogen peroxide, are yet to emerge. Perhaps the most significant application of the methylotrophic yeast will be

References:


[1]Hitzeman, R. A., et al. Nature (1981) **293**:717-722.
[2]Romanos, M., et al. Yeast (1992) **8**:423-488.
[3]Sreekrishna, K. and Kropp, K. (Ed. Wolf.) Chapter 4 (1996) 203-252.
[4]Veenhuis, M., et al. Adv. Microb. Physiol. (1983) **24**:2-82.
[5]Anthony , C. The Biochemistry of Methylotrophs. (1982) Academic Press, Inc., New York.
[5]Cregg, J. M., et al. Biotechnology (1987) **5**:479-485.
[7]Lal, S. K., et al. (1997) personal communication.
[8]Clare, J. J., et al. Biotechnology (1991) **9**:455-460.
[9]Lal, S. K., et al. Gene (1997) **190**:63-67.


as a host organism for the production of heterologous proteins. As described above, high-level expression has been achieved employing the tightly regulated and highly expressed *AOX1* regulatory sequences in *P. pastoris*. Moreover, this yeast possesses genetic and physiological features that make it an excellent host for production of recombinant proteins. In the future it may even be possible to target foreign proteins to the peroxisomes in these yeasts to protect them from proteolysis. Phillips Petroleum Company had been licensing the *P. pastoris* expression system since 1988, however it is now commercially available from Invitrogen Corporation, CA. With this tool now in the hands of a growing number of scientists, this use of the methylotrophic yeasts is emerging as their most noteworthy application.


[10]Cregg, J. M., et al. Biotechnology (1993) **11**:905-910.
[11]Hagenson, M. J., Enzyme Microb. Technol. (1989) **11**:650-656.
[12]Wegner, G. H. FEMS Microbiol. Rev. (1990) **87**:279-284.
[13]Payne, M. S. et al. Gene (1995) **167**:215-219.
[14]Trant, J. M. Arch. Biochem. Biophys. (1996) **326**:8-14.
[15]Steinlein, L. M. Prot. Exp. & Puri. (1995) **6**:619-624.
[16]Masure, S. et al. J. Interferon Cyto. Res. (1995) **15**:955-963.
[17]McGrew, J. T. et al. Gene (1997) **187**:193-200.
[18]Vedvick, T. et al. J. Ind. Microbiol. (1991) **7**:197-202.
[19]Vozza, L. A. et al. Biotechnology (1996) **14**:77-81.
[20]Paifer, E. et al. Yeast (1994) **10**:1415-1419.



**II. Laboratory of Microbiology, Wageningen Agricultural University, P.O. Box 8033, 6700 EJ Wageningen, The Netherlands. Communicated by W.J. Middelhoven <Wout.Middelhoven@algemeen.micr.wau.nl>.**


## Essay: Assimilation of organic acids: the pH as determining factor.

It has long been known that organic acids are taken up by microorganisms in two different ways. In some cases transport mechanisms for the free acid are operating. It is clear that these processes are favoured by a low pH, at which the substrate is largely present as undissociated acid. The substrates of many other transport mechanisms, however, are the anions. These prevail in the culture medium at higher pH. It can be expected that some organic acids are preferably assimilated at low pH and others at higher pH. This of course will depend on the nature of the microorganism studied.

For this reason the use of assimilation tests for taxonomic purposes requires careful standardization of the pH, especially when organic acids are the substrate. This is necessary to ensure reproducibility of the results. Until 1984 all growth media used for assimilation tests were carefully adjusted to pH 5.5 (van der Walt and Yarrow, in The Yeasts, 1984). Unfortunately this practice has been abandoned in some cases. Galacturonic and quinic acids are administered as free acids, and saccharic acid as a monopotassium salt, without pH adjustment (D. Yarrow, personal communication). However, galactonic acid is supplied as the completely neutralized calcium salt.

The pH of media with galacturonic or quinic acid, both prepared according to the instructions of CBS Delft, i.e. 0.5% in YNB, is as low as 2.9! In my opinion negative growth results reflect sensitivity to acid rather than failure to assimilate the compound involved.

The pH of 0.5% monopotassium saccharate in YNB is 3.8. Recently some firms introduced calcium saccharate which is cheaper. I guess that the pH of the growth medium prepared with it will be about 5.5. If people are using calcium saccharate as the substrate, the pH should be carefully adjusted to 3.8 in order to render results comparable to those of CBS Delft.

The importance of the pH in growth media containing organic acids is illustrated by two examples, both from my own experience. Some years ago, one of my students isolated a yeast strain growing on pectin and polygalacturonate as the sole source of carbon and energy. I identified it as *Trichosporon laibachii*, without any doubt. To my surprise, this yeast species is unable to assimilate the monomer, galacturonate (Barnett, Payne and Yarrow, 1990). However, in my hands the type strain grew readily on it, as well as on both polymers! The clue to this controversy was supplied by David Yarrow who wrote me that galacturonate medium should not be neutralized. *Trichosporon laibachii* (and other yeasts?) is either unable to grow at pH 2.9 or to take up the free galacturonic acid.

Another example regards lactate assimilation. We isolated yeast strains from maize silage responsible for its aerobic deterioration. This process involves oxidation of lactic acid. Surprisingly, two of the most numerous species, i.e. *Candida*

*milleri* and *Saccharomyces exiguus/Candida holmii* responded negatively in the growth test on lactate YNB pH 5.5, but if the pH was lowered to 4.0 growth occurred. In these species the initiation of lactate assimilation requires a low pH and possibly the presence of free lactic acid. We did not study this in detail. In this case the taxonomic test does not reflect the true biochemical potency of the yeast species involved.

This may also be true of yeasts unable to assimilate galacturonic and quinic acids. It can be expected that the growth tests performed at pH 2.9 give false negative results. Of course, the main objective of the tests, the distinction of yeast taxa, is met, but the data could supply more information if the tests were performed at the standard pH of 5.5. For example, yeasts producing enzymes hydrolyzing pectin and polygalacturonate can be expected to grow on the monomer galacturonate. Screening of the yeast collection for production of pectinases, which are enzymes of great industrial importance, could be facilitated if the data on galacturonate assimilation were reliable. Now they are not, and species like *Trichosporon laibachii* are overlooked.

In some bacteria quinic acid is metabolized via protocatechuate (3,4-dihydroxybenzoate). The same pathway can be expected to be operative in yeasts. Hence, assimilation of quinate could be an indication for aromatic catabolism. As growth on quinic acid indicates acid tolerance and quinate assimilation at the same time, strains not growing on quinic acid nevertheless may be able to assimilate hydroxybenzoates and other benzene compounds. Correct performance of the growth test at pH 5.5 could have given an indication for the assimilation of this important group of naturally occurring compounds.

During the last years several hundreds of yeast strains have been tested in the new way, i.e at pH 2.9 or pH 3.8, for growth on galacturonic and quinic acids and on hemi-saccharate, respectively. This can not be changed anymore and we will have to live with anomalies like pectin-assimilating yeasts (e.g. *Trichosporon laibachii*) apparently unable to assimilate the monomer galacturonate. It is very important that in future descriptions of yeast species the substrates should be named correctly, in order to avoid confusion. Hence galacturonate should be replaced with "galacturonic acid" and quinate with "quinic acid". Saccharate could either be named "hemi-saccharate" or "saccharate pH 3.8". Galactonate is correct, as are the anionic substrates used before 1984.

In August in Bled Slovenia, during the 18th ISSY, I discussed this matter with the editors of both yeast monographs, Drs. Barnett, Kurtzman, and Fell. I asked them to name these substrates correctly and to pay attention to the altered practice by careful amendment of the chapters dealing with materials and methods. I feel that it might be useful to inform my collegues world-wide on this matter.

Comments on this issue are welcome!

## Essay: Phyllosphere yeasts in an arid climate and their biodegradative abilities.

Plants are inhabited by many microorganisms, yeasts included. This ecological niche was named the "phylloshere" by my former collegue Dr. Jakoba Ruinen[1] in 1956. She studied the microbiology of plant leaves collected in the wet tropics, especially in Bogor, Indonesia. Most yeasts she detected were basidiomycetes, taxa forming ballistospores included.[2]

Some years ago I spent my holidays on the Canary Islands Fuerteventura and Lanzarote. I wondered whether yeasts could also grow on plants growing in a very dry climate. The weather on these islands is usually dry and sunny and rainfall is scarce. However, dew in the early morning is a daily event giving the plants some relief from the constant sea-breeze.

To my surprise all 24 plant species collected were inhabited by one or more yeast species, sometimes in large numbers. The taxa were not the same as those observed by Ruinen.[2] Ascomycetes were the most common ones. About 50% of the isolates turned out to be *Debaryomyces hansenii*. Ascomycetous black yeastlike fungi were also detected: *Hormonema dematioides*, *Hortaea werneckii* and a recently described novel species, i.c. *Hormonema schizolunatum*.[3] Basidiomycetous isolates belonged to *Cryptococcus albidus*, *Cr. laurentii*, *Rhodotorula glutinis* and *Rh. mucilaginosa*, species frequently isolated from plants.[2] In addition, a rare basidiomycetous yeastlike fungus, *Cerinosterus cyanescens*, was found.

Attempts were made to study the biodegradative potency of the yeast strains isolated, especially with regard to plant constituents. For this purpose the plant was considered from a strictly chemical viewpoint, i.e as a collection of mainly macromolecular components showing some structural organisation.

Microorganisms growing on plants are encountered by some lipid barriers, such as the cuticle and the plasma membrane, which prevent leaking-out of the cell contents. All yeast strains isolated showed lipase activity as was deduced from their growth on olive oil or lecithin, or by enzymatic tests. D. *hansenii* and some other species were notable for the oxidative attack of lipids, as was shown by growth on the model substrate n-hexadecane.

Polysaccharides, of which cellulose is the most important one, give plants their solid structure. As the model substrate carboxymethyl cellulose was not assimilated by any of the strains studied, the lignocellulose complex of plants can supposed to be resistant to yeast enzymes. However, hemicelluloses like pectin and xylan were degraded by most black yeastlike fungi and by *Cryptococcus* sp.

About half of the isolated strains were proteolytic. This was deduced from their assimilation of casein. Almost all strains assimilated RNA as sole carbon source, but DNA only as sole source of nitrogen. The latter is probably caused by a failure of the yeasts to grow at the expense of deoxyribose. The assimilation of individual amino acids and of several naturally occurring benzene compounds which are known to support growth of many yeasts[4] was also reported in a paper dealing with "chemical yeast ecology".

[1]J. Ruinen. 1956. Occurrence of *Beyerinckia* in the "phyllosphere". Nature **177:**220-221.

[2]J. Ruinen. 1963. The phyllosphere. II. Yeasts from the phyllosphere of tropical foliage. Antonie van Leeuwenhoek **29:**425-438.

[3]W.J. Middelhoven and G.S. de Hoog. 1997. *Hormonema schizolunatum*, a new species of dothideaceous black yeasts from the phyllosphere. Antonie van Leeuwenhoek **71:**297-305.

[4]W.J. Middelhoven. 1993. Catabolism of benzene compounds by ascomycetous and basidiomycetous yeasts and yeastlike fungi. A literature review and an experimental approach. Antonie van Leeuwenhoek **63**:125-144.

Recent publications.

1. W.J. Middelhoven. 1997. Identity and biodegradative abilities of yeasts isolated from plants growing in an arid climate. Antonie van Leeuwenhoek **72:**81-89.

2. W.J. Middelhoven and F. Spaaij[1] . 1997. *Rhodotorula cresolica* sp. nov., a cresol-assimilating yeast species isolated from soil. Int. J. Syst. Bacteriol. **47**:324-327.
[1]Labor für Schimmelpilze und Hefen, Milanweg 1, 72076 Tübingen, Germany

A cresol-assimilating yeast strain of an undescribed species belonging to the genus *Rhodotorula* was isolated from soil. The strain differs from the described species of the genus in its assimilation pattern of carbon and nitrogen compounds, mol% G + C and low percentage of DNA-DNA-homology. The type strain is CBS 7998. The species is very similar to *Rh. pilatii*, but can be distinguished by its failure to assimilate L-sorbose and lactose, and by its growth with L-lysine, cadaverine, creatine and creatinine as sole nitrogen sources. In addition to the cresols, a lot of other benzene compounds are assimilated as sole carbon sources.

3. W.J. Middelhoven and G.S. de Hoog[1]. 1997. *Hormonema schizolunatum* sp.nov., a new species of dothideaceous black yeasts from the phyllosphere. Antonie van Leeuwenhoek **71**:297-305.
[1]Centraalbureau voor Schimmelcultures, P.O.Box 273, NL-3740 AG Baarn, The Netherlands

Two black yeast strains from plants from the Canary Islands (Spain) are described and illustrated. Absence of Woronin bodies at simple septal pores, local coralloid terminal hyphal cells, indeterminate thallus maturation, the presence of budding cells and local conversion to meristematic growth all indicate a relationship to the Dothideaceae (Dothideales, Ascomycota). Morphological properties were consistent with the genus *Hormonema* Lagerberg et Melin. Results of PCR-ribotyping supported this classification. The isolates were judged to belong to a hitherto undescribed species, characterized in particular by curved conidia soon developing transverse septa. The physiological profile of this species is described.

The type strain, isolated from leaves of *Salvia canariensis* L. var. *candidissima* Bolle (fam Lamiaceae), is CBS 707.95. The other strain, isolated from *Arundo donax* L. (fam. Poaceae) is CBS 706.95.

## III. Department of Food Science and Technology, Wiegand Hall, Oregon State University. Communicated by A.T. Bakalinsky <bakalina@bcc.orst.edu>.

1. D. Avram and A.T. Bakalinsky. 1996. Multicopy *FZF1* (*SUL1*) suppresses the sulfite sensitivity but not the glucose derepression or aberrant cell morphology of a *grr1* mutant of *Saccharomyces cerevisiae*. Genetics **144**:511-521.

An ssu2 mutation in *Sacccharomyces cerevisiae*, previously shown to cause sulfite sensitivity, was found to be allelic to *GRR1*, a gene previously implicated in glucose repression. The suppressor *rgt1*, which suppresses the growth defects of *grr1* strains on glucose did not fully suppress the sensitivity on glucose or non-glucose carbon sources, indicating that it is not strictly linked to a defect in glucose metabolism. Because the *Cln1* protein was previously shown to be elevated in *grr1* mutants, the effect of *CLN1* overexpression on sulfite sensitivity was investigated. Overexpression in *GRR1* cells resulted in sulfite sensitivity, suggesting a connection between *CLN1* and sulfite metabolism. Multicopy *FZF1*, a putative transcription factor, was found to suppress the sulfite sensitive phenotype of *grr1* strains, but not the glucose derepression or aberrant cell morphology. Multicopy *FZF1* was also found to suppress the sensitivity of a number of other unrelated sulfite-sensitive mutants, but not that of *ssu1* or *met20*, implying that *FZF1* may act through *Ssu1p* and *Met20p*. Disruption of *FZF1* resulted in sulfite sensitivity when the construct was introduced in single copy at the *FZF1* locus in a *GRR1* strain, providing evidence that *FZF1* is involved in sulfite metabolism.

2. D. Avram and A.T. Bakalinsky. 1997. *SSU1* encodes a plasma membrane protein with a central role in a network of proteins conferring sulfite tolerance in *Saccharomyces cerevisiae*. J. Bact. **179**:5971-5974.

The *S. cerevisiae SSU1* gene was isolated based on its ability to complement a mutation causing sensitivity to sulfite, a

methionine intermediate. *SSU1* encodes a deduced protein of 458 amino acids containing nine or ten membrane-spanning domains but shares no significant similarity to other proteins in public databases. An Ssu1-GFP fusion protein was localized to the plasma membrane. Multicopy suppression analysis, undertaken to explore relationships among genes previously implicated in sulfite metabolism, suggests a regulatory pathway in which *SSU1* acts downstream of *FZF1* and *SSU3*, which in turn act downstream of *GRR1*.

3. H. Park and A.T. Bakalinsky. 1997. Ethanol production from spent cherry brine. J. Ind. Micro. Biotech. **19**:12-17.

Spent cherry brine is an acidic byproduct of maraschino cherry processing and typically consists of variable amounts of glucose and fructose of up to 11% fermentable solids, 0.5-1.5% $CaCl_2$, up to 0.4% sulfur dioxide, sorbitol, and lesser amounts of other cherry constituents. Disposal of brine represents a significant cost to processors because of its high biological oxygen demand. As an alternative, brine was tested as a substrate for ethanol production. Initially, the toxic level of sulfur dioxide was reduced by raising brine pH to 8.0 to precipitate calcium sulfite. Because alkalinization was subsequently found to result in a 10-fold reduction in phosphorous, brines were titrated with phosphoric acid to pH 6.0 prior to inoculation with *Saccharomyces cerevisiae*. All strains of *Saccharomyces cerevisiae* tested were able to ferment all lots of $Ca(OH)_2$-treated and phosphorus-enriched brines efficiently. One lot of brine containing 10% (w/v) fermentable sugar yielded 4.7% (w/v) ethanol in four days.

Successful defense of a doctoral dissertation.

4. D. Avram. 1997. Genetic aspects of sulfite tolerance in *Saccharomyces cerevisiae*. Ph.D. dissertation. Oregon State University.

Sulfite is a normal but potentially toxic metabolite in *S. cerevisiae* and other organisms where it is produced as an intermediate of reductive sulfate assimilation. *S. cerevisiae* has a basal tolerance to sulfite presumably due to formation of a non-toxic adduct with acetaldehyde, and by reduction via sulfite reductase. The present study was undertaken to explore additional mechanisms for sulfite detoxification in *S. cerevisiae* using molecular genetic methods. A regulatory pathway for sulfite detoxification was found, in which *Ssu1*, a plasma membrane protein with 9 or 10 membrane-spanning domains, and no significant similarity to proteins present to date in public databases, is the putative effector. *Ssu1p* resembles a transporter that may function in sulfite efflux rather than import, since ssu1 mutants as well as mutants in genes situated upstream of *SSU1* in the pathway were found to be sensitive rather than resistant to sulfite. *SSU1* was placed downstream of *FZF1* based on multicopy suppression analysis. *FZF1* encodes a putative *C2H2*-type zinc finger transcription factor, previously implicated in sulfite tolerance. *SSU1* promoter-*lacZ* fusion analysis defined Fzf1p as a transcriptional activator of *SSU1*, confirming a role for the *Fzf1* protein in transcriptional activation, previously suggested solely on the basis of sequence analysis. Transcription from the *SSU1* promoter was lower in an *fzf1* background, and it was strongly activated in wild-type when *FZF1* was expressed in multicopy. Another protein, *Ssu3*, may be required for *Fzf1*-induced activation, because transcription from the *SSU1* promoter was lower in an *ssu3* background. The *SSU3* gene was previously implicated in sulfite tolerance, but its function remains unknown. The *Fzf1* protein was shown to bind directly and specifically to the *SSU1* promoter. The first zinc finger appeared to be essential for DNA binding; the fourth and fifth zinc fingers were not. Another gene, *GRR1*, previously shown to be involved in glucose repression and cell cycle regulation, was also found to cause sulfite sensitivity when mutated. Based on multicopy suppression analysis, *GRR1* was placed in the pathway upstream of *SSU1* and *FZF1*, and is presumed to regulate both, and to be involved in additional routes of sulfite detoxification.

## IV. Seccion Enologia, Catedra de Ciencia y Tecnologia de los Alimentos, Facultad de Quimica, Universidad de la Republica, Av. Gral. Flores 2124, 11.800 Montevideo, Uruguay. Communicated by F.M. Carrau.

The following papers are being published since our last communication:

1. Neirotti,E., G.Perez, F.M. Carrau and O. Gioia. 1995. Native killer yeasts isolated from vineyards and vineries ecosystems. Arq. Biol.Tecnol. **38**:961-968.

Native yeasts presents in grapes and must fermentations were isolated and quantitatively examined in three wine producing regions of Uruguay. The origin and development of killer yeasts during wine fermentation were studied and their killer types classified. Eighteen samples of grapes and 31 wine fermentations were analyzed during three different vintages. The results showed that in grapes and fresh musts more than 99% of the yeasts population consisted of neutral and non-*Saccharomyces* strains. *Saccharomyces* killer and neutral strains dominated in wine fermentations of the two traditional wine

producing regions. By contrast, in a newer region (vines of 15 years old) neither killer nor neutral strains were detected in wine fermentations. Results obtained with enrichment liquid medium suggested that the origin of *Saccharomyces* wine killer yeasts was the vines.

2. K. Medina, F.M. Carrau. O. Gioia, and N. Bracesco. 1997. Nitrogen availability of grape juice limits killer yeast growth and fermentation activity during mixed culture fermentation with sensitive yeast. Appl. Environ. Microbiol. **63**:2821-2825.

The competition between selected or commercial killer (K) strains of type K2 and sensitive (S) commercial strains of *Sacharomyces cerevisiae*, was studied under various conditions in sterile grape juice fermentations. The focus of this study was the effect of yeast inoculation levels and the role of assimilable nitrogen nutrition on killer activity. A study of the consumption of Free Amino Nitrogen (FAN) by pure and mixed cultures of K and S cells showed no differences between the profiles of nitrogen assimilation in all the cases, and FAN was practically depleted in the first two days of fermentation. The effect of addition of assimilable nitrogen and size of inoculum was examined in mixed K and S strain competitions. Stuck and sluggish wine fermentations were observed to depend on nitrogen availability when the killer strain was in a small killer/sensitive cell proportion (1:10 to 1:100). A relationship between the initial assimilable nitrogen content of must and the proportion of killer cells during fermentation was shown. An indirect relation was found between inoculum size and the percentage of killer cells; a smaller size of inoculum resulted in a higher proportion of killer cells in grape juice fermentations. In all cases, wines obtained with pure culture fermentations were preferred by sensory analysis to mixed culture fermentations. The reasons why killer cells do not finish the fermentation in these competitive conditions with sensitive cells are discussed.

3. F.M.Carrau. 1997. The emergence of a new Uruguyan wine industry. J. Wine Research (in press).

Although the traditional viticulture of Uruguay is practically unknown in the international wine market, wine sales are now increasing in some of the most important markets of Europe and North America. Because of a strong domestic consumption and a quiescent research and development situation during past decades, the pioneer vision of high quality that was born in the 1880s was not realised. The strategy of promoting the typical grape Tannat (Harriague) best known for making Madiran best reds in the south-west of France is starting to result in successful competitive reds, only found in Uruguay. The relationship of the first vines of Tannat planted in the 1870s by the Frenchman Harriague, with a British family who developed the most technically advanced winery of those days, including a bacteriological laboratory, is presented. With the programme of training for winemakers and the recently started Research & Development projects in the University of the Republic it is hoped that the Uruguayan wine industry, based on quality wines of a good price will continue opening markets and reach the target of 300 thousand cases by the year 2000. The country could occupy an interesting position within the quality wines of the New World in the near future.

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

Recent publications:

1. Boekhout, T., van Belkum, A., Leenders, A.C.A.P., Verbrugh, A.H., Mukamurangwa, P., Swinne, D. and Scheffers, A. 1997. Molecular typing of *Cryptococcus neoformans*: taxonomic and epidemiological aspects. Int. J. Syst. Bacteriol. **47**:432-442.

Pulsed-field gel electrophoresis (PFGE), randomly amplified polymorphic DNA (RAPD) analysis, serotype, and killer toxin sensitivity patterns of a wide range of saprobic, clinical, and veterinary isolates of both varieties of *Cryptococcus neoformans* were examined. *C. neoformans* var. *neoformans* and *C. neoformans* var. *gattii* differed in chromosomal makeup, RAPD patterns, and killer sensitivity patterns. These results suggest that there are two separate species rather than two varieties. No clear genetic or phenotypic differences were observed among the clinical, saprobic, and veterinary isolates within each taxon. The serotypes differed substantially in their RAPD characteristics. Geographical clustering was observed among the isolates of *C. neoformans* var. gattii, but not among the isolates of *C. neoformans* var. *neoformans*. The isolates of each taxon that originated from restricted geographical areas often had identical or similar karyotypes and RAPD patterns, suggesting that clonal reproduction had occurred. The combination of PFGE and RAPD analysis allowed us to distinguish almost all isolates. This combination of techniques is recommended for further research on epidemiological, ecological, and population issues.

2. Boekhout, T. & Scorzetti, G. 1997. Differential killer toxin sensitivity patterns of varieties of *Cryptococcus neoformans*. J. Med. Vet. Mycol. **35**:147-149.

Ten different killer sensitivity types are distinguished within *Cryptococcus neoformans*, namely four in var. *neoformans* and six in var. *gattii*. All strains of the var. *gattii* investigated were inhibited by killer toxins of *C. laurentii* CBS 139, whereas those of the var. *neoformans* were not. Killer sensitivity patterns are an easy-to-use method to differentiate between the two varieties of the clinically important yeast *C. neoformans*, and may be of help in epidemiological surveys.

3. Boekhout, T, van Belkum, A. 1997. Variability of karyotypes and RAPD types in genetically related strains of *Cryptococcus neoformans*. Curr. Genet. **32**:203-208.

Variation in karyotypes and RAPD patterns of genetically related strains of *Cryptococcus neoformans* were analyzed. Capsular and filamentous mutants usually differ in their karyotypes from wild-types, but the RAPD patterns were found to be similar. Karyotype differences were observed in most heterothallic matings, but RAPD patterns remained identical. After self-sporulation of a diploid strain, minor chromosomal length polymorphism and minor changes in the RAPD types occurred. Three mechanisms, either alone or in combination, may in varying degrees contribute to the karyotype variation pf *C. neoformans*: (1) mitotically induced changes; (2) karyotype changes as a results of meiotic recombination, and (3) mutagen-induced changes. The present data do not support the meiotic maintenance hypothesis, which claims that the amount of CLP generated is inversely proportional to the frequency of meiosis.

4. van der Walt, J.P., Botha, A. & Smith, M.Th. 1997. *Lipomyces mesembrius* sp. nov., a member of the *L. starkeyi* species-complex. Antonie van Leeuwenhoek **71**:325-328.

*Lipomyces starkeyi* is known to be associated with three strains-clusters showing high mutual nDNA reassociation within each cluster, but which reassociate ambiguously with the type of *L. starkeyi*. Representative strains of *L. starkeyi* and Cluster α were examined for possible genetic exchange by the prototrophic selection technique. Since no genetic recombination was detected, the strains are presumed to be genetically isolated. Cluster α is consequently assigned to the rank of species as *Lipomyces mesembrius*. A description of the new species is given. *Lipomyces kononenkoae* ssp. *spencermartinsiae* has been raised to the rank of species as *L. spencermartinsiae*.

5. Meyer, W, Latouche, G.N, Daniel, H.M, Mitchell, T.G, Yarrow, D., Schönian, G., Sorrell, T.C. 1997. Identification of pathogenic yeasts of the imperfect genus *Candida* by polymerase chain reaction fingerprinting. Electrophoresis **18**:1548-1559.

6. Seifert, K.A, Samson, R.A., Boekhout, T. and Seize, G.L. 1997. *Remersonia*, a new genus for *Stilbella thermophila*, a thermophilic mould from compost. Can. J. Bot. **75**:1158-1165.

7. Verdoes,J.C., Wery, J., Boekhout, T. and van Ooyen, A.J.J. 1997. Molecular characterization of the glyceraldehyde-3-phosphate dehydrogenase gene of *Phaffia rhodozyma*. Yeast **13**:1231-1242.

8. Fell, J.W., Boekhout, T., Fonseca, A. and Sampaio, J.P. 1997. Basidiomycetous Yeasts. In: The Mycota (In press).

9. Müller, W.H., Stalpers, J.A., van Aelst, A.C., van der Krift, T.P., Boekhout, T. 1997. Field emission gun-scanning electron microscopy of septal pore caps of selected species in the *Rhizoctonia* s.l. complex. Mycologia (In press).

## VI. Department of Microbiology and Biochemistry, University of the Orange Free State, P.O. Box 339, Bloemfontein 9300, South Africa. Communicated by J.C. du Preez. <dpreezjc@micro.nw.uovs.ac.za>.

**UNESCO MIRCEN Yeast Biotechnology Course**. The United Nations Education, Science and Cultural Organisation (UNESCO) awarded the Department of Microbiology & Biochemistry, University of the Orange Free State, Bloemfontein, a Microbiological Resources Centre (MIRCEN) in 1996 with the purpose of serving the microbiological scientific needs of the Southern African region. This MIRCEN forms part of a limited number of such centres world-wide. The task of this centre is to provide training to regional scientists in the field of microbial biotechnology and to serve biotechnology in Southern Africa. The first course of this new Industrial Biotechnology MIRCEN will be hosted from 26 January to 6 February 1998 for a selected number of participants from Uganda, Zimbabwe, Tanzania, Botswana, Kenya and South Africa. The course will cover Yeast Biotechnology, a topic especially relevant to the development of biotechnology in Africa. The course lecturers will be from this

department, from two other South African universities (Rhodes University and the University of Stellenbosch) as well from South African Breweries and Anchor Yeast. Special guest lecturers will be Prof. Peter Biely, Slovak Academy of Sciences, Slovakia, Prof. Johan Thevelein, Catholic University of Leuven, Belgium and Prof. John Villadsen, Technical University of Denmark, Lyngby. For more information please see our web page:

http://www.uovs.ac.za/nat/mkboc/index.htm

Papers recently published or in press.

1. Kilian, S.G., Sutherland, F.C.W., Meyer, P.S. and du Preez, J.C. 1996. Transport-limited sucrose utilization and neokestose production by *Phaffia rhodozyma*. Biotechnol. Lett. **18**:975-980.

2. Sutherland, F.C.W., Lages, F. Lucas, C., Luyten, K. Albertyn, J., Hohmann, S., Prior, B.A. and.Kilian, S.G. 1997. Characteristics of Fps1-dependent and -independent glycerol transport in *Saccharomyces cerevisiae*. J. Bacteriol. **179**:24.

Eadie-Hofstee plots of glycerol uptake in wild type *Saccharomyces cerevisiae* W303-1A grown on glucose showed the presence of both saturable transport and simple diffusion whereas an *fps1Δ* mutant displayed only simple diffusion. Transformation of the *fps1Δ* mutant with the *glpF* gene, which encodes glycerol transport in *Escherichia coli*, restored biphasic transport kinetics. Yeast-extract-peptone-dextrose-grown wild type cells had a higher passive diffusion constant than the *fps1Δ* mutant and ethanol enhanced the rate of proton diffusion to a greater extent in the wild type than in the *fps1Δ* mutant. In addition, the lipid fraction of the *fps1Δ* mutant contained a lower percentage of phosholipids and a higher percentage of glycolipids than that of the wild type. Fps1p, therefore, may be involved in the regulation of lipid metabolism in *Saccharomyces cerevisiae*, affecting membrane permeability in addition to fulfilling its specific role in glycerol transport. Simultaneous uptake of glycerol and protons occurred in both glycerol- and ethanol-grown wild type and *fps1Δ* cells and resulted in the accumulation of glycerol at an inside-to-outside ratio of 12:1 to 15:1. Carbonyl cyanide *m*-chlorophenylhydrazone prevented glycerol accumulation in both strains and abolished transport in the *fps1Δ* mutant grown on ethanol. Likewise, 2,4-dinitrophenol inhibited transport in glycerol-grown wild type cells. These results indicate the presence of an Fps1p-dependent facilitated diffusion system in glucose-grown cells and an Fps1p-independent proton symport system in derepressed cells.

3. Kock, J.L.F., Jansen van Vuuren, D., Botha, A., van Dyk, M.S., Coetzee, D.J., Botes, P.J., Shaw, N., Friend, J., Ratledge, C., Roberts, A.D. and Nigam, S. 1997. The production of biologically active 3-hydroxy-5,8,11,14-eicosatetraenoic acid (3-HETE) and linoleic acid metabolites by *Dipodascopsis.* Syst. Appl. Microbiol. **20**:39-49.

3-Hydroxy-5,8,11,14-eicosatetraenoic acid (3-HETE) is a biologically active compound which has a signal transducing role in human neutrophils. When 12.5 mg/l arachidonic acid (AA) was fed to sexual reproductive cells of *Dipodascopsis uninucleata* and *D. tóthii* the former transformed approximately 4% of AA to 3-HETE, while the latter produced only trace amounts of 3-HETE. The 3-HETE was only associated with cellular ethanol extracts of both species. *D. uninucleata* utilized 69% of the AA after 6h while *D. tóthii* utilized 33%. The residual or unused AA was mainly present in the cells of *D. tóthii* (96%) and *D. uninucleata* (98%) and only small amounts (4% and 2%) in their respective supernatants. Extraction with 80% aqueous ethanol succeeded in removing respectively 92% and 45% of AA present in the cells of *D. tóthii* and *D. uninucleata*. The poor AA extraction from cells of *D. uninucleata* contributed to the presence of AA in the neutral lipid fraction which is insoluble in ethanol. If efficient AA extraction can be implemented, approximately 67% and 31% unused AA can be recovered from *D. tóthii* and *D. uninucleata* respectively which can be recycled for biotransformation purposes. Although no traces of other 3-hydroxy fatty acids were detected, enzyme extracts from *D. uninucleata* metabolised exogenous 18:2 in a manner consistent with the presence of a prostaglandin endoperoxide synthase similar to that in fetal calf blood vessels.

4. van der Walt, J.P., Botha, A. and Smith, M.Th. 1997. *Lipomyces mesembrius* sp. nov., a member of the *L. starkeyi* species complex. Antonie van Leeuwenhoek **71**:325-328.

*Lipomyces starkeyi* is known to be associated with three strains-clusters showing high mutual nDNA reassociation within each cluster, but which reassociate ambiguously with the type of *L. starkeyi.* Representative strains of *L. starkeyi* and Cluster α were examined for possible genetic exchange by the prototrophic selection technique. Since no genetic recombination was detected, the strains are presumed to be genetically isolated. Cluster α is consequently assigned to the rank of species as *Lipomyces mesembrius.* A description of the new species is given. *Lipomyces kononenkoae* ssp. *spencermartinsiae* has been raised to the rank of species as *L. spencermartinsiae.*

5. Jeffery, J., Kock, J.L.F., Botha, A., Coetzee, D.J. and Botes, P.J. 1997. The value of lipid composition in the taxonomy of the Schizosaccharomycetales. Antonie van Leeuwenhoek (Accepted for publication).

In this study, the lipid fractions i.e. neutral (NL), phospho- (PL) and glycolipids (GL) with associated fatty acids (FAs) of 54 strains, representing the Schizosaccharomycetales, were analyzed during stationary growth phase and compared. Trace amounts of linoleic acid (18:2) were present in most of the strains representing *Schizosaccharomyces*. An increased percentage 18:2 was observed in the PL fraction when compared to the NL fraction. This is possibly related to membranes requiring polyunsaturated FAs for fluidity. On the basis of the percentage oleic acid (18:1) and 18:2 FAs in the different lipid fractions, the Schizosaccharomycetales can clearly be divided into two groups i.e. Group 1 (represented by the genus *Hasegawaea*) comprising strains producing relatively large amounts of 18:2 and relatively low amounts of 18:1 when compared to Group 2 (represented by the genus *Schizosaccharomyces* comprising *Schizosaccharomyces octosporus* and *Schizosaccharomyces pombe*). These results are in accordance with 18S and 26S rRNA base sequence analyses and emphasize the difference between the genera *Hasegawaea* and *Schizosaccharomyces*. Utilizing gas chromatography-mass spectrometry analyses, it was found that these strains were all capable of producing gamma-linolenic acid. This further emphasizes the uniqueness of this order in the Dikaryomycota.

6. Mothibeli, A., Kock, J.L.F., Botha, A., Coetzee, D.J., Botes, P.J. and Pretorius, E.E. 1997. Lipomycetaceous yeasts from the Northern Province of South Africa. South African J. Sci. **93**:45-47.

A total of 27 lipomycetaceous strains were isolated from different soils in the Northern Province of South Africa and characterised to species level. *Lipomyces starkeyi* and *L. tetrasporus* were widespread in this region whereas *L. kononenkoae* and representatives of *Kawasakia, Smithiozyma, Waltomyces, Zygozyma* and the anamorphic genus *Myxozyma* with lipomycetaceous affinities, were not detected. During the present study two strains representative of *Dipodascopsis uninucleata* were uncovered from soil in the Giyani area. This is the first report of this species' occurrence in the Southern Hemisphere and recovery from soil. On the basis of cellular lipid content, isolates representative of *D. uninucleata* could clearly be distinguished from those representing the genus *Lipomyces*.

7. Venter, P., Kock, J.L.F., Kumar, S., Botha, A., Coetzee, D.J., Botes, P.J., Bhatt, R.K., Schewe, T. and Nigam, S. 1997. Production of 3R-hydroxy-polyenoic fatty acids by the yeast *Dipodascopsis uninucleata*. Lipids (accepted for publication).

8. Kock, J.L.F. and Botha, A. 1997. Fatty acids in fungal taxonomy. In: Frisvad, J.C., Bridge, P.D., Arora, D.K. (eds.). Chemical Fungal Taxonomy. In: *Handbook of Applied Mycology,* Vol. 6, London: Marcel Dekker, Inc.

9. Kock, J.L.F., Nigam, S. and Botha, A. 1997. The production of biologically active eicosanoids by yeasts. Sinzinger, H., Vane, J.R., Samuelson, B., Paoletti, R., Ramwell, P.W., Wong, P.Y.-K. (eds.). Recent Advances in Prostaglandin Thromboxane and Leukotriene Research. New York: Plenum Publishing Corporation.

10. Prior, B.A. and Hohmann, S. 1997. Glycerol production and osmoregulation. In F.K Zimmermann and K.-D. Entian (eds.). Yeast sugar metabolism: Biochemistry, genetics, biotechnology and applications. pp. 313-337. Technomic Publishing Co., Inc., Lancaster, PA, USA.

11. Prior, B.A. and Kötter, P. (1997). Pentose utilization by yeasts. In F.K Zimmermann and K.-D. Entian. Yeast sugar metabolism: Biochemistry, genetics, biotechnology and applications. pp. 435-457. Technomic Publishing Co., Inc., Lancaster, PA, USA.

12. Schoch, C., Brüning, A. R. N. E., Entian, K.-D., Pretorius, G. H. J. and Prior, B. A. (1997). A *Saccharomyces cerevisiae* mutant defective in the kinesin-like protein Kar3 is sensitive to NaCl stress. Current Genetics (in press).

Several mutants of *Saccharomyces cerevisiae* showing poor growth in the presence of elevated concentrations of NaCl were isolated to identify genes involved in the osmostress response. One of these mutants (WAY.5-4A-11; *osr11*) which showed a clear 2:2 segregation of the salt-stress phenotype upon tetrad analysis when crossed to a wild type strain has been characterised. The mutation responsible for poor growth under salt-stress was recessive. The corresponding gene was cloned by complementation of the mutant phenotype and a 3.5 kb fragment was isolated. Sequences of this fragment matched *KAR3,* a gene previously identified to be involved in karyogamy and mitosis. Allelism of *OSR11* to *KAR3* was confirmed by tetrad analysis and disruption mutants showed the same NaCl-phenotype as the original *osr11* mutation. The disruption mutant was more

sensitive to high sucrose concentrations than the original mutant was to high glucose concentrations. In a different genetic background (W303-1A), the *kar3* disruptants were less sensitive to osmostress than the WAY.5-4A strain. Heat stress, nitrogen starvation and cultivation on ethanol failed to affect the growth of *osr11* and *kar3* mutants, pointing to a possible specific involvement of *KAR3* in the osmotic stress response. Microscopic studies showed that cell division of the *kar3* mutants was impaired and NaCl stress conditions aggravated the phenotype.

## VII. Department of Food Science and Technology, The University of New South Wales, Sydney NSW, Australia 2052. Communicated by G.H. Fleet <G.Fleet@unsw.edu.au>.

Doctor of Philosophy theses completed under the supervision of Graham Fleet, UNSW.

1. Charoen Charoenchai. 1995. Growth and metabolic activities of wine yeasts during batch and high cell density fermentations.
2. Jian Zhao. 1995. Degradation of nucleic acids during the autolysis of *Saccharomyces cerevisiae*.
3. Bryan Todd. 1995. Microbiological and chemical changes during the fermentation and ageing of sparkling wines.
4. Ly Nguyen. 1996. The production and properties of the alkali soluble glucan of the cell wall of *Kluyveromyces marxianus*.
5. Wanna Praphailong. 1996. Growth, metabolic and ultra-structural properties of food spoilage yeasts cultured under different environmental conditions.

Recent publications


6. Gao, C,. and G.H. Fleet. 1995. Degradation of malic and tartaric acid by high density cell suspensions of wine yeasts. Food Microbiol. **12**:65-71.
7. Hernawan, T. and G.H. Fleet. 1995. Chemical and cytological changes during the autolysis of yeasts. J. Ind. Microbiol. **12**: 65-71.
8. Todd, B.J., J. Zhao and G.H. Fleet. 1995. HPLC measurement of guanine for the determination of nucleic acids (RNA) in yeasts. J. Microbiol. Methods **22**:1-10.
9. Kopecka, M., G.H. Fleet and H.J. Phaff. 1995. Ultrastructure of the cell wall of *Schizosaccharomyces pombe* following treatment with various glucanases. J. Struct. Biol. **114**: 140-152.
10. Roostita, R. and G.H. Fleet. 1996. The occurrence and growth of yeasts in Camembert and Blue-veined cheeses. Int. J. Food Microbiol. **28**:393-404.
11. Roostita, R. and G.H. Fleet. 1996. Growth of yeasts in milk and associated changes to milk composition. Int. J. Food Microbiol. **31**: 205-219.
12. Mian, M.A., G.H. Fleet and A.D. Hocking. 1997. Effect of diluent type on viability of yeasts enumerated from foods or pure culture. Int. J. Food Microbiol. **35**: 103-107.
13. Charoenchai, C., G.H. Fleet, P.A. Henschke and B.E.N. Todd. 1997. Screening of non-*Saccharomyces* wine yeasts for the presence of extracellular hydrolytic enzymes. Aust. J. Grape and Wine Research **3**:208-212.
14. Praphailong, W. and G.H. Fleet. 1997. The interactive effects of environmental factors on the growth of spoilage yeasts. Food Microbiol. (in press ).
15. Praphailong, W., G.M. Heard, M. Rohinda and G.H. Fleet. 1997. Assessment of Biolog system for the identification of foodborne yeasts. Letter. Appl. Microbiol. **24**: 455-459.

16. Fleet, G.H. Wine. 1997. In, FUNDAMENTALS IN FOOD MICROBIOLOGY Eds. M.P. Doyle, L.R. Beuchat and T.J. Montville. American Society for Microbiology, Washington DC pp.671-694.

17. Fleet, G.H. 1997. Microbiology of alcoholic beverages. In MICROBIOLOGY OF FERMENTED FOODS second edition, Ed. B.J. Wood. Chapman & Hall, Glasgow. In press.

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

Five groups of yeast researchers participate in a yearly meeting, exchanging results, techniques, tips, abstracts books from International Meetings, etc. The following are the contribution of three groups among the five since the last Yeast Newsletter.

### Group 'Biogenesis of RNA'. M. Caizergues-Ferrer, Yeast Ribosomes Biogenesis Laboratory, LBME du CNRS, 118 route de Narbonne, 31062 Toulouse cedex.

Publications for 1997:

1. Gulli, M.P., Faubladier, M., Sicard, H. and Caizergues-Ferrer, M. 1997. Mitosis-specific phosphorylation of gar2, a fission yeast nucleolar protein structurally related to nucleolin. Chromosoma, **105**, 532-541.

2. Léger-Silvestre, I., Gulli, M.P., Noillac-Depeyre, J., Faubladier M.,Sicard, H., Caizergues Ferrer, M. & Gas, N. 1997. Ultrastructural changes in the *S. pombe* nucleolus following the disruption of the gar2+ gene whichencodes a nucleolar protein structurally related to nucleolin. Chromosoma, **105**:542-552.

3. Venema, J., Bousquet-Antonelli, C., Gélugne, J.P., Caizergues-Ferrer, M. & Tollervey, D. 1997. Rok1 is a potential RNA helicase required for pre-rRNA processing. Mol. Cell. Biol. **17**:3398-3407.

4. Ganot, P., Caizergues-Ferrer, M.and Kiss T. 1997. The family of box ACA small nucleolar RNAs is defined by an evolutionarily conserved secondary structure and ubiquitous sequence elements essential for RNA accumulation. Genes Dev., **11**:941-956.

5. Bousquet-Antonelli, C., Henry, Y., Gélugne, J.P., Caizergues-Ferrer, M.and Kiss, T. 1997. A small nucleolar RNP protein is required for pseudouridylation of eukaryotic ribosomal RNAs. EMBO J., **16**:4770-4776.

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

Publications for 1997.

6. François, J., Blazquez, M.A., Rino, J. & Gancedo, C. (1997) Storage carbohydrates in the yeast *Saccharomyces cerevisiae*. Yeast sugar metabolism (F.K. Zimmermann & K.D Entian ed.) pp 285-311, Technomics Publishing Co, PA.

7. Parrou, J.L., Teste, M.A. & François, J. (1997) Effects of various types of stresses on reserve carbohydrates metabolism in *Saccharomyces cerevisiae*: Genetic evidences for a stress-induced recycling of glycogen and trehalose. *Microbiology*,143, 1381-1900.

8. Daran; J.M., Bell, W. & François, J. (1997) Physiological effects of genetic alterations leading to a reduced synthesis of UDP-glucose in *Saccharomyces cerevisiae*. *FEMS Microbiol. Lett*., 153, 89-96.

9. Gonzalez, B., François, J. & Renaud, M. (1997) A rapid and reliable method for metabolite extraction in yeast using boiled buffered ethanol. *Yeast*, **13**, 1347-1356.

10. Parrou, J.L. & François, J. (1997) A simplified procedure for a rapid and reliable assay of both glycogen and trehalose in whole yeast cells. *Anal. Biochem*., **248**, 186-188.

11. Vorisek, J., Gas, N. & Denis-Duphil, M. (1997) Cerium-base ultracytochemical localisation of aspartate transcarbamylase activity in the cell membrane complex of *Saccharomyces cerevisiae*. Micron, **28**, 221-230.

In the press.

12. Alexandre, H., Plourde, L., Charpentier, C. & François, J. Lack of correlation between trehalose accumulation, cell viability and intracellular acidification as induced by various stress in Saccharomyces cerevisiae, while trehalose synthesis is enhnaced in a mutant with reduced ATPase activity. Microbiology (in the press).

The following an Abstract that will be presented at the International Symposium on Progress in Basic, Applied and Diagnostic Histochemistry", Prague, December 10-12. 1997.

13. P. Benoist[1], R. Antonelli[2], J. Vorisek[3], A. Pliss[4], P. Feau[2], I. Raska[4], and M. Denis-Duphil[2].The cytoplasmic localization of the yeast Ura2 multifunctional protein.

[1]Instituto de Investigaciones Biomedicas, C.S.I.C. 4, Arturo Duperier, 28029 Madrid Spain.
[2]Dep. de Génie Biochimique, UMR-CNRS 5504. INSA. 31077 Toulouse, France.
[3]Institute of Microbiology, Acad. Sciences. Videnska 1083, 14220 Praha 4.
[4]Department of Cell Biology, Institute of Experimental Medicine, Acad. Sciences. Albertov 4, 12800 Praha 2. Czech Republic.

In *Saccharomyces cerevisiae,* the Ura2 protein catalyzes the first two steps of the pyrimidine pathway via carbamyl-phosphate synthetase and aspartate-transcarbamylase (ATCase) reactions. This multifunctional protein was considered to be in the nucleoplasm since the orthophosphate produced by the ATCase reaction was precipitated by lead inside yeast nuclei[1]. In a recent paper, however, it has been shown that the above ultracytochemical approach revealed non specific lead-polyphosphate precipitates[2]. In order to localize the Ura2 protein itself, the present study used three immunocytochemical approaches: 1. Indirect immuno-fluorescence, 2. Green fluorescence of an Ura2-GFP (green fluorescence protein) fusion protein, 3. Immunolabelling on ultrathin sections of embedded yeast cells. In all three approaches, the Ura2 protein was seen to be highly enriched in the cytoplasm whereas the signal found in the interior of nuclei, mitochondria or vacuoles was absent or at the background level. These results, compatible with the membranous localization of ATCase activity of the Ura2 protein recently shown, make the intranuclear localization of the protein unlikely.

[2]Nagy et al. 1989, J. Biol. Chem. :8366
[2]Vorisek et al. 1997, Micron **28**:221.

**Laboratory of Protein Engineering, Institut de Pharmacologie et de Biologie Structurale, CNRS 205, Route de Narbonne, 31077 Toulouse cédex, France. J.M. Masson. E. Meilhoc <meilhoc@opium.ipbs.fr>.**

Our group is involved in the two different following thematics: 1) Development of a new yeast host system for heterologous protein production. *Schwanniomyces occidentalis* presents many interesting features (i.e secretion of large proteins, no hyperglycosylation of proteins, etc.) and we try to set up genetic tools (transformation, expression vectors, etc.) essential for the subsequent use of this non conventional yeasts. 2) Expression of the opioid μ-receptor in *Saccharomyces cerevisiae.* Development of a yeast-based bioassay to study the coupling ligands-μ receptor and receptor-protein G.

Recent Publications

14. P. Costaglioli, E. Meilhoc and J.M. Masson. 1994. High efficiency electrotransformation of the yeast *Schwanniomyces occidentalis,* Current Genetics **27**:26-30.

15. P. Gourdon, I. Janatova, E. Meilhoc, R.D. Klein, P. Costaglioli and J.M. Masson 1995. Sequence analysis of the ADE2 gene coding for phosphoribosylaminoimidazole carboxylase in *Schwanniomyces occidentalis,* Yeast **11**:1289-1293.

16. P. Costaglioli, E. Meilhoc, I. Janatova, R.D. Klein and J.M.Masson. 1997. Secretion of invertase from *Schwanniomyces occidentalis*. Biotechnology letters **19**:623-627.

17. I. Janatova, P. Gourdon, E. Meilhoc, R.D. Klein and J.M. Masson. Replication of plasmids based on the homologous *ADE2* gene and the 2 μm *ori* in *Schwanniomyces occidentalis*. (submitted).

## IX. Instituto de Investigaciones Biomedicas, CSIC. Arturo Duperier, 4, 28029-Madrid, Spain. Communicated by J.M. Gancedo.

The following papers have been published recently.

1. E. Peñalver, L. Ojeda, E. Moreno and R. Lagunas. 1997. Role of the cytoskeleton in endocytosis of the yeast maltose transporter. Yeast **13**:551-549.

Certain components of the cytoskeleton play a role in yeast fluid-phase endocytosis as well as in endocytosis of the a-factor when this pheromone is bound to its 7-transmembrane segment receptor. The yeast maltose transporter is a 12-transmembrane segment protein that, under certain physiological conditions, is degraded in the vacuole after internalization by endocytosis. In this work, the possible role of the cytoskeleton in endocytosis of the transporter has been investigated. Using mutants defective in P-tubulin, actin and the actin-binding proteins SacG and Abp5, as well as nocodazole, which inhibits formation of microtubules, we have shown that actin microfilaments are involved in endocytosis of the maltose transporter whereas microtubules are not.

2. P. Lucero, E. Peñalver, E. Moreno and R. Lagunas. 1997. Moderate concentrations of ethanol inhibit endocytosis of the yeast maltose transporter. Appl. Environ. Microbiol. **63**:3831-3836.

The maltose transporter in *Saccharomyces cerevisiae* is degraded in the vacuole after internalization by endocytosis upon nitrogen starvation in the presence of a fermentable substrate. This degradation, known as catabolite inactivation, is inhibited by the presence of moderate concentrations (2 to 6%, vol/vol) of ethanol. We have investigated the mechanism of this inactivation and have found that it is due to the inhibition of the internalization of the transporter by endocytosis. The results also indicated that this inhibition is due to alterations produced by ethanol in the organization of the plasma membrane protein which also affects to endocytosis of other plasma membrane proteins. Apparently, endocytosis is particularly sensitive to these alterations compared with other processes occurring at the plasma membrane.

## X. Russian Collection of Microorganisms (VKM), 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., Ikeda, R., Shinoda, T. & Nakase, T. 1997. Antifungal activity of *Bullera alba* (Hanna) Derx. Mycoscience **38**:25-29.

A strain of *Bullera alba* that secretes a killer toxin inhibitory (at pH values ranging from 3 to 7) to many ascomycetous and basidiomycetous yeast-like fungi was discovered. Its killer phenotype was incurable. The toxin was relatively thermostable and resistant to many proteases, and it was identified as a microcin. It inhibited the growth of some pathogenic yeasts and was the most active against *Cryptococcus neoformans*.

2. Golubev, W.I. & Churkina, L.G. 1997. Sensitivity to mycocins and physiological characteristics of authentic strains of *Rhodotorula mucilaginosa* synonyms. Mikrobiologiya **66**:254-261.

*Rhodotorula mucilaginosa* strains were found to differ in their sensitivity to mycocins produced by species of *Bensingtonia*, *Rhodotorula*, *Sporidiobolus* and *Sporobolomyces*. The sensitivity patterns in many cases correlated with morphological and physiological properties. Cluster analysis using these characteristics revealed a heterogeneity of the species *Rh. mucilaginosa*. Authentic strains of some species included in the list of *Rh. mucilaginosa* synonyms formed separate clusters.

## XI. Japan Collection of Microorganisms, The Institute of Physical and Chemical Research (RIKEN), Wako, Saitama 351-01, Japan. Communicated by M. Hamamoto <hamamoto@ulmus.riken.go.jp> and T. Nakase <nakase@ulmus.riken.go.jp>.

The following articles have been published recently.

1. Takashima, M. & Nakase, T. 1997. A phylogenetic analysis of three group I introns found in the nuclear small subunit ribosomal RNA gene of the ballistoconidiogenous anamorphic yeast-like fungus *Tilletiopsis flava*. Genes & Genet. Syst. **72**:205-214.

There are three group I introns in the nuclear small subunit ribosomal RNA gene (SSU rDNA) of the ballistoconidiogenous

anamorphic yeast-like fungus *Tilletiopsis flava* JCM 5186. The size of these sequences were 325nt (position 516), 335nt (position 1199) and 437nt (position 1506), respectively. The introns at position 516 (T.flav516) and position 1199 (T.flav1199) belonged to subgroup IB3, and that of position 1506 (T.flav1506) belonged to subgroup IC1. The results of comparison with other group I introns found in SSU rDNA of eucaryotes showed that the positions 516 and 1199 were common positions to IB3 group I introns of fungi and green algae, and that positions 943, 1506 and 1512 were those to IC1 group I introns of fungi, and green and red algae. It is indicated that the insertion position of introns have close relationship with the nature of the subgroup to which they belonged. For phylogenetic analysis, we employed 9 IB3 introns, in which 7 were at position 516 and 2 were at position 1199, and 25 IC1 introns. The maximum likelihood tree based on the conserved region alignment showed that group I introns of subgroup IB3 were phylogenetically distant from those of subgroup IC1. T.flav516 (basidiomycete) constituted a subcluster with R.dacr516 (basidiomycete) and M.albo516 (ascomycete). T.flav1199 was located at the closer position of C.chlo1199 (green alga) than other IB3 introns at position 516. T.flav1506 was located at the subcluster, which was constituted by the 1506 introns found in SSU rDNA of fungi (B.yama1506, P.cari1506 and P.inou1506) and those of green algae (C.elli1506, C.mira1506, G.spir1506 and M.sacl1506) with IC1 introns at the position 1512 (D.parv1512 and C.sacc1512). The analysis of flanking regions showed that both 5' and 3' flanking sequences were well conserved in each insertion site, and indicated that the ancestors of the intron at different site had been inherited from the different origin. Therefore, the two IB3 introns found positions 516 and 1199, T.flav516 and T.flav1199, were supposed to have the independent ancestors. Our results supported the theory of the diversity of group I introns that group I introns had been transferred horizontally to the distinct insertion site, and were inherited and diverged vertically.

2. Sugita T., Takashima M., Hamamoto M., Budhaka P. & Nakase T. 1997. *Bensingtonia sakaguchii* sp. nov. isolated from a leaf of *Bischofia javanica* in the Ogasawara islands. J. Gen. Appl. Microbiol. **43**:237-241.

During a survey on ballistoconidium-forming yeasts from leaves of plants collected in the Ogasawara Islands, Japan, we isolated two strains of a hitherto undescribed yeast from a leaf of Bischofia javanica in Euphorbiaceae, which collected in Chichi-jima, Ogasawara Islands, Japan. These isolates seemed to belong to the genus *Bensingtonia*, because they have Q-9 as the major ubiquinone, lack xylose in the cells, and produce kidney-shaped ballistoconidia. A new species, *Bensingtonia sakaguchii* Sugita, Takashima, Hamamoto et Nakase, is proposed for these two isolates, JCM 10047 (a type strain) and JCM 10048.

## XII. 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. A. Yokoyama and W. M. Ingledew. 1997. The effect of filling procedures on multi-fill fermentations. Master Brew. Assoc. Amer. Tech. Quart. **34**:320-327.

The development of large cylindroconical brewing tankage has enabled industry to ferment large volumes of wort in a smaller area of the plant. In such a case, fermentation tank volumes are normally larger than the volume held by the kettle(s) used in the brewhouse. Therefore, fermentation tanks are filled over a finite time period with multiple brews. Alterations in wort volume and timing in a simulated multi-fill procedure were investigated using very high gravity (VHG) wort with or without aeration. The objective was to ascertain the effect of a multi-fill process on the fermentation and to determine the optimal conditions that would give the most effective attenuation of VHG worts. For the commercial Saccharomyces uvarum lager yeast employed, sequential wort provision was found to affect the fermentation. This effect was due to yeast growth which is related to the supply of oxygen from the wort. The most effective condition for attenuation was found to be when air-saturated wort was provided at the time of initiation of the second yeast cell division. In this condition, maximum cell number, yeast dry weight, nitrogen assimilation and sterol synthesis were stimulated over that of a fermentor filled immediately using the same wort. Deaerated wort had a negative effect. Aerated wort provision earlier or subsequent to initiation of the second cell division either showed a slight adverse effect on the above parameters or did not influence the fermentation. The optimal chronological timing of wort provision was subject to change with temperature. The fact that fermentation behavior is affected by manipulation of the multi-fill process is an important factor in establishing wort production and fermentation schedules in a commercial plant.

2. S.H. Hynes, D.M. Kjarsgaard, K.C. Thomas and W.M. Ingledew. 1997. Use of virginiamycin to control the growth of lactic acid bacteria during alcohol fermentation. J. Indust. Microbiol. Biotechnol. **18**:284-291.

The antibiotic virginiamycin was investigated for its effects on growth and lactic acid production by seven strains of lactobacilli during the alcoholic fermentation of wheat mash by yeast. The lowest concentration of virginiamycin tested (0.5 mg Kg-1 mash), was effective against most of the lactic acid bacteria under study, but *Lactobacillus plantarum* was not significantly inhibited at this concentration. The use of virginiamyin prevented or reduced potential yield losses of up to 11 % of the produced ethanol due to the growth and metabolism of lactobacilli. However, when the same concentration of virginiamycin was added to mash not inoculated with yeast, *Lactobacillus rhamnosus* and *Lactobacillus paracasei* grew after an extensive lag of 48 hours and *L. plantarum* grew after a similar lag even in the presence of 2 mg virginiamycin Kg-1 mash. Results showed a variation in sensitivity to virginiamycin between the different strains tested and also a possible reduction in effectiveness of virginiamycin over prolonged incubation in wheat mash, especially in the absence of yeast.

3. K. Sosulski, S. Wang, M. Ingledew, F.W. Sosulski and J. Tang. 1997. Preprocessed barley, ryeand triticale as a feedstock for an integrated fuel alcohol-feedlot plant. Appl. Biochem. Biotechnol. **63-65**:59-70.

Rye, triticale and barley were evaluated as starch feedstock to replace wheat for ethanol production. Preprocessing of grain by abrasion on a Satake mill reduced fiber and increased starch concentrations in feedstock for fermentations. Higher concentrations of starch in flours from preprocessed cereal grains would increase plant throughput by 8-23% since more starch is processed in the same weight of feedstock. Increased concentrations of starch for fermentation resulted in higher concentrations of ethanol in beer. Energy requirements to produce on liter of ethanol from preprocessed grains were reduced, the natural gas by 3.5%-11.4%, whereas power consumption was reduced by 5.2-15.6%.

3. W.M. Ingledew. 1996. Nutrients, yeast hulls and proline in the wine fermentation. Die Wein-Wissenschaft (Vitic. Enol. Sci.) **51**:137-140.

The following thesis has been completed. Mr. Bayrock is continuing on a Ph.D. program studying continous cascade fermentation of ethanol - in a fuel context.

4. D. Bayrock. 1996. Fluidized bed drying of baker's yeasts. Department of Applied Microbiology and Food Science. University of Saskatchewan, Saskatoon, SK Canada S7N 5A8.

## XIII. Department of Food Microbiology and Toxicology, University of Wisconsin-Madison, 1925 Willow Drive, Madison, Wisconsin 53706-1187, U.S.A. Communicated by E.A. Johnson <eajohnso@facstaff.wisc.edu>.

1. W.A. Schroeder, P. Calo, M.L. DeClercq and E.A. Johnson. 1996. Selection for carotenogenesis in the yeast *Phaffia rhodozyma* by dark-generated singlet oxygen. Microbiology **142:**2923-2929.

Selection for carotenogenesis in *Phaffia rhodozyma* was achieved by exposure of yeast strains to dark chemical reactions that generate singlet oxygen. Incubation of a mixture of *P. rhodozyma* strains containing varying levels of carotenoids in hypochlorous acid or hydrogen peroxide resulted in weak selection for pigmented strains. However, the combination of hydrogen peroxide and hypochlorous acid was strongly selective for carotenogenesis and gave a monoculture of a carotenoid-hyperproducer. Exposure of the yeast to ozone for 10 to 20 min also selected for a hyperproducing strain. These selections were relieved by 1,4-diazabicyclo[Z.Z.2]-octane, a specific quencher of singlet oxygen or by L-ascorbic acid. Continuous growth of P. rhodozyma on agar plates in an ozone/air atmosphere for 5 d decreased astaxanthin and total carotenoid levels and increased the levels of carotenoid biosynthetic intermediates. Repeated rounds of random mutagenesis followed by ozone exposure yielded mutant strains with higher pigmentation than control cultures. Our results support the hypothesis that a primary function of carotenoids in *P. rhodozyma* is to protect against singlet oxygen generated in the natural environment of the yeast and that a practical method for preventing strain degeneration during industrial fermentations may be achieved by generation of singlet oxygen using simple chemical supplements or by bubbling ozone through *P. rhodozyma* cultures during fermentation.

2. W-L Gu, G-H An, and EA Johnson. 1997. Ethanol increases carotenoid production in *Phaffia rhodozyma.* J. Industr. Microbiol. Biotecbnol. **19:**114-117.

Addition of ethanol (0.2%) to cultures of the yeast *Phaffia rhodozyma* increased the specific rate of carotenoid production [(carotenoid)(cell mass)$^{-1}$(time)$^{-1}$]. The incremental increase in carotenoid synthesis with ethanol was highest in carotenoid-hyperproducing strains. Ethanol increased carotenoid production when it was added at various points during the lag and active growth phases. Ethanol increased alcohol dehydrogenase and hydroxy-methylglutaryl-CoA (HMG-CoA) reductase activities. Our results indicate that increased carotenoid production by ethanol is associated with induction of HMG-CoA reductase and possibly activation of oxidative metabolism.

3. An, G.-H., K. Chang, and E.A. Johnson. 1996. Effect of oxygen radicals and aeration on carotenogenesis and growth of *Phaffia rhodozyma* (*Xanthophyllomyces dendrorhous*). J. Microbiol. Biotechnol 6:103-109.

Mn(II)+succinate decreased the carotenoid formation of the yeast *Phaffia rhodozyma*, probably by scavenging O2. When duroquinone DQ), an internal and external $O_2$ generator, was added to medium, *P. rhodozyma* produced more amount of carotenoids. The increased carotenoid production was destroyed by oxygen radical (OR) scavengers, ascorbate+Cu(II) and dimethylsulfoxide. When sub-lethal concentrations of $H_2O_2$, an external OR source, and antimycin, an internal OR inducer, were used, the effect of $H_2O_2$ on carotenoid formation and composition was less significant than that of antimycin. Addition of superoxide dismutase, an external OR remover, rescued cells from death caused by the high concentration of DQ. In this condition, the yeast culture showed an increase in carotenoid content. Addition of DQ into *P. rhodozyma* culture in the stationary phase did not increase carotenoid production. Therefore, carotenoid formation was stimulated by internal ORs in the growing yeast. It was probably due to release of catabolite repression on carotenogenesis in the yeast. Aeration was important for carotenoid production but was not as effective as the internal OR producer, DQ.

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

1. S. Piombo G.B. Calleja[1], B.Y. Yoo, B.F. Johnson. In press. Ruptured fission yeast walls: structural discontinuities related to the cell cycle. Cell Biochem. Biophys.

[1]Diliman Institute, P-9 Dalan Roces, Area 14, UP Campus, Diliman, Lunsod Quezon, The Philippines
[2]Department of Biology, University of New Brunswick, Fredericton, New Brunswick E3B 5A3.

Distributions of rupture sites of fission yeast cells ruptured by glass beads have been related to a new morphomeric analysis. As before (Johnson et al., Cell Biophysics, 1995), ruptures were not randomly distributed nor was their distribution dictated by geometry. Rather, ruptures at the extensile end were related to cell length just as the rate of extension is related to cell length. The extensive patterns of early log, mid log, late log, and stationary phase cells from suspension cultures were found to approximate the linear growth patterns of Kubitschek & Clay (1986). The median length of cells was found to decline through the log phase in unbalanced manner.

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

Recent publications.

1. K. Riedel, M. Lehmann, K. Tag, R. Renneberg & G. Kunze. 1998. *Arxula adeninivorans* based sensor for the estimation of BOD. Anal. Lett. (in press.).

A microbial amperometric sensor for the determination of biochemical oxygen demand (BOD) using the yeast *Arxula adeninivorans* immobilized in polyvinylalcohol has been developed. The sensor with this microbial species has a wide substrate spectrum and allows BOD measurement with very short response times (70 sec), with an operation stability over 1 month, and a serial coefficient of $\pm$5% when a standard solution containing 275 mg/L BOD was employed. A linear range was obtained up to 550 mg/L BOD using a glucose standard. The BOD-sensor was used to determine the BOD of various waste water.

2. I. Kunze, C. Nielsson, K. Adler, R. Manteuffel, C. Horstmann, M. Bröker & G. Kunze. 1998. Correct targeting of a vacuolar tobacco chitinase in *Saccharomyces cerevisiae* - posttranslational modifications proceed in dependence on the host strain. Biochim. Biophys. Acta (in press).

The chitinase gene *FB7-1* of *Nicotiana tabacum* cv. samsun line 5 was expressed in the two *Saccharomyces cerevisiae* strains, INVSC2 and H4, under the control of the *GAL1* promoter from *S. cerevisiae* and a multicopy plasmid vector. Both yeast strains express the plant gene as enzymatic active proteins. In transformants of the strain INVSC2, 94% of the total plant chitinase is contained inside the cells, probably within the vacuole which has been confirmed by subcellular fractionation as well as immunohistochemical experiments. This retention inside the cells is due to the C-terminally located 7 amino acids long vacuolar targeting peptide of the prochitinase. When this sequence was removed, chitinase was transported into the culture medium. Pulse-chase experiments revealed that during translation in transformants of both yeast strains one chitinase polypeptide can be immunoadsorbed with specific antibodies. In the case of INVSC2-transformants newly formed chitinase is modified in a 60 minute chase to slightly increase its molecular mass, whereas in H4-transformants the molecular mass constantly remained 32 kDa. By Western blot analysis two chitinase corresponding polypeptides of 32 and 37 kDa were accumulated in the culture medium of both transformants carrying the chitinase gene without the vacuolar targeting sequence. The larger one was very likely O-glycosylated. Whereas both polypepitdes were also detected in cell extracts of

the H4-transformant, only the smaller one was found in the INVSC2-transformant. The plant chitinase passed through the endoplasmic reticulum on its way to the vacuole. The N-terminal signal peptide responsible for the uptake into the endoplasmic reticulum is cleaved correctly. However, cleavage of the vacuolar targeting peptide located at the C-terminus, to give the mature chitinase is obviously influenced by the genetic background of the host strain. In INVSC2-transformants chitinase accumulates in its mature form whereas both the polypeptides of H4-transformants retain their vacuolar targeting peptide. Our results demonstrate that in the case of plant class I chitinase, the plant sorting signal is recognized in yeast cells but posttranslational modifications are influenced by the host strain.

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

Recent publications or those which are due to be published.

1. F. Malík. 1997. Pure wine yeast cultures in modern enologic strategy. (in Slovak). Thesis, Authors Report, Slovak Technical University, Faculty of Chemical Technology, Bratislava, 38 pp.

The first part of the thesis deals with a wine survey of the author's scientific and professional papers published in the course of last 25 years on modern wine microbiological problems focused on specific aspects of isolation, cultivation, identification and characterization of liquid and active dry and immobilized pure wine yeast forms. The second part is dedicated to ecology of wine yeasts on primary and secondary habitats. Problems of modern propagation and application of active dry wine yeasts are discussed. New approaches to pure, mixed and associated wine yeast cultures in winemaking are dealt with in detail. Aspects of progressive philosophy in wine microbiology and complex views on recent and future events in winemaking and wine microbiology related to pure yeast starters are elucidated.

2. E. Minárik and O. Jungová. 1997. Novel activation of alcoholic grape must fermentation (in Slovak) Kvasný průmysl (Prague) **43:** (in press).

A combined Preparation of Fermaid® "E" containing important yeast nutrients [thiamin, yeast ghosts, $(NH_4)_2HPO_4$, $(NH_4)_2SO_4$] stimulating wine yeasts in alcoholic fermentation, was developed. It guarantees a smooth fermentation start, course, and completion even under quite unfavourable conditions, particularly in musts showing deficiencies in yeast nutrients.

3. E. Minárik. 1997. New procedure for utilizing sulphur dioxide in wine bottling (in Slovak) Kvasný průmysl (Prague) **43:**271.

Important advantages of direct $SO_2$ injection into empty bottles prior to wine bottling as proposed by Delfini are reported: 1) sulphur dioxide determined in advance may be injected in the empty bottle, 2) sterilization of the wine and the inner bottle walls are guaranteed, 3) intensive $SO_2$ effect on wine microorganisms. Molecular sulphur dioxide quickly kills yeast and bacterial cells. The binding ability of $SO_2$ with wine constituents (acetaldehyde, polyphenols) is very slow. Sulphur dioxide may be injected as concentrated water solution or in wine to be bottled. The anti-yeast effect of $SO_2$ in water solution or in wine at low pH is compared with that of a potassium metabisulphite solution.

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

Publications for 1996-1997.


1. Zimmer M., Blanchard S., Boze, H. Moulin G. and Galzy P. 1997. Glucose metabolism in the yeast *Schwanniomyces castellii*. Role of phosphoorylation site I and alternative respiratory pathway. Appl. Environm. Microbiol. **63**:2779-2784.
2. Gueguen Y., Chemardin P., Janbon G., Arnaud A., Galzy P. and Parfait A. 1996. Libération biologique d'arômes à partir de fruits tropicaux. Fruits (F) Numéro spécial: Technologie et Maîtrise de la Qualité **51:**299-305.
3. Gueguen Y., Chemardin P., Pien S., Arnaud A. and Galzy P. 1997. Enhancement of aromatic quality of muscat wine by the use of immobilized β-glucosidase. J. Biotechnol. **55**:151-156.

4. Vaysse L.., Dubreucq E., Pirat J.L, and Galzy P. 1997. Fatty hydroxamic acid biosynthesis in aqueous medium in the presence of the lipase-acyltransferase from *Candida parapsilosis.* J. Biotechnol. **53**:41-46.

5. Chevandier F., Dubreucq E. and Galzy P. 1997. Lipase-catalysed synthesis of propanediol monoesters in biphasic aqueous medium. Biotechnol. Lett., **19**:913-917.

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

We would like to thank Jure Piškur (Dept. of Microbiology, Technical University of Denmark, Lyngby), Paul Sniegowski (Dept. of Biology, University of Pennsylvania, Philadelphia) for fruitful collaboration in yeast research in 1997. G.I.N. is grateful to Peter Raspor and Sonja Smole Mozina for the invitation to give a lecture at the 18" ISSY, 27th August, 1997, Bled, Slovenia.

The following are our publications for 1996 and 1997.

1. G.I. Naumov. 1996. Genetic identification of biological species in the *Saccharomyces sensu stricto* complex. J. Ind. Microbiol. **17**:295-302.

2. E.S. Naumova, H. Turakainen, G.I. Naumov & M. Korhola. 1996. Superfamily of α-galactosidase *MEL* genes of *Saccharomyces sensu stricto* complex. Mol. Gen. Genet. **253**:111-117.

3. G.I. Naumov & D.G. Naumov. 1997. A superfamily of the α-galactosidase genes *MEL* in the yeast *Saccharomyces cerevisiae*. Doklady Biological Sciences. **353**:172-174.

4. G.I. Naumov & D.G. Naumoff.. 1997. Genetic Mapping of the new divergent family of α-galactosidase *MEL* genes in *Saccharomyces cerevisiae*. Biotechnologia. **1**:26-28 (in Russian).

5. G.I. Naumov. 1997. Natural diversity of yeasts is an inexhaustible gene pool for fundamental and applied research. Advances in Modern Biology (Uspehi Sovremennoj Biologii) **117**(2):185-195 (in Russian).

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

Wine *Saccharomyces cerevisiae* strains having high nDNA/nDNA homology with sibling species *S. paradoxus* or *S. bayanus* (Rodrigues de Sousa et al., Syst. Appl. Microbiol. **18:**44-51 - 1995) have been studied by genetic and karyotypic analyses. Despite the natural introgression the strains had the *S. cerevisiae-S. paradoxus* specific karyotype and belonged to the biological species *S. cerevisiae*. Their intraspecific hybrids were fertile having 28-99% ascospore viability and normal meiotic segregation of control markers while interspecific hybrids were sterile. The results demonstrate that molecular karyotyping is a reliable tool for delimiting strains of *S. bayanus* from *S. cerevisiae* and *S. paradoxus*. The latter two species, having nearly identical karyotypes, can be distinguished by genetic analysis. Several *Sarccharomyces sensu stricto* strains monitored earlier by different molecular methods (Guillamon et al., Int. J. Syst. Bacteriol. **44:** 708-714 - 1994; Molnar et al., System. Appl. Microbiol. **18:**135-145 - 1995) have been reidentified genetically as *S. bayanus* and *S. paradoxus*.

7. G.I. Naumov, E.S. Naumt,va. G. Marinoni & J. Piškur. 1997. Genetic hybridization analysis of yeasts *Saccharomyces castellii*, *Saccharomyces exiguus* and *Saccharomyces martiniae*. Genetika (submitted, in Russian).

Genetic lines for three biological species of the *Saccharomyces sensu lato* group, *viz*. *S. castellii*, *S. exiguus* and *S. martiniae*, were created. Homo- and heterothallic monosporic fertile strains were marked by different auxotrophic mutations. Conditions for hybridization of the *Saccharomyces sensu lato* yeasts were found. Mating of spores or haploid cells of auxotrophic complementary mutants occurred on complete YPD medium. Prototrophic hybrids were selected on minimal medium. Hybridization of diploid cells was found in *S. castellii.*

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

The philosophy of the research activities on yeast at the Center for Process Biotechnology is to combine physiological studies with advanced analytical techniques and mathematical modeling with the objective of increasing our understanding of *Saccharomyces cerevisiae*. Presently the research on yeast is focused on four aspects: (1) Minimization of glycerol formation under anaerobic conditions. The project aims at minimizing the formation of glycerol in the production of ethanol by applying Metabolic Engineering. (2) Mixed sugar utilization. The aim of the project is to study the mixed sugar utilization of S. *cerevisiae* in continuous cultures. The primary focus will be on the interaction between glucose and other sugars. (3) Glucose repression, in relation to utilization of other sugars, is studied. By means of genetic engineering, strains are constructed which are glucose derepressed, *i.e.* they can simultaneous utilize glucose together with other sugars. The physiology of "glucose-derepressed" recombinant *S. cerevisiae* strains is studied and compared with wild-type strains. (4) Production of recombinant proteins. The production of recombinant proteins by *S. cerevisiae* and other yeasts are studied using proteinase A and cutinase as the model system.

Recent publications:


1. Klein, C. J. L., Olsson, L., Rnrnnow, B., J. D. Mikkelsen and J. Nielsen. 1996. Alleviation of glucose repression of maltose metabolism by *MIG1* disruption in *Saccharomyces cerevisiae*. Appl. Environ. Microbiol. **62:**4441-4449.
2. Olsson, L., Larsen, M. E., Nielsen, J., Rønnow, B., and J. D. Mikkelsen. 1997. Silencing *MIG1* in *Saccharomyces cerevisiae*: Effects of antisense *MIG1* expression and *MIG1* gene disruption. Appl. Environ. Microbiol. **63:**2366-2371.
3. Nissen, T. L., Schulze, U., Nielsen J., Villadsen, J. 1997. Flux distribution in anaerobic, glucose limited continuous cultures of *Saccharomyces cerevisiae.* Microbiology **143:**203-218.
4. Klein, J. L., Olsson, L., and J. Nielsen. 1997. Glucose control in *Saccharomyces cerevisiae:* the role of *MIG1* on metabolic fluxes. Microbiology. In press.


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

The following is the summary of a recently completed dissertation.

1. M.J. Alves da Costa e Almeida. Characterization of the yeast flora of traditional bread doughs and their potential utilization in the baking industry.

In breadmaking, fermentation by yeasts is of primary importance for its leavening function and the possible contribution for the production of desirable flavour compounds. Although the baking industry generally uses *Saccharomyces cerevisiae*, in rural areas in Portugal bread is sometimes prepared with dough carried over from a previous making. Thus, the main objective of this work was to identify and characterize the yeast flora present in traditional bread doughs in order to select for strains of potential value for the baking industry. Thirty-four samples were randomly taken from home-made bread doughs at different locations in the country or at the same sites but from bread having distinct organoleptic characteristics and 95 strains were isolated. Only 75 were identified according to conventional methods used in yeast taxonomy and nine species representative of the yeast flora were found. By far, the predominant species, isolated from about 80% of the samples was *S. cerevisiae*, *Issatchenkia orientalis*, *Pichia membranaefaciens* and related species and *Torulaspora delbrueckii* were present in ca. 40% of the samples; *P. anomala* and *S. kluyveri*, were present in about 10% and *Kluyveromyces marxianus* and *I. occidentalis* present in 3%. It is noteworthy that some doughs had very specific yeast populations, some of them having two or three strains of a single species. All the isolates have been characterized with respect to their "killer" activity. About one quarter displayed killer activity, strains of *P. anomala* showing the broadest spectra.

Among the main characteristics desirable in a baking yeast are a high biomass production together with a rapid maltose fermentation and high fermentative capacity. Five strains of *S. cerevisiae* and three of *T. delbrueckii*, selected on the basis of these characteristics were used in this study. When these yeast strains were grown in a rich medium, Y.P.S. (with 2% of sucrose) and used in the dough, the leavening ability of the yeast cells ranged from 350 to 480 ml $CO_2.3h^{-1}$ dough. These values were very similar to those showed by the commercial strains and the highest ones belonged to a strain of *S. cerevisiae* (IGC 5320 [DB 56A]) and two strains of *T. delbrueckii* (IGC 5321 [DB 42B]) and (IGC 5323 [DB 62A]). When 3% of NaCl or 0.5% of maltose were added to the growth medium, we increased with the

increase of maltose concentration in the medium, we observed that fermentative activity was enhanced but maximum biomass decreased about 20 to 30%. The activity of α-glucosidase of the selected strains was studied in the different growth media and the strains of *T. delbrueckii* systematically showed the highest values. These values increased with the increase of maltose concentration in the medium. In what respects invertase and in the same experimental conditions, strains of *T. delbrueckii* (IGC 5321 [DB 42B]) and (IGC 5323 [DB 62A]) showed the same pattern.

Manufacturers of baker's yeast are in constant pursuit of strains with improved dough raising properties and capacity to retain fermentative ability during storage at low temperature in order to meet baking industry requirements such as the increasing use of frozen doughs. The same strains of *S. cerevisiae* and *T. delbrueckii* were used in this study. The objective was to test the influence of dough freezing and storage both on the leavening ability of the yeast cells and on their viability using two strains of commercial baker's yeast as a reference. Most of the studied strains displayed dough-raising capacities similar to the ones found in baker's yeasts. During storage of frozen doughs two strains of *T. delbrueckii* (IGC 5321 [DB 42B]) and (IGC 5323 [DB 62A]) presented approximately the same leavening ability for 30 days. Cell viability was not significantly affected by freezing, but when the dough was submitted to a bulk fermentation before being stored at -20°C there was a decrease in the survival ratio which depended on the yeast strain. Furthermore, the leavening ability after four days of storage decreased as the prefermentation period of the dough before freezing increased, except for strains of *T. delbrueckii*. These two strains retained the fermentative activity after 15 days of storage and 2.5 hours of prefermentation, despite showing a reduction of viable cells under the same conditions. The intracellular trehalose content was higher than 20% (w/w) in four of the yeasts tested: the two commercial strains of baker's yeast and the referred two strains of *T. delbrueckii*. However, the strains of *S. cerevisiae* were clearly more susceptible to freezing damages, indicating that other factors may contribute to the freeze tolerance of these yeasts. Summarizing, from the results obtained, we may conclude that two strains of *T. delbrueckii*, when compared to all the other strains studied, kept a strong capacity to produce $CO_2$ under all circumstances tested, showed high intracellular concentrations of trehalose and presented the highest tolerance to freezing. These properties make them candidates of potential value for the baking industry.

## XXI. Department of Genetics and Molecular Biology, Instituto de Fisiología Celular, Universidad Nacional Autónoma de México, Apartado 70-600, 04510, Mexico D.F. Communicated by A. Peña <apd@ifcsun1.ifisol.unam.mx>.

The following paper has been accepted for publication in YEAST.

1. M. Calahorra, G.A. Martínez, A. Hernández-Cruz, and A. Peña. Values of yeast cytoplasmic and vacuolar pH, the influence of monovalent cations.

The effects of monovalent cations on the internal pH of yeast were studied. Our former procedure was modified, inducing maximal alkalinization of the cells with 100 mM $NH_4OH$ instead of tris base. The pH values were lower than reported before (Peña *et al*., J. Bacteriol. 177:1017-1022, 1995). With glucose as substrate, the internal cytoplasmic pH reached higher values when incubating at an external pH of 6.0, as compared to pH 4.0. Monovalent cations added after approximately 5 min after glucose, produced a further increase of the internal pH, which was higher at a previous incubation pH of 4.0 than that observed at pH 6.0. The selectivity of the changes followed a similar order to that of the transport system for monovalent cations.

When incubating cells with glucose for more than 30 min, the initial changes of the internal pH appeared to be regulated by the cell. However, under the fluorescence microscope, it was observed that pyranine, which was confined to the cytoplasm during the first 15 min, was progressively concentrated in the vacuole. In this way, following the fluorescence changes of cells electroporated and then incubated with glucose or glucose plus potassium, allowed us to follow the internal pH of this organelle, obtaining values within the range reported by other authors. Also, in cells preincubated with glucose for 60 min, and electroporated afterward, the fluorescence of pyranine, which entered only the cytoplasm, allowed us to measure the pH of this compartment, showing that it was more alkaline than the vacuole. Moreover, the cytoplasmic pH increased upon the addition of glucose or potassium. The vacuolar pH, on the other hand, like the cytoplasmic, showed an increase upon the addition of potassium after glucose; however, the addition of glucose produced a decrease, rather than an increase of the vacuolar pH. In addition, the incubation of the cells with glucose with or without pyranine, produced the vesiculation of the vacuole.

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


1. Tornai-Lehoczki, J. and Dlauchy, D. 1996. An opportunity to distinguish species of *Saccharomyces sensu stricto* by electrophoretic separation of the larger chromosomes. Let. Appl. Microbiol. **23**:227-230.

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

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

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

The following are two abstracts from two B.Sc. projects done in my lab by two Erasmus/Socrates programme students, G. Marinoni (otherwise from University of Paris 7, France) and S. Paracchini (otherwise from University of Pavia, Italy). Both projects were presented as written reports in September and copies are available upon request.

### 1. S. Paracchini. *Saccharomyces kluyveri* as a model organism for studying catabolism of purines.

Purines are nitrogenous bases mainly found as nucleotides, and as constituents of nucleic acids. Purines play important roles also in many aspects of the metabolism. Their physiological importance is reflected in the careful regulation of the size of their pool through the de novo biosynthesis, salvage pathway and catabolic pathway. While the *de novo* biosynthetic and salvage pathways are relatively well characterized, less is known about the degradation pathway. So far very few studies have been conducted about purine degradation in yeast. Since *Saccharomyces cerevisiae* cannot utilize purines as nitrogen source the aim of this project was to find a yeast that can degrade purines and to develop a genetic system to study this catabolic pathway. *Saccharomyces kluyveri* can grow on purines as the only sources of nitrogen. Upon mutagenesis several mutants with a defect in degradation of purines were isolated and partially characterized.

### 2. G. Marinoni. Interspecific crosses of yeasts belonging to the Genus *Saccharomyces*.

Strains of *Saccharomyces cerevisiae* can exist as haploid, diploid or polyploid strains. Haploid strains can express one of the two mating types, a or α, but they can also switch from one mating type to another. In this case they are designated as homothallic. This switch is catalyzed by the product of the HO gene. If this gene is non-functional, the mating type becomes stable, and the strain is then heterothallic. Heterothallic strains are stable, as diploids or as haploids, while homothallic strains are only stable as diploids since the transient haploid cell quickly diploidizes as a result of the specific sex conversion and mating between the mother and daughter cell.

The genus *Saccharomyces* consists of ten species which belong to *Saccharomyces sensu stricto* (including *S. cerevisiae*), and *Saccharomyces sensu lato* (including species which have so far been only poorly studied) group. Among the characters used to delimit yeast species have been ascospore shape and ornamentation, antagonistic relationships (formation of killer toxins, sensitivity and resistance to toxins), peculiarities of life cycle (homo and heterothallism), growth on various carbon and nitrogen compounds, ability to ferment various sugars such as maltose, α-methylglucoside, sucrose, galactose or melibiose. However, the realization that nutritional characteristics and home and heterothallism are often controlled by a single gene has made these criteria unsatisfactory for taxonomic purpose. Therefore, it is still quite unclear which isolates represent real species, and what actually is a yeast species.

In this project, we have tried to study mating behaviour of various yeasts and a simple genetic system has been developed for some sensu late yeasts. Firstly, haploid strains were found among various collections or they were isolated upon mutagenesis. Then auxotrophic markers were introduced into these strains. This allowed us to perform interspecific crosses and select for hybrid strains on the minimal medium. All of tested haploid strains secreted a or a-like pheromones which were deti~cted by a halo assay based on mutant *S. cerevisiae* tester strains. When *sensu lato* strains were crossed to each other, hybrids were found only in a few cases. These hybrids were examined for their nuclear chromosomes and mitochondrial DNA (mtDNA). A cross between *S. castellii* (CBS 4310) and *S. exiguus* (CBS 2141) resulted in a hybrid which contained chromosomes from both parents and mtDNA from the *castellii* parent. This observation suggests that in the nature, it is likely that *Saccharomyces* isolates can freely mate and produce viable hybrids also in nature. Therefore, most of the modern genomes can be mosaic and of a polyphyletic origin. This makes it rather difficult to define what a yeast species is.

Abstract of a recently published paper.

### 3. J. Piškur. 1997. The transmission disadvantage of yeast mitochondrial intergenic mutants is eliminated in the *mgtl* (*ccel*) background. J. Bacteriol. **179**:5614-5617.

A trans-acting element, *MGTI* (also called *CCE1*), has previously been shown to be required in *Saccharomyces cerevisiae* for the preferential transmission of petite mitochondrial DNA (mtDNA) molecules over wild-type mtDNA molecules. In the present study a possible role of this nuclear gene in the transmission of mtDNA from various respiration-competent mutants was studied. Several of these mutants, lacking one or the other of two biologically active mitochondrial intergenic sequences, were employed in genetic crosses. When these deletion mutants were crossed to the parental wild-type strain in the *MGT1/CCE1* background, the progeny contained predominantly wild-type mtDNA molecules. When crosses were performed in the *mgtl/ccel* background, the parental molecules interacted in zygotes and underwent homologous recombination but wild-type and intergenic-deletion alleles were transmitted with equal frequencies.

## XXIV. Institute of Applied Microbiology, Universtät für Bodenkultur, IAM; Muthgasse 18, A-1190 Wien, Austria. Communicated by H. Prillinger <iam@mail.boku.ac.at>.

The following is an abstract of a recent publication.

1. Five new species of *Fellomyces*, *F. borneensis*, *F. chinensis*, *F. lichenicola*, *F. sichuanensis*, and *F. thailandicus* isolated from lichens growing on tree barks are described. System. Appl. Microbiol. **20**: in press.

Species delimitation was performed using RAPD-PCR and partial sequencing of ribosomal DNA. A fragment of 385 nucleotides, corresponding to the gene position 547 through 931 of the 18S rDNA in *Saccharomyces cerevisiae* was used. Based on phylogenetic data it was not possible to separate the ballistosporous genus *Kockovaella* from the sterigma forming genus *Fellomyces*. Species reproduce by enteroblastic budding or by forming one or more conidia on sterigmata per cell. Sterigmatoconidia are delivered by an end break in distal position of the sterigmata. Ubiquinone Q-10 is the major ubiquinone component in all species. The presence of xylose in purified yeast cell walls was chracteristic for all strains investigated. It was not possible to separate the five different *Fellomyces* species by phenotypic criteria of the physiological standard characterization only.

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

1. Romano, P., Suzzi, G., Comi, G., Zironi, R., Maifreni, M. 1997. Glycerol and other fermentation products of apiculate wine yeasts. J. Appl. Microbiol. **82**: 615-618.

Ninety-six strains of apiculate wine yeasts were studied for their ability to produce glycerol, acetaldehyde, ethyl acetate, sulphur dioxide and hydrogen sulphide in synthetic medium. *Hanseniaspora guilliermondii* produced smaller quantities of glycerol, acetaldehyde and hydrogen sulphide than *Kloeckera apiculata,* whereas the production of ethyl acetate and sulphur dioxide was found to be similar. Strains characterized by different capacities and properties were found for both species. The existence of apiculate strains differing in secondary compound production is of technological interest, as these yeasts constitute potenzial flavour producers. Selected strains of apiculate yeasts might favour an enhanced flavour formation and yield desirable characteristics to the final product.

2. Suzzi G., Romano, P., Sora, S. 1997. Flocculation expression in a strain of wine yeast *Saccharomyces cerevisiae*. Ann. Microbiol. Enz. **47**:53-62.

The genetic basis of wine yeasts flocculation was studied and in particular which genes determine thc high resistence to proteases. Spores of the flocculent homothallic strain 10450, phenotype B, was treated with EMS to obtain auxotrophic clones. Among these strain 450M crossed with a non flocculent heterothallic strain gave a flocculent diploid (*RE2*) that produced 2:2, 3:1 and and 1:3 segregations. Tetrad analysis of cross *RE2* showed that more than a single and dominant gene for flocculation was carried by strain 450M. In an attempt to verify further genetic bases of the results obtained with *RE2*, crosses between *RE2* segregants excluded the presence of a suppressor gene and showed that the flocculence of the wine strain 10450 was governed by the presence of two dominant genes. Crosses, constructed between segregants of *RE2* together with tester strains proved that the two genes were *FLO1* and *FLO5.* The flocculent phenotype of the segregants, did not show the typical flocculent phenotype described for *FLO1* and *FLO5* genes. These data suggest that the genetic basis of flocculent phenotypes of wine yeasts are very complex and a direct relationship between *FLO* genes and flocculent phenotypes does not exist.

3. Marchese, R., Romano, P. 1997. By-products formation during beer fermentation by high and low acetoin producing strains of *Saccharomyces cerevisiae*. Alcologia **9**:133-135.

Secondary products during the alcoholic fermentation of wort must produced by two *Saccharomyces cerevisiae* strains, characterised by high and low acetoin production, were determined. Higher alcohols and acetic acid were produced quite at the same levels by the two strains, whereas differences in acetaldehyde and ethyl acetate were recorded. Significantly different patterns in ethyl acetate were found, with about 30 mg/l produced by the low acetoin producing strain and only 9 mg/l by the high acetoin producing one. During the fermentation process both strains exhibited an increase in ethyl acetate production until the middle stage of fermentation, showing a difference in the second part of the process. The two strains yielded similar final amounts of ethanol, but, during the fermentation process the high acetoin producing strain formed higher amounts than the low producing one. These differences might be explained as due to a different fate of acetyl-CoA. From the technological point of view, the acetoin high producer phenotype, exhibiting a general lower flavour intensity, might be used for the fermentation of beverages which are characterized by a delicate flavour.

4. Suzzi, G., Romano, P., Polsinelli, M. 1997. Isolation and characterization of mutants resistant to amino acids analogues from *Saccharomyces cerevisiae* wine yeast strains. World J. Microbiol. Biotechnol.(in press).

Mutants resistant to amino acid analogues DL-thiaisoleucine, DL-4-azaleucine, 5.5.5-trifluoro-DL-leucine, L-O-methylthreonine, were isolated from *Saccharomyces cerevisiae* wine yeast strains. The fermentative production of secondary metabolites by the mutants was tested in grape must. Higher alcohols, acetaldehyde and acetic acid concentration varied depending on strain and analogue. Most of the mutants increased amounts of amyl alcohol produced. A remarkable variability in the level of n-propanol, isobutanol, acetaldehyde and acetic acid was observed. In the practical application the use of mutants resistant to amino acid analogues can improve the quality of wines by reducing or increasing the presence of some secondary compounds.

5. Romano, P., Paraggio, M., Turbanti, L. Stability in by-product formation as a strain selection tool of *Saccharomyces cerevisiae* wine yeasts. J. Appl. Microbiol. (in press).

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.

6. Romano, P., Brandolini, V., Ansaloni, C., Menziani, E. Characterization of wine yeasts for 2,3-butanediol production. World J. Microbiol. Biotechnol. (in press).

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 *Zygosaccharomyces 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.

## XXVI. Angewandte Molekularbiologie, Universität des Saarlandes, FB 13.3, Gebäude 2, Postfach 151150, D-66041 Saarbrücken, Germany. Communicated by M.J. Schmitt <mjs@microbiol.uni-sb.de>.

1. M.J. Schmitt, O. Poravou, K. Trenz & K. Rehfeldt. 1997. Unique double-stranded RNAs responsible for the anti-*Candida* activity of the yeast *Hanseniaspora uvarum*. J. Virol. **71**: in press.

Killer strains of the yeast *Hanseniaspora uvarum* contain cytoplasmic double-stranded RNAs (dsRNAs) of 4.7 kb L and 1.0 kb M species, which were shown to be separately packaged into icosahedral virus-like particles exhibiting RNA-dependent RNA polymerase activity. The L genome of the *H. uvarum* L-dsRNA virion HuV-L was shown to encode a 77 kDa major capsid protein. Peptide maps of the purified HuV coat protein and the 81 kDa major capsid protein from K1 killer viruses of *Saccharomyces cerevisiae* revealed distinctly different peptide patterns, suggesting significant sequence divergence at the level of the capsid-coding L-dsRNAs. In vitro transcripts from purified HuV-L particles showed no cross-hybridization to denatured $L_A$, $L_B$, or $L_C$, indicating that L from *H. uvarum* represents a unique L-dsRNA species. Weak, but clearly detectable cross-hybridization of the 1.0 kb dsRNA of HuV-M, encoding the secreted 18 kDa anti-*Candida* toxin, to the toxin-coding M genomes of *S. cerevisiae* K1, K2, and K28 killers indicated partial sequence homology among all of the M-dsRNAs tested.

2. K. Eisfeld & M.J. Schmitt. 1997. Construction and possible use of recombinant M-dsRNA satellite viruses in heterologous gene expression in yeast. Yeast **13**:237.

K28 killer strains of the yeast *S. cerevisiae* possess a cytoplasmically inherited, encapsidated double-stranded RNA genome (M-dsRNA) which contains the genetic information for a killer toxin precursor protein (preprotoxin) that is processed to

a non-glycosylated a/b-heterodimeric protein toxin during passage through the yeast secretory pathway. For replication and maintenance, the M killer virus depends on the coexistence of a second dsRNA virus (L-A) in the cell. In order to manipulate the satellite virus M genome, we used a full-length cDNA copy of the toxin-coding M28-dsRNA which was placed under transcriptional control of an SP6 bacteriophage promoter. This PCR-construct enabled us to generate mg amounts of viral M(+)strands in an in vitro run-off transcription system. By the means of biolistic and/or electroporation we transfected L-A non-killer strains and obtained M(+)ssRNA-containing yeast clones that stably expressed a strong K28 killer phenotype. Since all K+ transfectants were shown to possess the toxin-coding M28-dsRNA genome, it can be concluded that the M28(+)ssRNA in vitro transcript must have been recognized by the the viral Cap/Pol protein of L-A, and furthermore, subsequently packaged into functional toxin-secreting staellite viruses. Experiments will be described in which recombinant M28 viruses are constructed that are restricted (in cDNA sequence) to the very 5'-end bases of the M28(+)ssRNA and to the two viral packaging sites located at the 3'-end of the viral transcript. The K28 killer toxin gene has been removed and replaced by a short multiple cloning site which allows integration of foreign DNA. Therefore, recombinant killer viruses might be used as an alternative cloning system suitable for heterologous gene expression in yeast.

3. M.J. Schmitt & G. Schernikau. 1997. Construction of a cDNA-based $K_1/K_2/K_{28}$ triple killer strain of *Saccharomyces cerevisiae*. Food Technol. Biotechnol., in press.

By transforming a natural, dsRNA-based K1 killer strain of the wine yeast *Saccharomyces cerevisiae* with two multi-copy (2µ) vectors carrying cDNA copies of the $K_2$ and $K_{28}$ preprotoxin/immunity genes, a triple killer strain has been constructed that (i) simultaneously secreted three different killer toxins [$K_1$, $K_2$, $K_{28}$], (ii) expressed functional toxin immunities, and (iii) exhibited a strong and significantly broader killing spectrum than the single killer derivatives. Both plasmids were shown to be self-selective under conditions where the triple killer was cultivated in YEPD medium at pH 4.7. It is proposed that recombinant $K_1/K_2/K_{28}$ triple killers should be able to predominate in mixed yeast cultures and therefore might be useful in industrial wine fermentations to prevent stuck fermentations and/or killer yeast contaminations.

## XXVII. Department of Genetics, University of Debrecen, P.O.Box 56, H-4010 Debrecen, Hungary. Communicated by M. Sipiczki.

List of recent publications.


1. Sipiczki M.: Fission yeasts and polarity. 1996. Ann. Rev. Research Center for Pathogenic Fungi and Microbial Toxicoses, Chiba University, Japan, pp. 58-64.
2. Grallert, A., Miklos, L, Sipiczki M. 1997. Division site selection, cell separation and formation of anucleate minicells in *Schizosaccharomyces pombe* mutants resistant to cell wall lytic enzymes. Protoplasma **198**:218-229.
3. Benko, Z., Miklos, L, Carr, A., Sipiczki M. 1997.Caffeine-resistance in *S. pombe*: mutations in three novel *caf* genes increase caffeine tolerance and affect radiation sensitivity, fertility, and cell cycle. Curr. Genet. **31**:481-487.
4. Sipiczki M. 1997. Protoplast fusion. Molecular Genetics with the Fission Yeast *Schizosaccharomyces pombe*. EMBO Practical Course. Copenhagen, pp. 31-32.
5. Sipiczki M. 1997. Phylogenesis of fission yeasts. Molecular Genetics with the Fission Yeast *Schizosaccharomyces pombe*. EMBO Practical Course. Copenhagen, p. 82.
6. Sipiczki M., Miklos, L, Grallert, A. 1997. Polarity, spatial organization of cytoskeleton and morphogenesis in fission yeasts. Proceedings of the International Symposium on Theoretical Biophysics and Biomathemathics. (eds. L. Luo, Q. Li and W. Lee) Inner Mongolia University Press, Hohhot, China, pp. 129-132.
7. Miklos, L, Sipiczki M. 1995. Characterization of a novel temperature-sensitive cell cycle mutant in *Schizosaccharomyces pombe*. Forty-third Harden Conference. Programme and Abstracts. p. 67.
8. Grallert, A., Miklos, L, Ribar, B., Grallert, B., Sipiczki M. 1996. Analysis of cell separation mutants in *Schizosaccharomyces pombe*. Asian International Mycological Congress '96, Chiba (Japan), Proceedings pp. 15-16.

9. Miklos, I., Sipiczki M. 1996. Characterisation of a novel temperature-sensitive cell cycle mutatnt in *Schizosaccharomyces pombe*. Acta Microbiol. Immunol.. Hung. 43:253-254.

10. Grallert, A., Miklos, L, Sipiczki M. 1996. A *Schizosaccharomyces pombe* mutant defective in cytokinesis. Acta Microbiol. Immunol. Hung. **43**:254.

11. Sipiczki M., Miklos, I., Grallert, B. 1997. Polarity, spatial organisation of cytoskeleton and morphogenesis in the fission yeasts. The International Symposium on Theoretical Biophysiscs and Biomathematics. Hohhot(China), Program and Abstracts pp. 2-3.

12. Miklos, L, Varga, T., Nagy, k, Sipiczki M. 1997. Rearrangement of chromosomes during cell division in a wine yeast. 8th Eur. Gong. Biotechnol. Budapest (Hungary). Abstracts p. 193.

13. Batic, M., Czako-Ver, K., Pas, M., Pesti M., Sipiczki, M., Raspor, P. 1997. Chromium accumulation in the cells of *Schizosaccharomyces pombe* sensitive and tolerant mutants. 18th. International Specialized Symposium on Yeasts. Yeast Nutrition and Natural Habitats. Bled (Slovenia). Book of Abstracts P2-03.

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

Since our last report (Dec. 1996) the following publications have appeared.

1. Aalto, M.K., Jäntti, J., Östling, J., Keränen, S. and Ronne, H. 1997. Mso1p: a yeast protein that functions in secretion and interacts physically and genetically with Sec1p. Proc. Natl. Acad. Sci. USA **94**:7331-7336.

2. Haikara, A., Virkajärvi, I., Kronlöf, J. and Pajunen, E. 1997. Microbiological contaminations in immobilized yeast bioreactors for primary fermentation. Proc. 26th Congr. Eur. Brew. Conv., Maastricht, 24-29 May 1997, 439-446.

3. Kataja, K., Virkajärvi, I. and Linko, M. 1997. Yeast recycling in the main fermentation of beer. Mallas ja Olut 1997:4, 107-113.(English summary).

4. Kruszewska, J.S., Saloheimo, M,. Penttilä, M. and Palamarczyk, G. 1997. Isolation of the *Trichoderma reesei* c-DNA that encodes GTP: α-D-mannose-1-phosphate guanyl transferase and functions in *Saccharomyces cerevisiae*. Tricel 97, Ghent, 28-30 Aug. 1997, P45.

5. Lindborg, K. and Virkajärvi, I. (1997). The effect of aeration in continuous main fermentation of beer by an immobilised yeast bioreactor. Mallas ja Olut 1997:**4**:101-106 (English summary).

6. Ruohonen, L., Toikkanen, J., Tieaho, V., Outola, M., Söderlund, H. and Keränen, S. 1997. Enhancement of protein secretion in Saccharomyces cerevisiae by overproduction of Sso protein, a late-acting component of the secretory machinery. Yeast **13**:337-351.

7. Saloheimo, M., Nakari-Setälä, T., Tenkanen, M. and Penttilä, M. 1997. A novel *Trichoderma reesei* endoglucanase cDNA: isolation and expression in yeast. Tricel 97, Ghent, 28-30 Aug. 1997, P48.

8. Saloheimo, A., Aro, N., Ilmén, M. and Penttilä, M. 1997. A yeast-based cloning system for regulatory genes, applied for *Trichoderma* cellulases. Tricel 97, Ghent, 28-30 Aug. 1997, P47.

9. Sizmann, D., Kuusinen, H., Keränen, S., Lomasney, J., Caron, M.G., Lefkowitz, R.J. and Keinänen, K. 1996. Production of adrenergic receptors in yeast. Receptors and Channels **4**:197-203.

10. Virkajärvi, I. and Kronlöf, J. 1997. Long term stability of immobilized yeast reactors in main fermentation. ASBC Annu. Meet., Palm Springs, CA 21-25 June 1997. ASBC Newsletter **57**:21.

## XXIX. Dipartimento di Protezione e Valorizzazione Agroalimentare, University of Bologna, Microbiology section. via F.lli Rosselli, 107 42100 Reggio Emilia, Italy. Communicated by C. Zambonelli <gx4resb4@vm.cineca.it>.

Recent publications.

1. G. Montanari, C. Zambonelli, L. Grazia, G. Kamasheva, M.K. Shigaeva. 1996. *Saccharomyces unisporus* as the principal alcoholic fermentation micro-organism of traditional koumiss. J. Dairy Res. **63**:327-331.
2. L. Bertolini, C. Zambonelli, P. Giudici, L. Castellari. 1996. Higher alcohols production by cryotolerant *Saccharomyces* strains. Am. J. Enol. and Vitic. **47**:343-345.
3. C. Zambonelli, P. Passarelli, S. Rainieri, L. Bertolini, P. Giudici, L. Castellari. 1997. Technological properties and temperature response of interspecific *Saccharomyces* hybrids. J. Sci. Food Agric. 73, 7-12.
4. P. Giudici, C. Caggia, A. Pulvirenti, S. Rainieri. 1997. Karyotyping of *Saccharomyces* strains with different temperature profiles. J. Appl. Microbiol. (In press).

Our study examined the karyotype, the fermentation performance and the optimum growth temperature ($T_{opt}$) of 28 strains all identified as species belonging to *Saccharomyces sensu stricto*. The strains were isolated from uninoculated fermented musts at two temperature ranges: 20 to 40°C and approximately 0 to 6°C. The results demonstrated a correlation between the $T_{opt}$ and the chromosomes organisation. In particular, strains with $T_{opt}$ lower than 30°C showed only two bands in the region between 365 and 225 Kb, while those with a $T_{opt}$ greater than 30°C had three bands in this size range. From a taxonomic viewpoint, the $T_{opt}$ is more discriminant for the *Saccharomyces* species than the ceiling temperature of 37°C currently used to differentiate cryotolerant *Saccharomyces bayanus* and *Saccharomyces pastorianus* from non-cryotolerant *Saccharomyces cerevisiae* and *Saccharomyces paradoxus* strains.

The following communication and posters were presented at the 18th ISSY Yeast Nutrition and Natural Habitats 24- 29 August 1997 Bled, Slovenia.

5. P. Giudici, C. Caggia, A. Pulvirenti. Cryotolerant *Saccharomyces* strains and spoilage of refrigerated must. L7-3.
6. S. Rainieri, P. Giudici, C. Zambonelli. Enological properties of *Saccharomyces bayanus* and *Saccharomyces cerevisiae* interspecific hybrids. P7-05.
7. G. Montanari, L. Grazia. Galactose-fermenting yeasts as fermentation micro-organisms of traditional koumiss. P7-06.
8. L. Castellari, V. Tini, C. Zambonelli. Enological traits of cryotolerant and thermotolerant *Saccharomyces* strains. P7-07.

## XXX. Department of Food Science and Technology, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061-0418, U.S.A. Communicated by B. Zoeclein <bzoeckle@vt.edu>.

Recent publications.

1. B. W. Zoecklein,[1] J. E. Marcy,[1] J. M. Williams[2] and Y. Jasinski[1] Effect of native yeasts and selected strains of *Saccharomyces cerevisiae* on glycosyl glucose, potential volatile terpenes and selected aglycones of White Riesling (*Vitis vinifera* L.) wines.

Four strains of *Saccharomyces cerevisiae* and native yeasts were evaluated for their influence on White Riesling conjugated aroma precursors measured using three analytical procedures. Fermentation resulted in a decrease in glycosyl glucose with the greatest reduction occurring with native yeast fermentations. Potentially volatile terpene levels were similar among wines following fermentation, the exceptions being those fermented using the Fermiblanc strain and native yeasts which showed lower concentrations. Storing wines for 45 days on the yeast lees (*sur lie*) reduced the glycosyl glucose and potential volatile terpene content with slight differences among treatments. Gas chromatographic analysis of selected glycosidically bound monoterpene alcohols, oxides and aromatic alcohols showed a trend similar to the glycosyl glucose and potentially volatile terpenes following fermentation. Fermentation had a variable effect on the free linalool concentration. Levels of nerol and

geraniol decreased while free α-terpeniol, hotrienol, furanic and pyranic linalool oxides, benzyl alcohol and 2-phenylethanol increased. The concentrations of free volatiles following fermentation differed among yeasts, but differences were below the sensory thresholds reported for each compound.

2. B. W. Zoecklein,[1*] J. E. Marcy,[2] and Y. Jasinski[3]. Effect of fermentation, storage *sur lie* or post-Fermentation thermal processing on White Riesling (*Vitis vinifera* L.) glycoconjugates.

Four strains of *Saccharomyces cerevisiae* were evaluated for their influence on White Riesling glycoconjugates. Fermentation resulted in an average decrease of 66% in bound glycosides, as estimated by the analysis of glycosyl glucose. The greatest reduction occurred with the Fermiblanc strain, although the maximum difference among yeasts was only 7%. The concentration of bound monoterpene alcohols, oxides, and aromatic alcohols was diminished due to fermentation, although generally similar among yeasts. Following fermentation, the concentration of free volatile terpenes and aromatic alcohols differed slightly among yeasts, but differences were below the sensory thresholds reported for each compound. Post-fermentation thermal processing reduced the glycoconjugates by an average of 33% for all treatments, with the greatest reduction occurring with the VL1 strain. Storing wines for 45 days *sur lie* lessened the glycoside content uniformly among treatments by 52%.

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

The following are recetnly accepted publications.

1. J.W. Buck, M.A. Lachance and J.A. Traquair. 1998. Mycoflora of peach bark: population dynamics and composition. Can. J. Bot. In press.

Yeasts and filamentous fungi associated with smooth (non-lenticei) and lenticel bark of young and scaffold branches of peach (Pnrrms persica) were monitored using bark washing and direct/impression plating techniques and scanning electron microscopy during potential *Cystospora* canker infection periods. Total populations of fUngi were high in the fall but dropped in the winter and increased during the spring. Yeasts and yeast-like fUngi predominated in the spring and fall samples. The principal yeasts were Basidiomycetes in the form-genera *Cryptococcus*, *Rhodotorula* and *Sporobolomyces*. The yeast-like fungi were *Aureobasidium* and *Taphrina*. The principal mycelial fungi were in the form-genera *Alternaria*, *Epicoccum*, *Cladosporium*, *Coniothyrium* and *Libertella*. The canker pathogens, *Leucostoma persoonii* and *L. cincta* were observed mainly in the spring sampling. Lenticels supported greater fungal populations than smooth (non-lenticel) bark surfaces. The impact of fungal epiphytes, particularly the yeasts, on the potential biological control of peach canker is discussed.

2. M.A. Lachance, C.A. Rosa, W.T. Starmer, B. Schlag-Edler, J.S.F. Barker, and J. M. Bowles. 1998. *Metschnikowia continentalis* var. *continentalis, Metschnikowia continentalis* var. *borealis,* and *Metschnikowia hibisci*, new heterothallic haploid yeasts from ephemeral flowers and associated insects. Can. J. Microbiol. In press.

Several strains of three new taxa of haploid, heterothallic yeasts have been isolated from various ephemeral flowers and associated insects in North and South America and Australia. *Metschnikowia continentalis* comprises two varieties and is a close relative of *Metschnikowia hawaiiensis*. Like the latter, it produces giant ascospores and lives in association with the insects that colonize flowers in the Convolvulaceae. These species exhibit an unusual asymmetrical mating, but their rare asci are sterile. The varieties of *M. continentalis* undergo unlimited mating, but ascospores are rarely formed. *M. continentalis* var. *continentalis* was isolated in central Brazil and is thought to occur across South-America. *M. continentalis* var. *borealis* was recovered in the Great Lakes area and may represent a North-American population. *Metschnikowia hibisci* was found in flowers and insects of various *Hibiscus* species in the Australian states of New South Wales and Queensland, but appeared to be absent in Convolvulaceae growing in the same areas. The latter forms intermediate-sized ascospores, and one of its mating types forms conjugation tubes in the presence of cells of other *Metschnikowia* species. The three taxa share with *M. hawaiiensis* a large deletion in the D2 region of their large subunit ribosomal DNA, but in *M. hibisci*, the variable domain of the D2 region shares little, if any sequence similarity with others. The type cultures are as follows: *M. continentalis* var. *continentalis* strains UFMG96-173 ($h^+$, CBS8429) and UFMG96-179 ($h^-$, CBS8430); *M. continentalis* var. *borealis* strains UWO(PS)96-104.2 ($h^+$, CBS 8431) and UWO(PS)96-101.1 ($h^-$, CBS 8432); *M. hibisci* strains UWO(PS)95-797.2 ($h^+$, CBS8433) and UWO(PS)95-805.1 ($h^-$, CBS8434).

# International Commission on Yeasts

## Minutes of the Business Meeting of the ICY, 18th International Specialized Symposium on Yeasts, Bled, Slovenia, August 26, 1997

**Commissioners in attendance:** Graham Fleet (Chair), Australia; Sally A. Meyer (Vice Chair) & Clete Kurtzman, USA; Hansjoerg Prillinger, Austria; Leda Mendonca-Hagler, Brazil; André Lachance, Canada; Jorgen Stenderup, Denmark; Tibor Deak, Hungary; Alexander Rapoport, Latvia; Hans van Dijken & Lex Scheffers, The Netherlands; Isabel Spencer-Martins, Portugal, T. Lachowicz, Poland; Inna Bab'eva, Russia; Peter Biely, Slovakia; Peter Raspor, Slovenia; Bernard Prior & James du Preez, South Africa; Andrei Sibirny, Ukraine; Graham Stewart & David Berry, UK; Invited participant: Cecilia Leão, Portugal.

**1.** Graham Fleet called the meeting to order, welcomed everyone and thanked them for attending ISSY-18. He asked for approval of the Minutes of the 1996 ICY Business meeting which was held in Sydney, Australia, August 29, 1996. The Minutes were approved as published in the Yeast Newsletter, December, 1996. He announced that he had received regrets from several ICY Commissioners who could not attend this meeting. They include Byron Johnson, Canada; Matti Korhola, Finland; Sandro Martini, Italy; Martin Becker, Latvia; Haline Neujahr and B. Hahn-Hagerdal, Sweden; and Royall Moore, UK.

**2.** Graham Fleet stated the need to review the list of Commissioners. There appears to be names on the list of individuals who have retired or are no longer involved in yeast research. A Subcommittee composed of Graham Stewart, Peter Raspor and Sally Meyer was appointed to review the list of commissioners with Graham Fleet. It was recommended that individuals who are no longer interested in the ICY be asked to resign from the Commission and to nominate individuals that would take an active role. Graham stated the need for the Commissioners to 1) promote the aims of the ICY in their respective countries, 2) recruit young scientists, 3) dispense information about the ICY, meetings, the Yeast Newsletter, etc., and 4) organize sessions/meetings and assist organizers of meetings. He distributed a list that included countries and number of commissioners as well as the Statutes of the International Commission for Yeasts.

**3. New Commissioners** nominated and approved. Austria – Michael Breitenbach, Institute for Genetics & Algemeine Biology, University of Salzburg, nominated by Hansjoerg Prillinger. Hungary - Anna Maraz, Department Microbiology & Biotechnology, University of Horticulture & Food Industry, Budapest nominated by Tibor Deak to replace Dr. Novak. Italy - Patrizia Romano, Department of Biology, University Basilicata, Potenza, nominated by Alessandro Martini. Russia - Gennadi Naumov, State Institute for Genetics & Selection of Industrial Microorganisms, Moscow nominated by Inna Bab'eva. Ukraine - Mykhail V. Gonchar, Lviv Division of Institute of Biochemistry National Academy of Sciences of Ukraine, nominated by Andrei Sibirny.

**4.** Dr. Inna Bab'eva resigned from the ICY. It was proposed and unanimously supported that she be given emeritus status on the ICY.

**5. Report on meetings.**

**1996, ISY-9, Sydney Australia.** Graham Fleet discussed the banquet problems and apologized to those who did not receive adequate food. Since many attendees did not receive an adequate amount of food provided by the caterers a refund was requested. This has been a real hassle and has taken many months of discussion. A particular problem has been that Graham has to work through the Australian Biotechnology Association (ABA) who were the official hosts of the joint IBS-10 and ISY-9 symposia. A refund of the "food costs" for the dinner has been obtained. This amounted to $40 per head which has been deposited into a trust account of the Australian Biotechnology Association. The president of the Association was to write to all those who attended the dinner offering an apology and the option of (i) refund of $28.00/head (which would be the amount available after bank charges), or (ii) retention of the funds by ABA to be used to support a student scholarship. However, at this stage, it appeared that none of the ISY-9 delegates who attended the dinner had received such a letter from the ABA. The meeting agreed that any refunds to ISY-9 delegates should be used for future activities of the IYC and should not revert to the ABA. Graham noted that about 500 people attended the dinner and about one fifth of these were likely to be ISY-9 delegates. At best, this would generate about $4000 refund. Graham also noted that the ABA had not provided him a financial statement of the outcome of the joint symposia, but was under the impression it had not made significant profit, and had just broken even. This was mainly due to a less than expected registration of delegates to the IBS-10. The Commissioners agreed that any monies generated by refunds to ISY-9 delegates should be placed in an account for operational expenses of ICY.

*Note added*: Since the Bled meeting, Graham has written to the president of ABA on these matters, but has not yet received a formal response (18-11-97).

**1997 - ISSY-18 - Yeast Nutrition & Natural Habitats.** Peter Raspor reported that ISSY-18 had 179 registrants, 73 lectures and 117 posters. Thirty-seven countries were represented.

**1998 - ISSY-19 - Yeast in the Production and Spoilage of Food and Beverages.** August 30-September 3. Cecilia Leão is organizing the meeting and welcomed any suggestions for topics to be included in the meeting. It will be held at the University of Minho, Braga, Portugal. It was recommended that the First Announcement be mailed by November 1997. It was brought to the attention of the Commissioners that the Sixth International Mycological Congress will be held the week before ISSY-19, August 23-28 in Jerusalem.

**1999 - ISSY-20 - Cell Surface structures and membrane phenomena.** Peter Biely along with the Czech & Slovak Yeast Commission will organize a meeting to be held May 24-28 at Smolenice Castle.

**1999 - The general meeting of the IUMS** will be held in Sydney Australia, August 16-20. The ICY should participate and organize symposia. Suggested topics included 1) Biodiversity, 2) Sporulation and 3) Yeasts: Genus/Species Concepts. Commissioners André Lachance, Dave Berry, Isabel Spencer-Martins and Sally Meyer agreed to work with Graham to determine the appropriate topics for the symposia. The number of symposia ICY may organize will be determined by the IUMS Organizing Committee.

**2000 - ISY-10.** August 27-31. Hans Van Dijken, Lex Scheffers and colleagues from The Netherlands are organizing this meeting. Sponsorship is being sought to provide funding for graduate students and postdocs. The site of the meeting will be the Papendal Congress Centre in Arnhem where ISSY-16 was held in 1993. It was announced that the International Biotechnology Symposium will take place the following week in Berlin.

**2001?** - Andrei Sibirny will organize an ISSY on The ecology, genetics, biochemistry and biotechnology of nonconventional yeasts.

**6. Other Business**

**6.1** A proposal from Patrizia Romano was offered, to set up a network of people interested in Food & Beverage Yeasts, and possibly communicate this through the Yeast Newsletter. She agreed to work with André Lachance to provide information. The meeting was very supportive of this concept and indicated it could be developed for other specialized areas of yeasts.

**6.2** André said a few words about the Yeast Newsletter. No new people have been added and there have been a few retirements. Distribution and costs have remained about the same.

**6.3** Jorgen Stenderup said there is an EC Medical Mycology group that should be made aware of the ICY. There may be individuals there interested in ICY activities.

**6.4** Bernard Prior told that UNESCO has funding for training courses and we should look into the possibility of getting funds for a yeast course.

**7.** Graham Fleet thanked everyone for attending and contributing to the ICY business meeting. He thanked Peter Raspor for organizing an outstanding specialized symposium and for providing lunch for the commissioners. Attendees agreed unanimously. The meeting was adjourned at 1:50 pm.

Graham H. Fleet, Chair, ICY.

## Network: yeasts in food and beverages

The International Commission on Yeasts intends to set up some specialist groups in various areas of yeast research to promote scientific interaction world-wide. The first of these groups is "Yeasts in food and beverages". An information network will be created, calling on people who work in this research area and who are interested in discussing specialized topics, such as yeasts in fermented products (*e.g.* dairy, beer, wine, bread, meat, etc.), spoilage yeasts, methods for yeast isolation, enumeration and identification, methods of yeast control, yeast physiology, biochemistry, genetic engineering, probiotic yeasts, yeasts as sources of food additives and ingredients, yeasts involved in biocontrol, and economic consequences of yeasts in food and beverages.

The network will be supervised by a coordinator working in consultation with the ICY, to collect and disseminate information of interest to the members. This will include information regarding conferences, workshops, various proposals and all the materials of interest on the proposed net-work. An annual report will be inserted in the Yeast Newsletter as a specific section reporting recent publications on "Yeasts in food and beverages". Meetings will be organized in order to focalize specific problems of concern. Where appropriate, symposia organized by the ICY will include a section dedicated to the topic: "Yeasts in food and beverages" with the aim of stimulating constructive discussion and the exchanging of latest developments in "Yeasts in food and beverages". Young researchers will be invited to give short contributions on their specific research work. "Mobility-net": Another aim will be the exchange of researchers and experiences with a view to harmonizing research methods and eventually creating interdisciplinary work groups. In this context it might be possible to set up a help-net for needy countries, organizing research mobility by the experts.

Readers interested in the network are invited to send recent publication references with a brief summary (Yeast Newsletter style) and any relevant information. Please send your information and your complete address and E-mail to the network coordinator:

Patrizia Romano,
Dipartimento di Biologia, Difesa
e Biotecnologie Agro-Forestali
Università della Basilicata, Via Anzio 10
85100 Potenza, Italy

Tel/Fax: +39-971-474435
E-mail: pot2930@iperbole.bologna.it

# Recent meetings

## 26th Annual Conference on Yeasts of the Czech and Slovak Commission for Yeasts, Smolenice, Slovakia, May 21-23, 1997

The traditional event of the Czech and Slovak Commission for Yeasts, the 1997 Annual Conference on Yeasts, took place during May 21-23 in the Smolenice Castle near Bratislava, the Capital of Slovakia. The conference was attended by 25 scientists from the Czech Republic, 37 participants from Slovakia and three distinguished guests from neighbouring countries. The oral program was divided into four parts. The first part was devoted to lectures given by foreign guests in English. The lectures of the three subsequent sessions in Biochemistry, Molecular biology and genetics, and Cell surfaces, were presented in Czech and Slovak. The oral program was complemented by 32 posters. The titles of all contributions are listed below.

**Plenary lectures of foreign guests:**

H. Prillinger (Austria): Yeasts associated with termites: a phenotypic and genotypic characterization and use of coevolution for dating evolutionary radiations in Asco- and Basidiomycetes.

P. Raspor (Slovenia): Flocculation in yeast cultures.

T. Deak (Hungary): Biodiversity of yeasts in agri-ecosystems.

**Plenary lectures in yeast biochemistry**

E. Hostinová: Molecular, genetic and enzymic characterization of yeats amylolytic systems.

A. Solovicová, J. Ševčik, E. Hostinová, J. Gašperík: X-Ray structural analysis of yeast glucoamylases prepared by protein engineering.

P. Biely, M. Vršanská, L. Kremnicky: Hemicellulolytic and pectolytic systems of yeasts.

R. Zeman, F. Adámek: Factors affecting the production of phenylacetylcarbinol in the yeast *Saccharomyces coreanus*.

**Plenary lectures in molecular biology and genetics**

F. Cvrčková: Strategy of using yeasts to study the genes and proteins of higher eukaryotes.

V. Žarský: Isolation of a plant regulator of the Rab GTP-ases by complementation of a yeast mutation.

J. Nosek, L. Tomáška: Linear mitochondrial DNA of the yeast *Candida parapsilosis*.

G. Gavurníková, L. Šabová, J. Kolarov: Transcription regulationof the *AAC1* gene in *Saccharomyces cerevisiae*.

V. Vondrejs, M. Bartuněk, O. Janoušková, Z. Palková: Sensitivity of cells to zymocines and its evaluation.

G.A.P. Rojas: Papilla tumors on yeast colonies.

Z. Storchová: Mechanisms of adaptive reversions in the yeast *Saccharomyces cerevisiae*.

**Plenary lectures in the session on cell surface**

R. Kollár, B.B. Reinhold, E. Petráková, H. Yeh, G. Aswell, E Cabib: Yeast cell wall architecture.

M. Kopecká, M. Gabriel, A. Svoboda: Ultrastructural changes in the *ts* mutants of budding yeasts as a result of defects in the function of the actin cytoskeleton.

I. Slaninová, S. Sestak, V. Poláková, A. Svoboda, V. Farkaš: Osmotic shock effects on cytoskeleton and enzymes of the cell wall biosynthesis in *Saccharomyces cerevisiae*.

G. Kogan: Cellular polysaccharides of pathogenic strains of the genus *Candida*.

**Posters**

K. Sigler, A. Pokorná, M. Opekarová, N. Stadler, N. Radovánovič. M. Höfer: Reasons of high sensitivity of membrane transports to oxidative stress in the aerobic yeast *Rhodotorula glutinis*.

M. Opekarová, K. Sigler: In vivo phosphorylation of the specific translocator for arginin V in *Saccharomyces cerevisiae*.

K. Sigler, N. Stadler, B. Densteinová, M. Höfer, V. Petrov, C. Slayman: Yeast plasma membrane $H^+$-ATP-ase as the target of the oxidative stress.

J. Šubík, D. Papajová, M. Obernauerová: The sensitivity of yeasts to mucidine depends on the activity of the products of genes *PDR3* and *PDR5*.

I. Hikkel, Y. Gbelská, J.Šubík: Isolation and molecular characterization of the gene *KICOX18*.

K. Horváthová, R. Teplý, A. Ragnini, R.J. Schweyen: MRS15 - a new initiation factor in yeasts.

V. Vlčková, Z. Mikulovská, I. Fridrichová, J.A.P. Henriques, J. Brozmanová: The role of the *PSO3* gene of *S. cerevisiae* in reparations of oxidatively damaged DNA.

M. Závodná, M. Slaninová, V. Vlčková: Comparison of the effect of metabolic activation of the m-phenyldiamine promutagene by plant and human activation systems on *S. cerevisiae* as an indicator organism.

M. Slaninová, M. Závodná, V. Vlčková: Effect of the bioactivation of aromatic amines by algae on genetic changes in yeasts.

L. Sabová, B. Sokoliková, J. Kolarov: A repressor dependent on the carbon source controls the transcription of the hypoxic gene *AAC3* in *Saccharomyces cerevisiae*.

F. Cvrcková: *SEC19*: A new phenotype, a new function?

P.A. Rojas-Gil, M. Bartosová, Z. Storchová, V. Vondrejs: Colony growth of *Saccharomyces cerevisiae* and topology of the formation of papillae.

Z. Palková, B. Janderová, J. Gabriel, B. Zikánová, M. Pospišek, J. Forstová: Communications between yeast colonies.

E. Kutejová, D. Perečko, V. Leksa: Isolation and characterization of a yeast mitochondrial protease.

V. Raclavský, M. Gabriel, R. Novotný, M. Kopecka, J. Novotná: Chloropromazin causes changes in chemical composition and ultrastructure of cell walls and in polarization of actin patches in *Saccharomyces cerevisiae*.

M. Gabriel, M. Kopecka, A. Svoboda, D. Horký: Effect of cytochalasin D on actin ring and morphogenesis in fission yeast *Schizosaccharomyces japonicus* var. *versatilis*.

A. Tomšiková: Growing significance of non-*Candida* species as opportunist pathogens.

F.J. Záhradnik: Kinetic properties of extracellular acid phophatase of a *Candida* sp.

P. Patáková, J. Fiala: Investigation of cell components of *Saccharomyces cerevisiae* by flow cytometry.

D. Šmogrovičová, Z. Domeny, P. Gemeiner, J. Patáková: Effect of the oxygen concentration on vitality of immobilized yeast and beer quality.

Z. Dömény, D. Šmogrovičová, P. Gemeiner, R. Szöke: Continuous beer fermentation by immobilized yeast.

H. Mikula, M. Rosenberg, L. Krištofiková, P. Sulo: Production of L(-)-malic acid using *Endomyces magnusii*.

V. Stollarová: Comparison of yeast populations in well-maintained and neglected vineyards.

E. Sláviková, R. Vadkertiová: Diversity of yeast and yeast-like species found in the Morava river.

J. Šajbidor, E. Breierová, M. Lamačka: Effect of phenpropimorph-derivatives on growth and sterol composition of some pathogenic yeasts.

E. Breierová, E. Slaviková, E. Stratilová: Relationship between the occurrence of yeasts and their resistance to stress conditions.

B. Kosiková, E. Slaviková: Growth of *Saccharomyces cerevisiae*, *Rhodotorula rubra* and *Bullera alba* in the presence of lignin fractions from beech wood prehydrolysates.

J. Šandula, E. Sláviková, G. Kogan, E. Machová: Structure and antigenic similarities of glucomannan from *Candida utilis*, *Hansenula jadinii* and *H. petersonii*.

L. Kremnický, P. Biely: Selectivity of induction of xylanolytic and mannanolytic system of *Aureobasidium pullulans*.

V. Puchart, M. Vršanská, P. Biely: Attempts to solubilize the cell-wall-bound β-glucosidase of the yeast *Cryptococcus albidus*.

I. Jančová, N. Kolarová: Enzymic synthesis of α-galactooligosaccharides by a yeast α-galactosidase.

P. Polčic, L. Sabová, J. Kolarov: Mitochondrial $H^+$ transport in *Saccharomyces cerevisiae* induced by fatty acids, requires an intact ADP/ATP translocator.

Future activities of the Czech and Slovak Commission for Yeasts will include the 27th Annual conference on Yeasts which will be held during May 13-15, 1998, in the Smolenice Castle. In May 1999 the Commission plans to organize an International Specialized Symposium on Yeasts dedicated to Yeast Cell Surfaces and Membrane phenomena. Three tentative areas were proposed to be the main subjects of the meeting: Membrane transport, Biogenesis and structure of cell walls, and Cytoskeleton.

Communicated by Peter Biely.

## 18th International Specialized Symposium on Yeasts. Yeast nutrition and natural habitats. Bled, Slovenia.

The connection between ecology and nutrition seems to be so obvious that we often push it aside with the belief that there is really not much to be elucidated in this direction. However, the International Yeast Commission recommended that a forum be provided for open debate among yeast researchers about the impact of ecology and nutrition on physiology, genetics, molecular biology, taxonomy, engineering, technology, or health.

The 18th ISSY on Yeast nutrition and natural habitats, took place from August 24 to 29, in Bled, a beautiful location in alpine Slovenia. Over 200 participants from 37 countries attended the symposium, which included 76 lectures and 115 posters. For the first time in the history of ISSY meetings, a questionnaire was used to evaluate the scientific and organizational level of the meeting. Through 20 questions the scientific program and lectures were evaluated as high as 93% and the technical level, depending on the issue, was given marks of 80-90%. Interesting results were obtained regarding the first source of information on the 18th ISSY: 67% of participants received the first announcement from the organizer, 15% from friends, 4% via internet, and 12 % from other sources. Forty participants were from Italy, Austria, Hungary, and Croatia in our region, and another 40 were from other continents.

We can now conclude that a specialized yeast meeting oriented towards yeast ecology and nutrition was a most necessary meeting in the current context. It was also a highly needed meeting for tomorrow because it opens new questions about yeasts, their natural habitats, and the artificial habitats created by human needs, technology and pollution.

The problem of yeast ecology is not new, but with an increased interest in the issue of biodiversity, environments where yeasts were not traditionally studied are now being explored. Traditionally recognized niches certainly also harbor some unknown communities which could be of technological or medical importance.

The 18th ISSY was opened on Sunday afternoon, August 24, 1997, with a short ceremony and a **plenary session** (chaired by G.H. Fleet, Australia and P. Raspor, Slovenia), with two plenary lectures: *C.P. Kurtzman, USA*: Species diversity in natural habitats predicted from nucleic acid sequence analysis and *J.A. Barnett, UK*: The contribution of taxonomists to the understanding of yeast nutrition.

The scientific program continued in nine sessions. Each session consisted of 5 session lectures and 3 short oral presentations. Additional contributions were presented as posters. All posters were displayed during the whole meeting, which was much appreciated by participants. An evening round table entitled "From the known sequence of yeast genome back to its function in natural habitats" was chaired by *C.P. Kurtzman*. Some starting points for discussion were given in the lecture of *C.V. Bruschi* entitled: “From the known sequence back to the natural habitats: The case study of *Saccharomyces cerevisiae*. “

The nine sessions were as follows.

**Yeasts in conventional, unconventional and extreme habitats.** Chaired by Marc-André Lachance, Canada and Inna P. Bab'eva, Russia. Yeasts in aquatic environments (*A.N. Hagler, Brazil*), Biodiversity of yeasts in man-made environments, *(T. Deak, Hungary*), Yeasts associated with termites (*H. Prillinger, Austria*), Responses of yeasts to osmotic stress (*B. Prior, South Africa*), Yeast cell response to dehydration stress, (*A. Rapoport, Latvia*), Yeast communities from different geographical regions as possible reservoirs of conventional and unconventional yeasts: Patagonia (*M. van Broock, Argentina*),

subtropical deserts (*I.Y. Chernov, Russia*), polar circle latitude *(I.P. Bab'eva, Russia*), etc.

**Organic and inorganic nutrition in yeasts.** Chaired by: Hans van Dijken, The Netherlands and Johan M. Thevelein, Belgium. Signal transduction in relation to nutrition in the yeast *Saccharomyces cerevisiae* (*J.M. Thevelein, Belgium*), Regulation of nitrogen repression in *Saccharomyces cerevisiae (E.G. ter Schure, The Netherlands*), Nutrition and cell cycle progression in individual *Saccharomyces cerevisiae* cells (*D. Porro, Italy*), The role of transport in glucose metabolism (*M.C. Walsh, The Netherlands),* Nutrition and cellular ultrastructure of methylotrophic yeasts (*A.A. Sibirny, Ukraine*), Unconventional inorganic nutrient in yeast: chromium (*M. Batic, Slovenia*), Metabolic studies of *Saccharomyces cerevisiae*: anaerobic redox reactions and nitrogen metabolism *(E. Albers, Sweden).*

**Physiology and energy metabolism in yeasts.** Chaired by: Andrei A. Sibirny, Ukraine and D. Porro, Italy. Physiology and energetics of energy limitation and energy excess in *Saccharomyces cerevisiae (C. Larsson, Sweden),* Physiological and stoihiometric aspects of mixed sugar utilisation in yeast *(L. Olsson, Denmark*), The impact of carbon metabolism on recombinant protein production in yeast (*D. Mattanovich, Austria*), Effect of environmental conditions on the fermentative capacity of bakers' yeast (*J. T. Pronk, The Netherlands*), The role of transport in glucose metabolism (*M. Walsh*, *The Netherlands*), Phospholipid synthesis in yeast - an interplay of organelles (*G. Daum, Austria*), Energy metabolism of extreme salt-tolerant *Candida (Yarrowia) lipolytica* yeast species *(R. Zvyagilskaya, Russia*), etc. Physiological changes in *Candida* population induced by osmotic pressure and ionic strength, (*J. Paca, Czech Republic*). Influence of some organic nitrogen compounds on nutrients uptake in yeast *Saccharomyces cerevisiae* (*T.M. Lachowicz, Poland*).

**Ecophysiology of yeasts.** Chaired by: Isabel Spencer Martins, Portugal and M. J. Schmitt, Germany. Molecular biology and possible applications of toxin secreting yeasts and their dsRNA viruses (*M. J. Schmitt, Germany*), Responses of *Zygosaccharomyces bailii* to acidic environments (*C. Leao, Portugal*), Physiological responses of yeasts to high salinity environments (*A. Blomberg, Sweden),* Metabolic characteristics of wine strains during spontaneous and inoculated fermentation (*P. Romano, Italy*), Genetic polymorphism of *Saccharomyces* yeasts in natural habitats (*G. Naumov, Russia*), Zinc deficiency inhibits fungal growth and causes changes in yeast wall morphology (*S. Brul, The Netherlands*), Cadmium resistance of *Schizosaccharomyces pombe* (*M. Zimmermann, Germany*), Cloning of a new *FLO* gene from the flocculating *Saccharomyces cerevisiae* IM1-8B strain, (Reboredo, N. H., Spain).

**Methodology of yeasts detection and characterisation in simple and complex environments.** Chaired by: H. Prillinger, Austria, Italy and A. N. Hagler, Brazil. Progress in conventional methods for detection and enumeration of foodborne yeasts (*L.R. Beuchat, USA*), Isoenzymes for yeast identification (*C. Pais, Portugal*), Detection and characterization of basidiomycetous yeasts from marine environments (*J.W. Fell, USA*), ITS spacer analysis in yeast taxonomy (*L.C. Mendonca-Hagler, Brazil*) Ribosomal DNA sequence analysis as a tool for the identification of endophytic yeasts from woody plants *(W. Schweikofler, Austria*), Identification of yoghurt-spoiling yeasts with oligonucleotide probes and RAPD-PCR (*D. Kosse, Germany*), Identification of food spoilage yeasts with Fourier transform infrared-spectroscopy (*M. Kuemmerle, Germany*), Genetic and molecular diversity in *Saccharomyces cerevisiae* natural populations (*D. Cavalieri, Italy*).

**Yeasts as human and animal pathogens.** Chaired by: Julia Douglas, UK and Sally A. Meyer, USA. The pathogenic yeast *Cryptococcus neoformans*: ecology, biology, pathogenicity and virulence (*I. Polacheck, Israel*), Heterogeneity of *Candida parapsilosis* and *C. haemulonii (S. A. Meyer, USA*), Expression of virulence factors of *Candida albicans* in vaginitis (*F. De Bernardis, Italy*), Allergens of the moulds *Cladosporium herbarium* and *Alternaria alternata* (*M. Breitenbach, Austria*), Expression of the different aspatyl proteinase genes (SAP) in *Candida albicans* (*M. Monod*, Switzerland), The world of molecular cross-reactivity among fungal allergens (*R. Crameri, Switzerland*), Invasive growth of yeasts, (*M.G. Lambrechts, South Africa),* Comparison of molecular typing methods for *Candida albicans* strain delineation,(*S. Senesi, Italy)*.

**Yeasts in food production and spoilage.** Chaired by: Graham G. Stewart, UK and Tibor Deak, Hungary. High gravity brewing and ethanol tolerance studies *(G.G. Stewart, UK),* Some genetic aspects of film-forming yeasts of *Saccharomyces* genus (*M. Budroni, Italy*), Cryotolerant *Saccharomyces* strains and spoilage of refrigerated must,*(P. Giudici, Italy),* Investigation of the yeast flora in dairy products: A case study of kefyr (*M.T. Wyder, Switzerland*), Differences of *Saccharomyces cerevisiae* strains in the formation of undesirable volatile sulphur compounds during grape must fermentation in dependence on assimilable nitrogen (*D. Rauhut, Germany*), Cryotolerant *Saccharomyces* strains and spoilage of refrigerated must (*P. Giudici, Italy*), Occurrence and technological properties of yeasts isolated from mould ripened cheeses, *(van den Tempel, Denmark)*, Ewes' cheese browning: a serious problem associated with pigment producing yeasts, (*A. Carreira, Portugal),*The yeast flora of maize silage (*W. J. Middelhoven, The Netherlands*).

**Yeast interactions in natural environments.** Chaired by: Graham H. Fleet, Australia and Zoran Zgaga, Croatia. Yeast-yeast interactions: Yeasts as a yeast habitat (*M.A. Lachance*), Killer yeasts as competitors during Tokaj wine fermentation (*A. Maraz, Hungary*), Yeast-fungi interactions: Stored grain ecosystems - Implications for biocontrol (*J. Schnuerer, Sweden*), Yeast-bacteria interactions: Ecology of yeasts and bacteria in vineyards and during winemaking - molecular and physiological studies with practical conclusions (*J. Gafner, Switzerland*), Yeast transformation and horizontal gene transfer (*Z. Zgaga, Croatia*), Biological inhibition of lactic bacteria in white wines (*A. Caridi, Italy*), Interactions between yeasts and soil millipedes (*B.A. Byzov),* A model of growth and toxin-induced death in mixed cultures of killer and sensitive yeasts strains, *(Peinado, J.M, Spain).*

**Extracellular enzymes in yeast nutrition and ecology.** Chaired by: Peter Biely, Slovakia and A. Scheffers, The Netherlands. Yeasts and their enzyme systems degrading cellulose, hemicelluloses and pectin (*P. Biely, Slovakia*), Genetic, enzymatic and structural characterization of glucoamylase from *Saccharomycopsis fibuligera (E. Hostinova*, *Slovakia*), Production and properties of ß-glucosidase by wine-related yeasts *(I. Rosi, Italy),* Characterisation of *Saccharomyces cerevisiae* cell wall proteins and their impact of yeast extracellular metabolism**,**

**(***Mrša, V, Croatia),* Production and partial characterization of an amylase from *Phaffia rhodozyma, (Diaz, A.,Spain*), Effect of oxygen on glucoamylase production encoded by STA genes, (*Balogh, I.Hungary)*.

P. Raspor and Cecilia Leão, Portugal, chaired the final session, during which *G.H. Fleet, Australia,* addressed the question: Yeasts - what reactions and interactions really occur in natural habitats?. Dr. Fleet discussed current views on yeasts and their habitats especially from the perspective of foods and beverages, which naturally flowed into the theme of the next ISSY meeting in Portugal.

The program was successful as it allowed new ideas to be exchanged and new contacts to be established, which is expected to foster further work in the area of yeast ecology. During the symposium, the International Commission on Yeasts (IUMS) reviewed some future meetings, for instance, in 1998, in Portugal, where the subject of yeasts in food production and spoilage will be discussed, in 1999, in the Slovak Republic, dealing with yeast envelope, and finally, to finish this century, in the Netherlands for the general meeting (ISY2000).

Present at the meeting of the ICY were C. Kurtzman and S. Meyer USA, Mendonca-Hagler, Brasil, I. Spencer-Martins, Portugal, H. Prilinger Austria, T. Deak, Hungary, P. Biely, Slovakia, C. Leao, Portugal, P. Raspor, Slovenia, L. Scheffers, the Netherlands, I. Bab'eva, Russia, A. Stenderup, Denmark, G. Fleet, Australia, A. Sybirny Ukraine, A. Lachance, Canada, A. Rapaport, Latvia, T. Poland, H. van Dijken and J.du Preez, South Africa, D. Berry and G.G. Stewart, UK. The minutes of the meeting are reported separately.

Peter RASPOR, Chair, 18th ISSY.

# Forthcoming meetings

## 1998 Yeast Genetics and Molecular Biology Meeting, University of Maryland, July 28 - August 2, 1998

The 1998 "Yeast Meeting" will be held on the campus of the University of Maryland at College Park. The facilities provide excellent meeting space and superior air-conditioned housing with nearby dining. This East-coast venue is within driving distance of many major metropolitan areas, and is accessible from three international airports. In addition, the museums and monuments of Washington, D.C., are just a short Metro ride away. This meeting provides an important forum for presenting and learning about recent research advances on *S. cerevisiae*, *S. pombe*, and related yeast species. As at past meetings, there will be platform and workshop sessions on a wide variety of timely subjects, including: Cell Growth and Development. Chromosome & Genome Structure & Function. Protein Localization & Degradation. Gene Expression. Cell Cycle & Mating. Calls for Abstracts will be mailed in late December only to those on the YGM mailing list. If you wish to be added to the mailing Iist to receive the Call for Abstracts for this meeting, contact:

Marsha Ryan
Genetics Society of America
9650 Rockville, Maryland
Bethesda, MD 20814-3998

Tel (301) 571 1825
Fax (301) 530 7079
Email: mryan@genetics.faseb.org
http://genome-www.stanford.edu/Saccharomyces/yeast98/

## Sixth International Mycological Congress - IMC 6, August 23-28, 1998, Jerusalem, Israel

I take pleasure in inviting you to attend the Sixth International Mycological Congress - IMC 6 scheduled to take place from August 23-28, 1998 in Jerusalem at the ICC Jerusalem international Convention Center. You can expect excellent science combined with an enjoyable holiday. The Congress Program encompasses a wide array of themes structured of symposia sessions and workshops, daily plenary lectures, social activities and a special program for accompanying persons. Israel has a long tradition of Mycological and Phytopathological research that goes back to the beginning of the century. We have presently extensive investigations in Mycology including Medical Mycology, Phytopathology, Biotechnology and Symbiotic Systems. Jerusalem is a center of biblical, ancient and modern history, and the birthplace of great religions. The city is rich in archaeology, culture and natural beauty. It enjoys an ideal Mediterranean climate and is a Perfect place to combine science with travel. This international Congress will offer an opportunity to visit Israel's institutions and Centers for mycological research and establish personal contact with Israel's mycologists. Looking forward to welcoming you in Jerusalem! Yours sincerely, Margalith Galun.

**Organizing committee**: M. Galun, President; I. Barash, Vice-President; Z. Eyal, General Secretary; A. Sztejnberg, Treasurer.

**Scientific committee**: Y. Koltin, Chair, Y. Elad, R. Fluhr, Y. Hadar, T. Katan, M. Kupiec, I. Polacheck, O. Yarden.

**International Mycological Association**: F. Oberwinkler, President; M. Blackwell, Secretary-General; M.E. Noordeloos, Treasurer.

**Tentative Program**: Opening address: Genomics and Mycology - S. Oliver (UK). Plenary lectures: Bioprospecting - L. Nisbet (UK); Molecular systematics and evolution - J.W. Taylor (USA); Gene regulation and morphogenesis - W. Timberlake (USA); Fungal diversity - D.L. Hawksworth (UK); Medical Mycology - J. E. Edwards (USA); Symbiosis and Parasitism,

synonymous or distinct? - D.H.S. Richardson (Canada). Symposia and workshops: A. Fungal diversity; B. Cell biology; C. Fungal Genetics; D. Fungal Development and morphogenesis; E. Fungal-host interactions; F. Medical mycology; G. Technology; H. Ecology and biosystematics; Teaching Mycology; Computer networks and information systems; Specific taxonomic groups (We invite suggestions for workshops on specific taxonomic groups).

Travel programs of great interest for participants and accompanying persons are also planned.

IMC6 Congress secretariat
P.O. Box 50006
Tel Aviv 615002
Israel

Tel: 972 3 5140014
FAX: 972 3 5175674/5140077
Compuserve: ccmail: MYCOL@Kenes
Internet: Mycol@Kenes.ccmail.compuserve.com
WWW: http://Isb380.plbio.1su.edu/ima/index.html

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

**Invitation.** On behalf of the Organizing Committee, I have the pleasure to invite the yeast scientific community to attend the 19th ISSY On "Yeast in the Production and Spoilage of Foods and Beverages". It is the second time that such a meeting is held in Portugal. The first was organized in Lisboa by Prof. N. van Uden in 1986 (13" ISSY). Braga, the venue of the next Specialized Symposium, is located in the heart of Minho region, 50 Km from Porto. It is an old town founded by the Romans, being the administrative center of the Minho region. The Minho forms the north-west corner of Portugal, also known as the Costa Verde (Green Coast) by virtue of its outstanding natural beauty. The river Minho wich gives its name to the region, is the natural border between the provinces of Minho, in Portugal, and Galicia, in Spain.

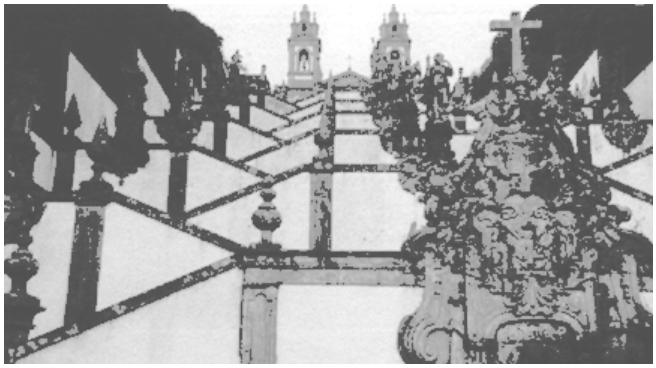
**Organizing Committee.** Judite Almeida, Helena Cardoso, Margarida Casal, Fernanda Cassio, Manuela Côrte-Real, Cecilia Leão, Virgilio Loureiro, Maria C. Loureiro-Dias, Candida Lucas, Amândio Madeira-Lopes, Célia Pais, Maria João Sousa, Isabel Spencer-Martins.

**Local Organizing Committee**. Department of Biology, University of Minho

**International Advisory Committee**. Larry Beuchat (USA), Tibor Deak (Hungary), Graham H. Fleet (Australia), Bärbel Hahn-Hägerdal (Sweden), Cletus P. Kurtzman (USA), Cecilia Leão (Portugal), Maria C. Loureiro-Dias (Portugal), Sally A. Meyer (USA), Bernard Prior (South Africa), Peter Raspor (Slovenia), Isabel Spencer-Martins (Portugal), Graham G. Stewart (UK).

The scientific programme will focus on the most recent developments in the field of ecology, systematics, physiology, molecular biology and genetics of food-related yeasts. We look forward to your contribution to a successful of the 19th ISSY and we would like to stimulate, in particular, the attendance of younger scientists.

**Scientific Programme.** The programme, supported by an International Scientific Committee, will consist of invited



lectures, contributed papers in thematic workshops and poster sessions. A few posters will be selected to be presented as oral short communications. The following topics will be included: Food ecology and differential diagnostic of yeasts. Stress in food environments: yeast physiology and molecular biology. Food-borne yeasts: metabolism and regulation. .Yeast in food spoilage: quality control in the production chain. Molecular typing and rapid identification methods. Fermented foods. Alcoholic fermentations. Genetic improvement of food-processing yeasts.

**Location and Accommodation.** The 19th ISSY Symposium will take place in the Campus de Gualtar, University of Minho, Braga.. Participants will be accommodated in three to five-stars hotels located in a beautiful park (Bom Jesus) overlooking the Campus (30 minutes walking distance). A shuttle service will be provided between the hotels and the Symposium venue. The official language of the Symposium will be English.

**Further Information**. The Second Announcement will be distributed in March 1998 and will include: Scientific and Social Programmes. Registration and Accommodation Forms. Deadlines for submission of Abstracts.

Cecília Leão

## Symposium "Yeast as a cell factory", Vlaardingen, The Netherlands, 30 November - 2 December 1998

For information on this symposium please consult our web site or write to:

Dr. Jack Pronk
Kluyver Laboratory of Biotechnology
Delft University of Technology
Julianalaan 67, 2628 BC Delft
The Netherlands.

Telephone; +31 215 783214
Fax: +31 215 782355
E-mail: j.t.pronk@stm.tudelft.nl
WWW: http://www.ecyeastsymp.com

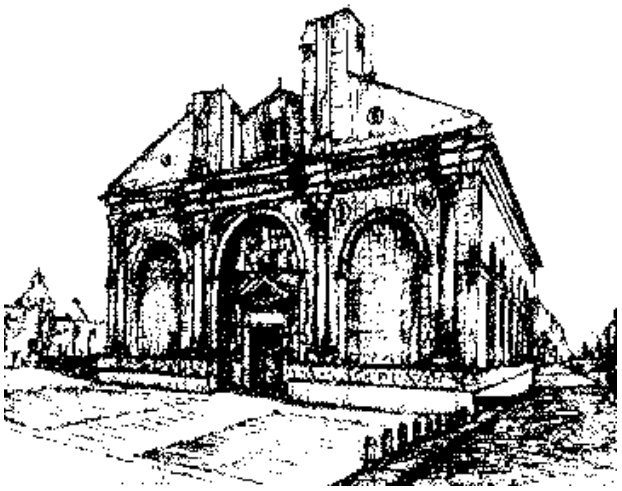
## Nineteenth International Conference on Yeast Genetics and Molecular Biology, Rimini, Italy, on 26-30 May, 1999

You are cordially invited to attend the Nineteenth International Conference on Yeast Genetics and Molecular Biology to be held in Rimini, Italy, on 26-30 May, 1999

**Scientific Committee**: L. Frontali, L.Alberghina, C.V. Bruschi, L. Donini, I. Ferrero, G. Lucchini, A. Martini, M. Polsinelli, J. Pulitzer. Organizing Committee: L. Frontali, Chair, C.V. Bruschi, Secretary, N. Altamura, E, Berardi, I. Ferrero, C. Galeotti, G. Lucchini, E. Martegan, D. Porro.

Symposia. Cell cycle and checkpoints. DNA replication, recombination and repair. Signal transduction and growth control. Biotechnology and industrial applications. Cell architecture and morphogenesis. Nucleo-cytoplasmic interactions. Intracellullar dynamics and transport. Telomeres, silencing and ageing. Genomics. Workshops and posters: Gene expression. Chromosome structure and function. Metabolism and metabolic regulation. Meiosis and sporulation. Organelles. New tools. Functional analysis of the yeast genome. Cell cycle and DNA replication. Recombination and repair. Biodiversity in yeasts. Signal transduction and stress response. Protein trafficking. Cell wall and morphogenesis. Others.

**Location**. Rimini is a historical city situated on the Adriatic coast. The symbol of its past is the Templo Malatestiano (see illustration) built by Leon Battista Alberti for Sigismondo Malatesta in the years from 1447 to 1470. Its unfinished facade is considered as a sort of "manifesto" of Italian Renaissance Architecture. In recent years Rimini has become a very popular sea-side resort. The hotels are known to offer reasonable rates and the quality of food is very good. The "Palacongressi", Italy's largest Conference Center, has a complete range of facilities including several halls and wide spaces well-suited to our Conference. It is our intention to keep the cost of participation as low as possible, especially for students, as we are keen to attract young researchers to the conference. Rimini can be reached by



train from Bologna (1 hr), Milan (3 hrs) and Rome (4 hrs), which have international airports. Excursions will be organized during and after the Conference to Ravenna (50 km) and to Urbino (70 km). A wide ranging programme of social events will be also offered to all the registered accompanying persons.

**Second announcement**. A second circular will be distributed in early spring 1998 to all those who request it. The circular will contain the preliminary scientific programme as well as information for early registration and accommodation.

**Exhibition**. There will be an exhibition of scientific and professional material. More information will be provided in the second announcement.

To receive the second circular or indicate your interest in presenting a poster, please write, by February 15 1998 to:

Secretariat, 19th International Conference
on Yeast Genetics and Molecular Biology
OIC srl, Via A. La Marmora, 24
I-50121 Florence, Italy

Tel: +39/55/50 351
Fax: +39/55/50 01 912
E-mail: oic@dada.it
WWW: http://www.icgeb.trieste.it/yeast99/

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

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

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

# Brief News Item

### Change of address: Isabel Spencer-Martins

My address has recently changed to the following:

Dr. Isabel Spencer-Martins
SA Biotecnologia, Faculdad de Ciências e Tecnología — Tel. 351-1-2954464
Universidade Nova de Lisboa, Quinta da Torre — Fax. 351-1-2948530
2825 Monte de Caparica, Portugal — E-mail: ism@mail.fct.unl.pt

# Yeasts on the Internet

### YEAST e-mail directory and related sites

The latest version of the YEAST e-mail directory is now available. I invite you all to download the latest version of this list and verify that you are on it (once, and only once).

I also want to let those of you who do not participate in the YEAST/bionet.molbio.yeast discussion newsgroup that this is now a moderated newsgroup, meaning that this group only has "real" valid yeast-related messages, and no "unwanted" messages. Further information on how to participate to this newsgroup is available from:

http://www.bio.net:80/hypermail/YEAST/

You can participate to this newsgroup via e-mail, Usenet or the WWW (at the abobe URL)

Please do not hesitate to contact me if you have any questions about this directory or the newsgroup.

YEAST e-mail list, version 12.02 (29-OCT-1997). This list has 1615 entries. It can be retrieved by anonymous FTP to ncbi.nlm.nih.gov in the /repository/yeast directory or, if you are using a WWW viewer, at this URL:

ftp://ncbi.nlm.nih.gov/repository/yeast/yeaster.lst

This page is now part of an on-going effort to represent all of the NIH yeast related resources.

These are present on the following page: http://www.nih.gov/sigs/yeast/index.html

The list is **no longer** e-mailed to the people on the list, as it is now too long, and fails to reach too many of the recipients because of its size. An announcement of the updates present on the list will be e-mailed a few times a year, to inform the people on the list but also to ensure that the addresses of the people on the list are still valid. Names are removed if they fail to reach their destination.

The above FTP URL at NCBI will always be the most up-to-date version of the list, and other internet services may reflect this list, or may be behind schedule.

Users are reminded that, as a courtesy to others on the list, the directory should **not** be used as a general mailing list, and that people can post to YEAST/bionet.molbio.yeast if they want to propagate information to a YEAST interested audience. If you have any questions about how to participate in the YEAST/bionet.molbio.yeast newsgroup, please ask me about it.

Please forward all corrections, additions, or comments to:

B.F. Francis Ouellette,
GenBank Coordinator — Tel: (301) 496-2477 ext 247
NCBI/NLM/NIH Building 38A — Fax: (301) 435-2433
Bethesda, MD 20894, USA — E-mail: francis@ncbi.nlm.nih.gov

## PomBase, a *Schizosaccharomyces pombe* database

A new compilation of *S. pombe* data in ACEDB for UNIX computers is now available by anonymous FTP (see below).

This release of PomBase (4-3) has been completely rebuilt from ace files at the Sanger Centre. Old versions of PomBase should be discarded and the latest release downloaded by ftp.

PomBase now includes:

**DNA sequences**. 1207 S. pombe DNA sequences from EMBL and EMNEW including 121 cosmid sequences from systematic genome sequencing projects. All cosmids have the results of BLASTN and BLASTX searches of the EMBL/EMNEW and SWIR databases respectively (Note. SWIR is a non-redundant compilation of SWISS-PROT, PIR and WORMPEP).

The sequence display incorporates the Blixem tool for BLASTX homology. Pick a BLASTX homology block (light blue) with the right hand mouse button to reveal a menu; choose "Analyse in Blixem". This will give a multiple alignment of peptide sequences to a 3 phase translation of the query sequence.

**Papers**. 4218 paper references, including abstracts.

**Protein records.** 1691 full text records of *S. pombe* proteins from SWISSPROT and TREMBL
16466 peptides showing homology to *S. pombe* proteins.

**Gene designations**. ~1280 new gene predictions from the sequencing project.

**Enzyme**. Release 21.0 (October 1996) of the ENZYME database.

**Prosite**. Release 13.0 (November 1995) of the Prosite Pattern Database.

**Genetic maps**. from: Munz, P., Wolf, K., Kohli, J. and Leupold U. (1989). Genetics overview in Nasim A. et al. (Eds), Molecular Biology of the fission yeast, Academic Press, London, pp. 1-30.

**Physical maps**. Views of the ICRF physical maps (Hoheisel, J. et al., (1993). High Resolution Cosmid and P1 Maps Spanning the 14Mb genome of the fission yeast *S. pombe*. Cell **73**:109-120.) and correlations with the restriction fingerprinting maps from the Sanger Centre. A map "Sanger_I" shows the production status of maps undergoing sequencing.

Get PomBase by anonymous FTP from:
ftp.sanger.ac.uk in directory pub/PomBase/
(ftp://ftp.sanger.ac.uk/pub/PomBase/)

| | |
|---|---|
| README | This file |
| NOTES | Further notes - please get and read this file unless you know exactly what you want to do. |
| INSTALL | The installation script |
| ace4****/ | Subdirectory with binaries and source files |
| spombe/ | Subdirectory with S. pombe ace files |
| doc/ | Subdirectory containing documentation |

Information about the Pombe sequencing project can be obtained from the Sanger Centre Pombe pages:
http://www.sanger.ac.uk/Projects/S_pombe/

PomBase is also accessible via the HGMP-RC for registered users running X-windows at:
http://www.hgmp.mrc.ac.uk/
under the Genome Databases menu

Valerie Wood, *S. pombe* Genome Project
Yeast Genetics, The Sanger Centre
Wellcome Trust Genome Campus
Hinxton, Cambridge CB10 1SA

Tel: 01223 4954
Fax:: 01223 494919
E-mail: <val@sanger.ac.uk>
WWW: http://www.sanger.ac.uk/Projects/S_pombe