

Y E A S T

A Newsletter for Persons Interested in Yeast

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Editorial

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

Final preparations for the 10th general ISY are underway. This is the main opportunity, provided through the International Commission on Yeasts, for researchers in all aspects of yeast biology to meet and exchange latest developments in research. Dr. Scheffers, general secretary of the symposium, informs us that he has received an impressive number of abstracts from which oral and poster presentations are to be selected. The format of the Symposium will include plenary sessions in the morning, followed in the afternoon by concurrent sessions expanding on the topics presented in the morning, as well as evening workshops.

***FEMS Yeast Research* A New Journal**

The FEMS series of journals will be adding one more title to its list, namely *FEMS Yeast Research*, under the Editorship of Lex Scheffers and Teun Boekhout. The new journal, to be published by Elsevier, is intended to cover yeast research in the broadest sense, including basic and applied research, and all aspects of yeasts, ranging from physiology and taxonomy to functional genomics and industrial applications. We wish them success with this new endeavour.

M.A. Lachance
Editor

ESSAY

Crabtree effect, respiratory deficiency and ferrous ions as metallic cofactor of arginase in some ascomycetous yeasts

Many ascomycetous and a few basidiomycetous yeasts are able to ferment glucose and other sugars, with ethanol and carbon dioxide as the products. In many species this reaction is shown only at low oxygen tension, but some ascomycetous yeasts do ferment also under aerobic conditions. This phenomenon is named the Crabtree effect. It is caused by catabolite repression of enzymes of the respiratory chain and the tricarboxylic acid cycle, which are necessary for respiration. The mitochondria if present are impaired. In old-fashioned terms the Crabtree effect is defined as suppression of respiration by fermentation. The growth of Crabtree-positive yeasts on glucose is biphasic: a rapid initial phase during which the glucose is fermented is followed by a slower one during which ethanol is respired.

Crabtree-positive yeasts are subject to the so-called petite mutation which occurs spontaneously or induced by acriflavin dyes (Ephrussi et al., 1949). Petite mutants produce small colonies on agar plates, do not have a complete respiratory chain and do not grow at the expense of non-fermentable substrates. This is caused by an irreversible impairment of mitochondrial synthesis resulting in an obligatory fermentative sugar catabolism, like that exerted by the Crabtree effect. Crabtree effect and respiratory deficiency have been studied from a systematic point of view by Bulder (1963) and De Deken (1966a and b). De Deken (1966b) concluded that stable respiratory deficient mutants could only be obtained from Crabtree-positive yeasts. In Crabtree-negative yeasts the petite mutation is lethal (Bulder, 1964). After a few generations the cultures die. It is tempting to compare these old literature data with recent ones dealing with the phylogeny of ascomycetous yeasts as presented by Kurtzman and Robnett (1998). Although only a limited number of fermentative ascomycetous yeasts have been studied for occurrence of respiratory deficiency and Crabtree effect, it can be concluded that most positive species are present in the *Saccharomyces* clade of the phylogenetic tree (Fig. 2 in Kurtzman and Robnett, 1998). These species according to today's nomenclature are: *Saccharomyces cerevisiae*, *S. pastorianus*, *Candida glabrata*, *Torulaspota globosa* and *Eremothecium coryli*. In *T. delbrueckii* and *Kluyveromyces thermotolerans* petite-positive as well as petite-negative strains appear to occur. *K. lactis* and *K. marxianus* are in the *Saccharomyces* clade but are remotely related to the positive species. Both of these are Crabtree and petite-negative. *Nadsonia fulvescens* is in the *Ascoidea/Nadsonia/Dipodascus* clade (Fig. 4). It is Crabtree-positive but petite mutants could not be obtained from this species (De Deken, 1963b). Out of the *Saccharomyces* clade a species both Crabtree and petite-positive is *Schizosaccharomyces pombe* of the "*Archiascomycete*" clade (Fig. 11). Some data on near relatives of this species are available. All strains of *S. versatilis* appear to be respiratory deficient, but *S. octosporus* is petite-negative (Bulder, 1963). Data on *Dekkera bruxellensis*, which belongs to the

Pichia/Issatchenkia/Saturnispora/Dekkera clade (Fig. 6), are contradictory.

In addition to effects on sugar catabolism, the susceptibility of yeasts to Crabtree effect and respiratory deficiency is associated with other characteristics. Petite-positive yeasts do not contain certain polyunsaturated fatty acids like linolenic acid which are present in petite-negative yeasts (Johnson and Brown, 1972). These yeasts also differ with respect to the location of ornithine transcarbamoylase (OTC). In petite-negatives this enzyme is mitochondrial while in petite-positives it is cytoplasmic (Legrain et al., 1977). This enzyme converts L-ornithine and carbamoyl phosphate into L-citrulline. In mammalian liver OTC is an essential step in the Krebs-Henseleit cycle which produces urea as the ultimate waste product of protein catabolism. In most plants and microorganisms OTC is part of the arginine biosynthetic pathway. It is present if no arginine is available. If cultures growing without arginine are supplied with this amino acid, synthesis of the first enzyme of its degradation soon is induced. In yeast this enzyme is arginase (Middelhoven, 1964) which hydrolyzes arginine to ornithine and urea. Under these growth conditions a complete Krebs-Henseleit cycle is present, both the enzymes catalyzing the conversion of ornithine into arginine and the enzyme producing ornithine and urea from arginine being active. Unlike in mammalian liver this situation is not desirable as urea synthesis costs two moles of ATP. The nitrogen in urea is available for biosynthetic purposes only if ammonia is liberated from it. In ascomycetous yeasts, like in other urease-negative organisms, this costs one mole of ATP. From this it is clear that operation of the Krebs-Henseleit cycle in microorganisms has to be avoided as it is futile and wasting energy. Petite-positive and negative yeasts follow different ways to achieve this. In the latter ones operation of the cycle is prevented by spatial separation of arginase and OTC, the latter being mitochondrial. In Crabtree-positive yeasts, however, both enzymes are cytoplasmic and a complete Krebs-Henseleit cycle appears to be operative. This is prevented by a unique regulatory device. In *S. cerevisiae* and some other Crabtree-positive yeasts arginase is binding to OTC thus inhibiting its catalytic activity. (Messenguy et al., 1971). In this way wasteful synthesis of urea is prevented. In Crabtree-negative yeasts this interaction of arginase and OTC is not necessary and was shown to be absent (Legrain et al., 1977).

Another difference between petite-positive and petite-negative yeasts may regard the metallic cofactor of arginase. In mammalian liver and several plants arginase is generally believed to be activated by manganese ions which in vitro can be replaced with some other bivalent cations. In *S. cerevisiae* ferrous ions are the natural activator of arginase (Middelhoven, 1969). Plenty of evidence has been presented for this. The native arginase extracted from the yeast cells is sensitive to 8-hydroxyquinoline, dimethylglyoxim and phosphate, inhibitors of ferrous arginase but not of yeast arginase activated in vitro with manganese

chloride. The pH-activity curves of native and of in vitro prepared ferrous arginase are identical and differ from those of other metallic yeast arginases prepared in vitro. Cell-free yeast extracts with high native arginase activity can only be obtained from yeast cultures poor in phosphate. In cultures generously supplied with phosphate the arginase is inhibited during the preparation of the cell-free extract by phosphate or polyphosphate present in the cells. Further evidence as to the role of ferrous ions in native yeast arginase was obtained from an experiment in which the yeast cells were grown in an iron-deficient growth medium supplied with growth-inhibiting amounts of manganese or cobaltous salts. Cell-free extracts prepared from these cultures also showed the characteristics of ferrous arginase (Middelhoven et al., 1969). Unfortunately the role of ferrous ions as metallic cofactor of *Saccharomyces* arginase has only been studied in one yeast species and has never been confirmed since its description now more than thirty years ago. Modern techniques like HPLC for isolation and purification of native arginase and mass spectroscopy for detection of the metal present in it are valuable tools to eliminate any uncertainty left, but nobody seems to be interested in the metallic cofactor of arginase in vivo. This may be caused by the troublesome preparation of cell-free extracts displaying high arginase activity without prior activation with metal salts. It is common practice to activate the enzyme with manganese salts prior to determination of the specific enzyme activity. Manganese ions are the best activators of arginase in vitro, but the optimum pH of manganese yeast arginase is much higher than that of the ferrous enzyme, viz. 9.5 and 8.5 respectively. At physiological pH of about 7 ferrous arginase is still active but the manganese enzyme is without activity.

From the above-cited literature data it is evident that the genetic factors determining a yeast to be Crabtree-positive or negative have effects beyond the regulation of sugar catabolism and energy generation. The Crabtree-positive yeasts, with a few exceptions, are found in the *Saccharomyces* clade of the ascomycetous yeasts (Kurtzman and Robnett, 1998). Crabtree-positive and -negative yeasts differ in the occurrence of stable respiratory deficient mutants (Bulder, 1963; DeDeken, 1966b), in fatty acid composition (Johnson and Brown, 1972), in localisation of ornithine transcarbamoylase (Legrain et al., 1977), in sensitivity of this enzyme to inhibition by arginase (Messenguy et al., 1971) and possibly in the metallic cofactor of arginase in vivo (Middelhoven, 1969).

These observations do raise some questions yet to be answered.

1. Are these characteristics distinguishing Crabtree-positive yeasts from negative species, as apparent in *S. cerevisiae* and closely related species, also shown by other species belonging to the same phylogenetic clade (e.g. *Eremothecium coryli*) or showing less relationship, such as *Schizosaccharomyces pombe*?

2. Is the activation of arginase by ferrous ions in vivo only shown by *S. cerevisiae*, or is it a general property of all Crabtree-positive yeasts distinguishing these from Crabtree-negative species?

3. Is activation of arginase by ferrous ions in vivo of general occurrence in the fungal realm, hitherto overlooked for lack of the proper techniques and without significance for the distinction of Crabtree-positives and -negatives?

As the author is approaching the age of retirement and does not dispose of facilities necessary to give an answer to the questions as to the yeast arginase in vivo, he would be grateful if he could be contacted by people able and willing to resolve this problem by continuing the research in the indicated way.

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- Messenguy, F., M. Penninckx and J.-M. Wiame (1971) Interaction between arginase and ornithine carbamoyltransferase in *Saccharomyces cerevisiae*. *European Journal of Biochemistry* **22**:277-287
- Kurtzman, C.P. and C. Robnett (1998) Identification and phylogeny of ascomycetous yeasts from analysis of nuclear large subunit (26S) ribosomal partial sequences. *Antonie van Leeuwenhoek* **73**:331-371
- Middelhoven, W.J. (1964) The pathway of arginine breakdown in *Saccharomyces cerevisiae*. *Biochimica et Biophysica Acta* **93**:650-652
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- Middelhoven, W.J., M.A. de Waard and E.G. Mulder (1969) The ferrous ion as the activator of arginase in vivo. II. Experiments on the replacement of ferrous ions in native yeast arginase by other cations in vivo. *Biochimica et Biophysica Acta* **191**:122-129

The following papers appeared recently.

1. W.J. Middelhoven, C.P. Kurtzman and A. Vaughan-Martini. In press. *Saccharomyces bulderi* sp. nov. a yeast that ferments gluconolactone. Antonie van Leeuwenhoek.

An unknown yeast species was isolated from maize silage and was determined to be novel on the basis of morphological and physiological characteristics, nucleotide sequence of domain D1/D2 of LSU rDNA and from its electrophoretic karyotype. *S. bulderi* (type strain CBS 8638, NRRL Y-27203, DBVPG 7127) is closely related to *S. barnettii* and

S. exiguus from which it can be distinguished by having a double vitamin requirement of biotin and thiamine and by no or slow aerobic growth on raffinose, a sugar that on the contrary is fermented rapidly. Gluconolactone is rapidly fermented with ethanol, glycerol and carbon dioxide being the main products.

2. W.J. Middelhoven, G. Scorzetti and J.W. Fell. 2000. *Trichosporon veenhuisii* sp. nov., an alkane-assimilating anamorphic basidiomycetous yeast. Int. J. Syst. Evol. Microbiol. **50**:381-387.

A morphological and physiological description of an alkane-assimilating anamorphic basidiomycetous yeast species, named *Trichosporon veenhuisii*, is presented. The ability to assimilate several aliphatic and aromatic compounds as sole

source of carbon and energy is reported. The phylogenetic position within the genus, based on nuclear base sequencing of the D1/D2 region of the large subunit of rDNA is discussed. The type strain is CBS 7136.

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

Recent acquisitions.

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

The following articles have appeared or are in press.

1. D. Begerow, R. Bauer & T. Boekhout. 2000. Phylogenetic placements of ustilaginomycetous anamorphs as deduced from nuclear LSU rDNA sequences. Mycol. Res. **104**:53-60.

In order to integrate ustilaginomycetous anamorphs into the general phylogenetic system of Ustilaginomycetes, partial nuclear large subunit ribosomal DNA sequences of 56 teleomorphic and 19 anamorphic species of the Ustilaginomycetes were analysed. Maximum parsimony and neighbour joining confirm the new suprageneric system of Ustilaginomycetes and indicate that (i) the species of *Pseudozyma* represent anamorphs of Ustilaginales parasitizing grasses. (ii) *Pseudozyma prolifica*, the type of *Pseudozyma*, is very closely related to *Ustilago maydis*, (iii) *Pseudozyma tsukubaensis* is probably synonymous

with *Ustilago spermophora*, (iv) the species of *Malassezia* represent a group of its own within the Exobasidiomycetidae, (v) *Tilletiopsis cremea*, *T. lilacina*, and *T. washingtonensis* belong to the Entylomatales and (vi) *T. flava*, *T. fulvescens* and *T. minor* are members of the Georgerfischeriales. Like all *Tilletiopsis* species tested, *T. albescens* and *T. pallescens* are members of the Exobasidiomycetidae, but they cannot be ascribed to any of the known orders of this subclass. The description of the Malasseziales is emended.

2. T. Boekhout, J.W. Fell, A. Fonseca, H.J. Prillinger, K. Lopandic & H. Roelijmans. 2000. The basidiomycetous yeast *Rhodotorula yarrowii* comb. nov. Antonie van Leeuwenhoek (in press).

Sequence analysis of the D1/D2 domains of the large subunit rDNA of *Cryptococcus yarrowii* (CBS 7417) indicates that this species does not belong to the hymenomycetous fungi, but instead is of urediniomycetous affinity. Therefore, the name change *Rhodotorula yarrowii* comb. nov. is proposed. The cell wall of the species contains xylose, a character considered by most

authors to indicate fungi of hymenomycetous affinity. However, our results show that xylose may occur in minor amounts in the cell walls of urediniomycetous fungi. A high mannose content of the cell walls may be a more reliable character for urediniomycetous yeasts.

3. T. Boekhout, B. Theelen, M. Diaz & J.W. Fell. 2000. Molecular epidemiology and subspecific classification of *Cryptococcus neoformans*. Abstracts SGM/SAM Millennium Meeting 10-14 April 2000, University of Warwick.

4. W.H. Müller, B.M. Humbel, A.C. van Aelst, T.P. van der Krift & T. Boekhout, 1999. The perforate septal pore cap of basidiomycetes. In: *Plasmodesmata* (Eds. A.J.E. Bel & W.J.P. van Kesteren) Springer. p. 119-127.
5. W.H. Müller, A.J. Koster, B.M. Humbel, U. Ziese, A.J. Verkleij, A.C. van Aelst, T.P. van der Krift, R.C. Montijn and T. Boekhout. 2000. Automated electron tomography of the septal pore cap in *Rhizoctonia solani*. *J. of Structural Biology* (in press).
6. W.H. Müller, J.A. Stalpers, A.C. van Aelst, M.D.M. de Jong, T.P. van der Krift and T. Boekhout. 2000. The taxonomic position of *Asterodon*, *Asterostroma* and *Coltricia* inferred from the septal pore cap ultrastructure. *Mycol Res.* **104** (in press).
7. G.I. Naumov, M.T. Smith & G.S. de Hoog. 1999. Genetic interpretation of speciation and lifecycle in *Galactomyces* fungi. *Microbiology* **68**:362-364.

Data on the genetic and molecular classification of the genus *Galactomyces* (teleomorph of *Geotrichum*) are summarized. This genus contains five sibling species of the *Galactomyces geotrichum* - *Ga. citri-aurantii* complex and the close species *Ga. reessii*. Genetic data pertaining to the life cycles

of *Galactomyces* species were analyzed. Unlike *Ga. citri-aurantii*, the type species of *Ga. geotrichum sensu stricto* seems to produce ascospores as a result of karyogamy rather than of meiosis.

8. M.Th. Smith, G.A. Poot & A.W.A.M. de Cock. 2000. Re-examination of some species of the genus *Geotrichum*. *Antonie van Leeuwenhoek* **77**:71-81.

The nutritional physiology and the growth rate of thirty-four strains representing species of *Geotrichum* without known teleomorph states were examined. From twenty-seven strains the mol% G+C were calculated from the melting curves. The first derivatives of the melting curves of seven strains, including the type strain of *Geotrichum clavatum*, demonstrated the presence of two peaks, 12% away from each other; the

remaining strains showed only a single broad peak. DNA homology values among strains of the former group were high, indicating their conspecificity. The strains of the latter group could be subdivided into six DNA homology groups, four of which could be identified with recognized species and two may represent novel taxa. A combined key of *Geotrichum* and its teleomorph states *Galactomyces* and *Dipodascus* is present.

9. J.P. van der Walt, M.Th. Smith & H.J. Roelijmans 1999. Four new species in *Lipomyces*. *Syst. Appl. Microbiol.* **22**: 229-236.

Announcement.

Mr. D. Yarrow left the CBS at the end of May and has been succeeded as curator of the yeasts by Dr. V. Robert.

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

The director of Japan Collection of Microorganisms (JCM) has changed over from Dr. Takashi Nakase to Dr. Hiroyuki Osada because of a mandatory retirement for Dr. T. Nakase. Dr. H. Osada is administratively directing JCM.

The following articles have been published recently.

1. Sugita, T., Kagaya, K., Takashima, M., Suzuki, M., Fukazawa, Y. & Nakase, T. 1999. A clinical isolate of *Candida palmioleophila* formerly identified as *Torulopsis candida*. *Jpn. J. Med. Mycol.* **40**:21-25.
2. Nakase, T., Suzuki, M., Sugita, T., Suh, S.-O. & Komagata, K. 1999. Three new species of anamorphic yeasts phenotypically and phylogenetically related to *Candida mesenterica*: the description of *Candida fungicola* sp. nov., *Candida sagamina* sp. nov., and *Candida fukazawae* sp. nov. isolated from fruit bodies of mushrooms. *Mycoscience* **40**:465-476.

3. Sugita, T., Ca-ete-Gibas, C. F., Takashima, M. & Nakase, T. 1999. Three new species of *Bullera* isolated from leaves in the Ogasawara Islands. *Mycoscience* **40**:491-501.
4. Takashima, M. & Nakase, T. 1999. Molecular phylogeny of the genus *Cryptococcus* and related species based on the sequences of 18S rDNA and internal transcribed spacer regions. *Microbiol. Cult. Collect.* **15**:35-47.
5. Mikata, K., Ueda-Nishimura, K., Goto, S., Kurtzman, C. P., Suzuki, M., Yarrow, D. & Nakase, T. 1999. Reidentification of yeast strains deposited as *Candida agrestis*, with a description of *Candida kofuensis* sp. nov. *Microbiol. Cult. Collect.* **15**:49-57.
6. Sugita, T. & Nakase, T. 1999. Nonuniversal usage of the leucine CUG codon in yeasts: investigation of basidiomycetous yeast. *J. Gen. Appl. Microbiol.* **45**:193-197.
7. Suzuki, M., Suh, S.-O., Sugita, T. & Nakase, T. 1999. A phylogenetic study on galactose-containing *Candida* species based on 18S ribosomal DNA sequences. *J. Gen. Appl. Microbiol.* **45**:229-238.
8. Suzuki, M. & Nakase, T. 1999. A phylogenetic study of ubiquinone Q-8 species of the genera *Candida*, *Pichia* and *Citeromyces* based on 18S ribosomal DNA sequence divergence. *J. Gen. Appl. Microbiol.* **45**:239-246.
9. Hamamoto, M., Tamura, M. & Nakase, T. 2000. Emended descriptions of *Tilletiopsis washingtonensis*, *Tilletiopsis cremea* and *Tilletiopsis lilacina*. *Int. J. Syst. Evol. Microbiol.* **50**:925-930.
10. Hamamoto, M. & Nakase, T. 2000. Phylogenetic analysis of the ballistoconidium-forming yeast genus *Sporobolomyces* based on 18S rDNA sequences. *Int. J. Syst. Evol. Microbiol.* **50**:1373-1380.

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

The following are abstracts of articles that were published recently and are in press.

1. Košíková, B., Sláviková, E. 2000. Biotransformation of lignin biopolymers by yeast microorganisms. *Lenzinger Berichte* **79**:60-64.

Due to lack of information about the interaction of yeasts with lignin biopolymers the effect of lignin derived from organosolv pulping on the growth of yeast species *Sporobolomyces roseus*, *Rhodotorula rubra*, and *Bullera alba*, isolated from natural microflora, was examined. The production of biomass was significantly increases in the presence of oxidized lignin preparations. In this study a relationship between surface tension of lignin preparations and growth of yeasts tested was

revealed. GPC analysis and ¹³C NMR spectroscopy was applied for characterization of lignin fractions isolated from culture media by extraction with organic solvents. The revealed structural changes of lignin biopolymer tested caused by the yeasts indicate demeth(oxy)lation and degradation of aromatic rings, similar to that observed with lignin-degrading hyphal fungi. The observed structural changes confirm biotransformation of lignin biopolymer by *Sp. roseus* particular when lignin was only source of carbon.

2. Sláviková, E., Vadkertiová, R. 2000. The occurrence of yeasts in the forest soils. *J. Basic Microbiol.* (in press)

One hundred and eighty one yeast strains were isolated from 180 soil samples collected in three types of forest. The samples were taken during one year. The yeast species found were similar in spite of distinct forest types. *Cryptococcus laurentii*, *Cystofilobasidium capitatum*, *Leucosporidium scottii*, *Rhodotorula aurantiaca*, and *Trichosporon cutaneum* were the

predominant species in both deciduous and coniferous forests. The number of yeasts ranged from 1.5 x 10³ to 1.1 x 10⁴ CFU/g soil. We found that yeasts occurred unevenly in soils during the year. The lowest number of yeasts was ascertained in December and the highest one in May.

3. Sláviková, E., Stollárová, V., Minárik, E. 1999. II. Taxonomy and ecology. In: Biely P. (Ed.) Forty years of activity in Czech and Slovak yeast research. Bratislava, VEDA, 9-19.

4. Šajbidor, J., Breierová, E., Lamačka, M., Bohov, P. 2000. Influence of methyl fenpropidine on growth, sterol content and fatty acid composition in five strains of *Candida albicans*. Folia Microbiol. (in press)

The effect of methyl fenpropidine on growth, lipid contents, sterol and fatty acid composition in 5 strains of *Candida albicans* was investigated. The sensitivity of strains decreased in the order: wild strains > erg⁺, ade⁺, nys^R, > ade⁺, nys^R, erg⁻ (defective Δ8-7 isomerase) > ade⁺, nys^R, erg⁻ (defective Δ5 desaturase). The sterol profile results show that the presence of inhibitor influenced fecosterol isomerisation, episterol

dehydrogenation, zymosterol transmethylation, ergosterol reduction and squalene epoxidation. Methyl fenpropidine induced changes in fatty acid composition. Reduction of palmitic and oleic acid content with concomitant elevation of stearic, linoleic and linolenic acid level was found. Index of lipid unsaturation slightly increased. Morphological changes of wild strains of yeasts were observed after fungicide treatment.

5. Šajbidor, J., Breierová, E., Lamačka, M. 2000. Effect of six new fungicides on the base of allylamines, piperazines and piperidines on growth of some pathogen yeasts. Z. Naturforsch. C (in press).

The antifungal activity of the six newly synthesised compounds against *Candida albicans*, *Cryptococcus neoformans*, *Malassezia pachydermatis* and *Malassezia furfur* was tested. Minimum inhibitory concentrations (MICs) for the most effective derivatives were in the range from 1.3 to 47.4 mg.l⁻¹ and were 2-3 times lower compared to systemic fungicide - bifonazol. Growth inhibition was accompanied with ergosterol

reduction, but direct correlation between ergosterol content and MICs was not observed. Sterol alterations in *C. albicans* suggested inhibition of C24 sterol methyl transferase, Δ14-reductase and Δ8-7 isomerase. The presence of inhibitor in growth medium induced decrease (*M. furfur*) and rise (*C. albicans*) of unsaturation index in total lipids.

6. Šandula, J., Kogan, G., Kačuráková, M., Machová, E. 1999. Microbial (1→3)-β-D-glucans, their preparation, physico-chemical characterization and immunomodulatory activity Carbohydr. Polym. **38**:247-253.

(1→3)-β-D-glucans have the ability to stimulate the immune system and are classified as biological response modifiers (BRMs). Glucans from the two technologically important species, baker's yeast *Saccharomyces cerevisiae* and filamentous fungus *Aspergillus niger* were isolated and characterized. Water-insoluble yeast glucan has a low branched structure with the ratio of the glucose units in the side chains and the backbone 1:8. Using ultrasonic treatment and subsequent chemical derivatization, water-soluble derivatives (carboxymethyl and sulfoethyl) of yeast glucan were obtained with high yield. The glucan isolated from *Aspergillus niger* forms a complex with chitin and is relatively resistant to solubilization. The yield of its

carboxymethylated derivative was only 30%. A method of determination of the relative ratio of α- and β-glycosidic linkages in the glucans using FTIR spectroscopy has been developed as well as the method of ultrasonic purification of the isolated glucans. Using ultrasonic treatment, glucan derivatives with decreased molecular weight (90-100 kDa) have been prepared, which showed broader application possibilities in comparison with the initial high molecular weight derivatives (300-600 kDa). It has been found that the derivatives prepared revealed high mitogenic and comitogenic activities, as well as radioprotective and antimutagenic effects.

7. Babincová, M., Machová, E., Kogan, G. 1999. Carboxymethylated glucan inhibits lipid peroxidation in liposomes. Z. Naturforsch. **54c**:1084-1088

Protective capabilities of carboxymethylated (1→3)-β-D-glucan from *Saccharomyces cerevisiae* cell wall against lipid peroxidation in phosphatidylcholine liposomes induced by OH⁻ radicals produced with Fenton's reagent (H₂O₂/Fe²⁺) and also by microwave radiation were studied using absorption UV-VIS spectrophotometry. Significant decrease in the conjugated diene production, quantified as Klein oxidation index, was observed at 500 mW/g specific absorbed power of microwave radiation with

2.45 GHz frequency at a moderate amount of added glucan. Increase of oxidation index was accompanied with enhanced carboxyfluorescein leakage as a result of liposome membrane destabilization. This process was markedly suppressed in the presence of glucan in the liposome suspension. Therefore, glucan may be considered as a potent protector against microwave radiation-induced cell damage.

8. Chorvatovičová, D., Machová, E., Šandula, J., Kogan, G. 1999 Protective effect of yeast glucomannan against cyclophosphamide-induced mutagenicity *Mutation Research* **444**:117-122.

Glucomannan (GM) isolated from *Candida utilis* with molecular weight 30 kDa was administered either intraperitoneally or orally prior to cyclophosphamide (CP) injection and its effect on the frequency of micronuclei in polychromatic erythrocytes of mouse bone marrow was evaluated. Both ways of GM administration significantly ($p < 0.002$) decreased the clastogenic effect of CP. The protective effect was concentration dependent, with a higher decrease achieved by 200

mg/kg than by 100 mg/kg b. wt. The fact that GM was effective also on oral administration is indicative of the passage of GM molecules through the wall of the gastrointestinal tract. The important characteristics of glucomannan isolated from *Candida utilis* as a good solubility in water, relatively small molecular weight (30 kDa), antimutagenic effect exerted also by oral administration, could provide a great perspective for its utility as natural protective agent.

V. Collection de Levures d'Intérêt Biotechnologique (CLIB), Laboratoire de Microbiologie et Génétique Moléculaire, INA-PG INRA, BP01, F-78850 Thiverval-Grignon, France. Communicated by Nguyen H-V <clib@grignon.inra.fr>.

The following have been recently published or submitted.

1. Nguyen, H.V., Lepingle, A., and Gaillardin, C. 2000. Molecular typing demonstrates homogeneity of *S. uvarum* strains and reveals the existence of hybrids between *S. uvarum* and *S. cerevisiae*, including the *S. bayanus* type strain CBS 380. *Syst. Appl. Microbiol.* **23**:71-85.

PCR/RFLP of the NTS2 sequence of rDNA was shown to be suitable for differentiating *Saccharomyces sensu stricto* species. We previously showed that, within the presently accepted *S. bayanus* taxon, strains formerly classified as *S. uvarum* represented a distinct subgroup (Nguyen and Gaillardin, 1997). In this study, we reidentified 43 more strains isolated recently from wine, cider and various fermentation habitats, and confirmed by karyotyping, hybridization and mtDNA analysis the homogeneity of strains from the *S. uvarum* subspecies. Molecular typing of nuclear and mitochondrial genomes of strains preserved in collections, and often originating from beer like *S. pastorianus*^{NT}, revealed the existence of hybrids between *S. uvarum* and *S. cerevisiae*. Surprisingly, *S. bayanus*^T

CBS380 appeared itself to be a hybrid between *S. uvarum* and *S. cerevisiae*. This strain has a mitochondrial genome identical to that of *S. uvarum*, and a very similar karyotype with 13 isomorphic chromosomes, six of which at least hybridise strongly with *S. uvarum* chromosomes or with a *S. uvarum* specific sequence. However, four of the chromosome bands of *S. bayanus* T beard Y' sequences indistinguishable from those of *S. cerevisiae*, a feature that is not observed among presently isolated *S. uvarum* strains. Because of the hybrid nature of *S. bayanus*^T and of the scarcity of similar hybrids among present days isolates, we propose to reinstate *S. uvarum* as a proper species among the *Saccharomyces sensu stricto* complex.

2. Nguyen, H.V., Pulvirenti, A. & Gaillardin, C. Rapid differentiation of the closely related *Kluyveromyces lactis* var. *lactis*, *K. lactis* var. *drosophilarum* and *K. marxianus* by PCR/RFLP of the rDNA non transcribed spacer 2. *Can. J. Microbiol.* (submitted).

PCR/RFLP of the NTS2 of rDNA was applied to differentiate two closely related species *Kluyveromyces lactis* var. *lactis* (referred to as *K. lactis*) and *K. marxianus*. Using specific primers, the NTS2 region was amplified from DNA of both *K. lactis* and *K. marxianus* type and collection strains. *AluI* restriction of amplified fragments generated patterns characteristic for each species. The NTS2 region from *K. lactis* var. *drosophilarum* and related species *K. aestuarii*, *K. africanus*, *K. dobzhanskii* and *K. wickerhamii* could also be amplified with the same primers but *AluI* patterns generated were clearly

different. PCR/RFLP of the NTS2 appears thus to be a convenient method for rapid identification of *K. lactis* and *K. marxianus*, frequently found in dairy products. This test was validated therefore on *K. lactis* and *K. marxianus* from natural habitats. We showed actually that most, if not all, yeast strains collected from whey and scoring blue on X-gal glucose plates were either *K. lactis* or *K. marxianus*. For application purpose, we propose here an approach for screening quickly *K. lactis/marxianus* and *Saccharomyces cerevisiae* from dairy products using X-gal coloured and lysine growth media.

3. Genomic exploration of 13 hemiascomycete yeasts.

The CLIB collection has participated in a project with five other french laboratories associated with the Centre National de Séquençage (GENOSCOPE) to sequence partly and randomly the genome of the following yeast species: *Candida tropicalis*, *Debaryomyces hansenii*, *Hansenula polymorpha*, *Kluyveromyces lactis*, *K. marxianus*, *K. thermotolerans*, *Pichia sorbitophila*,

Saccharomyces exiguus, *S. kluyverii*, *S. servazzii*, *S. uvarum*, *Yarrowia lipolytica*, *Zygosaccharomyces rouxii*. **Aims:** The species were chosen for their phylogenetic relationship and their potential biotechnological uses. For each species 2500 to 5000 random sequenced tags (RST) were obtained. The sequences

were analysed: (1) comparison between various yeast genomic organization mainly with the *S. cerevisiae* completely sequenced genome, (2) studying synteny conservation between these genomes, (3) discovery of yeast new genes. **The CLIB contribution:** Scientists from our collection performed the shot gun cloning and the computer analysis of the resulting RSTs of the five following species: *Debaryomyces hansenii*, *Saccharomyces exiguus*, *S. kluyverii*, *S. servazzii*, and *Yarrowia*

lipolytica. **Results:** Gain from the comparison of the new sequences to databases includes: discovery of novel genes, about 1100-1400 per species; information on the synteny and the conservation of genome organisation; indication on the evolution of the genome structure. **Publications** will be available grouping specific papers on each species as well several general ones reporting the results of the whole comparison.

VI. Sezione di Microbiologia Applicata e Collezione dei Lieviti Industriali DBVPG, Dipartimento di Biologia Vegetale & Biotecnologie Agroambientali. Borgo XX Giugno, 74; 06121 Perugia, Italy. Communicated by A. Vaughan-Martini <avaughan@unipg.it>.

Dear Yeast Newsletter friends: Please note a few changes in our institutional name (see above). Telephone numbers have also had some variations in the sense that if one telephones from outside of Italy, one should **not** drop the "0" before the local area code as before. Tel. +39 075 585 6479. Fax. +39 075 585 6470.

Below are some recent publications by our group.

1. P. Buzzini and J. Rossi. 1999. Semi-continuous and continuous riboflavin production by *Candida saitoana* 4253 calcium alginate immobilized cells in concentrated rectified grape must. World J. Microbiol. Biotechnol. **15**:139.
2. P. Buzzini and A. Martini. 1999. Production of carotenoids by strains of *Rhodotorula glutinis* cultured in raw materials of agro-industrial origin. Biores. Technol. **71**:41.
3. P. Buzzini, A. Bolano, P. F. Ganter, A. Martini, G. Cardinali. 1999. Ecological distribution of the yeast species *Myxozyma mucillagina* in extreme habitats of different continents. Proc. XIX Int. Conference on Yeast Genetics and Mocular Biology. Rimini (Italy), May 25-30 1999, p. 366.
4. P. Buzzini. 2000. An optimization study of carotenoid production by *Rhodotorula glutinis* DBVPG 3853 from substrates containing concentrated rectified grape must as the sole carbohydrate source. J. Ind. Microbiol. Biotechnol. **24**:41, 2000.
5. Cardinali G, Liti G, Martini A. 2000. Non-radioactive dot-blot DNA reassociation for unequivocal yeast identification. Int J Syst Evol Microbiol **2**:931-936.
6. Pellegrini L, Cardinali G, and Martini A. 1999. Selection of *Saccharomyces cerevisiae* strains able to ferment at supraoptimal temperatures. Ann. Micr. Enz. **49**:55-65.
7. Ciani, M., Fatichenti, F. 1999. Selective sugar consumption by apiculate yeasts. Lett. Appl. Microbiol. **28**:203-206.
8. Ferraro, L., Fatichenti, F. and Ciani M. Pilot scale vinification process by immobilised *Candida stellata* and *Saccharomyces cerevisiae*. Process Biochem. (In press).

Sequential grape juice fermentation first with immobilized *Candida stellata* and then with an inoculum of *Saccharomyces cerevisiae* was carried out at pilot scale and under non-sterile conditions in order to evaluate the dynamics of yeast microflora and their influence on the analytical profile of wine. Non-*Saccharomyces* yeast were adequately controlled while *S. cerevisiae* wild strains were consistently present after

three days of fermentation and could compete with the inoculated *S. cerevisiae* strain. However, the metabolism of immobilized *C. stellata* cells strongly influenced the analytical profile of wines with a consistent increase in glycerol (70%) and succinic acid content in comparison with values for a *S. cerevisiae* fermentation control.

9. M. Ciani, L. Menghini, F. Mariani, R. Pagiotti, A. Menghini and F. Fatichenti. Antimicrobial properties of essential oil of *Satureja montana* L. on pathogenic and spoilage yeasts. *Biotechnol. Letters* (in press).

The essential oil of *Satureja montana* L. had a broad-spectrum of antimicrobial activity against 46 species of yeasts. This high and diffused activity could be used to control potential pathogenic and spoilage yeasts. The assay of MIC toward some

pathogenic and spoilage yeasts showed a range values from 0.10 to 0.25 $\mu\text{l ml}^{-1}$. The MIC and growth rate reduction assay were effective tests for quantitative evaluation of antimicrobial activity.

10. W.J. Middelhoven, C.P. Kurtzman, A. Vaughan-Martini. *Saccharomyces bulderi*, a yeast that ferments gluconolactone. *Antonie van Leeuwenhoek* (in press).

11. A. Vaughan-Martini, P. Pollacci & S. Barcaccia. 1999. A reconsideration of species designations of some strains of the Industrial Yeasts Collection DBVPG assigned to the genus *Torulaspora*. *Ann. Microbiol.* **49**:67-77.

12. A. Vaughan-Martini, P. Angelini & A. Maartini. 1999. Yeasts associated with the surface of various species of *Tuber* Micheli Proceedings of the Vth International congress: Science and Cultivation of truffles. Aix-en-Provence, France. 4-6 March in press.

A preliminary survey of the yeast ecology of the surface of truffles was conducted considering 7 species of *Tuber* collected in Italy and China. Results revealed that yeast were present on all samples and were represented by over 20 different species, belonging to both ascomycetous and basidiomycetous taxa. Among them, some of the most commonly soil-associated taxa, such as *Cryptococcus humicolus*, *Leucosporidium scottii*, *Trichosporon dulcitum* and *Williopsis saturnus* were isolated. Other less expected species, such as *Cystofilobasidium*

infirmominiatum and *Saccharomyces paradoxus* were also present. An overview of some physiological properties of the strains isolated as well as the numerical presence suggests that the presence of yeast cells on the surface of *Tuber* may not be casual. On the contrary, some still unknown yeast metabolites could possibly be involved in *Tuber* mycorrhization or development and/or impart positive organoleptic characteristics to the final product.

13. A. Vaughan-Martini, P. Angelini & G. Cardinali. Use of conventional taxonomy, electrophoretic karyotyping and DNA/DNA hybridization for the classification of fermentative apiculate yeasts *Int. J. Syst. Bacteriol.* (in press).

A taxonomic study was conducted considering strains of the genera *Hanseniaspora*/ *Kloeckera* held in the Industrial Yeasts Collection (DBVPG) of the Dipartimento di Biologia Vegetale of the Università di Perugia, Italy. Standard phenotypic as well as molecular criteria were considered in an effort to revisit the classification of these strains, some of which have been in the Collection for 48 years. Results of salient physiological tests

showed that some of the DBVPG and type strains could not be identified by current taxonomic keys. Electrophoretic karyotypes were identical for some species, with the type strains of the 7 accepted species showing only 5 distinct chromosomal patterns. DNA/DNA hybridization analyses, using a non-radioactive dot blot technique, allowed for the distinction of taxa. The taxonomic implications of these results are discussed.

VII. Escola Sup. Agr. Luiz de Queiroz, University of São Paulo, and Fermentec S/C Ltda, Rua Treze de Maio, 768 - Sala 153, Edifício Sisal Center, 13400-900 Piracicaba, São Paulo. Communicated by C.E. Garcia.

The following is a summary of work currently in progress under the supervision of Prof. Dr. L.E. Gutierrez.

1. C.E. Garcia. Effect of the population of contaminant bacteria isolated from industrial alcohol fermentation process on the yeast flocculation.

Yeast flocculation in the industrial alcohol fermentation process is one of the most serious problems, because it causes reduction in the fermentative yield, delay in fermentation time, and increases the difficulty of yeast separation by centrifugation. This research is carried out to quantify the bacterial population of some strains of *Lactobacillus* which causes the yeast

flocculation, as being the main cause in the flocculation yeast-bacteria. It is known that some strains of *L. fermentum* cause yeast flocculation, so, in this work, the possibility that other species of *Lactobacillus* can also cause yeast flocculation has been evaluated. Several strains of *Lactobacillus* isolated from industrial scale ethanol fermentation tanks were used. It was

concluded that *L. fructivorans* also causes yeast flocculation. A specific bacterial population size is required for yeast flocculation to occur. This is true also for *L. fermentum*.

Consequently the control of *Lactobacillus* population is a very important aspect of fermentation yield.

VIII. Escola Superior de Agricultura “Luiz de Queiroz”- USP, Department of Biological Science. 13418-900 Piracicaba (SP), Brazil. Communicated by L.C. Basso <lucbasso@esalq.usp.br>

The following is a recently prepared doctoral dissertation.

1. Lopes, M.L. Study of chromosomal polymorphism in yeast selected from industrial process of ethanol production. Instituto de Biociências de Rio Claro, UNESP, Brazil, 132 pp.

Electrophoretic karyotyping was used for characterization and identification of yeasts in samples collected from industrial ethanol fermentations. It was possible to identify changes in yeast populations not only in relation to wild yeasts but also on numerical and structural changes in chromosomes of a selected strain used as starter (PE-2). Chromosomal rearrangements were more frequently in bands with high electrophoretic mobility and altered intensity. The main types were changes of size (69.5%), new bands (25.0%) and disappearance of bands (5.5%). Although such changes occurred

in all distilleries and in different periods of the season, no selective advantage could be attributed to any chromosomal rearrangements. These observations indicate that changes in electrophoretic karyotyping of PE-2 strain are neutral or of low adaptive value. Such strain as well as other isolated from industrial environment sporulated abundantly. Moreover, the products of sporulation of PE-2 strain showed the same chromosomal rearrangements described for isolates of this strain rescued from industrial fermentation. The possibility of sporulation of this strain during industrial process is discussed.

The following doctoral dissertation was successfully defended.

2. Alves, D.M.G. Physiological responses of two *Saccharomyces cerevisiae* strains to potassium during alcoholic fermentation. Instituto de Biociências de Rio Claro (UNESP), Brazil, 118 pp.

This work describes some physiological differences between *Saccharomyces cerevisiae* strains without (Fleischmann) and with (PE-2) survival ability in industrial process of fuel alcohol fermentation. Sucrose hydrolysis, sugar consumption, ethanol production, intra- and extracellular contents of glycerol and potassium, succinic and malic acids formation, external pH, trehalose yeast content and cell growth and viability were monitored during an 8 hours fermentation, using different contents of potassium in the media. The data showed a distinct behaviour for each strain, suggesting that the acidification due to larger amounts of organic acids produced by Fleischmann strain could

be responsible for the greater stressing condition observed. Potassium exhibits a protective action to the yeast, allowing a re-uptake of the succinic acid produced. Malic acid was produced in higher amounts than succinic acid at the beginning of fermentation, being re-absorbed at the final stage. PE-2 strain produced less glycerol and organic acids, restored trehalose content at the end of fermentation and presented higher viability, supporting the stressing condition of the fermentation. These results could help to explain why Fleischmann strain is unable to survive industrial process with cell re-use.

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

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

1. Gomes, N.C. M., C.A. Rosa, P.F. Pimentel, V.R. Linardi, and L. C. S. Mendonça-Hagler. 1999. Uptake of free and complexed silver ions by yeasts isolated from a gold mining industry in Brazil. J. Gen. Appl. Microbiol. **45**:121-124.
2. Rezende, R.P., J.C.T. Dias, C.A. Rosa, F. Carazza, and V.R. Linardi. 1999. Utilization of nitriles by yeasts isolated from a Brazilian gold mine. J. Gen. Appl. Microbiol. **45**:185-192.
3. M.A. Dias, H.F. Castro, P.F. Pimentel, N.C.M. Gomes, C.A. Rosa, and V.R. Linardi. 2000. World J. Microbiol. Biotech. **16**: 107-108.

4. Dias, J.C.T., R.P. Rezende, C.A. Rosa, M.A. Lachance, and V.R. Linardi. 2000. Enzymatic degradation of nitriles by *Candida guilliermondii* UFMG-Y65. *Can. J. Microbiol.* **46**:525-531.

Candida guilliermondii UFMG-Y65, isolated from a gold mine, was able to utilize different nitriles and the corresponding amides as sole source of nitrogen, at concentrations up to 2 M. Resting cells cultivated on YCB-acetonitrile medium showed nitrile hydrolyzing enzyme activities against acrylonitrile and benzonitrile. These enzymes were inducible and intracellular; the optimum pH was 7.0-8.0, and the optimum temperature

25°C-30°C. Liquid chromatographic analysis indicated that *C. guilliermondii* UFMG-Y65 metabolized 12 mM benzonitrile to 11 mM benzoic acid and 10 mM acrylonitrile to 7.9 mM acrylic acid. The results suggest that *C. guilliermondii* UFMG-Y65 may be useful for the bioproduction of amides and acids, and for the bioremediation of environments contaminated with nitriles.

5. C. Pataro, J. B. Guerra, M. L. Petrillo-Peixoto, L. C. Mendonça-Hagler, V. R. Linardi, and C. A. Rosa. 2000. Yeast communities and genetic polymorphism of *Saccharomyces cerevisiae* strains associated with artisanal fermentation in Brazil. *J. Appl. Microbiol.* **88** (in press).

Yeast communities and genetic polymorphism of prevalent *Saccharomyces cerevisiae* strains isolated from the spontaneous fermentation of the sugarcane juice during the production of *aguardente* in three distilleries in the state of Minas Gerais, Brazil, were studied. *S. cerevisiae* was the prevalent species during the process of *aguardente* production, but *Schizosaccharomyces pombe* was predominant in old fermentations in one distillery. Transient yeast species were found in a variable number, probably due to the daily addition of

sugarcane juice, and they were different for each of the three distilleries studied. PFGE and PCR analysis of the predominant strains of *S. cerevisiae* isolated from the fermented must showed a high degree of genetic polymorphism among the three distilleries. A high molecular variability of *S. cerevisiae* strains was also observed among strains isolated from the same vat at different fermentation ages. Our results showed that there was a succession of genetically different strains of *S. cerevisiae* during the process of *aguardente* production.

X. Institute for Fermentation, Osaka (IFO), Osaka 532-8686, Japan. Communicated by K. Ueda-Nishimura <nishimura-kumiko@ifor.jp>.

Recent publications.

1. Ueda-Nishimura, K. & Mikata, K. 1999. A new yeast genus, *Tetrapisispora* gen. nov.: *Tetrapisispora iriomotensis* sp. nov., *Tetrapisispora nanseiensis* sp. nov. and *Tetrapisispora arboricola* sp. nov., from the Nansei Islands, and reclassification of *Kluyveromyces phaffii* (van der Walt) van der Walt as *Tetrapisispora phaffii* comb. nov. *Int. J. Syst. Bacteriol.* **49**:1915-1924.

Seven strains of three new species were isolated from soil, flowers and leaves in the Nansei Islands, Japan. In physiological characteristics and nuclear DNA base composition (30-32 mol% G+C), these isolates most closely resemble *Kluyveromyces phaffii*; but on the basis of DNA-DNA hybridization and electrophoretic karyotyping they were categorized into three new species different from *K. phaffii*. Phylogenetic analysis using 18S rRNA gene sequences showed that the three new species and *K. phaffii* were highly related to

one another and phylogenetically separate from the members of other species. Based on phylogeny and physiological characters, it is proposed that the three new species represent a novel taxon and should be designated *Tetrapisispora iriomotensis* sp. nov. (type strain IFO 10929), *Tetrapisispora nanseiensis* sp. nov. (type strain IFO 10899), and *Tetrapisispora arboricola* sp. nov. (type strain IFO 10925), while *Kluyveromyces phaffii* becomes *Tetrapisispora phaffii* comb. nov.

2. Mikata, K., Ueda-Nishimura, K., Goto, S., Kurtzman, C.P. Suzuki, M., Yarrow, D. & Nakase, T. 1999. Reidentification of yeast strains deposited as *Candida agrestis*, with a description of *Candida kofuensis* sp. nov. *Microbiol. Cult. Collect.* **15**:49-57.

IFO 10103 and IFO 10932 (= JCM 2321) deposited as *Candida agrestis* RIFY 4611^T (= No. 611, S. Goto) and IFO 10931 (= CBS 8058) deposited as *Candida* sp. RIFY 4841 formed spheroidal to ovoidal chlamydospores and were morphologically similar to *Candida pulcherrima* the anamorph of *Metschnikowia pulcherrima*. However, these strains differed from *M. pulcherrima* in lactate assimilation, growth at 34°C, and pulcherrimin pigmentation. An analysis of domain D1/D2 26S *Saturnispora zaruensis* from its morphological, physiological and

rDNA sequences from currently accepted ascomycetous yeasts demonstrated that the strains represent a new species, which is described here as *Candida kofuensis* sp. nov. Phylogenetic placement of this new species in the *Metschnikowia* clade was further analyzed from 18S rDNA sequence divergence. In additional comparisons, IFO 10933 (= JCM 10341 = NRRL Y-17640 = CBS 8055), which is believed to have originated from *C. agrestis* RIFY 4611^T has been reidentified as molecular characters.

3. Mikata, K., & Ueda-Nishimura, K. 2000. Reclassification of *Pichia membranifaciens* sensu Kurtzman. *Antonie van Leeuwenhoek* **77**:159-171.

Forty strains which were stocked as *Pichia membranifaciens* sensu Kurtzman and 9 strains stocked as *Candida valida*, anamorphs of *P. membranifaciens*, in the Institute for Fermentation, Osaka (IFO) were reclassified based on the data of base composition of nuclear DNA, DNA/DNA hybridization, coenzyme Q system, electrophoretic karyotype, and base sequence of 18S rDNA. *P. membranifaciens* complex was assigned into 3 groups: (I) *P. membranifaciens* group, including 25 strains with high DNA homologies to the type strain of *P. membranifaciens* (72-98%); (II) *P. manshurica* group,

including 18 strains with high DNA homology of 79.4-95.0% to the type strain of *P. manshurica*; and a group including the remaining 6 strains, which had low DNA homology to the above two species. GC content was 42.9-45.3 mol% for the *P. membranifaciens* group, 40.0-42.0 mol% for the *P. manshurica* group, and 27.2-44.7 mol% for the remaining group. All three groups had ubiquinone Co Q-7. Of the 6 anomalous strains, IFO 0162 was identified as *Pichia deserticola*, and IFO 0839 and IFO 0840 were identified as *Issatchenkia occidentalis*; but IFO 0842, IFO 0843, and IFO 1788 were thought to be unknown strains.

4. Ueda-Nishimura, K. & Mikata, K. 2000. Two distinct 18S rRNA secondary structures in *Dipodascus* (Hemiascomycetes). *Microbiology* **146**:1045-1051.

The nucleotide sequences of 18S rRNA gene from ascomycetous yeast-like fungi, in the genera *Dipodascus*, *Galactomyces*, and *Geotrichum* were determined, and the tested strains were separated into two groups by sequence length. In group 1, the length and secondary structure of 18S rRNA corresponded to those of typical eukaryotes. In group 2, the 18S rRNA gene sequences were about 150 nucleotides shorter than those of most other eukaryotes, and the predicted secondary structure lacked helices 10 and E21-5. Many substitutions and

some deletions in group 2 18S rRNA gene were not only found in variable regions but also in regions that are highly conserved among ascomycetes. Despite the considerable differences in 18S rRNA gene sequence and secondary structure between group 2 and other fungi, including group 1, phylogenetic analysis revealed that groups 1 and 2 are closely related. These findings suggest that a number of deletions occurred in the 18S rRNA of the common ancestor of group 2 strains.

XI. State Scientific-Research Institute for Genetics and Selection of Industrial Microorganisms, I-Dorozhnyi 1, Moscow 113545, Russia. Communicated by G.I. Naumov and E.S. Naumova <gnaumov@yahoo.com>.

We thank company J. Laffort & Cie (France) for financial support to prepare the manuscripts on genetics of wine yeasts. The following are publications for 2000.

1. G.I. Naumov, N.G. Tokareva, E.S. Naumova, I.P. Bab'eva. 2000. Discrimination of the soil yeast species *Williopsis saturnus* and *Williopsis suaveolens* by the polymerase chain reaction with universal primer N21. *Microbiology (Engl. Transl.)* **69**(2):280-285.

Thirty-five yeast strains of the genus *Williopsis* analyzed by the polymerase chain reaction with universal primer N21 were found to belong to two sibling species, *W. saturnus* and

W. suaveolens. Such affiliation of the strains studied agrees well with the results of genetic and physiological investigations.

2. G.I. Naumov. 2000. New variety *Saccharomyces bayanus* var. *uvarum* comb. nov. revealed by genetic analysis. *Microbiology (Engl. Transl.)* **69**(3) (in press).

In the paper a partial genetic isolation between populations (varieties) of the *Saccharomyces bayanus* species: *S. bayanus* var. *bayanus* and *S. bayanus* var. *uvarum* comb. nov. has been established by genetic hybridization analysis. Hybrids

of the two varieties were semi-sterile having low ascospore viability. The new variety has been earlier described by the author as a group of cultivars of wine cryophilic yeasts fermenting melibiose.

3. G.I. Naumov. 2000. Wild European species *Zygofabospora krassilnikovii* is an ancestor of the dairy yeast *Z. lactis*. *Dokl. Biol. Sciences* (in press).

Using genetic hybridization analysis, the relatedness of wild European and South-African lactose-negative yeasts *Zygofabospora krassilnikovii* (syn. *Kluyveromyces vanudenii*) with the dairy yeast *Z. lactis* has been established for the first

time. The two species can be crossed and give fertile hybrids. A partial genetic isolation of these species with North-American taxa *Z. drosophilorum* and *Z. phaseolospora* has been revealed.

4. G.I. Naumov, E.S. Naumova, I. Masneuf, M. Aigle, D. Dubourdieu. 2000. Genetical and molecular analyses of the phenomena of introgression among *Saccharomyces bayanus* and *S. cerevisiae*: wine, cider and beer strains. *Oenologie* 99, 6e Symposium International d'Oenologie, TecDoc, Paris, 218-220.
5. I. Masneuf, G.I. Naumov, M.L. Murat, N. Glumineau, D. Dubourdieu. 2000. Des hybrides *Saccharomyces cerevisiae*/*Saccharomyces bayanus* pour la vinification des vins de Sauvignon. *Oenologie* 99, 6e Symposium International d'Oenologie, TecDoc, Paris, 325-328.
6. E.S. Naumova, G.I. Naumov, F.I. Molina. 2000. Genetic variation among European strains of *Saccharomyces paradoxus*: results from DNA fingerprinting. *Syst. Appl. Microbiol.* **23**(1):86-92.
7. G.I. Naumov, I. Masneuf, E.S. Naumova, M. Aigle, D. Dubourdieu. 2000. Association of *S. bayanus* var. *uvarum* with some French wines: genetic analysis of yeast populations. *Research in Microbiol.* (in press).

Using genetic hybridization analysis, electrophoretic karyotyping and PCR-RFLP of the *MET2* gene, we found that the yeast *S. bayanus* var. *uvarum* is associated with certain type of wines produced in Val de Loire, Sauternes, and Jurançon. Average frequency of appearance of this yeast in the three regions of France was 41%, 7% and 77%, respectively. In contrast, we did not find *S. bayanus* var. *uvarum* in red wines produced in

Bordeaux area. The results of this study, as well as the findings already reported on Tokay (Slovakia), Muscat (Crimea, Ukraine) and Amarone (Italy) wines, allow to consider that distribution of *S. bayanus* var. *uvarum* yeast is connected with low temperature climatic conditions and/or wine technologies in which must fermentation, at least partly, carries out also at low temperatures (10-15°C).

8. G.I. Naumov, E.S. Naumova, I. Masneuf, M. Aigle, V.I. Kondrat'eva, D. Dubourdieu. 2000. Natural polyploidization of some cultured yeast *Saccharomyces* sensu stricto: auto- and allotetraploidy. *Syst. Appl. Microbiol.* (submitted).

Using genetic and flow cytometric analyses, we showed that wine strain S6U is an allotetraploid of *S. cerevisiae* x *S. bayanus*. Hybrid constitution of the strain and its meiotic segregants was confirmed by Southern hybridization analysis of their chromosomal DNAs using four *S. cerevisiae* cloned genes: *LYS2* (chr. II), *TRK1* (chr. X), *ARG4* (chr. VIII), *ACT1* (chr. VI)

and PCR/RFLP analysis of the *MET2* gene (chr. XIV). Monosporic progeny of strain S6U was highly viable in first generation but completely non-viable in the second one. According to the genetic analysis, sherry strain *S. cerevisiae* SBY 2592 was found to be an autotetraploid heterozygous for homo-heterothallism.

9. G.I. Naumov, S.A. James, E.J. Louis, E.S. Naumova, I.N. Roberts. 2000. Three new species in the *Saccharomyces* sensu stricto complex: *Saccharomyces cariocanus*, *Saccharomyces kudriavzevii* and *Saccharomyces mikatae*. *Int. J. Syst. Evol. Microbiol.* (in press).

On the basis of genetic analysis, molecular karyotyping and sequence analyses of the 18S rRNA and ITS region, three new *Saccharomyces* species, *Saccharomyces cariocanus* (with type strain NCYC 2890), *Saccharomyces kudriavzevii* (with type strain NCYC 2889) and *Saccharomyces mikatae* (with type strain NCYC 2888) are described. Genetic and molecular analysis did

not confirm earlier observed (Lemos *et al.*, 1995) conspecificity of *S. paradoxus* and *S. cariocanus*. The latter species has a post-zygotic isolation with representative strains from all known geographic populations of *S. paradoxus*: European, Far East Asian, North American and Hawaiian.

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

1. J.L.F. Kock, P.W.J. van Wyk, P. Venter, D.J. Coetzee, D.P. Smith, B.C. Viljoen & S. Nigam. 1999. An acetylsalicylic acid-sensitive aggregation phenomenon in *Dipodascopsis uninucleata*. *Ant. v. Leeuwenhoek* **75**:261-266

Aggregation of ascospores has been discovered in the yeast *Dipodascopsis uninucleata*. When this yeast is cultivated to reach the sexual reproductive stage, small ascospores are individually released from the tip of a sac-like ascus which then aggregate in orderly clusters. Acetylsalicylic acid (ASA) inhibited

ascospore release and subsequent ordered aggregation process. We suggest that novel ASA-sensitive oxidised fatty acids (3R-hydroxy-oxylipins) and small hooks located on the surface of these ascospores, are involved.

2. D.P. Smith, J.L.F. Kock, P.W.J. van Wyk, P. Venter, D.J. Coetzee, E. van Heerden, D. Linke and S. Nigam. 2000. The occurrence of 3-hydroxy oxylipins in the ascomycetous yeast family Lipomycetaceae. *S.A. J. of Sci.* (In Press).

The *in situ* occurrence and localisation of 3-hydroxy oxylipins (fatty acids) in selected members of the Lipomycetaceae were mapped with immunofluorescence microscopy. As was found previously in *Dipodascopsis uninucleata*, fluorescence was associated with the meiospores of all the strains tested. These results further highlight this yeast family as a rich source of 3-hydroxy oxylipins, which have potent biological activity in

mammalian cells and are hence of biological interest. In addition, immunogold labelling of *Dipodascopsis uninucleata* revealed lipid globules with a high affinity for the antibody specific to 3-hydroxy fatty acids. These lipids were produced during the generative stage. This observation might aid in the further optimisation and extraction of these novel compounds.

3. J.L.F. Kock, P. Venter, D.P. Smith, P.W.J. Van Wyk, P.J. Botes, D.J. Coetzee, C.H. Pohl, A. Botha, K-H. Riedel & S. Nigam. 2000. A novel oxylipin-associated "ghosting" phenomenon in yeast flocculation. *Ant. v. Leeuwenhoek* (In Press)

Research on the distribution of oxylipins (3-hydroxy fatty acids) in flocculant strains of the yeast *Saccharomyces cerevisiae* led to the uncovering of a novel "ghosting" phenomenon observed during assumed lectin-mediated aggregation. We found that intracellular oxylipin-containing osmophilic layers migrate through yeast cell walls in a

"ghostlike" fashion without visually affecting the cell wall structure or the layers. This migration resulted in the binding of these layers to cell walls of adjacent cells. Consequently, "ghosting" seems a prerequisite for flocculation to occur. However, "ghosting" alone may not be sufficient to ensure flocculation.

4. D.P. Smith, J.L.F. Kock, M.I. Motaung, P.W.J. van Wyk, P. Venter, D.J. Coetzee & S. Nigam. 2000. Ascospore aggregation and oxylipin distribution in the yeast *Dipodascopsis tothii*. *Ant. v. Leeuwenhoek* (In Press)

Upon cultivation of the yeast *Dipodascopsis tothii* in its sexual stage, small ascospores are released individually from the ascus tip, which then assemble in sheathed cluster balls. In contrast to *Dipodascopsis uninucleata*, this yeast produced smooth bean shaped ascospores with sheath-like appendages that assemble in a disordered sheathed ball of ascospores outside the

ascus. Strikingly, upon release, the ascus tip contained 3-hydroxy oxylipins, while the released ascospore clusters contained little or no 3-hydroxy oxylipins as indicated by immunofluorescence microscopy. In *D. uninucleata*, these oxylipins are concentrated on the spore surface and interspore matrix, but not on the ascus tip.

5. Welthagen, J.J. and B.C. Viljoen. 1999. The presence of yeasts during processing and ripening of Cheddar cheese. *Food Microbiology*. **16**:63-73.
6. Viljoen, B. C. 1999. The ecological diversity of yeasts in foods. *Food Australia*. **51**:22-25.

7. Viljoen, B.C. and G. Heard. 1999. *Saccharomyces cerevisiae*. International Food Microbiology Encyclopedia. Volume 2, pp.1918-1925.
8. Laubscher, P.J. and B.C. Viljoen. 1999. The resistance of dairy yeasts against commercially available cleaning compounds and sanitizers. *Food Technology and Biotechnology* **37**:281-286.
9. Viljoen, B.C., E. Laing and H. Wolter. 2000. Isolation and identification of yeasts associated with intermediate meats. *Food Technology and Biotechnology* **38**:69-76.

Biltong, cabanossi, dry sausage, salami are typical intermediate moisture meats produced and consumed to a large extent in South Africa. A study was undertaken with the objective of isolating and identifying the dominant yeasts associated with these meat products. Microbiological analyses were performed by the enumeration of all relevant microbial organisms on selective agar, and the isolation and identification of all visually distinct yeast colonies by means of conventional identification methods. A total of 11 different yeast species, representing nine genera, were present in the samples analysed. Although a broad spectrum of yeasts were found in the meats, *Debaryomyces hansenii* was

the most abundant yeast isolated. Other species encountered, were *Cryptococcus laurentii*, *Cryptococcus hungaricus*, *Torulaspora delbrueckii*, *Rhodotorula mucilaginosa*, *Sporobolomyces roseus*, *Debaryomyces vanriji*, *Trichosporon beigeli*, *Yarrowia lipolytica*, *Saccharomyces cerevisiae* and *Candida zeylanoides*. The chemical and physical composition of these intermediate moisture meat products was also established. Representative samples of biltong, cabanossi, dry sausage and salami were evaluated and the water activity, pH, % moisture and % salt content, measured.

10. Laubscher, W.D.F., B.C. Viljoen and J. Albertyn. 2000. The yeast flora occurring in the trachea of broiler chicken. *Food Technology and Biotechnology* **38**:77-79.

The occurrence of bacteria as natural occupants of the intestinal flora of poultry is well documented. The incidence and composition of the yeast microflora, however, have received little attention. A study was undertaken with the objective of identifying the predominant yeasts associated with the trachea of chickens, isolated from broilers of a poultry-processing plant. A

total of 38 representative yeast isolates were obtained and identified according to conventional methods. Species of *Bullera*, *Candida*, *Cryptococcus*, *Debaryomyces*, *Rhodotorula*, *Torulaspora*, *Trichosporon*, and *Zygosaccharomyces* were isolated at various stages of the broiler program.

11. Osei-Abunyewa, A., Hugo, A., Laing, E. and B.C. Viljoen. 2000. The growth and survival of yeasts in commercial salami. *Food Microbiol.* (accepted).

The survival of yeast during a pilot scale production of commercial salami, was investigated. A total of 108 distinctive yeast strains were isolated and identified during the processing of the salami. Initially, the number of yeasts remained below 10^3 cfu/g, but their numbers increased after the 12th day of maturation, reaching a maximum of 2.0×10^5 cfu/g at day 20. During maturation, the pH declined from 5.72 to 4.36, water content from 58% to 43%, while the salt content increased by 1%. The number

of lactic acid bacteria remained above 10^5 cfu/g throughout processing and maturation. Of the 108 yeast strains isolated, 22 strains were identified as members of the species *Debaryomyces hansenii*, being present in all samples taken. In descending numbers, *Rhodotorula mucilaginosa*, *Yarrowia lipolytica* and *Cryptococcus albidus*, were also frequently isolated during processing and maturation.

12. Tamás, M.J., Luyten, K., Sutherland, F.C.W., Hernandez, A., Albertyn, J., Valadi, H., Hong, L., Prior, B.A., Kilian, S.G., Ramos, J., Gustafsson, L., Thevelein, J.M., Hohmann S. 1999. Fps1p controls the accumulation and release of the compatible solute glycerol in yeast osmoregulation. *Molecular Microbiology* **31**: 1087-1104.
13. De Kock, S.H., du Preez, J.C. and Kilian, S.G. 2000. Anomalies in the growth kinetics of *Saccharomyces cerevisiae* strains in aerobic chemostat cultures. *J. Ind. Microbiol Biotechnol.* (in press).

Aerobic glucose-limited chemostat cultivations were conducted with *Saccharomyces cerevisiae* strains NRRL Y132, ATCC 4126 and CBS 8066, using a complex medium. At low dilution rates all three strains utilised glucose oxidatively with

high biomass yield coefficients, no ethanol production and very low steady-state residual glucose concentrations in the culture. Above a threshold dilution rate, respiro-fermentative (oxido-reductive) metabolism commenced, with simultaneous respiration

and fermentation occurring, which is typical of Crabtree-positive yeasts. However, at high dilution rates the three strains responded differently. At high dilution rates *S. cerevisiae* CBS 8066 produced 7 to 8 g ethanol.l⁻¹ from 20 g glucose.l⁻¹ with concomitant low levels of residual glucose, which increased markedly only close to the wash-out dilution rate. By contrast, in the respiro-fermentative region both *S. cerevisiae* ATCC 4126 and NRRL Y132 produced much lower levels of ethanol (only 3

to 4 g.l⁻¹) than *S. cerevisiae* CBS 8066, concomitant with very high residual sugar concentrations, which was a significant deviation from the Monod kinetics and appeared to be associated either with high growth rates or with a fermentative (or respiro-fermentative) metabolism. Supplementation of the cultures with inorganic or organic nutrients failed to improve ethanol production or glucose assimilation.

14. De Kock, S.H., du Preez, J.C. and Kilian, S.G. 2000. The effect of vitamins and amino acids on glucose uptake in aerobic chemostat cultures of three *Saccharomyces cerevisiae* strains. System. Appl. Microbiol. **23** (in press)

In the respiro-fermentative region of aerobic chemostat cultures at steady state, *Saccharomyces cerevisiae* CBS 8066 produced high concentrations of ethanol with concomitant low levels of residual glucose which followed Monod kinetics. By contrast, very high residual glucose concentrations were observed in cultures of *S. cerevisiae* strains ATCC 4126 and NRRL Y132 at dilution rates above 60% of the wash-out dilution rate, resulting in much lower ethanol concentrations, even though clearly glucose-limited at lower dilution rates in the respiratory region. The addition of a vitamin mixture resulted in decreased residual glucose concentrations in respiro-fermentative cultures of all three strains, but the effect was much more pronounced

with strains ATCC 4126 and NRRL Y132. *Meso*-inositol was mainly responsible for this effect, although with strain ATCC 4126 other vitamins as well as an amino acid mixture were also required to minimise the steady-state residual glucose levels. The residual glucose concentration in continuous culture was, therefore, greatly dependent on the growth factor requirements of the particular yeast strain, which apparently increased on increasing the dilution rate into the respiro-fermentative region. The strain differences with respect to growth factor requirements at high dilution rates, which were not evident at low dilution rates, had a profound effect on the kinetics of glucose assimilation in aerobic chemostat culture.

15. Du Preez, J.C., De Kock, S.H., Kilian, S.G. and Litthauer, D. 2000. The relationship between transport kinetics and glucose uptake by *Saccharomyces cerevisiae* in aerobic chemostat cultures. Antonie van Leeuwenhoek (accepted).

The steady-state residual glucose concentrations in aerobic chemostat cultures of *Saccharomyces cerevisiae* ATCC 4126, grown in a complex medium, increased sharply in the respiro-fermentative region, suggesting a large increase in the apparent k_s value. By contrast, strain CBS 8066 exhibited much lower steady-state residual glucose concentrations in this region. Glucose transport assays were conducted with these strains to determine the relationship between transport kinetics and sugar assimilation. With strain CBS 8066, a high-affinity glucose uptake system was evident up to a dilution rate of 0.41 h⁻¹, with a low-affinity uptake system and high residual glucose levels only evident at the higher dilution rates. With strain ATCC 4126, the high-affinity uptake system was present up to a dilution rate of about 0.38 h⁻¹, but a low-affinity uptake system was discerned

already from a dilution rate of 0.27 h⁻¹, which coincided with the sharp increase in the residual glucose concentration. Neither of the above yeast strains had an absolute vitamin requirement for aerobic growth. Nevertheless, in the same medium supplemented with vitamins, no low-affinity uptake system was evident in cells of strain ATCC 4126 even at high dilution rates and the steady-state residual glucose concentration was much lower. The shift in the relative proportions of the high and low-affinity uptake systems of strain ATCC 4126, which might have been mediated by an inositol deficiency through its effect on the cell membrane, may offer an explanation for the unusually high steady-state residual glucose concentrations observed at dilution rates above 52 % of the wash-out dilution rate.

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

We would especially like to draw attention to a recent publication by J.J.M. ter Linde *et al.* This paper is the result of a collaboration with colleagues at Leiden University (the Netherlands) and at the Stanford Medical School (USA). It describes the application of genome-wide-transcript profiling to aerobic and anaerobic chemostat cultures, to identify typical

‘aerobic’ and ‘anaerobic’ genes. A complete set of transcript levels has been determined for aerobic and anaerobic glucose-limited chemostat cultures grown at the same specific growth rate, temperature and pH. Data for YFG (your favourite gene) can be checked via the www, at <http://www.wimp.leidenuniv.nl/~yeast>.

1. ter Linde, J.J.M., H. Liang, R.W. Davis, H.Y. Steensma, J.P. van Dijken, and J.T. Pronk. 1999. Genome-wide transcriptional analysis of aerobic and anaerobic chemostat cultures of *Saccharomyces cerevisiae*. J. Bacteriol. **181**:7409-7413

Other recent publications.

2. Diderich, J.A., M. Schepper, P. van Hoek, M.A.H. Luttik, J.P. van Dijken, J.T. Pronk, P. Klaassen, H.F. Boelens, M.J. Teixeira de Mattos, K. van Dam, and A.L. Kruckeberg. 1999. Glucose uptake kinetics and transcription of *HXT* genes in chemostat cultures of *Saccharomyces cerevisiae*. *J. Biol. Chem.* **274**:15350-15359.
3. Flikweert M.T., M. Kuyper, A.J.A. van Maris, P. Kötter, J.P. van Dijken, and J.T. Pronk. 1999. Steady-state and transient-state analysis of growth and metabolite production in a *Saccharomyces cerevisiae* strain with reduced pyruvate-decarboxylase activity. *Biotechnol. Bioeng.* **66**:42-50.
4. Flikweert M.T., M. de Swaaf, J.P. van Dijken, and J.T. 1999. Growth requirements of pyruvate-decarboxylase-negative *Saccharomyces cerevisiae*. *FEMS Microbiol Lett.* **174**:73-79.
5. Van Hoek, P., E. de Hulster, J.P. van Dijken, and J.T. Pronk. 2000. Fermentative capacity in high-cell-density fed-batch cultures of baker's yeast. *Biotechnol. Bioeng.* **68**:517-523.
6. Overkamp, K.M., B.M. Bakker, P. Kötter, A. van Tuijl, S. de Vries, J.P. van Dijken, and J.T. Pronk. 2000. In vivo analysis of mechanisms for oxidation of cytosolic NADH by *Saccharomyces cerevisiae* mitochondria. *J. Bacteriol.* **182**:2823-2830.

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

Recent publications.

1. T. Wartmann, J. Erdmann, I. Kunze & G. Kunze. 2000. Morphology-related effects on gene expression and protein accumulation of the yeast *Arxula adenivorans*. *Arch. Microbiol.* (in press).

The dimorphism of the yeast *Arxula adenivorans* LS3 is regulated by cultivation temperatures. Up to 42°C the yeast grows as budding cells, which turn to mycelia at higher temperatures. To test whether the dimorphism is exclusively induced by high temperatures or also by other conditions, mutants were selected with a changed behaviour in dimorphism. After mutagenesis with N-methyl-N'-nitro-N-nitrosoguanidine, 5 of 25000 colonies formed a very rough surface consisting of mycelia at 30°C in contrast to the wild-type. These mutants allow

temperature-mediated and morphology-related effects on gene expression and protein accumulation to be distinguished. Budding cells and mycelia showed different expression of genes encoding secretory proteins at the same temperature. Mycelia secreted two-fold more protein than budding cells, including the enzymes glucoamylase and invertase. This indicated, that morphology is the decisive factor in the analysed processes, rather than temperature.

2. X.X. Yang, T. Wartmann, R. Stoltenburg & G. Kunze. 2000. Halotolerance of the yeast *Arxula adenivorans* LS3. *Antonie van Leeuwenhoek* (in press).

The non-pathogenic, dimorphic, ascomycetous yeast *Arxula adenivorans* LS3 is halotolerant. It can grow in a minimal medium containing up to 20% NaCl. The growth parameters are only weakly influenced by 10% NaCl. However, NaCl in a concentration higher than 10% causes a decrease in the specific growth rate, a longer adaptation phase and a lower cell count in the stationary growth phase. Concentrations of glycerol and trehalose, which differed 100-fold in magnitude in a salt free medium, are also influenced differently by salt. NaCl induces

accumulation of intracellular glycerol in exponentially growing cells but a reduced concentration of intracellular trehalose in stationary cells. Transcripts of the genes *ARFC3*, encoding a component of the replication factor C, and *GAA*, encoding a secretory glucoamylase, can be detected only in cells cultured in media with NaCl concentrations below 10%. Furthermore, NaCl in high concentration reduces the level of secreted proteins including glucoamylase and invertase.

3. M. Lehmann, K. Riedel, K. Adler & G. Kunze. 2000. Amperometric measurement of copper ions with a deputy substrate using a novel *Saccharomyces cerevisiae* sensor. *Biosensors & Bioelectronics* (in press).

The first microbial biosensor to detect Cu^{2+} by an amperometric method has been developed. For this purpose, recombinant *Saccharomyces cerevisiae* strains are suitable as the microbial component. These strains contain plasmids with the Cu^{2+} -inducible promoter of the *CUP1*-gene from *Saccharomyces cerevisiae* fused to the *lacZ*-gene from *E. coli*. On this sensor the *CUP1* promoter is first induced by the Cu^{2+} -containing probe and subsequently lactose is used as a deputy substrate to make the

measurement. If Cu^{2+} is present in the sample, these recombinant strains are able to utilize lactose as a carbon source, which leads to alterations in the oxygen consumption of the cells. The sensor measured Cu^{2+} in a concentration range between 0.5 and 2 mM CuSO_4 . In addition, an indirect amperometric measurement principle was developed which allows the detection of samples containing Cu^{2+} and fast biodegradable substances.

4. K. Tag, M. Lehmann, C. Chan, R. Renneberg, K. Riedel & G. Kunze. 2000. Measurement of biodegradable substances with a mycelia-sensor based on the salt tolerant yeast *Arxula adeninivorans* LS3. Sensors and Actuators B (in press).

The microbial sensor based on budding cells of the dimorphic yeast *Arxula adeninivorans* LS3 is one of the best suitable sensors for rapid measurement of biodegradable substances. However, *Arxula* is able to change its morphology (budding cells \leftrightarrow mycelia) in response to the cultivation temperature. The suitability of such mycelia was tested as microbial sensor component. The mycelia-based sensor possess a similar linear response, stability, limit of determination and

detection compared with the conventional budding cell-based sensor. However, differences exist in the storage capacity and substrate specificity: the mycelia-sensor has with six months a better storage stability as the conventional sensor, achieves a higher sensitivity for specific amino acids or sugars, shows a better correlation between sensorBOD and the BOD_5 -value with yeast extract as model wastewater and is more suitable for measurement of salt water.

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

The research activities on yeast at the Center for Process Biotechnology combines physiological studies with advanced analytical techniques and mathematical modelling with the objective of increasing our understanding of yeast. The following topics are studied: (1) Fermentation of complex substrates (metabolic engineering of the galactose and the xylose metabolism, mixed sugar utilisation and fermentation inhibitors);

(2) Yeast physiology (pyruvate metabolism in *Saccharomyces kluyveri*, modelling of the pyruvate node, transcriptome analysis of *Saccharomyces cerevisiae*, redox metabolism); (3) Metabolic network analysis (futile cycles, glucose repression, functional genomics); (4) Analytical biotechnology (measurement of intracellular metabolites, multiwave-length fluorescence, CE and combination sensors).

Recent publications.

1. Østergaard, S., Roca, C., Rønnow, B., Nielsen J., L. Olsson. 2000. Physiological studies in aerobic batch cultivations of *Saccharomyces cerevisiae* genetically engineered with respect to the *MEL1* gene. **68:252-259**.
2. Østergaard, S., Olsson, L., and J. Nielsen. 2000. Metabolic engineering of *Saccharomyces cerevisiae*. *Microbiology and Molecular Biology Reviews*. **64:34-50**.
3. Olsson, L. and J. Nielsen. 2000. The role of metabolic engineering in the improvement of *Saccharomyces cerevisiae*: Utilization of industrial media. *Enzyme Microbial Technology*. (In press).
4. Nissen T. L.; C. W. Hamann; M. C. Kielland-Brandt; J. Nielsen; J. Villadsen. 2000. Anaerobic and batch cultivations of *Saccharomyces cerevisiae* mutants impaired in glycerol synthesis. *Yeast* **16:463-474**.
5. Nissen T. L.; M. C. Kielland-Brandt; J. Nielsen; J. Villadsen. 2000. Optimisation of ethanol production in *Sacchaoromyces cerevisiae* by metabolic engineering of the ammonia assimilation. *Metabol. Eng.* **2:69-77**.

Recent PhD theses.

6. Nissen, T. L. 1999. Optimisation of ethanol production in *Saccharomyces cerevisiae* by metabolic engineering.
7. Østergaard, S. 2000. Metabolic engineering of the galactose metabolism in *Saccharomyces cerevisiae*.

XVI. Department of Molecular and Cellular Biology, University of California, Berkeley, California, 94720. Communicated by R.K. Mortimer.

We report the following publications (1992 - 1999)

1. Mortimer, R.K., Contopoulou, C. R. and King, J. S 1992. Genetic and physical maps of *Saccharomyces cerevisiae*. Edition 11, *Yeast* **8**:817-902.
2. Mortimer, R.K. 1993. Øvind Winge, founder of yeast genetics. In THE EARLY DAYS OF YEAST GENETICS. Cold Spring Harbor Laboratory Press, p. 16.
3. Mortimer, R.K. 1993. Carl Lindegren, Iconoclastic father of *Neurospora* and yeast genetics. In "The early days of yeast genetics". Cold Spring Harbor Laboratory Press, pp 17-38.
4. Mortimer, R.K. 1993. Some Recollection on Forty Years of Research in Yeast Genetics. In "The Early Days of Yeast Genetics". Cold Spring Harbor Laboratory Press, pp 173-185.
5. Mortimer, R., Romano, P., Suzzi, G., and Polsinelli, M. 1994. Genome renewal: a new phenomenon revealed from a genetic study of 43 strains of *Saccharomyces cerevisiae* derived from natural fermentations of grape musts. *Yeast* **10**:1543-1552.
6. Mortimer, R. 1994. Genetic analyses of *Saccharomyces* yeasts isolated from spontaneous fermentations of California grape musts. *American Vineyard* **8**:12-19.
7. Romano, P., Suzzi, G., Mortimer, R. and Polsinelli, M. 1995. Production of high levels of acetoin in *Saccharomyces cerevisiae* wine yeasts is a recessive trait. *J. Appl. Bacteriol.* **78**:169-174.
8. Mortimer, R. 1995. Genetic investigations of yeasts isolated from spontaneous fermentations of grape musts in California and Italy. *Practical Winery and Vineyard*, May-June issue 1995.
9. Mortimer, R., Romano, P., Suzzi, G. and Polsinelli, M. 1995. Heterothallism, homothallism and genome renewal in wine yeasts. Abstract 176B, Conference on Yeast Genetics and Molecular Biology, Seattle, August 1994.
10. Romano, P., Suzzi, G., Mortimer, R. and Polsinelli, M. 1995. The occurrence of high acetoin production in *Saccharomyces cerevisiae* wine yeasts as a possible process of genome renewal. (abstract) presented at the XVII International Conference on Yeast Genetics and Molecular Biology, Lisbon, June 10-17, 1995.
11. Mortimer, R., Török, T., Romano, P., Suzzi, G. and Polsinelli, M. 1995. *Saccharomyces cerevisiae* is present on the grapes and is introduced into the fermentation must at the time of crushing. Abstract presented at the XVII International Conference on Yeast Genetics and Molecular Biology, Lisbon, June 10-17, 1995.

12. Mortimer, R., Romano, P., Suzzi, G. and Polsinelli, M. 1995. Genome renewal: a natural selective mechanism for providing new phenotypes in *Saccharomyces cerevisiae*. Vth International Symposium on Oenology, Bordeaux, June 14-17, 1995.,pp. 284-286, A. Lonvaud-Funel, ed. Lavoisier TEC DOC, London, New York, 1996.
13. Polsinelli, M., Romano, P., Suzzi, G. and Mortimer, R. 1996. Multiple strains of *Saccharomyces cerevisiae* on a single grape vine. Letters in Appl. Microbiol.
14. Mortimer, R., de Carmejane, B. and Quinones, J. 1996. Studies on stuck fermentations. Practical Winery and Vineyard, September/October issue 1996
15. Török, T., Mortimer, R., Romano, P., Suzzi, G. and Polsinelli, M. 1996. Quest for wine yeasts - an old story revisited. J. Ind. Microbiology, **17**:303-313.
16. Mortimer, R., Prior, B. and Baccari, C. 1997. Development by breeding of high glycerol-producing strains of wine yeast. U. S. Patent Pending.
17. Cavaliere, D., Barberio, C., Casalone, E., Pinzauti, F., Sebastiani, E., Mortimer, R. and Polsinelli, M. 1998. Genetic and molecular diversity in *Saccharomyces cerevisiae* natural population. Food technol. biotechnol. **36**:45-50.
18. Mortimer, R. and Polsinelli, M. 1999. On the origins of wine yeast. Res. In Microbiol. **A150**:199-204.
19. Prior, B., Baccari, C. and Mortimer, R. 1999. Selective breeding of *Saccharomyces cerevisiae* to increase the levels of glycerol in wines. J. Int. Sci. Vigne Vin. Accepted for publication.
20. Mortimer, R. and Polsinelli, M. 1999. On the origins of wine yeasts. Research in Microbiology **150**:199-204.
21. Prior, B. A., Baccari, C. and Mortimer, R. 1999. Selective breeding of *Saccharomyces cerevisiae* to increase glycerol levels in wine. J. Int. des Sci. de la Vigne et du Vin. **33**:57-65.
22. Prior, B. A., Baccari, C. and Mortimer, R. 1999. Genetic Manipulation of glycerol levels produced by *Saccharomyces cerevisiae*. Oenologie 99 60th Symposium International d'Oenologie, Technique e Documentation, London, New York, Paris, 229-232.
23. Johnston, J., Baccari, C. and Mortimer, R. 1999. Genotypic characterization of strains of commercial wine yeasts by tetrad analysis. Research in Microbiology, submitted.
24. Cavaliere, D., McGovern, P., Mortimer, R. and Polsinelli, M. 2000. Ancient wine yeast. To be submitted to Nature.

XVII. Departamento de Bioquímica, CT Bloco A - Lab 547, Instituto de Química, Universidade Federal de Rio de Janeiro, Ilha do Fundão, 21949-900 Rio de Janeiro, Communicated by A.D. Panek.

Successful defense of Master's dissertations.

1. A.P. Rodrigues Torres. Involvement of Ca²⁺/calmodulin dependent protein kinase in response to environmental stresses in *Saccharomyces cerevisiae*.
2. M. Dias Pereira. Protection factors involved in acquisition of resistance to oxidative stress in *Saccharomyces cerevisiae*.

Papers recently published or in press.

3. Diniz Mendes, L; Bernardes, E, de Araujo, PS, Panek, AD, and Paschoalin, VM 1999. Preservation of frozen yeast cells by trehalose. *Biotech Bioeng* **65**:572-578.
4. Peixoto, DN, and Panek, AD 1999. Involvement of hexokinases in trehalose synthesis. *Biochem Mol Biol Int* **47**:873-880.
5. Ferreira, JC, de Araujo, PS, and Panek, AD In press. Inactivation of maltose permease and maltase in sporulating *Saccharomyces cerevisiae*. *Can J Microbiol*.

XVIII. Department of General and Environmental Microbiology, Institute of Biology, Faculty of Sciences, University of Pécs, H-7601 Pécs, P.O.B. 266., Hungary. Communicated by M. Pesti, <micro@ttk.jpte.hu>.

The following papers have been recently published.

1. J. Belágyi, M. Pas, P. Raspor, M. Pesti and T. Páli. 1999. Effect of hexavalent chromium on eukaryotic plasma membrane studied by EPR spectroscopy. *Biochim. Biophys. Acta* **1421**:175-182.

The effect of Cr(VI) anion on an ergosterol-producing strain of eukaryotic yeast *Candida albicans* and its mutant with ergosterol-less membrane was studied with EPR spectroscopy. 5- and 14-doxyl stearic acid spin probes were used to label the protoplast membrane after removal of the cell wall. In control experiments, the mutant strain exhibited larger rigidity in the membrane than its parental strain. Addition of Cr(VI), at a minimum inhibitory concentration of 0.6 mM, increased the rotational mobility of the spin labels significantly and decreased the temperature of the structural changes in both strains, in the

temperature range between 0 and 30 °C. The ergosterol-less mutant, having a membrane composition with increased polyunsaturated fatty acid content, exhibited higher Cr(VI) sensitivity. Treatment of the membrane with Cr(VI) for 10 min already resulted in an increase in membrane fluidity. An EPR signal of Cr(V) was detected which reached maximum amplitude after 120 min of treatment with Cr(VI). Further chemical reduction of Cr(V) in the absence of extracellular Cr(VI) led to a lack of detectable paramagnetic chromium intermediates within 200 min.

2. M. Pesti, Z. Gazdag and J. Belágyi. 2000. In vivo interaction of trivalent chromium with yeast plasma membrane, as revealed by EPR spectroscopy. *FEMS Microbiol. Lett.* **182**:375-380.

The in vivo effects of CrCl₃ on an ergosterol-producing 33 erg⁺ strain of the eukaryotic yeast *Candida albicans*, and on its ergosterol deficient erg⁻² mutant, were studied by using electron paramagnetic resonance spectroscopy. A 5-doxylstearic acid spin probe was applied to label the membranes. The absence of ergosterol, an increased accumulation of Δ⁸ sterols, a decreased fatty acid chain length and a lower proportion of unsaturated fatty acids of the erg⁻² mutant resulted in a higher membrane rigidity and an increased

sensitivity to Cr(III) than those of the parental 33 erg⁺ strain. Cr(III) significantly increased the fluidity of the spin labelled membranes, this being more pronounced for the erg⁻² mutant. The break in the slopes measured for the erg⁻² mutant was decreased (ΔAT ~ 4 °C) from 17 to 13 °C. Cr(III) treatment for 10 h caused a loss of metabolites adsorbing at 260 nm: this loss was 40 % for 33 erg⁺ and 60 % for erg⁻². This decriptification process might be the main cause of growth inhibition and cell killing by the impermeable Cr(III) ions.

XIX. Food Science and Technology Department, Biotechnical Faculty University of Ljubljana, Jamnikarjeva 101, 1000 Ljubljana, Slovenia. Communicated by P. Raspor <peter.raspor@bf.uni-lj.si>.

Recent publications.

1. Batič, M., Lenarčič, D., Štupar, J., Raspor, P. 1996. Rapid method for screening the tolerance of yeasts to zinc (II) and chromium (III). *J. Rapid Methods Autom. Microbiol.* **4**:265-278.

Rapid screening of the tolerance of yeasts to zinc (II) and chromium (III) was performed by an agar diffusion test. A rapid and reliable procedure for the determination of metal ion

concentration gradients on agar plates was developed. Different species of yeasts from the following genera were investigated: *Arthroascus*, *Bulera*, *Dekkera*, *Debaryomyces*, *Dipodascopsis*,

Eremothecium, *Candida*, *Hansenula*, *Kluyveromyces*, *Hormoacus*, *Geotrichum*, *Lipomyces*, *Pachysolen*, *Pichia*, *Saccharomyces*, *Schizosaccharomyces*, *Schizoblastosporion*, *Schwanniomyces*, *Sporobolomyces*, *Yarrowia*, *Torulaspora*, *Zygosaccharomyces* and *Williopsis*. The experimental conditions were defined as a constant volume of malt agar 32 cm³, and a temperature of 29°C. After periods of 24, 47 and 72 h, the concentration intervals of growth inhibition were

2. Batič, M., Raspor, P. 1998. Uptake and bioaccumulation of Cr(III) in yeast *Saccharomyces cerevisiae*. *Food Technol. biotechnol.* **36**:291-297.

The chromium uptake and bioaccumulation were studied for selected yeast *Saccharomyces cerevisiae* ZIM-198 after standardised cultivation in designed substrate with adequate amount of Cr(III) in the media. The impact of Cr(III) on biomass accumulation, RNA, metal accumulation, and organically bound chromium was studied in vivo. Efficiency of Cr(III) in yeast biomass was primarily studied in vitro to define the impact of pH and energy source. A strong correlation between energy supplementation and chromium uptake was found. The optimal pH value was found to be approximately 4. Selected pH minimised also the possibility of Cr(III) precipitation in the media. High Cr(III) concentrations (> 1.9 mmol L⁻¹) intensify the pH decrease in the media. Further studies in vivo in standardised media confirmed the impact of Cr(III) on yeast cell metabolism. It was proved that Cr(III) has an

3. Belagyi, J., Paš, M., Raspor, P., Pesti, M., Páli, T. 1999. Effect of hexavalent chromium on eukaryotic plasma membrane studied by EPR spectroscopy. *Biochim. Biophys. Acta, Biomembr.* **1421**:175-182.

The effect of Cr(VI) anion on an ergosterol-producing strain of eukaryotic yeast *Candida albicans* and its mutant with ergosterol-less membrane was studied with EPR spectroscopy. 5- and 14-doxyl stearic acid spin probes were used to label the protoplast membrane after removal of the cell wall. In control experiments, the mutant strain exhibited larger rigidity in the membrane than its parental strain. Addition of Cr(VI), at a minimum inhibitory concentration of 0.6 mM, increased the rotational mobility of the spin labels significantly and decreased the temperature of the structural changes in both strains, in the

4. Czakó-Vér, K., Batič, M., Raspor, P., Sipiczki, M., Pesti, M. 1999. Hexavalent chromium uptake by sensitive and tolerant mutants of *Schizosaccharomyces pombe*. *FEMS Microbiol. Lett.* **178**:109-115.

Lysine and leucine auxotrophic, heterothallic (h⁺, h⁻) strains of *Schizosaccharomyces pombe* were used to obtain chromium(VI)-sensitive and -tolerant mutants by ultraviolet radiation-induced and nitrosoguanidine-induced mutagenesis. The minimal inhibitory concentrations of K₂Cr₂O₇ on YEA media were 225 μM for the wild-type strain CW-6, 125 μM for the sensitive mutant CS-6.51 and 275 pM for the tolerant mutant CT-6.66. The mutants exhibited cross-sensitivity of various patterns to Cd²⁺, Cu²⁺, Ni²⁺, Zn²⁺ and VO₄³⁻. Cr(VI) was added to the actively growing cultures and the total chromium (TOCr) content of the cells was determined. The

determined, and the yeasts investigated were grouped in different classes according to their tolerance to Zn (II) and Cr (III). Yeasts were found to be tolerant to significantly higher zinc (II) concentrations in the malt agar medium (5.9 mM to 20 mM) in comparison to chromium (III) (1.5 mM to 6.9 mM). Yeasts showed inter- and intra-generic differences in zinc (II) and chromium (III) tolerance.

important effect on cell components. Total chromium concentration in the yeast cell increased in *Sacch. cerevisiae* when continuous growth in a concentration of 96 μmol L of Cr(III) in the medium was applied. The same tendency was detected for organically bound intracellular chromium where 43% higher concentration of chromium was determined under the same conditions of cultivation. The yeast detoxification capability for high intracellular chromium was reduced owing to the toxic action of environmental chromium loading pressure on yeast cell growth and viability. The cell detoxification system enables the yeast *Sacch. cerevisiae* to survive and grow at a moderate and higher concentration of inorganic chromium. In these processes, the distribution of excess inorganic chromium in the cell plays an important role.

temperature range between 0 and 30°C. The ergosterol-less mutant, having a membrane composition with increased polyunsaturated fatty acid content, exhibited higher Cr(VI) sensitivity. Treatment of the membrane with Cr(VI) for 10 min already resulted in an increase in membrane fluidity. An EPR signal of Cr(V) was detected which reached maximum amplitude after 120 min of treatment with Cr(VI). Further chemical reduction of Cr(V) in the absence of extracellular Cr(VI) led to a lack of detectable paramagnetic chromium intermediates within 200 min.

sensitive mutant exhibited a high bioaccumulation ability, with a dry biomass of 810 μg g⁻¹ after 30 min, while the tolerant mutant had a significantly lower ability than the wild-type strain. In PIPES buffer, washed, lysine-starved biomasses were treated with 75 μM Cr(VI) and after 2 h, the TOCr and the organically bound chromium (OBCr) were determined. Under these conditions, the sensitive and tolerant mutants had the same TOCr content, 50% of which was OBCr. The wild-type strain exhibited a lower TOCr content than that of its mutants and only 35% of this was OBCr. The Cr(VI)-sensitivity was due to a significantly increased uptake of Cr(VI).

5. Raspor, P., Batič, M., Jamnik, P.. 1999. Measurement of yeast viability/mortality in the presence of chromium(VI). *Food Technol. Biotechnol.* **37**:81-86.

The aim of this work was to develop a mathematical model - a set of equations for studying the effect of Cr(VI) on yeast viability/mortality. The model was built with parameters and a two-stream approach has been developed which includes four parameters that indicate viability like population condition (PC), cell viability in the buffer (VB), cell viability in the buffer supplemented with the effector (VEB), specific cell viability (VS), and three parameters that indicate mortality as follows:

6. Paš, M., Prah, S., Raspor, P. 1999. Effect of Cr(III) compounds on yeasts survival. *Zb. Bioteh. fak. Univ. Ljublj., Kmet.* **73**:187-194.

Chromium plays an important role in yeast metabolism, where its form and concentration in the micro- and macroenvironment of the yeasts are the crucial factors. The tolerance of yeasts *Saccharomyces cerevisiae* ZIM 753 (Industrial culture collection, Biotechnical faculty, Ljubljana), *Saccharomyces diastaticus* ZIM 152 and *Candida intermedia* ZIM 156 on Cr(III) compounds such as $\text{CrCl}_3 \cdot 6\text{H}_2\text{O}$, $\text{Cr}(\text{CH}_3\text{COO})_3$, $\text{KCr}(\text{C}_2\text{O}_4) \cdot 3\text{H}_2\text{O}$, $\text{Cr}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$, $\text{CrK}(\text{SO}_4) \cdot 12\text{H}_2\text{O}$, Cr-citrate in concentration range between 0,1 and 200 mM Cr(III) was determined. Yeast cells were propagated in malt extract liquid medium to the early stationary

cell mortality in the buffer (MB), cell mortality in the buffer supplemented with the effector (MEB) and specific cell mortality (MS). The parameters were calculated on the basis of primary results [the total cell number per mL (N_T) and the number of viable cells per mL (N_{CFU})]. The total cell number was obtained microscopically with a hemocytometer and yeast viability was determined with the colony count method (spread plate method).

phase, the biomass was washed and resuspended in the MES buffer (pH = 4.0). After addition of Cr(III) compounds in different concentrations the suspension was shaken 24 hours by 28°C. The survival of yeasts was determined by the CFU (colony forming unit) method on malt extract agar. The results indicate that the tolerance of yeasts to higher Cr(III) concentrations depends on the genera or the species of yeasts. However, the form of Cr(III) or Cr(III) compound, the yeasts were exposed to, is also the factor, which influences yeast survival.

7. Batič, M., Raspor, P.. 2000. Effect of cultivation mode on a bioprocess for chromium yeast biomass enrichment. *Pflügers Arch.* **439**(3, suppl.):R73-R75.

Defined cultivation media for yeast growth which contained 278.8 mM of glucose and 0.1 mM of chromium(III) added as $\text{K}_2\text{Cr}(\text{SO}_4) \cdot 2\text{H}_2\text{O}$ was used in batch and combined batch/fed-batch cultivation mode. In fed batch cultivation mode the rate of substrate addition remained constant during growth of yeast and corresponded to a growth rate of 0.25 h^{-1} . In both cases the growth and yeast activity was followed by on line measurement of optical density, pH and pO_2 at 30°C. At the end of the bioprocess the concentration of protein in yeast biomass was determined off line by the biuret reaction. Total and organically bound chromium was detected by ETA-AAS. Different cultivation modes affected the total cell protein concentration of yeast grown in media supplemented with chromium. In batch process the protein content represented 25.7% of dry yeast biomass, in contrast in the mixed bioprocess

this value was 16.9% one the same period of time. The influence of cultivation mode on chromium uptake was seen in total chromium accumulation which reached 8.68 ± 0.16 micromol g⁻¹ d.wt. in batch and 1.92 ± 0.04 micromol of chromium g⁻¹ of dry yeast biomass in combined batch/fed-batch cultivation mode. The opposite was observed for organically bound chromium. The 60% of total accumulated chromium was organically bound during yeast growth in combined batch/fed-batch mode. When yeast was grown in batch mode this value attained 13.5%. Results suggested that a combined batch/fed-batch mode of cultivation was more effective over a batch system in chromium biotransformation to organically bound chromium, regardless of the lower protein ratio determined in the yeast biomass.

8. Raspor, P., Batič, M., Jamnik, P., Josić, D., Milačič, R., Paš, M., Recek, M., Režić-Dereani, V., Skrt, M.. 2000. The influence of chromium compounds on yeast physiology. *Acta microbiol. immunol. Hung.* **47**(2/3):143-173.

Introduction
Chromium as an ion of choice for yeast metabolism
Chromium accumulation in yeasts biomass

Effect of chromium on yeasts viability
Effect of chromium on yeast genome
Conclusion

The following thesis was defended recently.

1. Jin, Y.L. 1999. Flocculation of brewing yeast: effect of zymolectin, cell surface hydrophobicity, and environmental factors, M.Sc, Department of Food Science and Technology, Dalhousie University.

The flocculation behavior of two *S. cerevisiae* strains expressing either *Flo1* or *NewFlo* phenotype were examined. The behavior of the two strains was examined after varying ethanol (0-10 %), pH (3.8-5.8), ionic strength (0.01-0.20 M) and temperature (5-25C). The flocculation behavior of *Flo1* cells was insensitive to ethanol and pH change. Flocculation of *NewFlo* cells significantly increased with increases in ethanol concentration ($P < 0.05$) and pH values ($P < 0.01$). Increasing ionic strengths and decreasing temperatures significantly ($P < 0.01$) retarded flocculation in both strains. The apparent activation energy of flocculation at pH 4 and 108 cells/ml was estimated to be 3.2 and 11.0 Kcal/mol for *Flo1* and *NewFlo* respectively, indicating distinct sensitivities to temperature. Interestingly, flocculation inhibition by urea was overcome by washing with 100 mM acetate buffer (20C, pH 4, containing 1 mM Ca^{++}) presumably due to the reversible unfolding of zymolectin molecules. A semi-empirical model was developed that indicated that the flocculation behavior is affected by the cell volume fraction for both *Flo1* ($r^2 = 0.981$) and *NewFlo* ($r^2 = 0.944$) strains. This semi-empirical model allows adjustment

of Helms values due to variation in cell volume fraction thus partially explaining reported variations of Helms values with respect to fermentation time. The cell surface hydrophobicity was determined via hydrophobic interaction chromatography using a Phenyl-Sepharose CL-4B column. As well, an anilinonaphthalene sulphonate (ANS) probe was employed to detect hydrophobic cavities on yeast cell surface. Presence of ethanol (0-10% v/v) in a pH 4.0 calcium containing sodium acetate buffer influenced CSH for both haploid and polyploid strains ($P < 0.05$). In the pH range of 3.8-5.8 (similar to pH changes during fermentation) CSH showed different responses for the two strains. Temperature (5-25C) had no effect on CSH of the polyploid strain ($P > 0.05$) but resulted in an increased tendency of hydrophobic association for the haploid strain at low temperatures ($P < 0.001$). Cell age, EDTA and alpha-methyl-D-mannopyranoside (a lectin blocking agent) all effected cell hydrophobicity. A relationship ($r = 0.684$) between CSH and flocculation of the two strains has been found over the conditions investigated above ($P < 0.001$).

The following paper was accepted recently.

2. Jin, Y-L. and Speers, R.A. 1999. The effect of environmental conditions on the flocculation of *Saccharomyces cerevisiae*. J. Am. Soc. Brew. Chem.. Accepted for publication.

The flocculation behavior of two *Saccharomyces cerevisiae* strains expressing either *Flo1* or *NewFlo* phenotype was examined. The behavior of the two strains was examined while varying ethanol (0-10.0ml/100ml), pH (3.8-5.8), ionic strength (0.01-0.20 M) and temperature (5-25C). The flocculation behavior of *Flo1* cells was insensitive to ethanol and pH changes. *NewFlo* cells exhibited significantly increased flocculation with increases in ethanol concentration ($p < 0.05$) and pH value ($p < 0.01$). Increasing ionic strength and decreasing temperature significantly ($p < 0.01$) retarded flocculation in both strains. The apparent activation energy of flocculation at pH 4.0 and 1×10^8 cells/ml was estimated to be

3.2 and 11.0 kcal/mol for *Flo1* and *NewFlo* strains respectively, indicating distinct sensitivities to temperature. Interestingly, flocculation inhibition by urea was reversed by washing with 100 mM acetate buffer (20°C, pH 4.0, containing 1.0 mM Ca^{2+}) presumably due to the reversible unfolding of zymolectin molecules. A semi-empirical model was developed that indicated that the flocculation behavior is affected by the cell volume fraction for both *Flo1* ($r^2 = 0.97$) and *NewFlo* ($r^2 = 0.93$) strains. This semi-empirical model allows adjustment of Helms values due to variation in cell volume fraction thus partially explaining reported variations of Helms values with respect to fermentation time.

Poster presentation.

3. J.W-C. Hsu, R. A. Speers and A.T. Paulson. 2000. Orthokinetic flocculation of *Saccharomyces cerevisiae*: Comparison of experiment and theory. Winnipeg, MB. Agrifood 2000. July 15-19, 2000. Winnipeg, MB.

The flocculation behavior of two *S. cerevisiae* strains was examined in a laminar flow field by measurement of a fundamental flocculation parameter, the orthokinetic capture coefficient (OCC). The strains examined, a haploid (LCC1209) and a production strain (LCC125) are reported to express the

Flo1 and *NewFlo* phenotypes, respectively. The flocculation tendency of 5.0 micrometer diameter polystyrene microspheres was also examined. The initial orthokinetic capture coefficient values of these three dilute, disperse colloidal suspensions were measured as a function of shear rate (5.9-225 /s) and

temperature (5-45°C). The OCC of pronase treated cells was also measured over similar shear rate and temperature ranges. Measured OCC values were sensitive to shear rate. For example, for the LCC1209 strain, increasing shear rates (from 5.9 to 46.3 /s) resulted in a rapid decline in the orthokinetic capture coefficient. Shear rates in the range of 100-250 /s had little effect on the capture coefficient values. When sheared at 9.3 /s the capture coefficient exhibited a slight increase as the temperature was increased to 45°C. A theory which predicts

capture coefficient values due to zymolectin interactions was modified from that developed by Long et al. (1999. *Biophys. J.* 76:1112). This theory along with estimates of: (1) cell wall density of zymolectin and mannose structures, (2) cell wall collision contact area, (3) collision time and (4) the forward rate coefficient of binding were used to predict theoretical capture coefficients, which were then compared to measured values.

Recently submitted manuscript.

4. Jin, Y-L, Ritcey, L.R., Speers A.R. and Dolphin, P.J. 2000. Effect of cell surface hydrophobicity, charge and zymolectin density on the flocculation of *Saccharomyces cerevisiae*. Submitted.

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

The following contributions were recently published.

1. D. Šmogrovičová, Z. Dömény. 1999. Beer volatile by-product formation at different fermentation temperatures using immobilised yeasts. *Process Biochem.* **34**:785-794.

Beers produced by bottom fermenting yeast *Saccharomyces cerevisiae* entrapped in calcium pectate and L-carrageenan contained lower amounts of diacetyl and higher alcohols at all temperatures studied (from 5 to 20°C). Ester formation was lower at temperatures from 5 to 15°C and acetaldehyde formation at temperatures from 5 to 12°C. The contents of total nitrogen and free amino nitrogen were higher at all temperatures studied due to lower amino acid uptake by entrapped cells. The character of beers produced by yeasts

adsorbed on DEAE-cellulose at different temperatures was similar to beers produced by free yeasts. The concentration of diacetyl in beers fermented by entrapped yeast decreased as the temperature was increased. In contrast, the diacetyl concentration increased with increasing temperature using free yeast and yeast immobilised on DEAE-cellulose. The concentration of acetaldehyde, higher alcohols and esters increased as the temperature was increased in all beers.

2. P. Bafrcová, D. Šmogrovičová, I. Sláviková, J. Pátková, Z.D Dömény. 1999. Improvement of very high gravity ethanol fermentation by media supplementation using *Saccharomyces cerevisiae*, *Biotechnol. Lett.* **21**:337-341.

The final ethanol concentration achieved was increased by 17% (to 103 g ethanol/l) when excess assimilable nitrogen was added to the batch very high gravity (VHG) ethanolic fermentations by *Saccharomyces cerevisiae*. The supplementation of the media with 12 g yeast extract l⁻¹, 0.3 cell

walls l⁻¹, 3 g glycine l⁻¹ and 20 g soya flour l⁻¹ led to halving reduction of the fermentation time to 28 h. The ethanol productivity was enhanced by more than 50% to achieved value 3.3 g l⁻¹ h⁻¹.

3. J. Zigová, M. Mahle, H. Pachold, M. Malissard, E.G.Berger, D. Weuster-Botz. 1999. Fed-batch production of a soluble β -1,4-galactosyltransferase with *Saccharomyces cerevisiae*. *Enz. Microb. Technol.* **25**:201-207.

A feedback-controlled fed-batch process for the recombinant production of a soluble human β -1,4-galactosyltransferase (NdrGal-T) with *Saccharomyces cerevisiae* was developed and scaled up to the pilot scale. A 5000 U NdrGal-T fermentation run was performed on a 300 l scale. Indirect feedback control of the glucose feeding with RQ data at a set-point of RQ = 1.1 resulted in higher NdrGal-T

activities (30 U/l) than direct feedback control of glucose with on-line flow-injection analysis (9 U/l). This increase in final activity of NdrGal-T by a factor of 42 compared to published data makes the fed-batch production of glycosyltransferases with a *Saccharomyces cerevisiae* expression system competitive with cell-culture systems.

4. E. Minárik. 2000. Acetic acid and acetic acid ester formation in wine. *Kvasný průmysl* **46**: (in press).

The formation of acetic acid and acetic acid ester in grape wine is produced not only by acetic acid bacteria but also by some yeast species, particularly by film-forming *Hansenula anomala*, *Candida krusei*, *C. vini* and *Pichia* sp. In order to minimize volatile acid and ester formation, selected active dry

wine yeast cultures are recommended in grape must fermentations. Reduced oxygen access to young wine and careful hygiene in winemaking are basic requirements to achieve wines with minimal volatile acid and ester formation.

XXII. School of Biological Sciences, University of East Anglia, Norwich NR4 7TJ, England, Communicated by J.A. Barnett <J.Barnett@uea.ac.uk>.

Current publications.

1. J A Barnett, R W Payne & D Yarrow. 2000. YEASTS: CHARACTERISTICS AND IDENTIFICATION - 3RD EDITION. Cambridge University Press (ISBN 0 521 573963) Publication date: 25 May 2000. Price: £200 (introductory offer £175 until 31 July 2000).
2. J A Barnett, R W Payne & D Yarrow. 2000. YEAST IDENTIFICATION PC PROGRAM - VERSION 5 (ISBN 0 9513148 9 0). Updated to identify 678 species of yeast using the data from the above new book. Available for IBM PC and compatibles with MS-Windows 95, 98 or NT, or with MS/PC-DOS and 512 KB of free RAM . Available from 25 May 2000. Price as version 4. Enquiries to J A Barnett.
3. J A Barnett. 2000. A history of research on yeasts 2: Louis Pasteur and his contemporaries, 1850-1880. *Yeast* **16**: (June 2000).
4. In preparation: A history of research on yeasts 3: 1880-1900.

XXIII. Centro de Estudos de Insetos Sociais – Unesp, Rio Claro, São Paulo, Brasil. Communicated by F.C. Pagnocca <pagnocca@rc.unesp.com.br>.

Summary of our current research, funded by FAPESP (Fundação de Amparo a Pesquisa do Estado de São Paulo) and CNP (Conselho Nacional de Pesquisas).

1. Killer toxin production and plant polysaccharides metabolism by yeasts from nests of leaf-cutting ants

The production of killer factor, the ability to degrade plant polysaccharides (starch, pectin, cellulose and lignin) and to assimilate their hydrolysis products (maltose, galacturonic acid, cellobiose and xilose) were investigated in 152 isolates from laboratory nests of the leaf-cutting ant *Atta sexdens*. Of these isolates 46 came from the newer sponge, 21 from the older sponge, 26 from the waste deposit (discharged material), 6 from *Eucalyptus alba* leaves used for the nest foraging, 20 from the ant's body, and 33 from the floor of containers in which ants nests were maintained. Of the 152 isolates, 45 were killer against at least one of the 6 sensitive standard strains. Isolates from newer sponge, waste deposit and ant's body presented a larger number of positive killer reactions than did isolates from the floor of containers. None of the isolates from leaves presented killer activity. Yeast isolates from newer sponge, older sponge, waste deposit, or leaves were also able to kill each other. Seventy isolates showed killer activity. The waste deposit showed the higher number of positive killer reactions, followed by newer sponge, older sponge and leaves. Killer activity was especially observed in isolates from newer

sponge against those from the newer and older sponge, as well as in isolates from waste deposit against those from newer sponge, older sponge and leaves. Since killer activity was observed in most of yeast isolates, killer activity may be an important factor in yeast colonization and maintenance inside the nests. The killer factor seems to be responsible for the predominance of *Candida colliculosa* in older sponge and *C. famata* in waste deposit. The characterization of strains, as defined by Polonelli et al. (1983), showed predominance of strain types 511, 888 and 811. Of the 152 isolates, 120 (79%) degraded polysaccharides, indicating that this ability is important for yeast to survive inside ants' nests. Pectin was degraded by 81 (53%) isolates, 51 isolates (35%) were able to degrade starch, 14 (9%) degraded cellulose, and none of the isolates degraded xylan or lignin. Isolates from newer sponge (63%) or waste deposit (58%) were more active on pectin degradation than on degradation of the other polysaccharides. On the other hand, isolates able to degrade pectin or starch predominated in older sponge (48 and 33%, respectively), ants' body (50 and 55%, respectively), and floor of containers (39

and 51%, respectively). Thus, our results suggest that yeast living in ants' nests play a part in plant polysaccharide degradation, especially pectin and starch. The effect of this polysaccharide degradation to the symbiosis between ants and their cultured fungus was assessed by determining the ability of yeast isolates to assimilate polysaccharide degradation products. Many of the isolates assimilated maltose (96%), cellobiose (96%) or xylose (90%), indicating that degradation of starch, cellulose, and xylan may provide important carbon sources to the maintenance of yeast inside ants nests. By degrading starch and cellulose, and assimilating their hydrolysis products, yeast may compete with the ant symbiotic

fungus. Since ants' nutrition relies on their fungus, yeast metabolic activity on starch and cellulose may represent an important energetic loss for the ants. However, few isolates assimilated galacturonic acid (31%), indicating that pectin degradation does not provide an essential carbon source for the surviving of most of the yeast isolates. Instead, pectin degradation by yeast may contribute to the maceration of plant tissue, which is necessary to the ants and their symbiotic fungus to get access to the internal nutrients of vegetation. Thus pectin degradation by yeast may represent an advantage too the nutrition of the ants and their symbiotic fungus.

XXIV. Department of Soil Biology, Lomonosov Moscow State University, Vorobjevy Gory, Moscow 119899, Russia. Communicated by I.P. Bab'eva

The following papers were recently published or have been prepared for publication.

1. Bab'eva I.P., Lisichkina G.A., Maximova I.A., Reshetova I.S., Terenina E.E., Chenov I.Yu. 2000. New yeast species *Candida anutae* sp. nov. from fruit bodies of agaricous fungi. *Microbiology* **69**:276-279.
2. Naumov G.I., Tokareva N.G., Naumova E.S., Bab'eva I.P. 2000. Differentiation of soil yeasts *Williopsis saturnus* and *Williopsis suaveolens* by PCR method with nonspecific primers. *Microbiology* **69**:280-285.
3. Bab'eva I.P., Lisichkina G.A. New species of psychrophilic basidiomycetous yeasts *Leucosporidium fasciculatum* sp.nov. *Microbiology*, in press.

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

Current research.

1. H. Park and A.T. Bakalinsky. *SSU1* mediates sulfite efflux in *Saccharomyces cerevisiae*.

Ssu1p, a plasma membrane protein involved in sulfite metabolism in *Saccharomyces cerevisiae*, was found to be required for efficient sulfite efflux. An *SSU1* null mutant accumulated significantly more sulfite than wild-type, whereas cells expressing multicopy *SSU1* accumulated significantly less. Cells expressing FZF1-4, a dominant allele of a transcriptional activator of *SSU1* that confers sulfite resistance, also accumulated less sulfite. β -galactosidase activity in the FZF1-4 strain carrying an *SSU1::lacZ* fusion was found to be 8.5-fold higher than in a strain carrying wild-type FZF1, confirming that the heightened resistance was correlated with

hyperactivation of *SSU1*. Multicopy *SSU1* was also found to increase the sulfite resistance of a number of unrelated sulfite-sensitive strains by a factor of 3- to 8-fold. Rates of efflux of free sulfite from cells expressing multicopy *SSU1* or *FZF1-4* were significantly greater than that from wild-type or from an *SSU1* null mutant. Rates of efflux of bound sulfite from wild-type, an *SSU1* null mutant, an *FZF1-4* mutant, or cells expressing multicopy *SSU1* were not significantly different, suggesting that Ssu1p specifically mediates efflux of the free form of sulfite.

XXVI. Area de Bioquímica del Centro de Química, Instituto de Ciencias, Universidad Autónoma de Puebla, Apartado Postal 1613, 72000 Puebla Pue, México. Communicated by Irma Herrera-Camacho <pherrera@siu.buap.mx>

Recent publication.

1. Herrera-Camacho, R. Morales-Monterrosas, R. Quiróz-Alvarez. 2000. Aminopeptidase yscCo-II: a new cobalt-dependent aminopeptidase from yeast. Purification and biochemical characterization. *Yeast*. **16**:219-229.

Saccharomyces cerevisiae aminopeptidase yscCo-II (APCo-II) was purified to apparent homogeneity by gel filtration, affinity chromatography and anion-exchange chromatography. APCo-II is an hexameric cobalt-dependent metallo-enzyme with an estimated native molecular mass of 290 kDa. Enzyme activity is only detected in the presence of cobalt ions at pH 7.0. Substrate specificity studies indicate that

aminopeptidase yscCo-II cleaves only basic N-terminal residues. PMSF, Cu²⁺, 1,10-phenanthroline and bestatin were found to be very strong inhibitors of aminopeptidase yscCo-II activity. Kinetic studies indicated that the enzyme has a similar K_m and K_{aCo} (activation constant of cobalt) and, following extraction of cobalt from the enzyme, activity was recovered only after cobalt addition.

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

Current publications.

1. Golubev W.I., Staib F., 2000. Green and brown colour effects in tremellaceous yeast fungi on Staib agar. *Mycoses* **43**:1-5.

Fifty-eight species of basidiomycetous yeast fungi were examined for green and brown colour effects (GCE, BCE) on Staib agar (*Guizotia abyssinica* creatinine agar). In addition to 9% of *Cryptococcus laurentii* strains tested, only *Cryptococcus podzolicus* was GCE-positive. Out of 14 strains of this species, 13 showed a GCE and four showed a BCE. All GCE-positive strains utilized creatinine and creatine as a source of nitrogen but not of carbon. None of these strains could grow

at 37°C. Unlike *Cr. laurentii*, no strains of *Cr. podzolicus* assimilated glycine as a source of carbon. No association was found to exist between GCE and killer activity. Some tremellaceous creatinine-negative species (*Cryptococcus gastricus*) showed a BCE whereas some sporidiobolaceous creatinine-positive yeasts (*Leucosporidium*, *Rhodotorula*) produced neither GCE nor BCE.

2. Golubev W.I., 2000. Glucuronate medium for isolating tremelloid yeasts. *Mikrobiologiya* 69 (in press).

Glucuronate-containing agar is recommended for detection of tremelloid yeasts in natural cenoses. Both their

count and diversity revealed on this medium was much higher than on malt agar used commonly.

XXVIII. Department of Biology Faculty of Medicine Masaryk University, Joštova 10, 66243 Brno, Czech Republic, for Yeast Newsletter. Communicated by Marie Kopecká <mkopeccka@med.muni.cz>.

The following are papers published since our last communication.

1. Gabriel, M., Kopecká, M., Svoboda, A., Horký, D. 1996. The effect of Cytochalasin D on actin ring and septum formation in yeast *Schizosaccharomyces versatilis*. 44th Harden conference Biochem. Society Cirencester (UK) 8.-12. 9. Abstract Book p.52, 22.
2. Kopecká, M., Gabriel, M., Svoboda, A. 1996. Study of nuclei and cytoskeleton in yeast actin mutants. *Folia Microbiol.* **41**:284-285.
3. Gabriel, M., Horký, D., Svoboda, A., Kopecká, M. 1996. Morphological and functional restitution of the actin contractile ring after removal of cytochalasin D effects in *Schizosaccharomyces japonicus* var. *versatilis*. *Folia Microbiol.* **41**:280.
4. Svoboda, A., Slaninová, I. 1997. Colocalization of microtubules and mitochondria in the yeast *Schizosaccharomyces japonicus* var. *versatilis*. *Can. J. Microbiol.* **43**:945-953.
5. Kopecká, M., Gabriel, M., Svoboda, A. 1997. Defect in actin cytoskeleton functions in ts actin mutants of budding yeasts - an ultrastructural study. *Folia Microbiol.* **42**:645.

6. Gabriel, M., Kopecká, M., Svoboda, A., Horký, D. 1997. Effect of cytochalasin D on the actin ring and on morphogenesis in the fission yeast *Schizosaccharomyces japonicus* var. *versatilis*. *Folia Microbiol.* **42**:643.
7. Raclavský, V., Gabriel, M., Novotny, R., Kopecká, M., Novotná, J. 1997. Chlorpormazin induces changes in cell wall glucan and chitin content, in cell wall ultrastructure and in polarization of actin patches in *Saccharomyces cerevisiae*. *Folia Microbiol.* **42**:649.
8. Gabriel, M., Horký, D., Svoboda, A., Kopecká, M. 1998. Cytochalasin D interferes with contractile actin ring and septum formation in *Schizosaccharomyces japonicus* var. *versatilis*. *Microbiology (UK)* **144**:2331-2344.
9. Kopecká, M., Gabriel, M. 1998. The aberrant positioning of nuclei and the microtubular cytoskeleton in *Saccharomyces cerevisiae* due to improper actin function. *Microbiology (UK)* **144**:1783-1797.
10. Svoboda, A. 1999. Cytology. In: Forty Years of Activity in Czech and Slovak Yeast Research. Period 1990-1999. P. Biely, editor. Veda, Publ. House of the Slovak Academy of Sciences, Bratislava. pp. 20-29.
11. Slaninová, I., J. Kucsera, and A. Svoboda. 1999. Topology of microtubules and actin in the life cycle of *Xanthophyllomyces dendrorhous* (*Phaffia rhodozyma*). *Antonie van Leeuwenhoek* **75**:361-368.
12. Kopecká, M. 1999. Yeast Protoplasts - model for the study of mechanism of cell wall formation *de novo*. Review article. In: Annual Report of Research Center for Pathogenic Fungi and Microbial Toxicoses Chiba University Japan **2**:47-57.
13. Kopecká, M., Gabriel, M., Raclavský, V. 1999. Actin cytoskeleton and the cell wall assembly in yeast. *Folia microbiol.* **44**:107.
14. Slaninová, I., Sestak, S., Svoboda, A., Farkas, V. Cell wall and cytoskeleton reorganization as the response to hyperosmotic shock in *Saccharomyces cerevisiae*. *Arch. Microbiol.* (in press).

The following are lectures presented since our last communication.

15. Svoboda, A., Slaninová, I., Kopecká, M., Gabriel, M., and Necas, O. 1999. Restoration of the cytoskeleton during yeast protoplast regeneration. In: Proceedings of the 20th International Specialized Symposium on Yeasts: Yeast Cell Surfaces and Membrane Phenomena, ed. Biely, P., Bratislava, May 23 - 27, 1999, p.41.
16. Holubarová, A. and Svoboda, A. 1999. Meiosis and sporulation in fission yeasts: The effect of inhibitors. In: Proceedings of the 20th International Specialized Symposium on Yeasts: Yeast Cell Surfaces and Membrane Phenomena, ed. Biely, P., Bratislava, May 23 - 27, 1999, p.70.
17. Gabriel, M., Ishiguro, J., Kopecká, M., Svoboda, A. 1999. Actin and cytokinesis in fission yeasts. VII. Cytoskeletal club, Vranovská Ves, Czech Republic 17-19.3.1999.
18. Kopecká, M., Takeo, K., Yamaguchi, M., Ohkusu, M., Hata, K., Gabriel, M., Svoboda, A. 1999. Actin cytoskeleton in human fungal pathogen *Cryptococcus neoformans*. VII. Cytoskeletal club, Vranovská Ves, Czech Republic 17 - 19.3.1999.

19. Kopecká, M., Takeo, K., Yamaguchi, M., Ohkusu, M., Hata, K., Gabriel, M., Svoboda, A. 1999. Actin cytoskeleton in two human fungal pathogens *Aureobasidium pullulans* and *Cryptococcus neoformans*. In: Proceedings of the 20th International Specialized Symposium on Yeasts: Yeast Cell Surfaces and Membrane Phenomena, ed. Biely, P., Bratislava, May 23 -27,1999, p.38.
20. Gabriel, M., Ishiguro, J., Kopecká M. 2000. Study of *cps* mutants of *Schizosaccharomyces pombe*. XXVIII. Annual Yeast Conference Smolenice 15.-17. May 2000, pp. 47-48.
21. Kopecká, M., Gabriel, M., Svoboda, A., Takeo, K., Yamaguchi, M., Ohkusu, M., Hata, K., Yoshida, S.: Cytoskeleton and ultrastructure in some human fungal pathogens. XXVIII. Annual Yeast Conference Smolenice 15.-17. May 2000, pp. 45-46.
22. Gabriel, M., Ishiguro, J., Kopecká M. 2000. Cytoskeleton and morphological aberrations of septum formation and cytokinesis in *cps* mutant of fission yeast, *Schizosaccharomyces pombe*. Cytoskeletal club, Vranovská Ves, Czech Republic 15-17.3.2000, p.11.
23. Kopecká, M., Gabriel, M., Svoboda, A., Takeo, K., Yamaguchi, M., Ohkusu, M., Hata, K., Yoshida, S. 2000. Cytoskeleton in some pathogenic fungi. . Cytoskeletal club, Vranovská Ves, Czech Republic 15-17.3.2000, p.12.
24. Kopecká, M., Gabriel, M., Svoboda, A., Takeo, K., Yamaguchi, M., Ohkusu, M., Hata, K., Yoshida, S. 2000. Cytoskeleton in pathogenic fungi. 5.1.2000. Scripta medica Fac. Med. Masaryk Univ. Brno (in the press).

Lectures presented by Marie Kopecká during her 7-months stay as Guest Professor of Chiba University Research Center for Pathogenic Fungi and Microbial Toxicoses, Chiba, Japan 1998-1999.

25. Kopecká, M. 1998. Yeast cell wall and yeast protoplasts. Research center for pathogenic fungi and microbial toxicoses, Chiba University, Japan 8.9.1998.
26. Kopecká, M. 1998. Yeast cytoskeleton. Research center for pathogenic fungi and microbial toxicoses, Chiba University, Japan 27.10.1998.
27. Kopecká, M., Hata, K., Takeo, K. 1998. Searching for an actin cytoskeleton in *Aureobasidium pullulans*. 6th International Symposium of the Mycological Society of Japan Progress in Mycological Sciences in Japan and The United Kingdom, Chiba University Japan, 26-27.11.1998, p. 57.
28. Kopecká, M. 1998. Actin cytoskeleton and cell wall in Yeasts. Research Center for Pathogenic Fungi and Microbial Toxicoses, Chiba University, Chiba, Japan 1.12.1998.
29. Kopecká, M. Actin cytoskeleton in budding and fission yeasts. 1999. Tokyo University Komaba, Meguro-ku, Japan, 9.1.1999.
30. Kopecká, M. 1999. Yeast protoplasts - model for the study of mechanism of cell wall formation *de novo*. Tokyo University, Hongo Campus, Tokyo, Japan 12.1.1999.
31. Kopecká, M. 1999. Actin cytoskeleton in budding and fission yeasts and cell wall formation“. Research Center for Pathogenic Fungi and Microbial Toxicoses Chiba University, Japan 20.1.1999.

The following paper, whose abstract was given in the last issue, has now been published.

1. Lachance, M.A., Starmer, W.T., Bowles, J.M., Phaff, H.J., and Rosa, C.A. 2000. Ribosomal DNA, species structure, and biogeography of the cactophilic yeast *Clavispora opuntiae*. *Can. J. Microbiol.* **46**:195-210.

The following paper is in press.

2. M.A. Lachance, A. Pupovac-Velikonja, S. Natarajan, and B.Schlag-Edler. 2000. Nutrition and phylogeny of predacious yeasts. *Can. J. Microbiol.* **46**:495-505.

Yeast predation was studied with respect to the range of its distribution among ascomycetous yeasts, the range of yeast species that can be affected, and nutritional aspects of the phenomenon. The yeasts identified as predators belong to the *Saccharomycopsis* clade as defined on the basis of rDNA sequence relatedness. The 11 recognized species in the clade, plus three undescribed but related *Candida* species, were shown to be incapable of utilizing sulfate as sole source of sulfur, and all but two (*Saccharomycopsis capsularis* and *Saccharomycopsis vini*) were observed to penetrate and kill other yeasts under some conditions. Other unrelated sulfate transport-deficient yeasts (strains in the genera *Pichia* and *Candida* and the two known species of *Starmera*) are not predacious. The predacious species vary considerably as to the optimal environmental conditions that favour predation. Some

are inhibited by the presence of rich nitrogenous nutrients, organic sulfur compounds, or higher concentrations of ammonium nitrogen, whereas other species may be stimulated under the same conditions. An attempt was made to correlate prey susceptibility to the excretion of substances that stimulate the growth of predators, but no correlation was detected between the two phenomena. The range of susceptible prey covers both ascomycetes and basidiomycetes, and includes *Schizosaccharomyces pombe*, which was previously thought to be immune. The achlorophyllous alga *Prototheca zopfii* is not killed by predacious yeasts, but the initial steps of penetration have been observed in some cases. Predacious species attack other predacious species, and in some cases, young cultures may penetrate older cultures of the same strain.

The following presentations will be given at the 10th ISY, Papendal (Arnhem), The Netherlands, in August.

3. Marinoni, G., Kwon, S., and Lachance, M.A. 2000. The sexual cycle of giant-spored *Metschnikowia* species.
4. Lachance, M.A. 2000. Yeast biodiversity, how much and how many?

Network: Yeasts in Food and Beverages
Publications regarding in particular “Yeasts in fermented beverages”
Communicated by P. Romano <pot2930@iperbole.bologna.it>

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

1. Molnár, O., Messner R., Prillinger H., Stahl U., Slavikova E. 1995. Genotypic identification of *Saccharomyces* species using Random Amplified Polymorphic DNA analysis. *System. Appl. Microbiol.* **18**:136-145.

According to different molecular approaches the genus *Saccharomyces* was divided recently into 10 genotypically distinct species (*S.bayanus*, *S. castellii*, *S.cerevisiae*, *S.dairensis*, *S.exiguus*, *S.kluyveri*, *S.paradoxus*, *S.pastorianus*, *S.servazzii*, *S.unisporus*). This was corroborated by Random Amplified Polymorphic DNA – Polymerase Chain Reaction (RAPD-PCR) analysis in the present paper. Thirty two strains including the type strain of 20 *Saccharomyces* species defined originally by phenotypic characteristics (e.g. *S.chevalieri*, *S.diastaticus*, *S.ellipsoideus*) clustered with the

pattern of *S.cerevisiae*, fourteen (e.g. type strains of *S.globosus*, *S.heterogenicus*, *S.inusitatus*) with the pattern of *S.bayanus*, six including the type strains of *S.carlsbergensis* and *S.monacensis* with the pattern of *S.pastorianus* and two with the pattern of *S.paradoxus*. Two further strains isolated newly were identified to belong to *S.paradoxus*. In comparison with nuclear DNA/DNA hybridization or electrophoretic karyotyping, RAPD-PCR analysis turned out to be a simple and reliable method to separate *Saccharomyces* species at the genotypic level. In contrast to phenotypic characters genotypic

identification using RAPD-PCR analysis guarantees species specificity if type strains are included in the investigation. The ten *Saccharomyces* species arising from RAPD-PCR analysis are differentiated from each other to the maximal extent with

exception of the relationship between *S. bayanus* and *S.pastorianus*. In this case, the estimated similarity value of 45% is significantly higher than the background noise (0-20%), but less than the values within species (83 to 100%).

2. Molnár, O., Messner R., Prillinger H., Sheide K., Stahl U., Silberhumer H., Wunderer W. 1995. Genotypic identification of *Saccharomyces* strains used in beverage industry by means of Random Amplified Polymorphic DNA-Polymerase Chain Reaction (RAPD-PCR). *Mitteil. Kloster.* **45**:113-122.

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

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

France Institut des Produits de la Vigne, Laboratoire de Microbiologie et Technologies des Fermentations, INRA, 2 place Viala, 34060 Montpellier. Communicated by J.M. Sablayrolles <sablayro@ensam.inra.fr>.

1. Vidal S., Salmon J.M., Williams P., Pellerin P. 1999. *Penicillium daleae*, a soil fungus able to degrade Rhamnogalacturonan II, a complex pectic polysaccharide. *Enz. Microb. Technol.* **24**:283-290.

A strain of *P. daleae* has been isolated from a forest soil sample for its ability to degrade monomeric rhamnogalacturonan-II (mRG-II), a complex polysaccharide ubiquitous in the primary plant cell wall. This unusual pectic polysaccharide is resistant to all known pectinolytic enzymes used in the fruit- and vegetable-processing industry. *P. daleae* has been cultured in minimal medium supplemented with 0.5% mRG-II as the sole carbon source. *P. daleae* growth was

supported by the degradation of 75% of the initial mRG-II. Sequential degradation of mRG-II led to a resistant core after 31 days of culture, representing 25% of the initial molecule which has been fully characterized. *P. daleae* is a potential source of new pectinases whose mode of action requires further elucidation. Such enzymes seemed to be exposed to the surface of fungal cell walls or were present in the periplasmic compartment.

2. Fornairon C., Mazauric J.P., Salmon J.M., Moutounet M. 1999. Observations on the oxygen consumption during maturation of wines on lees. *Journal International des Sciences de la Vigne et du Vin*, **33**(2):79-86.

Traditional enological practices ("Bâtonnage" or "microoxygénation" techniques) during wine aging on yeast lees include limited repetitive additions of small amounts of oxygen to the wines. Such empirical practices are generally associated with a limited homogenisation of wine and lees. In this study, the potential relationship between oxygen consumption and the presence of wine lees during wine aging was investigated. Strong oxygen uptake rates by yeast lees were observed during wine aging at 16°C on total yeast lees obtained after fermentation of either synthetic medium or red and white grape musts. These specific oxygen utilization rates by yeast lees is always comprised between 3 and 11 $\mu\text{g O}_2 \text{ h}^{-1} 10^{-9}$ cells from the second to the sixth month of aging. The initial levels

of specific oxygen utilization rates and the time-decay of these rates along wine aging were very dependent on yeast strains. However such oxygen utilization rates by yeast lees could be responsible for the total dissolved oxygen depletion from wines in less than 20 hours at 16°C during aging on total lees. Such results were of particular importance to evaluate the exact timing of oxygen additions during wine aging on lees. Further experiments had to be done to determine the biological or chemical nature of such oxygen consumption by lees. Such oxygen consumption by yeast lees may lead to final reaction products which may exert strong organoleptic effects on the final quality of wines.

3. Guo W., Salmon J.M., Baumes R., Tapiero C., Gunata Z. 1999. Purification and characterization of an *Aspergillus niger* apiosidase from an enzyme preparation. *J. Agric. Food Chem.* **47**(7):2589-2593.

A beta-apiosidase was isolated and purified to electrophoretic homogeneity from an enzyme preparation (Klerzyme 2000), through ammonium sulfate precipitation, gel filtration chromatography, ion-exchange chromatography, and

HPLC on ion exchange and exclusion columns. The enzyme was not inhibited by glucose and ethanol. This enzyme hydrolyzed the intersugar linkages of apiofuranosylglucosides, aroma precursors from grape.

4. Salmon J.M., Fornairon-Bonnefond C., Mazauric J.P., Moutounet M. 2000. Oxygen consumption by wine lees : impact on lees integrity during wine ageing. *Food Chem.* (in press).

During wine ageing on lees, periodic stirring of the lees and repetitive additions of small amounts of oxygen to the wines is generally performed on a traditional and empirical basis. Although this technological step is thought to have a significant impact on the organoleptic composition of the final product, few reports dealt with the effect of the interaction between oxygen and lees during such a process. During simulation of wine ageing on lees, we demonstrated that some membrane lipids of yeast lees in contact with dissolved oxygen, even at very low concentration, were submitted to a mild oxidation. These oxidation reactions led first to the production

of lipid peroxides and then to the production of unknown end products, which were suspected to be kept within the membranes of lees. These oxidation reactions were primarily due to the production of reactive oxygen species (ROS), and totally explained the capacity of yeast lees to consume oxygen during wine ageing. The lipid oxidations within the plasma membrane were responsible for strong modifications of plasma membrane order. One can expect that some of the final products of oxidation reactions may play a favourable role in the organoleptic equilibrium of wines aged on lees.

5. Dequin S., Baptista E., Barre P. 1999. Acidification of grape musts by *S. cerevisiae* wine yeast strains genetically engineered to produce lactate. *Am. J. Enol. vit.* **50** (1):45-50.

Engineered *Saccharomyces cerevisiae* strains overexpressing a bacterial lactic dehydrogenase (LDH) have been described to perform mixed lactic acid-alcoholic fermentation under laboratory conditions (7). The acidification properties of these strains might be of great value for fermented beverages and especially in enology to solve problems of insufficient acidity in hot regions. To assess the relevance of this model during alcoholic fermentation under enological conditions, eight wine strains expressing the LDH gene on a multicopy plasmid were constructed. The level of L(+) lactic acid produced and the resulting acidification were shown to be influenced by the strain and to a greater extent by the grape must composition. Wines obtained by lactic acid-alcoholic fermentation of seven grape musts contained 2.6 to 8.6 g/L of

L(+) lactic acid. In comparison with classic alcoholic fermentation, lactic acid-alcoholic fermentation resulted in a decrease of 0.2 to 0.35 pH units in wines containing 5 to 8 g/L of lactic acid. Two grape musts with moderate or low acidity levels (pH 3.36 and pH 3.75) were acidified effectively (50% increase in total acidity, 0.17 and 0.27 pH unit decrease) by the production of 5 g/L of L(+) lactic acid by an engineered wine yeast strain. A reduction of 0.25 percent (vol/vol) of alcohol was also observed as a result of the diversion of carbon towards L(+) lactic acid. The volatile acidity was unchanged in the wine obtained with the engineered strain compared to the control strain. Despite a slower fermentation rate, the ability to ferment grape musts and the growth characteristics were not affected.

6. Remize F., Roustan J.L., Sablayrolles J.M., Barre P. and Dequin S. 1999. Glycerol overproduction by engineered *Saccharomyces cerevisiae* wine yeast strains leads to substantial changes in by-product formation and to a stimulation of fermentation rate in stationary phase. *Appl. Environ. Microbiol.* **65**(1):143-149.

Six commercial wine yeast strains and three non-industrial strains (two laboratory strains and one haploid derived from a wine yeast strain) were engineered to produce high amounts of glycerol with lower ethanol yield. Overexpression of the *GPD1* gene encoding a glycerol-3-phosphate dehydrogenase resulted in a 1.5- to 2.5-fold increase in glycerol production and a slight decrease in ethanol formation under conditions simulating wine fermentation. All the strains overexpressing *GPD1* produced a higher amount of succinate and acetate, with marked differences in the level of these compounds between industrial and non-industrial engineered strains. Acetoin and 2,3-butanediol formation was

enhanced with significant variation between strains and with the level of glycerol produced. Wine strains overproducing glycerol at moderate levels (12 to 18 g/l) almost completely reduced acetoin to 2,3-butanediol. A lower biomass concentration was attained by *GPD1* overexpressing strains, probably due to high acetaldehyde production during the growth phase. Despite the reduction in cell number, complete sugar exhaustion was achieved during fermentation in sugar-rich medium. Surprisingly, the engineered wine yeast strains exhibited a significant increase in the fermentation rate in stationary phase, which reduced the time of fermentation.

7. Remize F., Sablayrolles JM and Dequin S. 2000. Re-assessment of the influence of yeast strain and environmental factors on glycerol production in wine. *J. Appl. Microbiol.* **87** (In press).

Increasing glycerol production is a matter of concern for wine makers to improve the quality of certain wines. We have compared the impact of strain and relevant environmental factors influencing glycerol production under the same conditions i.e. standardized conditions simulating enological fermentation. Glycerol production of 19 industrial wine strains ranged from 6.4 to 8.9 g l⁻¹ and varied significantly between strains. The production of acetate and succinate was also found to differ substantially depending on the strain but no significant strain-dependent variation was observed for acetaldehyde. Interestingly, high glycerol production was not correlated to high production of acetate or acetaldehyde that

are undesirable in wine. A detailed study with two low- or two high-glycerol producers strains showed that temperature and the initial concentration of nitrogen had little effect on the amount of glycerol formed, although agitation or a nitrogen source composed mainly of ammoniacal nitrogen slightly enhanced glycerol production. The influence of environmental factors remained minor while the predominant factor for glycerol variability in wine was attributed to the strain. Taking into account winemaking constraints, the results indicate that achieving high glycerol content in wine requires the selection or improvement of yeast strains rather than the control of growth and cultivation conditions.

8. Remize F., Andrieu E. , Dequin S. 2000. Engineering of the pyruvate dehydrogenase by-pass in *S. cerevisiae*. Role of the cytosolic Mg²⁺ and mitochondrial K⁺ acetaldehyde dehydrogenases Ald6p and Ald4p in acetate formation during alcoholic fermentation. *Appl. Environ. Microbiol.* In press.

Acetic acid plays a crucial role in the organoleptic balance of many fermented products. We have investigated the factors controlling the production of acetate by *S. cerevisiae* during alcoholic fermentation by metabolic engineering of the enzymatic steps involved in its formation and its utilisation. The impact of reduced pyruvate decarboxylase (PDC), limited acetaldehyde dehydrogenase (ACDH), or increased acetoacetyl CoA synthetase (ACS) levels in a strain derived from a wine yeast strain was studied during alcoholic fermentation. In the strain deleted for the PDC1 gene exhibiting 25% of PDC activity of the wild type, no significant differences were observed in the acetate yield or in the amount of secondary metabolites formed. A strain overexpressing ACS2 and displaying a 4- to 7-fold increase in acetoacetyl CoA synthetase activity did not produce reduced acetate levels. In contrast,

strains disrupted for one or two copies of ALD6 encoding the cytosolic Mg²⁺-activated NADP-dependent Acdhp (exhibiting 60 and 30% of wild type ACDH activity) showed a substantial decrease in acetate yield (the acetate production was 75 and 40% of wild-type respectively). This decrease was associated with a rerouting of carbon flux towards the formation of glycerol, succinate and butanediol. The deletion of ALD4 encoding the mitochondrial K⁺-activated NAD(P)-linked ACDH had no effect on the amount of acetate formed. In contrast, a strain lacking both Ald6p and Ald4p exhibited a long delay in growth and acetate production, suggesting that Ald4p can partially replace Ald6p isoform. Moreover, the ald6 ald4 double mutant was still able to ferment high sugar amounts and to produce acetate, suggesting the contribution of other member(s) of the ALD family.

9. Julien A., Roustan J.L., Dulau L., Sablayrolles J.M. 2000. Comparison of nitrogen and oxygen demands of enological yeasts. Technological consequences. *Am. J. Enol. Vitic.* (In press).

Nitrogen and oxygen requirements of commercial yeasts were compared. Nitrogen demands were measured by calculating the amounts of ammoniacal nitrogen necessary to keep the fermentation rate constant during the stationary phase. Oxygen demands were studied by (i) running anaerobic fermentations and (ii) measuring the effect of adding 1 mg/l oxygen at the beginning of the stationary phase. Substantial

differences were found and their technological impact was discussed. Because of the importance of nitrogen and oxygen in enology, using such tests may be of great interest, especially when there are risks of (i) slow fermentations, due to nitrogen deficiencies or (ii) sluggish fermentations, mainly due to oxygen limitations.

10. Rachidi N., Barre P., Blondin B. 1999. Multiple Ty mediated chromosomal translocations lead to karyotype changes in a *Saccharomyces cerevisiae* wine yeast. *Mol. Gen. Gen.* **261**:841-850.

Enological *S. cerevisiae* yeasts display high chromosome length polymorphism but the molecular bases of this phenomenon have not yet been clearly defined. In order to gain further insight into the molecular mechanisms responsible for the karyotype alterations, we examined the chromosomal constitution of a strain shown to possess aberrant chromosomes. Our data revealed that the analyzed strain was bearing four rearranged chromosomes resulting from two

reciprocal translocations between chromosomes III and I, and chromosomes III and VII. The size of the chromosomal fragments exchanged through the translocations ranged from 40 to 150 kb. Characterization of the breakpoints indicated that the translocations involved the RAHS of chromosome III, a transposition hot-spot on the right arm of chromosome I and a region of the left arm of chromosome VII. An analysis of the junctions showed that Ty were present on all of them and

suggested that the translocations result from recombination between Ty transposable elements. The evidence of multiple translocations through Ty elements in a single strain suggests

that spontaneous Ty driven rearrangement could be quite common and play a major role in the alteration of karyotypes in natural or industrial yeasts.

11. Rachidi N., Martinez M.J., Barre P., Blondin B. 2000. *Saccharomyces cerevisiae PAU genes are induced by anaerobiosis. Molecular Microbiol.* **35**:1421-1430.

Saccharomyces cerevisiae PAU genes constitute the largest multigene family in yeast, with 23 members located mainly in subtelomeric regions. The role and regulation of these genes was previously unknown. We detected *PAU* gene expression during alcoholic fermentation. An analysis of *PAU* gene regulation using *PAU-lacZ* fusions and Northern analyses revealed that they were regulated by anaerobiosis. *PAU* genes display, however, different abilities to be induced by anaerobiosis and this appears to be related to their chromosomal localization: two subtelomeric copies are more weakly inducible

than an interstitial one. We show that *PAU* genes are negatively regulated by oxygen and repressed by heme. Examination of *PAU* gene expression in *rox1* and *tup1* strains indicates that *PAU* repression by oxygen is mediated by an unknown, heme-dependent pathway, which does not involve the Rox1p anaerobic repressor but requires Tup1p. Given the size of the gene family, *PAU* genes could be expected to be important during yeast life and some of them probably help the yeast to cope with anaerobiosis.

12. Rachidi N., Barre P., Blondin B. 2000. Examination of the transcriptional specificity of an enological yeast. A pilot experiment on the chromosome-III right arm. *Curr Genet.* **37**(1):1-11.

The adaptation of yeasts to industrial environments is thought to be largely dependent on gene expression specificity. To assess the transcriptional specificity of an enological strain, we performed a pilot experiment and examined the transcript level of 99 ORFs of the chromosome III right arm with two strains, an enological-derived strain and a laboratory strain, grown under different physiological conditions : respiration, standard alcoholic fermentation and enological alcoholic fermentation. The use of 99 single ORF-derived probes led to the detection of 49 transcripts, most of which were present at low levels and not regulated. Ethanol respiration induced

transcripts in a similar manner with both strains. While standard alcoholic fermentation led to only minor regulations, the enological fermentation conditions triggered a transcriptional response in the enological-derived strain alone. The known or predicted functions of several genes induced under enological conditions is related to alcoholic fermentation or stress, suggesting that their specific induction could reflect adaptation of the strain to the enological environment. Our data suggest that systematic transcriptional studies are an effective way to assess the molecular basis of yeast adaptation to industrial environments.

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1. Moroz O.M., Gonchar M.V., Sibirny A.A. 2000. Efficient bioconversion of ethanol to acetaldehyde using a novel mutant strain of the methylotrophic yeast *Hansenula polymorpha*. *Biotechnol. Bioeng.* **68**:44-51.

We report the isolation of mutant strains of the methylotrophic yeast *Hansenula polymorpha* that are able to efficiently oxidize ethanol to acetaldehyde in an intact cell system. The oxidation reaction is catalyzed by alcohol oxidase (AOX), a key enzyme in the methanol metabolic pathway that is typically present only in *H. polymorpha* cells growing on methanol. At least three mutations were introduced in the strains. Two of the mutations resulted in high levels of AOX in glucose-grown cells of the yeast. The third mutation introduced a defect in the cell's normal ability to degrade AOX

in response to ethanol; thus, stabilizing the enzyme in the presence of this substrate. Using these strains, conditions for bioconversion of ethanol to acetaldehyde were examined. In addition to pH and buffer concentration, we found that the yield of acetaldehyde was improved by the addition of the proteinase inhibitor phenylmethylsulfonyl fluoride (PMSF) and by permeabilization of the cells with digitonin. Under optimal shake-flask conditions using one of the *H. polymorpha* mutant strains, conversion of ethanol to acetaldehyde was nearly quantitative.

- Gonchar M.V., Maidan M.M., Moroz O.M., Woodward J.R., Sibirny A.A. 1998. Microbial O₂ - and H₂O₂-electrode sensors for alcohol assays based on the use of permeabilized mutant yeast cells as the sensitive bioelements. *Biosens. Bioelectron.* **13**:945-952.

Two types of alcohol-specific microbial/electrochemical biosensors have been developed using specially constructed mutant cells of the methylotrophic yeast *Hansenula polymorpha*. The cells were immobilized in a calcium alginate gel, and placed between two membranes on the surface of oxygen or hydrogen peroxide-electrodes. The O₂ electrode based biosensor contained mutant cells with strongly elevated alcohol oxidase activity. The peroxide electrode based biosensor consisted of catalase-defective mutant cells which produce hydrogen peroxide in the presence of alcohol. Both types of mutant cells were used in permeabilized form in order to release some components of the cellular respiration system, thus increasing the selectivity of the cellular respiration response to alcohol (cell/O₂-biosensor). Permeabilization also

increased sensitivity of the signal and shortened the response time (cell/H₂O₂-biosensor). Cell/O₂ biosensors were linear up to 1.2 mM for ethanol and 0.35 mM for methanol, cell/H₂O₂ biosensors were linear up to 4.0 mM for ethanol, and 1.2 mM for methanol. Results were reproducible, sample pretreatment was not required, and the sensors exhibited good operational and storage stability. The use of sucrose, dulcitol or inositol during the preparation of the sensors resulted in increased stability of cells during their lyophilization and storage in the dried state. Both biosensors had similar selectivity towards alcohols in the order of methanol (100%), ethanol (21%), and formaldehyde (12%). No signal was observed with glucose or glycerol as substrates.

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- Deak T., Chen J., Golden D.A., Tapia M.S., Tornai-Lehoczki J., Viljoen B.C., Wyder M.T., Beuchat L.R., in publication.

Comparison of dichloran glycerol (DG18) agar with general purpose mycological media for enumerating food-spoilage yeasts. DG18, originally developed to enumerate moderately xerophilic molds, are being used as a general medium to enumerate foodborne molds and yeasts. A collaborative study, with the participation of seven laboratories, was undertaken to compare DG18 agar with rose bengal chloramphenicol (DRBC) agar, tryptone glucose yeast extract chloramphenicol (TGYC) agar and plate count

chloramphenicol (PCAC) agar for enumerating fourteen species of common food-spoilage yeasts. There were no significant differences in mean CFU values between DRBC, PCAC and TGYC agar, while DG18 agar produced significantly less and smaller colonies. Growth of certain yeast species was particularly inhibited on DG18 agar. It cannot be recommended as a general enumeration medium for foodborne yeasts.

- Deak T et al., in publication.

Comparison of media for supporting colony formation by lyophilized yeasts. Evaluating earlier collaborative studies using lyophilized cultures, a question raised whether freezing and desiccation injuries of yeast cells would have an impact on subsequent growth on enumeration media. A collaborative

study was made to compare the performance of DRBC, TGYC, PCAC, DG18 agar, as well as acidified potato dextrose agar (APDA) and orange serum agar (OSA) for enumeration of fourteen strains of yeasts lyophilized in skim milk. Statistical analysis of results is in progress.

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- Romano P., Marchese, Laurita C., Turbanti L., Salzano G. 1999. Biotechnological suitability of *Saccharomyces ludwigii* for fermented beverages. *World J. Microbiol. Biotechnol.* **15**:451-454.

Nineteen *Saccharomyces ludwigii* strains were tested for the production of secondary products in grape must fermentation. A predominant metabolic pattern characterized by high production of isobutyl alcohol, acetoin and ethyl acetate was obtained. The occurrence of some strains producing enhanced amounts of these compounds suggests a potential utilization of this species for industrial applications. Feijoa juice was inoculated with a selected *S. ludwigii* strain in comparison to a control strain of *S. cerevisiae* and evaluation of the fermented products was carried out by 30

consumers with respect to the odour, flavour and taste. The sample fermented by *S. ludwigii* was characterized by a fresh odour with a fruity flavour, identified as flavour of apple and kiwi. This product was compared to apple juice, with a more acid taste. Despite the high concentrations of acetic acid, this beverage might be considered a potential summer refreshing drink, addressed to a target of consumers, who prefer fruit drinks leaving a slightly acid and little sugary taste in the mouth.

2. Reyes A., Paraggio M., Pesole G., Romano P. 1999. Genetic characterization of dsRNA plasmids in *Saccharomyces cerevisiae* wine yeast. *Food Technol. Biotech.* **37**(4):229-234.

Yeasts are responsible for the production of fermented foods and, in particular, *Saccharomyces cerevisiae* is considered as the principal wine yeast. The existence of dsRNA plasmids in the cytoplasm of yeast has been correlated with the production of a killer toxin lethal to other strains of the same species, making this character of great interest in enology. In the present study, we have analysed a total of 56 strains of *S. cerevisiae* from two different geographical areas of Italy: 27 of them came from the Basilicata region (South) while the remaining 29 strains were from Northern regions. These strains were assayed for the presence of dsRNA plasmids in their cytoplasm. The same strains were also tested for the production of the killer toxin. On the basis of length of

dsRNAs, most strains were classified as K₂-K₃. The K₂-K₃ type was much less common and K₁ killer was absent. These results were corroborated by plate assay and have been explained as a consequence of the pH of the fermenting musts. The existence of neutral strains that showed immunity to killer toxins has been mainly correlated to variation in length in the M genome of dsRNAs. However, the absence of viral particles or the M genome characterised most sensitive strains, while in some cases small M genomes were present. In those cases in which the M genome was present and the killer activity was lost, insertions/deletions affecting the killer preprotoxin gene has been suggested.

3. Paraggio M., Fiore C., Romano P. Yeast flocculation influence on volatile bouquet substances of wine. *Alcologia, European J. Alcohol Studies*, in press.

Forty flocculent strains of *Saccharomyces cerevisiae* were tested in Trebbiano grape must at 18°C for the flocculation and the production of higher alcohols, acetic acid, ethyl acetate and acetaldehyde. A significant variability in strain flocculation degree was determined, allowing the discrimination of the strains in 6 different groups. Of the 40 strains, 12 possessed the maximum flocculation level (F6), yielding on the flask bottom a coherent layer, which, after shaking, did not yield turbidity. As regards the fermentation behaviour, all the strains exhibited a similar fermentation energy and no significant differences were recorded between the different flocculent phenotypes. Regarding by-product formation, the flocculent strains exhibited a uniform behaviour in the production levels of acetaldehyde, ethyl acetate, whereas n-propanol and isobutanol were produced with significant variability, respectively from 5.98 to 26.18 mg/L for n-propanol and from 9.45 to 34.38 mg/l for isobutanol. Other compounds,

such as isoamyl alcohol and acetic acid, were formed with a wide variability, the amounts produced depending on the strain which performed grape must fermentation. Isoamyl alcohol was formed from 47.15 to 127.86 mg/L and acetic acid from 103 to 694.56 mg/l. The results of this work revealed that strain metabolic variability led to the individuation of different behaviours correlated with differing levels of flocculation. The major difference was observed in the production of isoamyl alcohol and acetic acid, the low productions being always correlated to a high degree of strain flocculation. It appears that the choice of the flocculent strain determines not only a major efficiency in the technological removal of yeast cells, but also different proportions and concentrations of some volatile compounds. Studies are in progress in our laboratory to ascertain on pilot scale fermentation the correlation of flocculent phenotypes with strain aromatic properties.

The following conference abstracts have been published recently.

4. Romano P., Marchese R., Paraggio M., Caruso M., Lipani G., Capece A. 1999. Phenotypic and genotypic variation of *Saccharomyces cerevisiae* natural strains from Aglianico wine. Proc. XIX Int. Conf. "Yeasts Genetics and Molecular Biology", Rimini (Italy) 25-30 May, *Curr. Genetics* **35**(3-4):371.
5. Romano P., Salzano G., Caruso M., Marchese R., Paraggio M. 1999. Fingerprinting of yeast starter cultures by metabolic/molecular characterization. Proc. ECB9, Bruxelles (Belgium) July 1999.
6. Romano P., Brandolini V., Borsetti E., Marchese R. 1999. Analysis of copper-resistance in *Saccharomyces cerevisiae* wine yeast. Proc. Congr.. Sydney (Australia) 16-20 August, 252.
7. Sipiczki M., Paraggio M., Miklos I., Antunivics Z., Romano P. 1999. Isolation and characterisation of Tokay wine yeasts. Proc. Congress.. Sydney (Australia) 16-20 August, 251.
8. Romano P. 1999. Selection of starter cultures for winemaking. Proc. 13th Int. Congr. Hungarian Soc. Microbiol., Budapest (Hungary) 30 August- 1 September.

9. Greco I., Romano P. 1999. Molecular biology strategies as biotechnological tools in wine quality. Proc. 13th Int. Congr. Hungarian Soc. Microbiol., Budapest (Hungary) 30 August- 1 September.
10. Bellavia C., Romano P., Capece A., Caruso M. 1999. Differenziazione di lieviti vinari isolati dall'area del Vulture: analisi per due anni consecutivi. Proc. 5° Naz. Congr. Biodiversità e Sistemi Ecocompatibili, Caserta (Italy) 9-10 September.
11. Romano P., Caruso M., Fiore C., Ricciardi A. 1999. Characterization of apiculate strains isolated from Aglianico grape musts from Basilicata region in South Italy. Proc. 7th Int. Conf. Food Microb. Hyg. (ICFMH), Veldhoven (The Netherlands), 13-17 September, pp. 680-683.

The Netherlands Department of Food Technology and Nutritional Sciences, University and Research Centre, Wageningen. Communicated by P. Breeuwer <pieter.breeuwer@micro.fdsi.wau.nl>.

1. Breeuwer, P., Abee T. 2000. Assessment of the intracellular pH of yeast cells employing fluorescence ratio imaging analysis. J. Microbiol. Methods **39**:253-264.

The intracellular pH (pH_m) of *Saccharomyces cerevisiae* was measured employing fluorescence ratio imaging microscopy (FRIM). The yeast cells were fluorescently labeled with the pH dependent probe 5(and-6)-carboxyfluorescein (cF) or 5(and-6)-carboxyfluorescein succinimidyl ester (cFSE), and subsequently attached to ferric nitrate pretreated glass slides. The labeled and adhered cells could still divide and were metabolically active. Measurement of the pH_m was performed during continuous perfusion of the cells with buffer or medium. Cells labeled with cF are highly fluorescent and in non-energized cells the pH_m could be easily measured. However, in

energized yeast cells cF was accumulated in the vacuoles and/or exported to the extracellular environment, most likely by an energy-dependent transport system, thus limiting the time period over which the pH_m can be effectively measured. Therefore, cFSE (which conjugates with aliphatic amines in the cytoplasm) was applied to prevent translocation of fluorescent probe to the vacuole and/or extracellular environment. The continuous perfusion in combination with the cFSE labeling of the immobilized cells was successfully applied to determine the effect of low and high pH, and addition of glucose on the pH_m of individual yeast cells over a long time period.

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1. Van der Westhuizen T.J., Augustyn O.P.H., Pretorius I.S. 1999. The value of long-chain fatty acid analysis, randomly amplified polymorphic DNA and electrophoretic karyotyping for the characterization of wine yeast strains. S. African J. Enol. Viticulture **20**:3-10.

Wine yeast strains of *Saccharomyces* had previously been classified into several different species or varieties. This classification system was based mainly on sugar fermentation and assimilation patterns. Subsequently, most of these species were reclassified as *Saccharomyces cerevisiae*. The assignment of the majority of wine yeast strains to a single species does, however, not imply that all strains of *S. cerevisiae* are equally suitable for wine fermentation. These physiological strains of *S. cerevisiae* differ significantly in their fermentation performance and their ability to contribute to the final bouquet and quality of the various types of wine and distillates. Therefore, to ensure strain authenticity, security and proper

strain management, it is of cardinal importance to have reliable taxonomic techniques available to identify and characterize individual strains of commercial cultures. In this study, 18 commercial wine yeast strains were characterized in order to evaluate and compare three taxonomic techniques, namely long-chain fatty acid analysis, randomly amplified polymorphic DNA (RAPD) and electrophoretic karyotyping. As a single identification technique, electrophoretic karyotyping seems to be the most useful method for routine fingerprinting of wine yeast strains. However, we propose that the combined use of these three techniques provides the most reliable means of differentiating among commercial wine yeast strains.

2. Schoeman H., Vivier M.A., Dicks L.M.T., Pretorius I.S. 1999. The development of bactericidal yeast strains by expressing the *Pediococcus acidilactici* pediocin gene (*pedA*) in *Saccharomyces cerevisiae*. *Yeast* 15:647-656.

The excessive use of sulphur dioxide and other chemical preservatives in wine, beer and other fermented food and beverage products to prevent the growth of unwanted microbes holds various disadvantages for the quality of the end-products and is confronted by mounting consumer resistance. The objective of this study was to investigate the feasibility of controlling spoilage bacteria during yeast-based fermentations by engineering bactericidal strains of *Saccharomyces cerevisiae*. To test this novel concept, we have successfully expressed a bacteriocin gene in yeast. The pediocin operon of *Pediococcus acidilactici* PAC1.0 consists of four clustered genes, namely *pedA* (encoding a 62 amino acid precursor of the PA-1 pediocin), *pedB* (encoding an immunity factor), *pedC* (encoding a PA-1 transport protein) and *pedD* (encoding a protein involved in the transport and processing of PA-1). The *pedA* gene was inserted into a yeast expression/secretion cassette and introduced as a multicopy episomal plasmid into a laboratory strain (Y294) of *S. cerevisiae*. Northern blot analysis confirmed that the *pedA* structural gene in this construct ($ADHI_P$ - $MFaI_S$ -*pedA*- $ADHI_T$,

designated *PEDI*), was efficiently expressed under the control of the yeast alcohol dehydrogenase I gene promoter ($ADHI_P$) and terminator ($ADHI_T$). Secretion of the *PEDI*-encoded pediocin PA-1, was directed by the yeast mating pheromone α -factor's secretion signal ($MFaI_S$). The presence of biologically active antimicrobial peptides produced by the yeast transformants was indicated by agar diffusion assays against sensitive indicator bacteria (e.g., *Listeria monocytogenes* B73). Protein analysis indicated the secreted heterologous peptide to be approximately 4.6 kDa which conforms to the expected size. The heterologous peptide was present at relatively low levels in the yeast supernatant but pediocin activity was readily detected when intact yeast colonies were used in sensitive strain overlays. This study could lead to the development of bactericidal yeast strains where *S. cerevisiae* starter cultures not only conduct the fermentations in the wine, brewing and baking industries but also act as biological control agents to inhibit the growth of spoilage bacteria.

3. Pretorius I.S., Van der Westhuizen T.J., Augustyn O.P.H. 1999. Yeast biodiversity in vineyards and wineries and its importance to the south african wine industry. A review. *S. African J. Enol. Viticulture* 20:61-74.

The art of winemaking is as old as human civilization and the use of yeast in this complex ecological and biochemical process dates back to ancient times. Traditionally, yeasts associated with grape berries were simply allowed to ferment the sugars to ethanol, carbon dioxide and other minor, but important, metabolites. Spontaneous fermentations are still being used in *boutique* wineries that depend more on vintage variability. Various microbes found on the surface of grape skins and the indigenous microbiota associated with winery surfaces participate in these natural wine fermentations. Yeasts of the genera *Kloeckera*, *Hanseniaspora* and *Candida* predominate in the early stages, followed by several species of *Metschnikowia* and *Pichia* (including those species that were previously assigned to the genus *Hansenula*) in the middle stages when the ethanol rises to 3-4%. The latter stages of natural wine fermentations are invariably dominated by the alcohol-tolerant strains of *Saccharomyces cerevisiae*. However, other yeasts, such as species of *Brettanomyces*, *Kluyveromyces*, *Schizosaccharomyces*, *Torulasporea* and *Zygosaccharomyces* also may be present during the fermentation and can occur in the resultant wine. By contrast, the rule, rather than the exception, for modern wineries depending on reliable fermentation and the production of wines with predictable quality, is the use of specially selected starter cultures of *Saccharomyces*. However, the use of such cultures

may not necessarily prevent the growth and metabolic activity of indigenous, winery associated strains of *S. cerevisiae* or other wild yeasts such as *Kloeckera apiculata*, *Hanseniaspora uvarum*, *Candida stellata* and *Torulasporea delbrueckii*. It is therefore clear that both spontaneous and inoculated wine fermentations are affected by the diversity of yeasts associated with the vineyard (natural habitat) and winery (man-made niche). In light of this, focused taxonomic surveys within an ecological framework are essential to preserve and exploit the hidden oenological potential of the untapped wealth of yeast biodiversity in our wine-producing regions. To achieve this, yeast taxonomists need to continue to isolate and characterize new yeast species and strains, while wine microbiologists develop improved identification techniques that differentiate more efficiently among individual strains. At the same time such biological surveys will complement strain development and the current international effort of molecular biologists to assign a biological function to the products of each of the 6000 genes identified by computer analysis of the nucleotide sequence of the 16 chromosomes of a laboratory strain of *S. cerevisiae*. Furthermore, only when we have a much better understanding of yeast biodiversity, biogeography, ecology and the interaction within yeast communities will we be able to optimally harness gene technology that will benefit both the wine producer and the consumer.

- Gagiano M., Van Dyk D., Bauer F.F., Lambrechts M.G., Pretorius I.S. 1999. Mss10p, Mss11p and Muc1p are part of a signal transduction pathway downstream of Mep2p regulating invasive growth and pseudohyphal differentiation in *Saccharomyces cerevisiae*. *Mol. Microbiol.* **31**:103-116.

In *S. cerevisiae*, a network of signal transduction pathways governs the switch from yeast-type growth to pseudohyphal and invasive growth that occurs in response to nutrient limitation. Important elements of this network have been identified, including nutrient signal-receptors, GTP-binding proteins, components of the pheromone-dependent MAP kinase cascade and several transcription factors. However, the structural and functional mapping of these pathways is far from being complete. Here we present data regarding three genes, *MSS10*, *MSS11* and *MUC1*, which form an essential part of the signal transduction

network establishing invasive growth. Both *MSS10* and *MSS11* are involved in the co-regulation of starch degradation and invasive growth. Mss11p acts downstream of Mep2p, Ras2p, and Mss10p and regulates transcription of both *STA2* and *MUC1*. Regulation of *MUC1* mediates the effect of Mss10p and Mss11p on invasive growth. In addition, our results suggest that the activity of Mss10p is independent of the invasive growth MAP kinase cascade, but that Mss11p acts either downstream of, or in conjunction with, Ste12p. We also show that starch metabolism in *S. cerevisiae* is subject to regulation by components of the MAP kinase cascade.

- Vivier M.A., Sollitti P., Pretorius I.S. 1999. Functional analysis of multiple AUG codons in the transcripts of two coregulated yeast genes, *STA2* and *MUC1*. *Mol. Gen. Genetics* **261**:11-20.

A scanning ribosome will usually initiate translation as soon as it encounters the first favourable AUG codon and only 10% of eukaryotic transcripts have more complex arrangements. These relatively few complex transcripts normally have structural features such as multiple AUGs and significant secondary structures. However, the functional relevance of these features have rarely been established. We present here a study into the functionality of the multiple AUGs in the leaders of the *STA2* and *MUC1* transcripts of the budding yeast *Saccharomyces cerevisiae*. Each of these genes contains a putative upstream open reading frame, whereas *STA2* has an additional two in-frame AUG codons 5' of the major cistron. The *STA2* gene (a representative model of the polymorphic

STA1-3 gene family), encoding an extracellular glucoamylase, is evolutionary linked to and transcriptionally coregulated with the *MUC1* gene, that encodes a mucin-like protein essential for pseudohyphal growth and cell-adhesion in *S. cerevisiae*. We show that utilization of the alternative translational start-sites of *STA2* cause the glucoamylases to differ at their N-termini, resulting in differences of their localization patterns. Mutant analysis revealed the presence of a novel secretion enhancing signal that might prove to be relevant to the alternative targeting mechanism recently uncovered in *S. cerevisiae*. We show that a short upstream open reading frame present in the leaders of *STA1-3* and *MUC1* is bypassed in a process of leaky scanning.

- Gagiano M., Van Dyk D., Bauer F.F., Lambrechts M.G., Pretorius I.S. 1999. Divergent regulation of the evolutionary closely related promoters of the *Saccharomyces cerevisiae* *STA2* and *MUC1* genes. *J. Bacteriol.* **181**:6497-6508.

The 5' upstream regions of the *Saccharomyces cerevisiae* glucoamylase-encoding genes, *STA1-3*, and of the *MUC1/FLO11* gene, which is critical for pseudohyphal development, invasive growth and flocculation, are almost identical and the genes co-regulated to a large extent. Besides representing the largest yeast promoters identified to date, these regions are of particular interest from both a functional as well as evolutionary point of view. Transcription of the genes seems indeed dependent on numerous transcription factors which integrate the information of a complex network of signaling pathways, while the very limited sequence differences between them should allow to study promoter

evolution on a molecular level. To investigate the transcriptional regulation, we compared the transcription levels conferred by the *STA2* and *MUC1* promoters under various growth conditions. Our data show that transcription of both genes responded similarly to most environmental signals, but also indicated significant divergence in some aspects. We identified distinct areas within the promoters that show specific responses to the activating effect of Flo8p, Msn1p (Mss10p/Fup1p/Phd2p) and Mss11p as well as to carbon catabolite repression. We also identified the *STA10* repressive effect as the absence of Flo8p, a transcriptional activator of flocculation genes in *S. cerevisiae*.

- Grossmann M.K., Pretorius I.S. 1999. Verfahren zur Identifizierung von Weinhefen und Verbesserung der Eigenschaften von *Saccharomyces cerevisiae*: eine Übersicht. *Die Weinwissenschaft* **54**:61-72.

8. Pretorius I.S. 1999. Engineering designer genes for wine yeasts. *Australian and New Zealand Wine Indust. J.* **14**:42-47.

Winemaking is continuously subjected to the forces of *market-pull* and *technology-push* and new trends in the global wine market continue to call for a diversity of innovations, including the genetic improvement of traditional wine yeast starter cultures that would allow higher quality end-products as well as more cost-effective and environmentally friendly winemaking practices. However, two decades after the first successful transformation of *Saccharomyces cerevisiae* with foreign genetic material, there is still no recombinant wine yeast that is used at a commercial scale to produce wine. With the announcement of the complete nucleotide sequence of the yeast genome two years ago, the question is asked anew whether the disclosure of the yeast's genetic blueprint will now possibly lead to practical benefits for both the wine producer and consumer. Although gene technology has already contributed enormously to our basic understanding of the yeast's biochemical and physiological processes during wine fermentations and to the genetic fingerprinting of the various wine yeast strains, it will be unwise to entertain unrealistic expectations over short-term benefits. The information and technology that currently exist for laboratory strains of

S. cerevisiae, have yet to be expanded to the much more complex genome of industrial wine yeast strains before dramatic break-throughs can be expected. Simultaneously, creative technical strategies are required to comply with the strict statutory regulations that pertain to the use of genetically modified organisms (GMOs) and to address the negative overreaction of some consumer groups. Despite the daunting challenges, it will be equally unwise to pretend that, given the availability of the complete genome and proteome of *S. cerevisiae*, there will be no innovative developments that could be of great benefit to both the wine producer and the wine consumer. However, notwithstanding the staggering potential of genetic engineering, the question remains: Will consumers accept gene technology? Will the wine industry adopt it? It is of vital importance that every person who cares about the well being of the wine industry, including the winemakers and wine connoisseurs, are provided with the relevant facts concerning the potential benefits and risks involved so that informed decisions can be made about where we should be going from here with genetic engineering.

9. Lilly M., Lambrechts M.G., Pretorius I.S. 2000. The effect of increased yeast alcohol acetyltransferase activity on the flavor profiles of wine and distillates. *Appl. Environ. Microbiol.* **66**:744-753.

The distinctive flavor of wine, brandy and other grape-derived alcoholic beverages is affected by many compounds, including esters produced during alcoholic fermentation. The characteristic fruity odors of the fermentation bouquet are primarily due to a mixture of hexyl acetate, ethyl caproate (apple-like aroma), iso-amyl acetate (banana-like aroma), ethyl caprylate (apple-like aroma) and 2-phenylethyl acetate (fruity, flowery flavor with a honey note). The objective of this study was to investigate the feasibility of improving the aroma of wine and distillates by over-expressing one of the endogenous yeast genes that controls acetate ester production during fermentation. The synthesis of acetate esters by the wine yeast *Saccharomyces cerevisiae* during fermentation is ascribed to at least three acetyltransferase activities, namely alcohol acetyltransferase (AAT), ethanol acetyltransferase (EAT) and iso-amyl alcohol acetyltransferase (IAT). To investigate the effect of increased AAT activity on the sensory quality of Chenin blanc wines and distillates from Colombar base wines, we have over-expressed the alcohol acetyltransferase gene (*ATF1*) of *S. cerevisiae*. The *ATF1* gene, located on chromosome XV, was cloned from a widely used commercial wine yeast strain of *S. cerevisiae*, VIN13, and placed under the control of the constitutive yeast

phosphoglycerate kinase gene (*PGK1*) promoter and terminator. Chromoblot analysis confirmed the integration of the modified copy of *ATF1* into the genome of three commercial wine yeast strains (VIN7, VIN13 and WE228). Northern blot analysis indicated constitutive expression of *ATF1* at high levels in these yeast transformants. The levels of ethyl acetate, iso-amyl acetate and 2-phenylethyl acetate increased 3- to 10-fold, 3.8- to 12-fold and 2- to 10-fold, respectively, depending on the fermentation temperature, cultivar and yeast strain used. The concentration of ethyl caprate, ethyl caprylate and hexyl acetate only showed minor changes, whereas the acetic acid concentration decreased by more than half. These changes in the wine and distillate composition had a pronounced effect on the solvent/chemical (associated with ethyl acetate and iso-amyl acetate), herbaceous and heads-associated aroma of the final distillate and the solvent/chemical and fruity/flowery character of the Chenin blanc wines. This study establishes the concept that the over-expression of acetyltransferase genes such as *ATF1* could profoundly affect the flavor profiles of wines and distillates deficient in aroma, thereby paving the way for the production of products maintaining a fruitier character for longer periods after bottling.

1. Smole-Mozina S., Rozman D., Komel R., Raspor P. 1995. Identification and genetic inter-relationship analysis of *Saccharomyces* yeasts by arbitrary primed PCR fingerprinting. *Acta Chim. Slov., Let.* **42**:459-464.

In the present investigation, we used arbitrary primed polymerase chain reaction (AP-PCR) to generate genomic fingerprints of *Saccharomyces* type strains and other isolates by several decamer oligonucleotide primers (50 - 90% G+C, Promega, Operon) and microsatellite DNA primers ((GTG)₅X, (GACA)₄X, MWG - Biotech). Genotypic relatedness of yeasts, evaluated on the basis of polymorphic RAPD markers, was expressed as calculated genetic similarity coefficients and

cluster analysis based on UPGMA method (NTSYS pc program). The estimated similarity values were between 43% - 88% for *Saccharomyces sensu stricto* strains, but much lower for *Saccharomyces sensu lato* yeasts. The results revealed high differentiation capacity of AP-PCR analysis in identification studies of very closely related strains of yeasts from the same species and good correlation of calculated relatedness with the results of more sophisticated molecular methods.

2. Smole-Mozina S., Rozman D., Raspor P. 1995. Molecular markers of *Schwanniomyces* (*Debaryomyces*) yeasts. *Zb. Bioteh. fak. Univ. Ljubl., Kmet.* (1990) **65**:73-80.

Two molecular genetic methods (karyotyping i.e. separation of intact chromosomes with pulsed field gel electrophoresis-CHEF PFGE and AP-PCR, i.e. PCR fingerprinting with randomly chosen oligonucleotide primers) have been tested for intra- and interspecific differentiation of some *Schwanniomyces* and closely related yeast strains. Because of the large chromosomes (1000-2500 kb), which require prolonged electrophoretic separation protocols, AP-

PCR fingerprinting revealed to be more effective for quick differentiation between *Schwanniomyces* yeast strains, but greater number of primers (deca-mer oligonucleotides with 50-80%G+C, microsatellite repeat sequences) should be tested for generation of strain specific and species specific RAPD markers. The result are discussed also in terms of classification system of *Schwanniomyces* yeasts.

3. Smole-Mozina S., Raspor P. 1997. Molecular techniques for yeast identification in food processing. *Food Technol. Biotechnol.* **35**(1): 55-61.

Yeasts are crucial agents in many important natural and industrial bioprocesses as well as in spoilage of foods and some diseases in humans and animals. Quick and reliable methods for yeast identification and classification are highly appreciated in all mentioned areas. Classical methods include morphological and physiologically-biochemical tests. They are time and material consuming, reliability and distinctive capacity of closely related isolates is low. Besides phenotypic characterization many molecular-biology analyses of yeast nucleic acids have been developed recently. They can give stable and unique electrophoretic profiles independently of the microbial cultivation conditions. In this article the application

of the yeast nuclear and mitochondrial DNA analysis for identification of food-borne yeasts is reviewed - the electrophoretic karyotyping, the study of the restriction fragment length polymorphism (RFLP) of mtDNA, and two techniques based on PCR amplification of yeast DNA: the amplification with non-specific primers (AP-PCR, arbitrarily primed PCR, RAPD, random amplified polymorphic DNA analysis) and PCR ribotyping - restriction analysis of amplified ribosomal RNA genes of tested yeasts. The advantages and limitations of the application of molecular methods based on DNA analysis for yeast characterization in research and routine industrial laboratories are discussed.

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

18SrDNA + ITS1 and 25SrDNA PCR products covering more than 95% of nuclear ribosomal DNA repeat unit of 28 *Saccharomyces sensu stricto* and *Torulaspota* yeasts and their anamorph forms were digested with HaeIII, MspI, HinfI and CfoI. Using combinations of two restriction enzymes, specific ribotyping patterns of 6 species were found. PCR

ribotyping offers a convenient tool for quick identification of yeast isolates, but specificity of ribotyping patterns should be checked with a larger number of strains to avoid misidentification because of lack of variation within different taxa or because of strain-specific ribotyping patterns of species type strains.

5. Smole-Mozina S., Eadez N., Raspor P. 1998. rDNA RFLPs and AP-PCR fingerprinting of type strains and grape-must isolates of *Hanseniaspora (Kloeckera)* yeasts. Food Technol. Biotechnol. **36**(1):37-43.

Molecular analyses of nucleic acid polymorphisms have proved to be very helpful in avoiding taxonomic ambiguities and simplifying yeast identification schemes. PCR-mediated typing assays have the advantage of a rapid amplification of yeast target DNA in the laboratory, thus enabling rapid analysis of DNA polymorphisms on different levels. We used two approaches - restriction analysis of amplified rDNA fragments (RFLPs of 2,550 bp long 18S-ITS1-5.8S-ITS2 and 3,350 bp long 25S rDNA amplicons) and AP-PCR fingerprinting of yeast DNA with six non-specific oligonucleotide primers for the characterization of *Hanseniaspora* (anamorph *Kloeckera*) yeasts. Species-specific restriction patterns were derived from type strains analysis.

Two restriction analyses of 18S-ITS1-5.8S-ITS2 rDNA and/or 25S rDNA fragment were sufficient for species delineation among *Hanseniaspora/Kloeckera* yeasts. They were used for rapid species identification of thirty three *Hanseniaspora/Kloeckera* strains isolated from grapes and musts at the start of spontaneous fermentation in two geographically distinct wine-producing subregions in Slovenia. All identified strains were *H. uvarum/K. apiculata*. AP-PCR fingerprinting revealed great heterogeneity of the isolates at the intraspecies level. No correlation between genetic similarity, calculated from AP-PCR fingerprints, and the geographical origin of *H. uvarum/K. apiculata* strains was evidenced.

6. Raspor P., Smole-Mozina S., Eadez N. 2000. Identification of yeasts from grape/must/wine system: *Methods in Biotechnology*, Vol. 14. Food Microbiology Protocols. J.F.T. Spencer and A.L. Ragout de Spencer ed., Humana Press Inc. Totowa, NJ. In press.

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Obituary

Robert G. Garrison Ph.D. - 1925-2000

Dr. Robert Garrison, mycologist, colleague, and friend, died February 17, 2000 due to complications arising from esophageal cancer. Bob was born in Pittsburg, Kansas. During World War II, Garrison served in the U.S. Army Medical Corps and was attached to maritime transport between the U.S. and the United Kingdom. He obtained his first degree at Pittsburg State University in 1949. His M.S. (1951) and Ph.D. (1954) were from Kansas State University, Manhattan, Kansas. Bob's professional career was primarily with the Veterans Administration Medical Services, initially in Nebraska, and then for thirty-three years at the V.A. Medical Center, Kansas City, Missouri, where he held the honor rank of Career Research Scientist. Concurrently he was professor of microbiology at the University of Kansas Medical Center and I was fortunate to have him as a scientific collaborator; formally for fourteen years but also well into his retirement after 1985. Bob's expertise was particularly evident in electron microscopy applied to the fungal specimen. As most readers will know, these are not the easiest of biological items to fix and stain. I particularly enjoyed the fact that Dr. Garrison would routinely subject pathogenic yeasts and yeast-like samples to three independent fixation regimens. He became well-known for his published micrographs and was consulted worldwide. Many investigators in the Americas and Europe were delighted to find that his kindly criticism of their own work was followed by volunteer pictures from Garrison's own laboratory, which they then published. At

the University of Missouri, Kansas City, he assisted several master's degree students and provided electron microscopy, which was inadequately acknowledged. During 1976, Dr. Garrison was Visiting Scientist, Government of France Award, in the Service de Mycologie, Institut Pasteur. He always expressed fond memories of that time. After his return to Kansas City, Bob continued to exchange scientific services and personal correspondence with Pasteur members. His hobbies in retirement included collecting coins and stamps, and genealogy. He is survived by daughter Grechen (Lawyer) and son Robert (Salesman), both of St. Louis. His wife passed away earlier this year.

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Forthcoming Meetings

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

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

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

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

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

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

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

The organizers are elaborating the programme on the basis of received abstracts. Over 300 abstracts have been submitted, from over 40 countries worldwide. The scientific committee has made a choice of abstracts for oral presentation in the afternoon sessions. Over 200 posters will be on display during the Symposium. A limited number of exhibitors has been admitted. On May 15 the number of registered participants had

passed 300 and since then is further growing. The organizers are looking forward to welcoming all at Papendal!

International Commission on Yeasts and Yeast-like Organisms. Tuesday 29 August, the International Commission on Yeasts will meet at Papendal during lunch. Members expecting to attend are kindly requested to let this know by e-mail to: <lex.scheffers@tnw.tudelft.nl>

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XIIIth meeting on The Biology of *Kluyveromyces* September 1-3, Leiden, The Netherlands

We cordially invite you to participate in the XIIIth meeting on The Biology of *Kluyveromyces* which will be held from Friday night September 1 till Sunday morning September 3 in Leiden. The place of venue will (almost certainly) be the Institute of Molecular Plant Sciences of the Leiden University, Clusius Laboratory, Wassenaarseweg 64, 2333 AL Leiden, The Netherlands.

For practical reasons announcements and further information will be provided solely by e-mail. Please inform colleagues in your lab. Those who are interested and have not received this announcement are requested to send a message to: <genetica@RULBIM.LeidenUniv.nl>.

Send Abstracts of your contribution by e-mail, preferentially as an attached Word document. Please indicate if you prefer an oral- or poster presentation. Deadline for sending abstracts is June 16. Authors will be notified before July 1st.

Leiden can easily be reached by car, train and plane. Schiphol Airport /Amsterdam Airport has a direct railroad connection with Leiden. Trains (direction The Hague, not Amsterdam) depart six times per hour and they will bring you to Leiden Centraal in 20 minutes (and vice versa). The price for a single fare is hfl 9.50.

For those arriving by car or railroad details may be found at www.wimp.LeidenUniv.nl/map.htm

This site provides printable maps.

Due to holidays and other meetings hotel space in Leiden is limited in September. We have taken options on rooms in Hotel Het Haagse Schouw and Golden Tulip Hotel.

Het Haagsche Schouw is at approximately 30 min walking distance from the Clusius laboratory and the railroad station. In addition to available public transport (busline 43) we will arrange a (limited) shuttle service. Rooms are hfl 130.- per night, excluding breakfast (hfl 15.-) and tourist tax (hfl 3.50 per day).

The Golden Tulip Hotel is across the railroad station and a 10 min walk from the Clusius laboratory. Rooms are hfl 205.- per night, excluding breakfast (hfl 25.-) and tourist tax (hfl 3.50 per day).

Options on the hotels expire on June 16, therefore those wishing a reservation should register before that date. If you wish to share a room please indicate with whom. After June 16 we will only provide a list of hotels.

Registration fee for the meeting will be hfl 220,- (or perhaps less, depending on the number of participants and the amount of sponsoring) and should be paid in cash on arrival. The fee covers meals on Friday and Saturday, coffee and tea, but not hotel and breakfast.

Further information will be mailed when available. For questions, registration and remarks use our e-mail address. Alternatively, you may reach the organizing committee by fax 31 71 52749 99.

The members of the organizing committee can be reached by phone on the following numbers:

Anneke van Dillen - 31 71 527 4944; Rolf Kooistra - 31 71 527 4952; Yde Steensma - 31 71 527 4947. If everything else fails you can send a letter to Anneke van Dillen, Clusius Laboratory, Wassenaarseweg 64, 2333 AL Leiden, The Netherlands.

To register, email the following information:

Name, Address/Affiliation, Phone, Fax, E-mail.

Title of abstract.

Hotel reservation: Hotel, Arrival date, Departure date, Shares room with__.

Credit card number, Expiration date.

This information is asked for hotel reservation but will have no further consequences with respect to cancellation.

Please e-mail this information as soon as possible to:

<genetica@RULBIM.LeidenUniv.nl>

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

The Symposium will be held in the conference hall of the main building of Lviv State University. Lviv (also known as: Lvov, Lwow, Lemberg, Leopoli) is the largest scientific, cultural and economic city in the Western Ukraine with population near 1 mln located in the geographical center of Europe. The topics will include (preliminary list): Systematics of NCY. Ecology. Methods of NCY Molecular Genetics; Chromosome Structure and Genome Organization. Genome Sequencing in NCY. Regulation of Gene Expression.

Metabolic Regulation. Organelles. *Saccharomyces* versus Non-*Saccharomyces*: Similarities and Differences. Membrane Structure and Functions. Stress Response. Heterologous Gene Expression. Biochemical Engineering. Industrial Applications. Medically Important Yeasts.

International Scientific Committee: Gerold Barth, Dresden Technical University, Germany (*Yarrowia lipolytica*). James M. Cregg, Oregon Graduate Institute, Portland, USA (heterologous gene expression, organelles). Graham H. Fleet,

University of New South Wales, Sydney, Australia (ecology). Laura Frontali, Rome University "La Sapienza", Italy (*Kluyveromyces*). Sergei G. Inge-Vechtomov, St. Petersburg University, Russia (genetics). Cornelis P. Hollenberg, Duesseldorf University, Germany (heterologous gene expression). Cletus P. Kurtzman, Center of Agricultural Research, Peoria, USA (systematics). Jesus Pla, Madrid University, Spain (*Candida albicans*). Andrei A. Sibirny, Institute of Biochemistry, Lviv, Ukraine (metabolic regulation). Suresh Subramani, University of California at San Diego, La Jolla, USA (organelles). Masamichi Takagi, The University of Tokyo, Japan (*Candida maltosa*). Yuri A. Trotsenko, Institute

of Biochemistry and Physiology of Microorganisms, Pushchino, Russia (biochemistry). Marten Veenhuis, Groningen University, The Netherlands (ultrastructure).

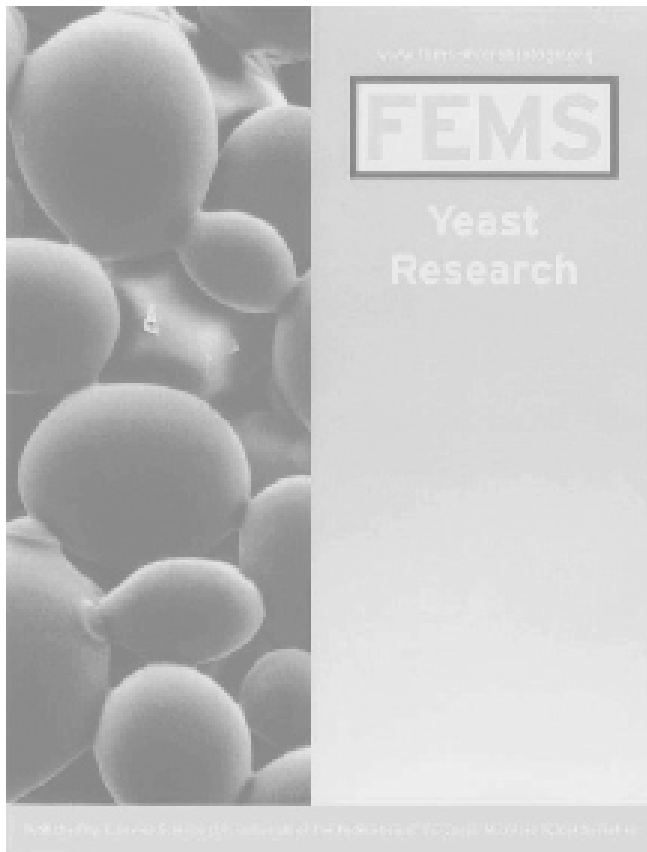
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New Journal - *FEMS Yeast Research* - Call for papers



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The journal will publish high-quality original research papers and mini-reviews that cover both yeast and yeast-like organisms. The editors aim to cover the entire field of yeast research in its broadest sense. The audience of the journal will include yeast researchers in academic institutions, but also those that use yeast as a model organism or work in industry. The following list of disciplines is not exclusive but these main topics are explicitly mentioned here to illustrate the wide spectrum of topics covered by the journal: physiology, taxonomy, phylogenetics, evolution, biodiversity, ecology, genetics, molecular biology, metabolic engineering, biotechnology, food microbiology, heterologous protein production and secretion, pathogenic yeasts, typing and diagnostics. In the field of physiology, the journal encourages submission of papers in rapidly developing fields like: cell cycle, morphogenesis, cell wall, organelle biosynthesis, cell ageing, metabolic regulation, transport, energetics, stress response, and signal transduction. Submissions dealing with genome sequencing and functional genomics are welcomed and greatly encouraged, as are papers in the important new area of comparative genomics.

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