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A Newsletter for Persons Interested in Yeast

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Editorials

23rd International Specialized Symposium on Yeasts, Budapest, August 2003

Congratulations to Tibor Deák, for his masterful organization of the recent ISSY. The excellent scientific program focused on interactions between yeasts and other organisms. We were treated also to the beautiful architecture of Budapest, a vibrant European city with a rich historic past, and to a delightful sample of Hungarian gastronomy, not to forget tastings of the famous Tokaj Aszu, five *puttonyos* no less. A summary of the highlights is presented under "Recent Events".

Learning from Yeast – A Symposium Honouring Herman Jan Phaff, Santiago de Compostela, Spain, September 2003

A symposium dedicated to the memory of Herman Jan Phaff was held during the annual congress of the Spanish Society for Microbiology at the Universidad de Santiago in Santiago de Compostela. On behalf of the participants, I offer my warmest thanks to Tomás Villa for putting together this symposium so successfully, and to the Ramón Areces Foundation for generous financial support of the event.

Vitamin-free Yeast Base

Every now and then, researchers involved with the characterization of yeasts in systematics or genetics are informed that one of their essential supplies may no longer be available. Recently, the word got out that cycloheximide may no longer be manufactured - apparently it still is and will be. Then the Difco catalogue stopped listing Vitamin-free Yeast Base as an available product. Clearly, few people use this medium, and those who do consume only a few grams each year, at best. However, even in such small quantities, the medium is highly impractical to prepare in an individual laboratory and is essential for the determination of vitamin requirements of yeasts. Many readers will therefore be delighted to learn that Vitamin-free Yeast Base is again available commercially. See the relevant entry under "Brief News Items".

A search for early Yeast Newsletter Issues

My collection of back issues of the Yeast Newsletter unfortunately only goes back to Volume XII No. 2 (1964). I would be most appreciative if someone who has access to earlier issues could allow me to obtain copies. Please contact me at <lachance@uwo.ca> if that is possible.

May the year 2004 bring you happiness and prosperity in research and all other aspects of your lives!

M. A. Lachance
Editor

I. Centraalbureau voor Schimmelcultures, Yeast Identification Service, Uppsalalaan 8, P.O.Box 85167 AD Utrecht, The Netherlands. Communicated by M.Th. Smith <smith@cbs.knaw.nl>.

Publications.

1. Boekhout, T. 2003. The 7th International Mycological Congress, Oslo, 2002. FEMS Yeast Res.3: I.
2. Boekhout, T. & Naumova, E. 2003) Pulsed field gel electrophoresis (PFGE) of yeasts. In: Epidemiological typing methods, Werkgroep epidemiologische typering, pp. 93-103.
3. Boekhout, T., Theelen, B., Houbraken, J., Robert, V., Scorzetti, G., Gafni, A., Gerson, U., Sztejnberg, A. 2003. Novel anamorphic mite-associated fungi belonging to the Ustilaginomycetes: *Meira geulakonigii* gen. nov., sp. nov., *Meira argovae* sp. nov. and *Acaromyces ingoldii* gen. nov., sp. nov. Int J Syst Evol Microbiol vol. 53, 1655-1664.
4. Boekhout, T., Theelen, B., Houbraken, J., Robert, R., Scorzetti, G., Gerson, U. & Sztejnberg, A. 2003. New anamorphic acaropathogenic fungi belonging to the Ustilaginomycetes: *Meira geulakonigii* Boekhout, Gerson & Sztejnberg, Gen. et Spec. Nov., *Meira argovae* Boekhout, Gerson & Sztejnberg Spec. Nov. and *Acaromyces ingoldii* Boekhout, Gerson & Sztejnberg Gen. et Spec. Nov. Int. J. Syst. Evol. Microbiol. 53: 1655-1664.
5. Borst, A., Theelen, B., Reinders, E., Boekhout, T., Fluit, A.C. & Savelkoul, P.H.M. 2003. AFLP as an identification method for medically important *Candida* spp., including *C. dubliniensis.* J. Clin. Microbiol. 41: 1357-1362.
6. Cadez N., Poot G.A., Raspor P. & Smith M.Th. 2003. *Hanseniaspora meyeri* sp.nov*, Hanseniaspora clermontiae* sp.nov, *Hanseniaspora lachancei* sp.nov and *Hanseniaspora opuntiae* sp.nov, novel apiculate yeast species. IJSEM 53: 1671-1680.
7. Horré, R., Schröteler, A., Marklein, G., Breuer, G., Siekmeier, R., Sterzik, B., Hoog, G.S. de, Schnitzler, N. & Schaal, K.P. 2003. Vorkommen von *Exophiala dermatitidis* bei Patienten mit zystischer Fibrose in Bonn. Atemw.-Lungenkrkh. 29: 373-379.
8. Iglesia B. de la, Wesselink, J.J., Rayward-Smith, V.J., Dicks, J., Robert, I.A., Robert, V. & Boekhout, T. 2003. Developing classification techniques from biological databases using simulated annealing. In: Metaheuristics: Computer Decision-Making. (Eds. Resende, M.G.C. & de Sousa, J.P.), Kluwer Academic Publisher, pp.347-368.
9. Kerrigan, J., Smith, M. T., Rogers, J. D., Poot, G. A. & Douhan, G. W. 2003. *Ascobotryozyma cognata* sp. nov., a new ascomycetous yeast associated with nematodes from wood-boring beetle galleries. Mycol. Res. 107: 1110-1120.
10. Kwon-Chung, K.J., Boekhout, T., Fell, J.W. & Diaz, M. 2002. *Cryptococcus gattii* (Vanbreus. & Takashio) Kwon-Chung & Boekhout comb. nov. (Fungi, Basidiomycota, Hymenomycetes, Tremellomycetoidea) and a proposal to conserve the name *Cryptococcus gattii.* Taxon 51: 804-806.
11. Rainer, J., Lackner, E. & Hoog, G.S. de 2003. Experiments on the competitive potential of opportunistic fungi.Trends Med. Mycol., Amsterdam pp. 85-89.
12. Reynolds, A.P., Dicks, J.L., Roberts, I.N., Wesselink, J.J., de la Iglesia, B., Robert, V., Boekhout, T. & Raywars-Smith, V.J. 2003. Algorithms for identification key generation and optimisation with application to yeast identification. In: Applications of Evolutionary Computing (G. Raisl. Et al. eds), Lecture Notes in Computer Science 2611. Springer Verlag, Berlin, pp. 107-118.
13. Smith MTh & Poot GA 2003. Genome comparisons in the genus *Dipodascus* de Lagerheim. FEMS Yeast Research 3: 301-311.
14. Trilles, L., Lazéra, M., Wanke, B., Theelen, B. & Boekhout, T. 2003. Genetic characterization of environmental isolates of the *Cryptococcus neoformans* species complex from Brazil. Med. Mycol. 41: 383-390.

Book published.

15. Boekhout, T. & Robert, R. (eds) 2003. Yeasts and Food. Behr's Verlag Hamburg, Germany, pp. 1-488.

Chapter 1. Boekhout, T. & Phaff, H.J. Yeast biodiversity and systematics. pp. 1-38.
Chapter 2. Deak, T. Detection, enumeration and isolation of yeasts. pp. 39-68.
Chapter 3. Kurtzman,C.P., Boekhout,T., Robert,V., Fell,J.W., Yarrow,D., Deak,T. Methods to identify yeasts. Pp 69-122.
Chapter 4. Vossen, J.M.B.M. van der, Rahaoui, H., de Nijs M.W.C.M. & Hartog, B.J. PCR methods for tracing and detection of yeasts in the food chain. pp. 123-138.
Chapter 5. Robert, V. Data processing. pp. 139-169.
Chapter 6. James, S.A. & Stratford, M. Spoilage yeasts with emphasis on the genus *Zygosaccharomyces*. pp. 171-191.
Chapter 7. Brul, S., Klis, F.M., de Nobel,H., Oomes, S.J.C.M., Coote, P., & Hellingwerf, K.J. Yeast stress response to food preservations systems. pp. 193-207.
Chapter 8. Fröhlich-Wyder, M.-T. Yeasts in dairy products. pp. 209-237.
Chapter 9. Samelis, J. & Sofos, J.N. Yeasts in meat and meat products. pp. 239-265.
Chapter 10. Fleet, G.H. Yeasts in fruit and fruit products. pp. 267-287.
Chapter 11. Bonjean, B. & Guillaume, L.-D.Yeasts in bread and baking products. pp. 289-307.
Chapter 12. Stratford, M. & James, S.A. Non-alcoholic beverages and yeasts. pp. 309-345.
Chapter 13. Dufour, J.-P., Verstrepen, K. & Derdelinckx, G. Brewing yeasts. pp. 347-388.
Chapter 14. Dequin, S., Salmon, J.-M., Huu-Vang, N. & Blondin, B. Wine Yeast's. pp. 389-412.
Chapter 15. Hanya, Y. & Nakadai, T. Yeasts and soy products. pp. 413-428.
Chapter 16. Schwan, R.F. & Wheals, A.E. Mixed microbial fermentations of chocolate and coffee. pp. 429-449.
Chapter 17. Nout. M.J.R. Traditional fermented products from Africa, Latin America and Asia. pp. 451-473.

Software.

16. Robert, V., Szoke, Sz. 2003. BioloMICS Web software. Version 2. Centraalbureau voor Schimmelcultures.

17. Robert, V., Szoke, Sz. 2003. BioloMICS software. Version 6. Centraalbureau voor Schimmelcultures.

Two new versions of our BioloMICS software have recently been released featuring a lot of new options and analysis modules. The BioloMICS Web software, freely accessible at

http://www.cbs.knaw.nl/yeast/BioloMICS.aspx

has been designed to be user-friendlier than the previous version. Many new features have been implemented such as a polyphasic online identification module including morphological, physiological and sequence data. Identification reports have been greatly improved. Identifications can Be performed against a yeast species database or against our CBS strains database (a new feature as well). Advance searching (complex queries using a combination of "And", "Or" & "Not") is also possible now for any of the fields of the database. A bibliographic and an extensive fungal taxonomic database can also be easily queried and will provide many details to the users. A quick and modified version of the Blastn software has also been implemented in the software and the user can search our large sequences database that contains virtually all fungal sequences available on Genbank/NCBI as well as a large array of our yet unpublished CBS sequences. Similarity ordering of the alignments is also possible allowing users to perform more reliable identifications than using the Blastn software of the Genbank/NCBI website. Online help movies (already available) and citation requirements can be view under the "Information" module. Your comments and remarks will be welcomed since we want to improve both our software and databases. This new version will be further improved in the next few months.

In press.

18. Ball, L.M., Bes, M.A., Theelen, B., Boekhout, T., Egeler, R.M., Kuijper, E.J. 2003. Significance of amplified fragment length polymorphism in the identification and epidemiology of *Candida* species colonization in children undergoing allogeneic stem cell transplantation. J. Clin. Microbiol.

19. Barreto de Oliveira, M.T., Baroni, F.C., Gambale, F.C., Lazera, M., Boekhout, T., Theelen, B., Hagen, F. & Paula, C.R. 2003) Serotypes, mating types and genetic diversity of *Cryptococcus neoformans* strains recovered in Brazil from clinical and environmental sources. J. Clin. Microbiol.

20. Boekhout, T. 2003. The 5th Conference on *Cryptococcus* and cryptococcosis, Adelaide, 2002. FEMS Yeast Res. 3: III-IV. 15th Congress of the International Society for Human and Animal Mycology (ISHAM), May 25-29 2003, San Antonio, TX, USA, 1st Trends in Medical Mycology, joint meeting of the 9th Congress of the European Confederation of Medical Mycology and the 7th Trends in Invasive Fungal Infections, September 28- October 1, Amsterdam, The Netherlands, and 47 th Annual meeting of the Japanese Society for Medical Mycology, Oct. 16-17, Tokyo, Japan. Fems Yeast Res.

21. Gupta, A.K., Batra, R., Bluhm, R., Boekhout, T. & Dawson, T.L. 2003) Skin diseases associated with *Malassezia* species. J. Am. Acad. Dermatol.

22. Hofmann, H., Choi, S.-M., Wilsmann-Theis, D., Horré, R., Bieber,Th. & Hoog, G.S. de: *Phialophora verrucosa* causing invasive chromoblastomycosis and sinusitis in a child from northern Africa. Mycoses.

23. Hölker, U., Bend, J., Pracht, R., Müller, T., Tetsch, L. & Hoog, G.S. de: *Hortaea acidophila*, a new acidophilic black yeast from lignite. Antonie van Leeuwenhoek.

24. Hoog, G.S. de & Guého, E.: Agents of white piedra, black piedra and tinea nigra. In: Topley & Wilson's Microbiolology and Microbial Infections. (Ed. R.Hay) 10th ed.

25. Kantarcio-lu, A.S. & Hoog, G.S. de: Infections of the central nervous system by melanized fungi: a review of cases presented between 1999 and 2004. Mycoses.

26. Marinelli, F., Brunati, M., Sponga, F., Ciciliato, I., Losi, D., Van Trappen, S., Mergaert, J., Swings, J., Göttlich, E., Hoog, G.S. de, Rojas, J.L. & Genilloud, O.: Biotechnological exploitation of heterotrophic bacteria and filamentous fungi isolated from benthic mats of Antarctic lakes. Appl. Microbiol. Biotechnol.

27. Nakagawa,Y., Robert,V., Kawarazaki,J., Epping,W., Smith,M.Th., Poot,G.A., Mizuguchi,I., Kanbe,T., Doi,M. 2003. Recurrent emergence of a less common yeast *Candida pararugosa* from a sarcoma patient. Medical Mycology.

28. Porteous, N.B., Redding S.W., Thompson, E.H., Grooters, A.M., Hoog, G.S. de & Sutton D.A.: Isolation of an unusual fungus in treated dental unit waterlines. J. Amer. Dental Assoc.

29. Taj-Aldeen, S.J., Al-Ansari, H.I., Boekhout, T. & Theelen, B. 2003) Co-isolation of *Trichosporon inkin* and *Candida parapsilosis* from a scalp white piedra case. Med. Mycol. (in press).

Submitted.

30. Bonifaz, A., McGinnis, M.R., Hoog, G.S. de, Mercadillo, P., Rodríguez-Cortés, O., Araiza, J. & Saúl, A.: *Cladophialophora carrionii*: a new etiologic agent of eumycetoma. Med. Mycol.

31. Horré, R., Jovani", B., Herff, S., Marklein, G., Zhou, H., Heinze, I., Hoog, G.S. de, Rüchel, R. & Schaal, K. P.: Wound infection due to *Absidia corymbifera* and *Candida albicans* with fatal outcome. Med. Mycol.

II. Department of Microbiology, Miami University, Oxford, Ohio 45056, USA. Communicated by J.K. Bhattacharjee <bhattajk@muohio.edu>.

We have received three US Patents based on novel DNA sequences of the cloned lysine genes as potential targets for rapid molecular (PCR) identification of the opportunistic fungal pathogen *Candida albicans*. We have also published several research papers on the complex structural and functional properties of the novel LYS2 and LYS5 genes of *C. albicans*.

U.S. Patents.

1. J.K. Bhattacharjee, R. Garrad, P. Skatrud and R. Perry. Methods and reagents for detecting fungal pathogens in a biological sample, 22 claims. U.S. Patent no. 5,919,617, awarded July 6, 1999.

2. J.K. Bhattacharjee and V. Bhattacherjee. Methods and reagents for detecting fungal pathogens in a biological sample, 24 claims. U.S. Patent no. 5,910,409, awarded June 8, 1999.

3. J.K. Bhattacharjee, K. Suvarna and V. Bhattacherjee. Reagents and kits for detecting fungal pathogens in a biological sample, 16 claims. U.S. Patent no. 6,455,248 B1, awarded Sept. 24, 2002.

Publications.

1. V. Bhattacherjee and J.K. Bhattacharjee, 1999. Characterization of a double gene disruption in the LYS2 locus of the pathogenic yeast, *Candida albicans*: Medical Mycology **37**: 411-417.

2. S. Guo, S.A. Evans, M.B. Wilkes and J.K. Bhattacharjee. 2001. Novel posttranstational activation of the LYS2-encoded aminoadipate reductase for biosynthesis of lysine and site-directed mutational analysis of conserved amino acid residues in the activation domain of Candida albicans. J. Bacteriol. **183**:7120-7125.

3. S. Guo and J.K. Bhattacharjee. 2003. Site-directed mutational analysis of the novel catalytic domains of "α-aminoadipate reductase (Lys2p) from *Candida albicans*. Mol. Gen. Genomics. **269**:271-279.

4. S. Guo and J.K. Bhattacharjee. 2003. Molecular characterization of the *Candida albicans* LYS5 gene and site-directed mutational analysis of the PPTase (Lys5p) domains for lysine biosynthesis. FEMS Microbiology Letters. **224**:261-267.

5. Bhattacharjee, J.K., G.R. Janssen and T.G. Gregg. 2003. Teaching evolution through molecular evidence. The Science Teacher (in press).

III. Laboratório de Microbiologia, Departamento de Botânica e Engenharia Biológica, Instituto Superior de Agronomia, 1349-017 Lisboa, Portugal. Communicated by M. Malfeito-Ferreira <mmalfeito@isa.utl.pt>.

Abstracts of papers published by the Laboratory of Microbiology, Department of Botany and Biological Engineering of the Instituto Superior de Agronomia.

1. Dias, L., Pereira-da-Silva, S., Tavares, M., Malfeito-Ferreira, M. and Loureiro, V. 2003. Factors affecting the production of 4-ethylphenol by the yeast *Dekkera bruxellensis* in enological conditions. Food Microbiology **20**:377-384.

The conversion of p-coumaric acid into 4-ethylphenol was studied in *Dekkera bruxellensis* ISA 1791 under defined conditions in synthetic media. The production of 4-ethylphenol occurred roughly between mid exponential growth phase and the beginning of the stationary phase. This behaviour was observed when glucose was the only energy and carbon source, the conversion rate being close to 90%. Ethanol, as the single energy source, yielded conversion rates close to 80% while in the presence of trehalose and acetic acid conversion rates lower than 10% were obtained. The production of 4-ethylphenol was not observed when the cells were maintained in buffer solution without carbon and energy sources. The precursor of 4-ethylphenol, p-coumaric acid, was not utilised as energy and carbon source. Furthermore, it was shown that 4-vinylphenol may be used as a precursor of 4-ethylphenol in the absence of p-coumaric acid. Growth and 4-ethylphenol production were inhibited by increasing concentrations of ethanol, being fully prevented at 13% (v/v) ethanol. The cultivation of strain ISA 1791 in mixed culture with *Saccharomyces cerevisiae*, in synthetic medium, showed that the cell numbers of *D. bruxellensis* increased from 10^4 CFU/ml to $5x10^9$ CFU/ml. Laboratory microvinifications of white and red juices inoculated with as low as 10 CFU/ml of *D. bruxellensis* and 10^7 cells/ml of *S. cerevisiae* showed growth of *D. bruxellensis* to levels of about $5x10^8$ CFU/ml. In addition, 4-ethylphenol production by *D. bruxellensis* was observed only after complete fermentation of the grape juices.

2. Dias, L., Dias, S., Sancho, T., Stender, H., Querol, A., Malfeito-Ferreira, M. and Loureiro, V. 2003. Identification of yeasts isolated from wine related environments and capable of producing 4-ethylphenol. Food Microbiology **20**:567-574.

The ability to produce 4-ethylphenol from the substrate *p*-coumaric acid in synthetic media was evaluated for several yeast species associated with wine production. Molar conversion rates as high as 90% were found by only *Dekkera bruxellensis*, *D. anomala* and by some unidentified strains isolated from wine related environments. Other unidentified strains produced traces of 4-ethylphenol. All unidentified strains showed the same cultural characteristics as *D. bruxellensis* when grown on DBDM (*Dekkera*/*Brettanomyces* differential medium) agar. The determination of long-chain fatty acid compositions and the utilization of peptide nucleic acid (PNA) probes specific for *D. bruxellensis* showed that the unidentified strains did not belong to this species. Further identification, by restriction pattern generated from PCR-amplification of the 5.8S rRNA gene and the two internal transcribed spacers (ITS), assigned the unidentified strains to *Candida cantarelli*, *C. wickerhamii*, *Debaryomyces hansenii*, *Kluyveromyces lactis* and *Pichia guilliermondii*. However, only some strains of *P. guilliermondii* were capable of converting *p*-coumaric acid into 4-ethylphenol with efficiencies close to those observed in *D. bruxellensis* and *D. anomala*.

3. Loureiro, V. and Malfeito-Ferreira, M. (2003). Spoilage yeasts in the wine industry. Int. J. Food Microbiol. **86**:23-50.

Yeasts play a central role in the spoilage of foods and beverages, mainly those with high acidity and reduced water activity (a_w). A few species are capable of spoiling foods produced according to good manufacturing practices (GMPs). These can survive and grow under stress conditions where other microorganisms are not competitive. However, many of the aspects determining yeast spoilage have yet to be clarified. This critical review uses the wine industry as a case study where the most serious microbiological problems are caused by yeasts. First the limitations of the available tools to assess the presence of spoilage yeasts in foods are discussed. Spoilage yeasts and factors promoting their colonisation in grapes and wines are discussed from the ecological perspective, demonstrating that a deeper knowledge of vineyard and winery ecosystems is essential to establish the origin of wine spoilage yeasts, their routes of contamination, critical points of yeast infection and, of course, their control. Zymological indicators are discussed as important tools to assess the microbiological quality of wines, although they are rarely used by the wine industry. The concepts of the susceptibility of wine to spoilage yeasts and wine stability are discussed based on scientific knowledge on the subject and on the industrial practices for monitoring yeast contamination. The discussion on acceptable levels of yeasts and microbiological criteria in the wine industry is supported by data obtained from wineries, wholesalers and the scientific literature. Finally, future directions for applied research are proposed, involving collaboration between scientists and industry technicians to improve the zymological quality of foods and its monitoring.

IV. Laboratory of Microbiology, Wageningen University, Hesselink van Suchtelenweg 4, 6703 CT Wageningen, The Netherlands. Communicated by W.J. Middelhoven <wout.middelhoven@wur.nl>.

The following papers appeared since June 2003.

1. W.J. Middelhoven, A. Fonseca, S.C. Carreiro, F.C. Pagnocca and O.C. Bueno. 2003. *Cryptococcus haglerorum*, sp. nov., an anamorphic basidiomycetous yeast isolated from nests of the leaf-cutting ant *Atta sexdens*. Antonie van Leeuwenhoek **83**: 167-174.

A yeast strain (CBS 8902) was isolated in the Centro de Estudos de Insetos Sociais of the Universidade Estadual de Sao Paolo (Brazil) from the nest of a leaf-cutting ant. Physiologically it resembles *Cryptococcus humicola* but sequencing of the D1D2 region of the 26S rRNA revealed phylogenetic relationship to the Cutaneum clade of the genus *Trichosporon.* CBS 8902 assimilated n-hexadecane and several benzene compounds when tested by the slant method.

2. W.J. Middelhoven. 2003. The yeast flora of the coast redwood, *Sequoia sempervirens*. Folia Microbiologica 48: 361-362.

Only few yeast species could be isolated from young shoots of the coast redwood, viz. *Debaryomyces hansenii* var. *fabryi* and *Trichosporon pullulans*. Perannual shoots were inhabited by *D. hansenii* var. *hansenii*. Enrichment cultures on malt extract, inoculated with soil from beneath the tree, yielded *D. hansenii* var. *fabryi* and *T. porosum*. The low biodiversity of the coast redwood forest is reflected in the yeast flora on and beneath the trees that appeared to be poor in species.

V. 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. Barnett, J.A. 2003. A history of research on yeasts 6: the main respiratory pathway. Yeast 20:1015-1044.
2. Barnett, J.A. 2004. A history of research on yeasts 7: enzymic adaptation and regulation. Yeast (in preparation).
3. Barnett, J,A. & van der Walt J.P. 2004. A history of research on yeasts 8: taxonomy. Yeast (in preparation).

VI. Molecular Genetics and Evolution Group, Research School of Biological Sciences, The Australian National University, G.P.O. Box 475, Canberra City, ACT 2601, Australia. Communicated by G.D. Clark-Walker <DCW@rsbs.anu.edu.au>.

Recent publication.

1. Clark-Walker, G.D. 2003. Kinetic properties of F1-ATPase influence the ability of yeasts to grow in anoxia or absence of mtDNA. Mitochondrion **2**:257–265.

A mechanism for hypoxia survival by eukaryotic cells is suggested from studies on the petite mutation of yeasts. Previous work has shown that mutations in the α, β and γ subunit genes of F_1-ATPase can suppress lethality due to loss of the mitochondrial genome from the petite-negative yeast *Kluyveromyces lactis*. Here it is reported that suppressor mutations appear to increase the affinity of F_1-ATPase for ATP. Extension of this study to other yeasts shows that petite-positive species have a higher affinity for ATP in the hydrolysis reaction than petite-negative species. Possession of a F_1-ATPase with a low K_m for ATP is considered to be an adaptation for hypoxic growth, enabling maintenance of the mitochondrial inner membrane potential, $\Delta\Psi$, by enhanced export of protons through F_1F_0-ATPsynthase connected to increased ATP hydrolysis at low substrate concentration.

VII. Department of Food Sciences, Università di Udine, via Marangoni 97, 33100 Udine, Italy. Communicated by M. Manzano <marisa.manzano@dsa.uniud.it>.

The following paper is in press.

1. M. Manzano, L. Cocolin, B. Longo, and G. Comi. PCR-DGGE differentiation of strains of *Saccharomyces sensu stricto*.

A quick molecular biology method based on the polymerase chain reaction (PCR) and Denaturing Gradient Gel Electrophoresis (DGGE) was developed for distinguishing strains belonging to the *Saccharomyces sensu stricto* group. Differentiation was obtained between *S. cerevisiae*, *S. paradoxus* and *S. bayanus/S. pastorianus* although no distinction was possible between *S. bayanus* and *S. pastorianus* using the amplification of the ITS regions. The ability to distinguish different strains of *Saccharomyces sensu stricto* group could allow for a better understanding the ecology of these species on grapes as well as in musts and wines and the method developed can be useful for the quick identification of *Saccharomyces sensu stricto* strains from numerous isolates.

VIII. State Scientific-Research Institute for Genetics and Selection of Industrial Microorganisms, I-Dorozhnyi 1, Moscow 117545, Russia. Communicated by G.I. Naumov and E.S. Naumova <gnaumov@yahoo.com>.

E.S.N. is grateful to the Organising Committee of the ISSY2003 (Budapest, Hungary) for the invitation to give an oral communication and for financial support to participate in the symposium. G.I.N. was awarded an FEMS Congress Attendance Grant (Ljublana, Slovenia). The following are publications for 2003 or in press.

1. Naumova E.S., Korshunova I.V., Naumov G.I. 2003. Molecular analysis of alpha-galactosidase *MEL* genes of the *Saccharomyces* sensu stricto complex. Molecular Biology (Moscow) 37(5).

To infer the molecular evolution of yeast *Saccharomyces* sensu stricto from analysis of the α-galactosidase *MEL* gene family, two new genes were cloned and sequenced from *S. bayanus* var. *bayanus* and *S. pastorianus*. Nucleotide sequence homology of the *MEL* genes of *S. bayanus* var. *bayanus* (*MEL*b), *S. pastorianus* (*MEL*pt), *S. bayanus* var. *uvarum* (*MEL*u), and *S. carlsbergensis* (*MEL*x) was rather high (94.1–99.3%), comparable with interspecific homology (94.8–100%) of *S. cerevisiae MEL*1–*MEL*11. Homology of the *MEL* genes of sibling species *S. cerevisiae* (*MEL*1), *S. bayanus* (*MEL*b), *S. paradoxus* (*MEL*p), and *S. mikatae* (*MEL*j) was 76.2–81.7%, suggesting certain species specificity. On this evidence, the α-galactosidase gene of hybrid yeast *S. pastorianus* (*S. carlsbergensis*) was assumed to originate from *S. bayanus* rather than from *S. cerevisiae*.

2. Naumov G.I., Gazdiev D.O., Naumova E.S. 2003. The finding of the yeast species *Saccharomyces bayanus* in Far East Asia. Microbiology (Moscow) 72 (6).

The genetic and molecular analyses of nine Far East Asian *Saccharomyces* isolates allowed us to identify three species *S. cerevisiae, S. paradoxus* and *S. bayanus.* The occurence of the last species in Far East Asia was documented for the first time. A new methodology for the molecular genetic differentiation of *Saccharomyces* sensu stricto species is described. The ecogeographical distribution of *Saccharomyces* yeasts is discussed.

3. Boekhout T., Naumova E. 2003. Pulsed field gel electrophoresis (PFGE) of yeats. In: Experimental approaches for assessing genetic diversity among microbial pathogens. Ed. by A. van Belkum et al., Wageninge, The Netherlands, pp. 93-103.

4. Naumova E.S., Naumov G.I., Nosek J., Tomaska, L. 2003. Differentiation of the yeasts *Williopsis, Zygowilliopsis* and *Komagataea* by karyotypic and PCR analyses. System. Appl. Microbiol., 26 (in press).

We used polymerase chain reaction with universal and microsatellite primers, and molecular karyotyping to evaluate the extent of divergence between the genomes of the yeasts currently assigned to the heterogeneous genus *Williopsis*. Pulsed-field gel electrophoresis of chromosomal DNAs indicates that *Zygowilliopsis californica, Komagataea pratensis, Williopsis mucosa, Williopsis salicorniae* species and *Williopsis* sensu stricto complex have clearly different karyotypes. In contrast, the latter six species, *Williopsis saturnus, W. beijerinckii, W. mrakii, W. suaveolens, W. subsufficiens* and *W. sargentensis*, show similar banding patterns and practically cannot be differentiated on the basis of their karyotypes. The data revealed that a PCR method employing the universal primer N21 is appropriate for the distinction of *Williopsis, Zygowilliopsis* and *Komagataea* yeasts. Unique fingerprints were generated with this primer for all 10 species studied while strains of the same species showed nearly identical profiles. The data of UP-PCR are in good agreement with genetic classification and provide support for the species status of the yeasts composing the *Williopsis* sensu stricto complex. Microsatellite primer $(GTG)_5$ allowing molecular typing of individual strains of the same species may be useful for investigating population structure of the saturn-spored yeasts.

5. Naumov G.I., Naumova E.S., Smith M. Th., de Hoog G.S. 2003. Ribosomal DNA sequencing and reinstatement of the genus *Arthroascus* von Arx. J. Gen. Appl. Microbiol. (in press).

Sequence analysis of the D1/D2 domain of 26S rDNA was conducted upon seven *Arthroascus* strains from different geographic localities. The European and Asian species *Arthroascus schoenii* was documented from the North-American continent and from the Island of Hawaii. We discuss the heterogeneity of the genus *Saccharomycopsis* sensu Kurtzman and Robnett 1995. On the basis of molecular and genetic data the genus *Arthroascus* von Arx is reinstated.

6. Naumova E.S., Naumov G.I., Smith M. Th., de Hoog G.S. 2003. Molecular and genetic bases for classification of predatory yeast *Arthroascus*. 23rd Int. Spec. Symp. on Yeasts (ISSY 2003), 26-29 August 2003, Budapest, Hungary, p. 21.

7. Naumov G.I., Naumova E.S., Kondratieva V.I., Kazaryan E.S. 2003. Genetic study of of predatory yeast *Arthroascus*. 23rd Int. Spec. Symp. on Yeasts (ISSY 2003), 26-29 August 2003, Budapest, Hungary, p. 98.

8. Naumova E.S., Naumov G.I., Barrio E., Querol A. 2003. Peculiarities of mtDNA of wine yeast *Saccharomyces bayanus* var. *uvarum*. 23rd Int. Spec. Symp. on Yeasts (ISSY 2003), 26-29 August 2003, Budapest, Hungary, p. 99.

9. Naumov G.I., Sukhotina N.N., Naumova E.S. 2003. Complex composition of the yeast *Zygofabospora/Kluyveromyces lactis*: genetic and molecular differentiation of sub-populations. 1st FEMS Congress of European Microbiologists, June 29-July 3, 2003, Ljubljana, Slovenia, p. 205.

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List of recent publications.

1. Bozsik, A., Szilagyi, Z., Benko, Z., Sipiczki, M. 2002. Marker construction and cloning of a *cut1*-like sequence with ARS activity in the fission yeast *Schizosaccharomyces japonicus*. Yeast **19**:485-498.

2. Szilagyi, Z., Grallert, A., Zilahi, E., Sipiczki, M. 2002. Isolation and characterization of fission yeast genes involved in transcription regulation of cell cycle events. Acta Microbiol. Immunol. Hung. **49**:285-287.

3. Naumov, G.I., Naumova E.S., Antunovics, Z., Sipiczki, M. 2002. *Saccharomyces bayanus* var. *uvarum* in Tokaj wine-making of Slovakia and Hungary. Appl. Microbiol. Biotechnol. **59**:727:730.

4. Szilagyi, Z., Grallert, A., Nemeth, N., Sipiczki, M. 2002. The *Schizosaccharomyces pombe* genes *sep10* and *sep11* encode putative general transcriptional regulators involved in multiple cellular processes. Mol. Genet. Genomics **268**:553-562.

5. Sipiczki, M. 2002. Taxonomic and physiological diversity of *Saccharomyces bayanus*. In "Biodiversity and Biotechnology of Wine Yeasts" (Ed. M. Ciani) Research Signpost, Kerala, pp. 53-69.

6. Martin-Cuadrado, A.B., Duenas, E., Sipiczki, M., Vazquez de Aldana, C.R., del Rey, F. 2003. The endo-β-1,3-glucanase Eng1p is required for dissolution of the primary septum during cell separation in *Schizosaccharomyces pombe*. J. Cell Sci. **116**:1689-1698.

7. Sipiczki, M. 2003. Tokaj yeasts. Vinohrad **41(3)**:8-9.

8. Antunovics, Z., Csoma, H., Sipiczki, M. 2003. Molecular and genetic analysis of the yeast flora of botrytized Tokaj wines. Bulletin de l'O.I.V. (Office International de la Vigne et du Vin Paris), **76**:380-397.

9. Sipiczki, M. 2003. *Candida zemplinina* sp. nov., an osmotolerant and psychrotolerant yeast that ferments sweet botrytized wines. Int. J. System. Evol. Microbiol. (in press).

10. Sipiczki, M. 2003. Fission Yeast Phylogenesis and Evolution. In "Molecular Biology of *Schizosaccharomyces pombe*" (Ed. R. Egel) Springer Verlag, Heidelberg, pp. 431-443.

Abstracts of papers presented at recent meetings.

11. Romano, P., Sipiczki, M., Capece, A., Paraggio, M., Lipani, G., Salzano, G. 2002. Analysis of *Saccharomyces cerevisiae* strains derived from spontaneous fermentation of Aglianico wine. 22nd International Specialised Symposium on Yeasts "Yeast Fermentations and other Yeast Bioprocesses". Pilanesberg National Park, South Africa. Programme and Abstracts, p. 77,

12. Sipiczki, M., Csoma, H. 2002. An investigation into the yeast flora of botrytized grapes in Tokaj. 22nd International Specialised Symposium on Yeasts "Yeast Fermentations and other Yeast Bioprocesses". Pilanesberg National Park, South Africa. Programme and Abstracts, p. 106.

13. Enczi, K., Bozsik, A., Sipiczki, M. 2002. Study of the dimorphic fission yeast *Schizosaccharomyces japonicus*. Acta Microbiol. Immunol. Hung. **49**:395.

14. Miklos, I., Koti, K., Sipiczki, M. 2002. Identification of cytokinesis genes in fission yeast. Acta Microbiol. Immunol. Hung. **49**:402-403.

15. Antunovics, Z., Sipiczki, M. 2002. Analysis and hybridization of *Saccharomyces bayanus* from Tokaj wine. Acta Microbiol. Immunol. Hung. **49**:409.

16. Antunovics, Z, Csoma H, Sipiczki, M. 2002. Molecular and genetic analysis of the yeast flora of botrytized Tokaj wines. XXVIIth World Congress of Vine and Wine. Bratislava, Slovakia. Abstracts, p. 71.

17. Sipiczki, M., Bozsik, A., Enczi, K. 2002. Cytological and genetic investigations into the regulation of dimorphism and growth polarity transitions in *Schizosaccharomyces japonicus*. VIII International Fungal Biology Conference. Guanajuato, Mexico. p. 77.

18. Romano, P., Sipiczki, M., Raspor, P. 2003. International master's studies in wine biotechnology. EoroMicroDay 2003, Book of Abstracts pp. 19-20.

19. Sipiczki, M., Miklos, I. 2003. Structural inheritance: the role of inherited structural elements in the polarisation of the fission-yeast cell. Yeast **20**:S69.

20. Miklos, I., Sipiczki, M. 2003. *spl1-1*: a citokinesis defect caused by a G-to-A transition in the loop of a proline-tRNA of *S. pombe*. Yeast **20**:S69.

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The following papers have been published recently.

1. J.C. Verdoes, G. Sandmann, H. Visser, M. Diaz, M. van Mossel and A.J.J. van Ooyen. 2003. Metabolic engineering of the carotenoid biosynthetic pathway in the yeast *Xanthophyllomyces dendrorhous* (*Phaffia rhodozyma*). Appl. Environ. Microbiol. **69**: 3728-3738.

The *crtYB* locus was used as an integrative platform for the construction of specific carotenoid biosynthetic mutants in the astaxanthin producing yeast *Xanthophyllomyces dendrorhous.* The *crtYB* gene of *X. dendrorhous*, encoding a chimeric carotenoid biosynthetic enzyme, could be inactivated by both single and double cross-over events resulting in non-carotenoid producing transformants. In addition, the *crtYB* gene, either linked to its homologous or a glyceraldehyde-3-phosphate dehydrogenase promoter, was overexpressed in a wild type and a β-carotene accumulating mutant of *X. dendrorhous.* In several transformants containing multiple copies of the *crtYB* gene the total carotenoid content was higher than in the control strain. This increase was mainly due to an increase of the β-carotene and echinone content, while the total content of astaxanthin was unaffected or even lower. Overexpression of the phytoene synthase-encoding gene (*crtI*) had a large impact on the ratio between mono- and bicyclic carotenoids. Furthermore we showed that in metabolic engineered *X. dendrorhous* strains the competition between the enzymes phytoene desaturase and lycopene cyclase for lycopene governs the metabolic flux either via β-carotene to astaxanthin or via 3,4-didehydrolycopene to 3-hydroxy-3'-4'-didehydro-β-ι-caroten-4-one (HDCO). The monocylic carotenoid torulene and HDCO, normally produced as minority carotenoids, were the main carotenoids produced in these strains.

2. H. Visser, A.J.J. van Ooyen and J.C. Verdoes. Metabolic engineering of the astaxanthin-biosynthetic pathway of *Xanthophyllomyces dendrorhous*. FEMS Yeast Research (in press).

This review describes the different approaches that have been used to manipulate and improve carotenoid production in *Xanthophyllomyces dendrorhous*. The red yeast *X. dendrorhous* (formerly known as *Phaffia rhodozyma*) is one of the microbiological production systems for natural astaxanthin. Astaxanthin is applied in food and feed industry and can be used as nutraceutical because of the strong antioxidant properties. However, the production levels of astaxanthin in wild type isolates are rather low. To increase the astaxanthin content in *X. dendrorhous* cultivation protocols have been optimized and astaxanthin hyperproducing mutants have been obtained by screening of classically mutagenized *X. dendrorhous* strains. The knowledge about the regulation of carotenogenesis in *X. dendrorhous* is limited yet in comparison to other carotenogenic fungi. The *X. dendrorhous* carotenogenic genes have been cloned and a *X. dendrorhous* transformation system has been developed. These tools allowed the directed genetic modification of the astaxanthin pathway in *X. dendrorhous*. The *crtYB* gene, encoding the bifunctional enzyme phytoene synthase / lycopene cyclase, was inactivated by insertion of a vector by single and double cross over events, indicating that it is possible to generate specific carotenoid biosynthetic mutants. Additionally, overexpression of *crtYB* resulted in the accumulation of β-carotene and echinone, which indicates that the oxygenation reactions are rate limiting in these recombinant strains. Furthermore, overexpression of the phytoene desaturase encoding gene (*crtI*) showed an increase in monocyclic carotenoids such as torulene and HDCO and a decrease in bicyclic carotenoids such as echinone, β-carotene and astaxanthin.

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

1. Farkaš,V. 2003. Structure and biosynthesis of fungal cell walls: Methodological approaches. Folia Microbiol. 48:469-478.

Fungal cell walls possess a characteristic chemical composition differentiating fungal cells from other cell types. For this reason, the mechanism involved in cell-wall formation represents a potential target for selective antifungal drugs for treating fungal diseases. This article reviews the history methods employed in chemical and structural analysis of fungal cell walls and in studies concerning their formation.

2. Sláviková E. and Vadkertiová R. 2003. The occurrence of yeasts in grass-grown soils. Czech Mycol. 54:239-247.

One hundred and fifty six yeast strains were isolated from 160 grass-grown soil samples collected in four different localities in Bratislava, Slovakia. The collection of soil took place in March, May, August, and October. *Cryptococcus laurentii*, *C. albidus*, *Cystofilobasidium capitatum*, *Sporobolomyces salmonicolor*, and *Trichosporon cutaneum* were the most frequently isolated species from the samples taken in the unpolluted localities Rusovce and Dúbravka. These species represented 92.1 % of total yeast counts found in these soil samples. *Cryptococcus laurentii*, *C.albidus*, *Cystofilobasidium capitatum*, *Debaryomyces castellii*, and *Rhodotorula glutinis* were the most frequently isolated species from the samples taken in the polluted localities Polianky and Mlynská Dolina. These species represented 93.3 % of total yeast counts there. Yeast densities ranged from 400 to 80.000 CFU/g soil. We found that yeasts occurred unevenly in soils during the year. The lowest average number of yeasts was found in August and the highest one in May.

3. Sláviková E. and Vadkertiová R. 2003. The diversity of yeasts in the agricultural soil. J. Basic Microbiol. 43:430-436.

One hundred and eleven yeast strains were isolated from 60 agricultural soil samples. The samples were taken from four various fields located in the southwest of Slovakia. *Cryptococcus laurentii*, *Candida maltosa*, *Metschnikowia pulcherrima*, and *Sporobolomyces salmonicolor* were the predominant species in the samples collected from all four types of fields. These species represented 78.4 - 86.6 % of the total yeast counts. The results obtained enabled comparisons to be made between forest and agricultural soil yeast population. We have found out that the yeast population in tilled soils was significantly reduced. The number of yeasts in the tilled soils ranged from 40 to 6.8 x 10^3 CFU/g soil and the average number reached approximately 1.12 x 10^3. This number is more than ten times lower in comparison with the forest soils.

4. Sláviková E. and Vadkertiová R. 2003 Effects of Pesticides on Yeasts Isolated from Agricultural Soil. Zeitschrift fur Naturforschung - in press.

The effect of six various pesticides on the growth of yeasts isolated from agricultural soil was investigated. Two herbicides (with the effective substances lactofen and metazachlor), two fungicides (with the effective substances fluquinconazole and prochloraz), and two insecticides (with the effective substances cypermethrin + chlorpyrifos and triazamate) were tested. It is evident that there are considerable differences in inhibition effects of studied pesticides. The fungicide with the effective substance prochloraz inhibited the growth of majority of yeast strains. Insecticide triazamate at concentration 0.6 mM restricted or inhibited growth of all tested strains. The strains of the genus *Cryptococcus* were the most sensitive to pesticides, while the strains of the species *Cystofilobasidium capitatum*, *Debaryomyces occidentalis* var. *occidentalis*, and *Trichosporon cutaneum* were the most resistant.

5. Márová I., Breierová E., Kočí R., Friedl Z., Slovak B., Pokorná J., Influence of exogenous stress factors on production of carotenoids by some strains of carotenogenic yeasts. Ann. Microbiol. In press.

The aim of this study was to comparise composition and content of carotenoids produced by some yeasts strains in optimal growth conditions and in the presence of exogenous stress factors. Nine strains of carotenogenic yeasts were grown aerobically on glucose medium. As the stress factors 10 mmol/l H_2O_2 and 5-10 % NaCl were used, which were added into media i) at the beginning of growth and ii) to the exponentially growing cells. Changes of growth parameters as well as carotenoid production (lycopene, α-carotene and β-carotene) were followed. Ergosterol production was followed as additional parameter of biomass quality. Analyzed strains partially differed in the spectrum of produced carotenoids; the highest content of â-carotene was detected in *S. salmonicolor* CCY 19-4-10. Stress factors added to yeast cultures resulted in different responses. As good producents of enriched biomas could serve above all strains *R. glutinis* and *S. salmonicolor* grown under salt stress. Carotenoids act as lipid-soluble membrane antioxidants whose production is considered as anadaptive mechanism against adverse stress effects. Ability of red yeasts to adapt by means of overproduction of industrially significant metabolites could be of increasing interest for potential biotechnological applications.

6. G. Kogan, J. Šandula, T. A. Korolenko, O. V. Falameeva, O. N. Poteryaeva, S. Ya. Zhanaeva, O. A. Levina, T. G. Filatova, V. I. Kaledin 2002 Increased efficiency of Lewis lung carcinoma chemotherapy with a macrophage stimulator - yeast carboxymethyl glucan. International Immunopharmacology 2:775–781.

The efficiency of chemotherapy of Lewis lung carcinoma with cyclophosphamide was affected by administration of the water-soluble yeast polysaccharide derivative carboxymethylated (1-3)-α-D-glucan (CMG) - a well-known macrophage stimulator. It was found that while cyclophosphamide showed 57% growth inhibition of the intramuscular tumor implants in comparison with the control group, its combined administration with CMG led to 75–90% inhibition. Similarly, increased inhibition of occurrence of lung metastases (up to 92–94%) was observed using the combined application of the two compounds. The stimulatory effect of CMG is not associated with the changed cellularity of peripheral blood, but is rather due to the obviously increased concentration of the intracellular inhibitor of cysteine proteases -stefin A and cystatin C in tumor tissue.

8. P.J. Rice, J.L. Kelley, G. Kogan, H.E. Ensley, J.H. Kalbfleisch, I.W.Browder, D.L. Williams. 2002 Human monocyte scavenger receptors are pattern recognition receptors for (1→3)-β-D-glucans. J. Leukoc. Biol. 72:140-146.

Glucans are cell wall constituents of fungi and bacteria that bind to pattern recognition receptors and modulate innate immunity, in part, by macrophage activation. We used surface plasmon resonance to examine the binding of glucans, differing in fine structure and charge density, to scavenger receptors on membranes isolated from human monocyte U937 cells. Experiments were performed at 25°C using a biosensor surface with immobilized acetylated low density lipoprotein (AcLDL). Inhibition of the binding by polyinosinic acid, but not polycytidylic acid, confirmed the in-teraction of scavenger receptors. Competition studies showed that there are at least two AcLDL binding sites on human U937 cells. Glucan phosphate interacts with all sites, and the CM-glucans and laminarin interact with a subset of sites. Polymer charge has a dramatic effect on the affinity of glucans with macrophage scavenger receptors. However, it is also clear that human monocyte scavenger receptors recognize the basic glucan structure independent of charge.

9. M. Babincová, Z. Bačová, E. Machová, G. Kogan. 2002. Antioxidant activity of carboxymethyl glucan: Comparative analysis. J. Medicinal Food 5:79-83.

Antioxidative capabilities of carboxymethylated (1-3)-α-D-glucan from *Saccharomyces cerevisiae* cell wall, α-tocopherol, and mannitol against lipid peroxidation in phosphatidylcholine liposomes induced by OH radicals produced with Fenton´s reagent (H_2O_2/Fe^{2+}) were studied using absorption UV-VIS spectrophotometry. It was found that (1-3)-α-D-glucan is an antioxidant with the scavenging ability lying between that of α-tocopherol, which is known to be incorporated in lipid bilayer, and the water-soluble antioxidant, mannitol.

10. E. Machová, S. Bystrický, A. Gáliková, G. Kogan, 2002 Preparation of a subcellular conjugate with the lipopolysaccharide from *Vibrio cholerae* 01 using β-D-glucan as matrix. Eur. J. Med. Chem. 37:681-687.

A conjugate consisting of detoxified lipopolysaccharide of *Vibrio cholera*e, a carrier polysaccharide matrix and an immunogenic protein has been synthesised and the reaction conditions have been optimised for obtaining a high degree of conjugation. The obtained construct showed reactivity with the antibodies against *V. cholerae* and can serve as a prospective candidate for preparation of subcellular anti-cholera vaccine.

11. E. Madrigal-Bujaidar, E. Madrigal-Santillán, N. Pages, G. Kogan, G. Chamorro. In: Toxines et recherches biomedicales Goudey-Perriere, F., Bon, C., Puisseux-Dao, S., Sauviat, M.-P., Eds.) Elsevier, Paris, Amsterdam, New York, Oxford, Shannon, Tokyo 2002, ISSN: 1631-9710, ISBN: 2-84299-445-0, pp. 123-132.

Aflatoxins are a group of heterocyclic compounds synthetized by the fungi *Aspergillus flavus* and *A. parasiticus*, which may contaminate a number of agricultural products (e.g., sorghum, wheat, corn, rye, and nuts, or livestock products (milk, meat and eggs). This type of contamination usually causes substantial agricultural and economic losses, besides posing possible harm to human health. Aflatoxin B_1 (AFB_1) in particular, a well known strong mutagen and hepatocarcinogen, is an indirect agent which is biotransformed into several metabolites; among these, the AFB1-8,9 epoxide is characterized as a highly reactive compound that may covalently bond to DNA to produce mutations. AFB_1 also induces various genotoxic events, for example, chromosomal aberrations, micronuclei (MN), and sister-chromatid exchanges (SCEs). Clearly, AFB_1 is a health problem as well as an economic one; yet the different physical and chemical methods that have been developed to inactivate it, have usually produced limited results, mainly related with modifications in the nutritive value of the treated food. Another approach to eliminate or reduce human exposure to the mycotoxin is by the use of substances known as antimutagens, which interfere with its genotoxic action. Studies with this aim in mind have been successfully made in a number of in vitro models, suggesting that it is pertinent to extend this type of research to in vivo mammalian models. In 1993, our laboratory evaluated the effect of ammonium hydroxide (1.5% at final concentration on a dry basis) on mice fed with AFB_1 contaminated corn. The experiment lasted 8 weeks, four of which the animals were fed with the aforementioned chemicals and a balanced diet; the last four weeks, they were given the same balanced diet, uncontaminated corn, and no antimutagen. A MN reduction was found starting at the first week, with a maximum effect of 60 % at week 4. SCEs were also reduced to 55 % at week 4; however, no complete recovery of the genotoxic damage was detected at the end of the experiment. Based on the usefulness of the model, a second study using the probiotic Saccharomyces cerevisiae (0.3%) was also carried out in mouse bone marrow for nine weeks: six weeks were taken to test the effect of the antimutagen, and the other three to check the

recovery of the animals. Observations of MN and SCEs were made at weeks 3, 6, and 9; the results showed a MN reduction of about 50 % starting at week 3, and of 60 % with respect to SCEs in week 6. The observed effect was probably related with either the adsorbent capacity or the chemical interaction of components from the yeast cell wall with AFB_1. The cell wall is mainly constituted by oligosaccharides: mannans (mannoses with bonds α-1,6 and with branches α 1,2 and α 1,3), glucans (glucoses with bonds α 1,6 and branches β 1,3 and β 1,2) and glucomannans. Thus, the next step in this line of research is to test the antigenotoxic capacity of these chemicals. We have recently finished an eight-week study in mouse bone marrow with mannan (from 50 to 500 mg/kg) in which four weeks were spent experimenting with the antimutagen, and the other four were taken up experimenting without the mutagen and the antimutagen. A MN inhibition was found with 500 mg/kg beginning at week 2, the maximum value was reached at week 4 (60%), and SCESs were reduced 50 % in weeks 4 and 8. No complete recovery was observed at the end of the experiment. Finally, research with the three putative antimutagens in mouse liver and intestine is currently under way in our laboratory using the unicellular electrophoresis (comet assay), a study which is being conducted parallel with the search for a possible chemical interaction between the oligosaccharides with AFB_1, applying attenuated total reflectance, HPLC and X-ray diffraction.

12. D.Slameňova, J. Lábaj, L. Kriková, G.Kogan, J. Šandula, N. Bresgen, P.Eckl. 2003. Protective effects of fungal (1?3)-b-D-glucan derivatives against oxidative DNA lesions in V79 hamster lung cells. Cancer Letters 198:153–160.

α-Glucans belong to the class of substances known as biological response modifiers with a broad range of activity. We have investigated two types of glucans: (1→3)- α-D glucan from the baker's yeast *Saccharomyces cerevisiae* and α-glucan–chitin complex from the mycelium of filamentous fungus *Aspergillus niger*. Since these fibrillar α-glucans are insoluble in water, their water-soluble derivatives -carboxymethyl glucan (CM-G), sulfoethyl glucan (SE-G), and carboxymethyl chitin-glucan (CM-CG) were prepared and tested. The aim of the present work was to investigate the protective effect of the prepared glucanderivatives against oxidative DNA damage induced by H_2O_2 and visible light-excited Methylene Blue in V79 hamster lung cells. The level of DNA damage (DNA strand breaks) was measured using the single cell gel electrophoresis, so called comet assay. Our findings demonstrate that all three tested glucans reduce oxidative DNA damage. The ability to reduce genotoxic activity increased in the order: CM-G ,SE-G ,CM-CG. We suggest that the analyzed glucans exhibit protective effects against oxidative damage to DNA as a consequence of scavenging of both, OH radicals and singlet oxygen.

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Recent publications.

1. Dlauchy, D., Tornai-Lehoczki, J., Fülöp, L. & Péter, G. (2003): *Pichia (Komagataella) pseudopastoris* sp. nov., a new yeast species from Hungary. Antonie van Leeuwenhoek **83**:327-332.

Four strains of an unknown yeast species were isolated from rotten willow samples, which were collected in Hungary. Although their phenotypic characteristics suggested that they were conspecific with *Pichia pastoris*, the investigation of their small (18S) and large (26S) subunit rDNA revealed that they belonged to an undescribed yeast species. The description of the new yeast species, *Pichia (Komagataella) pseudopastoris* is given.

2. Péter, G., Tornai-Lehoczki, J., Fülöp, L. & Dlauchy, D. (2003): Six new methanol assimilating yeast species from wood material. Antonie van Leeuwenhoek **84**:147-159.

Ten yeast strains representing six hitherto unknown methanol utilizing yeast species were isolated from tree exudate, bark and rotten wood samples. Following the sequencing of the D1/D2 region of their large (26S) subunit rDNA, the four ascosporogenous species were assigned to the genus *Pichia*, while the two anascosporogenous to the genus *Candida*. Although genetically clearly separated, three of the four new *Pichia* species are phenotypically very similar to *P. pini,* and they can be differentiated only by minor physiological and morphological characteristics. The description is given for the six new species (*C. suzukii, C. hungarica, P.trehaloabstinens, P. pilisensis, P. dorogensis* and *P. zsoltii*).

3. Tornai-Lehoczki, J., Péter, G., Dlauchy, D. (2003): CHROMagar Candida medium as a practical tool for the differentiation and presumptive identification of yeast species isolated from salads. International Journal of Food Microbiology **86**:189-200.

CHROMagar *Candida* medium was used to study the diversity of yeast biota of salad samples, and to presumptively identify the isolates. This medium was originally developed for the selective isolation and presumptive identification of some clinically important yeast species such as *Candida albicans*, *Candida tropicalis*, *Candida krusei*, and *Candida glabrata* on the basis of differences in colour and surface of colonies. Ninety three yeast strains representing 33 species from the culture collection and 39 fresh isolates from different mayonnaise-based mixed salads showed a wide range of hue of colony colours ranging from white to yellow, orange, red, pink, purple, blue, green, etc., as well as different morphological appearances on the CHROMagar *Candida* medium. Therefore, CHROMagar *Candida* medium facilitates the detection of mixtures of yeast species from different samples on a single isolation plate and this medium can be a practical method for the differentiation and rapid presumptive identification of many yeast species occurring frequently in different kind of foods.

XIII. Research Institute for Viticulture and Enology, Matúškova 25, 831 01 Bratialava, Slovakia, Communicated by E. Minárik.

The following papers were recently published or in press.

1. E. Minárik. 2003. New activators of alcoholic and malolactic fermentation (sunmary). Vinič a víno 3:17-18 (in Slovak).

Recently used activators simulating alcoholic and malolactic fermentation in grape must and wine are mainly yeast nutrients. The activators for both fermentations comprise either yeast nutrients (ammonia salts, amino acids, long-chain fatty acids), or substances able to detoxicate the medium (yeast ghosts, cellulose). Macromolecules such as mannoproteins show important efficiency towards proteins, phenolics and tartrate. Yeast mannoproteins may be regarded as also recommended by the Office International de la Vigne et du Vin (O.I.V.) in Paris.

2. E. Minárik. 2003. Starter cultures of lactic acid bacteria (sunmary). Vinič a víno 3:63 (in Slovak).

By the use of malolactic starter cultures (*Oenococcus oeni*) controlled L-malic acid decomposition may be attained. Basic requirements are: low $S0_2$ content (up to 10-15 mg/L free sulphur dioxide), pH level above 3.1, wine temperature 19-20°C and alcohol content of the wine below 12% Vol.

3. E. Minárik. 2003. Dimethyl dicarbonate (DMDC) - an efficient antiseptic agent in winemaking (summary) Vinohrad 41:24 (in Slovak).

By the use of DMDC that is authorized in some countries, wines containing residual sugar may be stabilized. In countries of the European Union DMDC is authorized for the stabilization of soft drinks. DMDC is cited in the Codex Alimentarius as a common additive and is also recommended for sweet grape wines. Though DMDC is not authorized in all countries of the European Union, imported wines containing DMDC may not be refused.

XIV. Alkomohr Biotech Ltd. and Department of Biosciences, Division of General Microbiology, POB 56 (Viikinkaari 9), FIN-00014 University of Helsinki, Finland. Communicated by M. Korhola.

We have this year published one sour dough yeast and lactic acid bacteria article. I presented a poster based principally on that work at the very interesting ISSY 23 in Budapest, Hungary.

1. Simonson, L., Salovaara, H. and Korhola, M. 2003. Response of wheat sour dough parameters to temperature, NaCl and sucrose variations. Food Microbiol. 20:193-199.

Fermentation temperature, NaCl level and sucrose level of a wheat sourdough were varied according to a Box-Behnken response surface design. The effect on yeast increase, LAB increase and sourdough acidity were investigated. Yeast and LAB growth increased with temperature in the range from 15 to 27°C. Optimum growth temperature of two *C. milleri* strains, isolated earlier from the same sourdough as used in this study, was between 26 and 28°C in pure culture. Decreasing temperature affected yeast growth to largely the same extent as LAB. Increasing NaCl addition had a negative effect on yeast growth throughout the range (0 to 3.2%). A low level of NaCl (up to 0.7%) stimulated LAB growth but higher levels decreased LAB growth drastically, and to a much greater degree than yeast growth. Sucrose addition had a stimulatory effect on both yeast and LAB growth. Sourdough TTA increased with sucrose addition throughout the range (0 to 6%) and was largely due to the increase in acetic acid accumulation.

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

Recent publications.

1. Sulo P, Spirek M, Soltesova A, Marinoni G, Piskur J. 2003. The efficiency of functional mitochondrial replacement in *Saccharomyces* species has directional character. FEMS Yeast Res. 4:97-104.
2. Lundgren S, Gojkovic Z, Piskur J, Dobritzsch D. 2003. Yeast β-alanine synthase shares structural scaffold and origin with di-zinc dependent exopeptidases. J Biol Chem. In press.
3. Vernis L, Piskur J, Diffley JF. 2003. Reconstitution of an efficient thymidine salvage pathway in *Saccharomyces cerevisiae*. Nucleic Acids Res. 31:e120.
4. Dobritzsch D, Gojkovic Z, Andersen B, Piskur J. 2003. Crystallization and preliminary X-ray analysis of beta-alanine synthase from the yeast *Saccharomyces kluyveri*. Acta Crystallogr D Biol Crystallogr. 59:1267-1269.
5. Langkjaer RB, Casaregola S, Ussery DW, Gaillardin C, Piskur J. 2003. Sequence analysis of three mitochondrial DNA molecules reveals interesting differences among *Saccharomyces* yeasts. Nucleic Acids Res. 31:3081-91.

6. Spirek M, Yang J, Groth C, Petersen RF, Langkjaer RB, Naumova ES, Sulo P, Naumov GI, Piskur J. 2003. High-rate evolution of *Saccharomyces sensu lato* chromosomes. FEMS Yeast Res. 3:363-73.

7. Mikkelsen NE, Johansson K, Karlsson A, Knecht W, Andersen G, Piskur J, Munch-Petersen B, Eklund H. 2003. Structural basis for feedback inhibition of the deoxyribonucleoside salvage pathway: studies of the *Drosophila* deoxyribonucleoside kinase. Biochemistry. 42:5706-5712.

8. Moller K, Bro C, Piskur J, Nielsen J, Olsson L. 2002. Steady-state and transient-state analyses of aerobic fermentation in Saccharomyces kluyveri. FEMS Yeast Res. 2:233-244.

9. Marinoni G, Piskur J, Lachance MA. 2003. Ascospores of large-spored *Metschnikowia* species are genuine meiotic products of these yeasts. FEMS Yeast Res. 3:85-90.

10. Gojkovic Z, Rislund L, Andersen B, Sandrini MP, Cook PF, Schnackerz KD, Piskur J. 2003. Dihydropyrimidine amidohydrolases and dihydroorotases share the same origin and several enzymatic properties. Nucleic Acids Res. 31:1683-92.

11. Knecht W, Petersen GE, Sandrini MP, Sondergaard L, Munch-Petersen B, Piskur J. 2003. Mosquito has a single multisubstrate deoxyribonucleoside kinase characterized by unique substrate specificity. Nucleic Acids Res. 31:1665-1672.

12. Langkjaer RB, Cliften PF, Johnston M, Piskur J. 2003. Yeast genome duplication was followed by asynchronous differentiation of duplicated genes. Nature 421:848-852.

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

Recent publications.

1. K. Bacigálová, K. Lopandic, M.G. Rodrigues, A. Fonseca, M. Herzberg, W. Pinsker and H. Prillinger. 2003. Phenotypic and genotypic identification and phylogenetic characterisation of *Taphrina* fungi on alder. Mycological Progress 2:179-196.

All *Taphrina* species are dimorphic with a mycelium stage biotrophic on vascular plants and a saprophytic yeast stage. European species of *Taphrina* on *Alnus* species (Betulaceae) were identified using morphological, physiological and molecular characteristics, the latter including determination of PCR fingerprints and of nucleotide sequences from selected nuclear ribosomal DNA regions. PCR fingerprinting gives a good overview of species identification, as do nucleotide sequences, which in addition, help to clarify phylogenetic relationships. *Taphrina alni* is a homogeneous species that exhibited more than 50% similarity in PCR fingerprinting with three different primers. Morphologically, it produces tongue-like outgrowths from female catkins of *Alnus incana. Taphrina robinsoniana* from *A. rugosa* and *A. serrulata* in North America is phylogenetically closely related to *T. alni*, but the two species could be separated by their PCR fingerprints, partial sequences of 26S rDNA (D1/D2) and ITS1/ITS2 sequences. *T. epiphylla* and *T. sadebeckii* are two phylogenetically closely related species. *T. epiphylla* causes witches brooms in crowns of *A. incana*. In addition, *T. epiphylla* forms slightly yellow white-grey leaf spots in midsummer on *A. incana.* Yellow white-grey leaf spots up to 10 mm on *A. glutinosa* are characteristic for *T. sadebeckii.* Both species can be separated well by PCR fingerprinting. Different from *T. epiphylla, T. sadebeckii* is genotypically more heterogeneous. Only two out of three different primers showed similarity values above 50% in different European strains of *T. sadebeckii.* Although genetic variability was not detected in complete sequences of the 18S ribosomal DNA of *T. sadebeckii*, ITS1/ITS2 sequences appeared to be more heterogeneous too. *Taphrina tosquinetii* is a genotypically homogeneous species causing leaf curl on *Alnus glutinosa*. It was not possible to distinguish the yeast phases from different *Taphrina* species on *Alnus* using morphological and physiological characteristics only.

2. Lopandic, K., Sugita, T., Middelhoven, W.J., Herzberg, M., Fell, J.W., Zelger, S., Prillinger, H. 2004. *Trichosporon caseorum* sp. nov. and *Trichosporon lactis* sp. nov., two basidiomycetous yeasts isolated from cheeses. Frontiers in Basidiomycota Mycology (in press).

Two isolates from unripened soft cheese and fresh cheese from the Salzburg region (Austria) were identified as new members of the genus *Trichosporon* by a polyphasic approach including phenotypic, chemotaxonomic and genotypic characterisation. Although genotypic differences at the ribosomal DNA level (18S-, 26S-rDNA and ITS) were insignificant, differences in the RAPD-PCR patterns allowed the separation of the isolates at the species level. *T. caseorum* sp. nov. and *T. lactis* sp. nov. are closely related to the human pathogens *T. ovoides* and *T. inkin* on the phylogenetic trees based on 26S rRNA and 18S rRNA encoding genes and ITS regions. Both species could be distinguished phenotypically too and show strong ability to decompose mutagenic and toxic compounds such as phenol, hydroquinone, pyrogallol, cresole and cinnamate.

XVII. 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>.

Original scientific paper

1. Jamnik P., Raspor P. 2003. Stress response of Yeast *Candida intermedia* to Cr(VI). J. Biochem. Mol. Toxicol. 17(6) in press.

Stress response of the yeast *Candida intermedia* - ZIM 156 exposed to chromium(VI) was investigated. Yeast cells were treated with Cr(VI) in concentrations of 50, 100, 300 and 500 μM in the mid-exponential growth phase. Monitoring of some bioprocess parameters during growth, specifically pO_2 showed that Cr(VI) addition, specifically in concentration of 100 and partially 50 μmol/L increased metabolism intensity, which is connected to induced stress responses. Furthermore, oxidation of 2`,7`-dichlorofluorescin indicated increased intracellular oxidant level, specifically at 100 μM Cr(VI) concentration. Antioxidant defense systems were further investigated. Catalase and superoxide dismutase activity was not increased in the cells exposed to the both Cr(VI) concentrations, which indicate that catalase and superoxide dismutase do not participate in cell defense systems. In contrast intracellular glutathione content in reduced form increased significantly in the cells exposed to 100 μmol Cr(VI)/L. Therefore, we demonstrated that glutathione plays an important role in the stress response of yeast *Candida intermedia* to Cr(VI).

2. Raspor P., Fujs Š., Banszky L., Maraz A., Batic M. 2003. The Involvement of ATP sulfurylase in Se(VI) and Cr(VI) reduction processes in the fission yeast *Schizosaccharomyces pombe*. Appl. Microbiol. Biotechnol. 63:89-95.

The response of *Schizosaccharomyces pombe* towards the oxyanions selenate [Se(VI)] and dichromate [Cr(VI)] was investigated in order to establish the involvement of the yeast ATP sulfurylase in their reduction. An ATP sulfurylase-defective/selenate-resistant mutant of S. pombe (B-579 Se(R) -2) and an ATP sulfurylase-active/selenate-sensitive strain of *S. pombe* (B-579 Se(S)) were included in this study. The inhibitory effect of Se(VI) and Cr(VI) oxyanions on growth and bioaccumulation was measured. The sensitive strain showed natural sensitivity to selenate while the resistant mutant tolerated a 100-fold higher concentration of selenate. These results indicate that selenate toxicity to microorganisms is connected with the reduction of selenate to selenite. Both strains showed similar sensitivity to Cr(VI) and in this study there was no evidence that ATP sulfurylase participates in the reduction process of Cr(VI).

3. Paš M., Milačič R., Drašlar K., Pollak N., Raspor P. 2003. Uptake of chromium(III) and chromium(VI) compounds in the yeast cell structure. *Biometals* - in press.

The study presented in this article investigated the influence of different Cr(III) and Cr(VI) compounds in the cultivation medium on the uptake and localization of chromium in the cell structure of the yeast *Candida intermedia*. The morphology of the yeast cell surface was observed by the scanning electron microscopy. Results demonstrated that the growth inhibitory concentration of Cr(III) in the cultivation medium induced changes in the yeast cell shape and affected the budding pattern, while inhibitory concentration of Cr(VI) did not cause any visible effects on morphological properties of the yeast cells. The amount of total accumulated chromium in yeast cells and the distribution of chromium between the yeast cell walls and spheroplasts were determined by atomic absorption spectroscopy. No significant differences were found neither in total chromium accumulation nor in the distribution of chromium in yeast cell walls and spheroplasts between the two of Cr(VI) compounds. Conversely, substantial differences between Cr(III) compounds were demonstrated in the total uptake as well as the localization of chromium in yeast cells.

4. Cadez N., Poot Gé A., Raspor P., Smith M. Th. 2003. *Hanseniaspora meyeri, Hanseniaspora clermontia*e, *Hanseniaspora lachancei* and *Hanseniaspora opuntia*e, novel apiculate yeast species. Int. J. Syst. Evol. Microbiol. 53:1671-1680.

Fourteen apiculate yeast strains isolated from various sources in South Africa, North America and Hawaiian islands were found genetically divergent from other *Hanseniaspora-Kloeckera* species using polymerase chain reaction of random-amplified polymorphic DNA (RAPD-PCR). After cluster analysis of the RAPD-PCR fingerprints five groups were recognized. DNA reassociation values among representatives of the groups and the strains of *Hanseniaspora-Kloeckera* species revealed that the strains represent five novel species. Four are described here as new species of *Hanseniaspora: H. meyeri* (type CBS 8734 T), *H. clermontiae* (type CBS 8821 T), *H. lachancei* (type CBS 8818 T) and *H. opuntiae* (type CBS 8733 T). The fifth novel species, which is only represented by a single strain, CBS 8772 is not introduced as a new taxon. Phylogenetic analyses of D1/D2 region of the 26S rDNA and internal transcribed spacer (ITS) regions with 5.8S rDNA sequences placed *H. meyeri, H. clermontiae, H. lachancei, H. opuntiae* and strain CBS 8772 close to *H. uvarum* and *H. guilliermondi*i. The key characteristics for standard physiological identification of *H. clermontiae* and *H. lachancei* were maximal growth temperature and assimilation of 2-keto-D-gluconate, respectively. However, *H. opuntiae* and strain CBS 877*2,* are physiologically indistinguishable from *H. guilliermondii* and *H. meyeri* from *H. uvaru*m. These three taxa can be identified either by ITS sequencing or PCR-RFLP of ITS regions using restriction enzymes *Mbo*II and *Hin*fI.

XVIII. CREM – Centro de Recursos Microbiológicos, Secção Autónoma de Biotecnologia, Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, 2829-516 Caparica, Portugal. Communicated by A. Fonseca <amrf@fct.unl.pt> and J. P. Sampaio <jss@fct.unl.pt>.

The following papers have been recently published (abstracts included in the last issue have been omitted).

1. Inácio, J., Behrens, S., Fuchs, B.M., Fonseca, Á., Spencer-Martins, I. and Amann, R. 2003. In situ accessibility of *Saccharomyces cerevisiae* 26S rRNA to Cy3-labeled oligonucleotide probes comprising the D1 and D2 domains. Appl. Environ. Microbiol. **69**:2899-2905.

Fluorescence in situ hybridization (FISH) has proven to be most useful for the identification of microorganisms. However, species-specific oligonucleotide probes often fail to give satisfactory results. Among the causes leading to low hybridization signals is the reduced accessibility of the targeted rRNA site to the oligonucleotide, mainly for structural reasons. In this study we used flow cytometry to determine whole-cell fluorescence intensities with a set of 32 Cy3-labeled oligonucleotide probes covering the full length of the D1 and D2 domains in the 26S rRNA of *Saccharomyces cerevisiae* PYCC 4455T. The brightest signal was obtained with a probe complementary to positions 223 to 240. Almost half of the probes conferred a fluorescence intensity above 60% of the maximum, whereas only one probe could hardly detect the cells. The accessibility map based on the results obtained can be extrapolated to other yeasts, as shown experimentally with 27 additional species (14 ascomycetes and 13 basidiomycetes). This work contributes to a more rational design of species-specific probes for yeast identification and monitoring.

2. Kirschner, R., Sampaio, J.P., Begerow, D., Chen, Z.-C. and Oberwinkler, F. 2003. *Mycogloea nipponica* – the first known teleomorph in the heterobasidiomycetous yeast genus *Kurtzmanomyces*. Antonie van Leeuwenhoek **84**: 109-114.

3. Gadanho, M., Almeida, J.M.F. and Sampaio, J.P. 2003. Assessment of yeast diversity in a marine environment in the South of Portugal by microsatellite-primed PCR. Antonie van Leeuwenhoek **84**: 217-227.

4. Libkind, D., Brizzio, S, Ruffini, A., Gadanho, M., van Broock, M and Sampaio, J.P. 2003. Molecular characterization of carotenogenic yeasts from aquatic environments in Patagonia, Argentina. Antonie van Leeuwenhoek **84**: 313-322.

Fifteen aquatic environments (lakes, lagoons and rivers) of glacial origin in the northern Andean Patagonia (Argentina) were surveyed for the occurrence of red yeasts. Subsurface water samples were filtered and used for colony counting and yeast isolation. A preliminary quantitative analysis indicated that total yeast counts ranged between 0 and 250 cells l^{-1}. A polyphasic approach including physiological and molecular methods was used for the identification of 64 carotenogenic yeast strains. The molecular characterisation of the isolates was based on the mini/micro satellite-primed technique (MSP-PCR) employing the $(GTG)_5$ and the M13 primers. Comparison of representative fingerprints of each group with those of the type strains of pigmented yeasts allowed the expeditious identification of 87.5% isolates. The sequence analysis of the D1/D2 domains of the 26S rDNA was employed to confirm identifications and in the characterization of the unidentified MSP-PCR groups. Teleomorphic yeast species were detected by performing sexual compatibility assays. The isolates corresponded to 6 genera and 15 yeast species, including four new yeast species of the genera *Cryptococcus* (1), *Rhodotorula* (1) and *Sporobolomyces* (2). *Rhodotorula mucilaginosa* was found in the majority of the samples and represented ca. 50% of the total number of isolates. However, this yeast was not detected in aquatic environments with very low anthropic influence. Other frequent yeast isolates were teleomorphic yeast species of *Rhodosporidium babjevae*, *R. kratochvilovae* and *Sporidiobolus salmonicolor*. This study represents the first report on red yeast occurrence and biodiversity in North-western Patagonia.

The following papers have been accepted for publication.

1. Inácio, J. and Fonseca, Á. Reinstatement of *Rhodotorula colostri* (Castelli) Lodder and *Rhodotorula crocea* Shifrine & Phaff, former synonyms of *Rhodotorula aurantiaca* (Saito) Lodder. FEMS Yeast Research.

Rhodotorula aurantiaca (Saito) Lodder is an anamorphic basidiomycetous yeast species that belongs to the so-called 'Erythrobasidium lineage' of the Urediniomycetes, according to molecular phylogenetic studies based on nucleotide sequence analyses of different ribosomal DNA regions. In the most recent editions of the yeast taxonomy treatises the species *Rhodotorula colostri* (Castelli) Lodder and *Rhodotorula crocea* Shifrine & Phaff were listed as synonyms of *R. aurantiaca*. Taxonomic heterogeneity within *R. aurantiaca* was demonstrated in a study based on whole-cell protein profiles and is also hinted at by the observed differences in physiological and biochemical characteristics among the different strains under that species name. We determined partial nucleotide sequences of the 26S rRNA gene (D1/D2 domains) of strains maintained in the CBS culture collection under *R. aurantiaca*, including the type strains of its synonyms. The results showed that *R. colostri* and *R. crocea* are clearly distinct from *R. aurantiaca* and from any other currently recognised basidiomycetous yeast species. Furthermore, phylogenetic analysis of the sequence data placed the former two species in separate lineages of the Microbotryomycetidae: *R. colostri* in the 'ruineniae clade' (Sporidiobolus lineage or Sporidiobolales) and *R. crocea* loosely linked to *R. javanica* (Microbotryum lineage).

2. Inácio, J., Rodrigues, M.G., Sobral, P. and Fonseca, Á. Characterisation and classification of phylloplane yeasts from Portugal related to the genus *Taphrina* and description of five novel *Lalaria* species. FEMS Yeast Research.

Taphrina Fries is a genus of dimorphic ascomycetes comprising more than 90 species distinguished by the specific infections they produce on different vascular plants. Their filamentous states are restricted to parasitised plant tissue whereas the yeast states are saprobic and can be grown on artificial media. The latter coincide with the anamorphic phases and have been given separate nomenclatural status by the erection of the genus *Lalaria* R.T. Moore. In its original circumscription *Lalaria* included only 23 yeast states of known species of Taphrina and its creation was then redundant. Here we describe five novel species in the genus *Lalaria* to accommodate a total of 44 yeast isolates obtained mainly from leaf surfaces (phylloplane) of different plants in Portugal: *Lalaria arrabidae* sp. nov. (one strain), *L. carpini* sp. nov. (one strain), *L. inositophila* sp. nov. (37 strains), *L. kurtzmanii* sp. nov. (one strain) and *L. veronaerambellii* sp. nov. (4 strains). *Lalaria inositophila* was notable for its widespread occurrence since it was recovered during two consecutive years from the leaves of miscellaneous plant species. In the absence of sexual states and of unequivocal associations to particular host plants, the taxonomic relationship of the novel species to the yeast states of *Taphrina* available from culture collections was verified by the comparative analysis of physiological and molecular characteristics. The latter included PCR-fingerprinting using single primers for microsatellite regions, sequencing of the 5' end of the 26S rRNA (LSU) gene (D1/D2 domains) and of the ITS1 and ITS2 rDNA spacer regions, and DNA-DNA hybridisation experiments. An emended description of the genus *Lalaria* is provided.

3. Sampaio, J.P., Inácio, J., Fonseca, Á., Gadanho, M., Spencer-Martins, I., Scorzetti, G. and Fell, J.W. *Auriculibuller fuscus* gen. nov., sp. nov. and *Bullera japonica* sp. nov., novel taxa in the Tremellales. International Journal of Systematic and Evolutionary Microbiology.

Seven phylloplane yeast strains collected in the Arrábida Natural Park (Portugal) and preliminarily identified as *Bullera alba*, the anamorphic stage of *Bulleromyces albus*, were investigated. Contrary to *Bulleromyces albus*, these isolates produced a brownish pigment when grown on potato dextrose agar. The pigment caused a darkening of the cultures and diffused into the culture medium. Mating studies revealed that the Arrábida isolates did not react with the different mating types of *Bulleromyces albus,* but were sexually compatible among them and produced mycelium with clamp connections, haustoria and transversally septate basidia that ejected the basidiospores. The various taxonomic criteria evaluated during the present study and the comparison with other sexual taxa of the Tremellales, indicated that this teleomorph should be classified in a new genus. Therefore, the genus *Auriculibuller* and the species *A. fuscus* (type PYCC 5690^T = CBS 9648^T) are proposed. In addition, during the course of this investigation a new *Bullera* species, *Bullera japonica* (type PYCC 4534^T = CBS 2013^T), was found among collection isolates formerly identified as *Bullera alba*. In a molecular phylogenetic analysis of the D1/D2 domains of the 26S rDNA and the ITS, the two taxa were found to be closely related but distinct at the species level.

XIX. Dipartimento di Biologia Vegetale, Università degli Studi di Perugia, Borgo XX Giugno 74, 06100 Perugia, Italy. Communicated by A. Vaughan-Martini.

We at the DBVPG Industial Yeasts Collection are happy to announce that we received a fantastic gift from Dr. Helen Vishniac, who sent out an SOS about a year ago, looking for a someone able to take on her collection of soil yeasts isolated from various parts of the world. DBVPG willingly answered the call and we are happy to report that most of the cultures have now been revitalized, lyophilized and frozen. Anyone who is interested in knowing more about these strains can look at our website *http://www.agr.unipg.it/dbvpg/* or contact us at <dbvpg@unipg.it>. Below are some recent publications by our group.

1. P. Buzzini, A. Martini. 2002. Extracellular enzymatic activity profiles in yeast and yeast-like strains isolated from tropical environments. J. Appl. Microbiol. 93:1020-1025.

2. P. Buzzini, A. Pieroni. 2003. Antimicrobial activity of extracts of *Clematis vitalba* L. towards pathogenic yeast and yeast-like microorganisms. Fitoterapia 74:397-400.

3. P. Buzzini, A. Martini, F. Cappelli, U. M. Pagnoni, P. Davoli. 2003. A study on volatile organic compounds (VOCs) produced by tropical ascomycetous yeasts. Antonie van Leeuwenhoek 84:301-311.

4. P. Buzzini, S. Berardinelli, B. Turchetti, G. Cardinali, A. Martini. 2003. Fingerprinting of yeasts at the strain level by differential sensitivity responses to a panel of selected killer toxins. Syst. Appl. Microbiol. 26:466-470.

5. P. Buzzini, A. Martini, U. M. Pagnoni, P. Davoli. 2003. Production of flavoured volatile organic compounds (VOCs) by *Candida oleophila* GK10. Optimization using factorial design and response surface. Enzyme. Microb. Technol. 33:535-754.

6. Blasi, E., Brozzetti, A., Francisci, D., Neglia, R., Cardinali, G., Bistoni, F., Vidotto, V. and F., B. 2001. Evidence for Microevolution in a Clinical Case of Recurrent *Cryptococcus neoformans* Meningoencephalitis. European J. Clin. Microbiol. Infect. Diseases 20:535-543.

7. Cardinali, G., Bolano, A. and Martini, A. 2001. A DNA Extraction and purification method for several yeast genera. Ann. Microbiol. 51:121-130.

8. Liti, G., Wardrop, F. R., Cardinali, G., Martini, A. and Walker, G. M. 2001. Differential responses to antimycin A and expressions of the Crabtree effect in selected *Kluyveromyces* spp. Ann. Microbiol 51:235-243.

9. Bolano, A., Stinchi, S., Preziosi, R., Bistoni, F., Allegrucci, M., Baldelli, F., Martini, A. and Cardinali, G. 2001. Rapid methods to extract DNA and RNA from *Cryptococcus neoformans*. FEMS Yeast Res. 1:221-224.

10. Cardinali, G. , A. Martini, R. Preziosi, F. Bistoni, and F. Baldelli. 2002. Multicenter comparison of three different analytical systems in the evaluation of DNA banding patterns from *Cryptococcus neoformans*. J. Clin. Microbiol. 40:2095-2100.

11. Cardinali, G., Liti, G. and Fatichenti, F. 2002. Non-radioactive dot blot DNA reassociation for yeast unequivocal identification. In "Laboratory manual in nonconventional yeasts. Genetics, Biochemestry, Molecular Biology and Biotechnology" K. Wolf, K.D. Breunig and G. Barth (eds.). Springer-Verlag. Berlin.

12. Cardinali, G., Bolano, A. and Fatichenti, F. 2002. Rapid method to extract DNA from capsulated yeast species. In "Laboratory manual in nonconventional yeasts. Genetics, Biochemestry, Molecular Biology and Biotechnology" K. Wolf, K.D. Breunig and G. Barth (eds.). Springer-Verlag. Berlin.

13. Martini, A., Vaughan-Martini, A.E. and Cardinali, G. 2002. Taxonomy rules and classification hints for non-taxonomists working with unknown or ill-identified yeast cultures. In "Laboratory manual in nonconventional yeasts. Genetics, Biochemestry, Molecular Biology and Biotechnology" K. Wolf, K.D. Breunig and G. Barth (eds.). Springer-Verlag. Berlin.

14. Cardinali G. 2003. Measure of species variability for a microbial taxonomy based on the relative resemblance. Rivista di Biologia/Biology Forum 96:271-292.

15. Cardinali G., Maraziti,F. and Selvi, S. 2003. Electrophoretic data classification for phylogenetics and biostatistics. Bioinformatics 19:2163-2165.

16. L. Zacchi and A. Vaughan-Martini. 2002. Yeasts associated with different species of insects collected in agricultural areas of Perugia, Italy. Ann. Microbiol. 52:237-244.

17. L. Zacchi and A. Vaughan-Martini. 2003. Distribution of three yeast and yeast-like species within a population of soft scale insects (*Saissetia oleae)* as a function of developmental age. Ann. Microbiol. 53:43-46.

18. L. Zacchi, P. Angelini and A. Vaughan-Martini. 2003. Yeast distribution in a truffle-field ecosystem. Ann. Microbiol. 53:275-282.

19. Vaughan-Martini, A. 2003. Reflections on the classification of yeasts for different end users in biotechnology, ecological studies or medicine. Internatl. Microbiol. 6:175-182.

20. Martini, A. 2003. Biotechnology of natural and winery-associated strains of *Saccharomyces cerevisiae.* Internatl. Microbiol. 6:207-210.

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

Publications since our last communication.

1. Davydenko, S. G., J. K. Juselius, T. Munder, E. Bogengruber, J. Jäntti, and S. Keränen (2003). Screening for novel essential genes of *Saccharomyces cerevisiae* involved in protein secretion. Yeast. In press.

2. Gupta, G.D., Free, S.J., Levina, N.N., Keränen, S. and Heath, I.B. 2003. Two divergent plasma membrane syntaxin-like SNAREs, *nsyn1* and *nsyn2,* contribute to hyphal tip growth and other developmental processes in *Neurospora crassa.* Fungal Genet. Biol. 40, 271-286.

3. Jäntti, J., M. K. Aalto, M. Öyen, L. Sundqvist, S. Keränen and H. Ronne (2002). Characterization of temperature sensitive mutations in yeast syntaxin homologues Sso1p and Sso2p, and evidence of a distinct function for Sso1p in sporulation. J. Cell Sci. 115:409-420.

3. Öyen, M, Jäntti, J, S. Keränen and H. Ronne (2003). Mapping of sporulation-specific function in the syntaxin SSO1 gene. Curr. Genet. *In Press*

4. Pitkänen, J.-P., Aristidou, A., Salusjärvi, L., Ruohonen, L. and Penttilä, M. (2003). Metabolic flux analysis of xylose metabolism in recombinant *Saccharomyces cerevisiae* using continuous culture. Metab. Eng. 5, 16-31.

5. Rautio, J. and Londesborough, J. (2003). Maltose transport by brewer's yeasts in brewer's worts. J. Inst. Brew. *in press.*

6. Reinman, M, J. Jäntti, K. Alfthan, S. Keränen, H. Söderlund and K. Takkinen (2003). Functional inactivation of the conserved Sem1p in yeast by intrabodies. Yeast. 20, 1071-1084.

7. Richard, P. (2003) The rhythm of yeast. FEMS Microbiol. Rev. 27, 547-557.

8. Richard. P., Verho, R., Putkonen, M., Londesborough, J. and Penttilä, M. (2003). Production of ethanol from L-arabinose by *Saccharomyces cerevisiae* containing a fungal L-arabinose pathway. FEMS Yeast Research 3, 185-189.

9. Salusjärvi, L., Poutanen, M., Pitkänen, J.-P., Koivistoinen, H., Aristidou, A., Kalkkinen, N., Ruohonen, L. and Penttilä, M. (2003). Proteome analysis of recombinant xylose-fermenting *Saccharomyces cerevisiae*. Yeast, 20, 295-314.

10. Toikkanen, J. H., Miller, K. H., Söderlund, H., Jäntti, J. and Keränen, S. (2003). The beta subunit of the Sec61p endoplasmic reticulum translocon interacts with exocyst complex in *Saccharomyces cerevisia*e. J. Biol. Chem. 278, 20946-20953. A Highlight commentary of this article appeared in Nature Reviews in Molecular Cell Biology 4, 344.

11. Torkko, J.M., Koivuranta, K.T., Kastaniotis, A.J., Airenne, T.T., Glumoff, T., Ilves, M., Hartig, A., Gurvitz, A. and Hiltunen, J.K. (2003). *Candida tropicalis* expresses two mitochondrial 2-enoyl thioester reductases that are able to form both homodimers and heterodimers. J. Biol. Chem. 278, 41213-41220.

12. Vasara, T., Keränen, S., Penttilä, M. and Saloheimo, M. 2002. Characterization of two *14-3-3* genes from *Trichoderma reesei:* interactions with yeast secretory pathway components. Biochim. Biophys. Acta 1590, 27-40**.**

13. Verho, R., Londesborough, J., Pentillä, M. and Richard, P. (2003) Engineering the cofactor regeneration for improved pentose fermentation in Saccharomyces cerevisiae. Appl. Environ. Microbiol. 69, 5892-5897.

14. Verho, R., Richard, P., Jonson, P.H., Sundqvist, L., Londesborough, J. and Penttilä, M. (2003) Identification of the first fungal NADP-GAPDH from *Kluyveromyces lactis*. Biochemistry 41, 13833-13838.

The following MSc. theses, supervised at VTT, have been presented.

15. Pedro Guimarães (2003) The effect of growth conditions on the kinetics of maltose transport by brewer's yeast. University of Minho, School of Engineering, Braga, Portugal.

16. Antti Kokko (2003) Functional studies of yeast fumarase. Department of Chemical Technology, Helsinki University of Technology, Finland.

17. Reetta Kuokka (2002) Applying genome-wide methods for a mutant characterization in a supersecretory *Saccharomyces cerevisiae* strain. Department of Engineering Physics and Mathematics, Helsinki University of Technology, Finland.

18. Satu Kuorelahti (2003) *Saccharomyces cerevisiae*-leivinhiivan transketolaasien rooli ksyloosifermentaatiossa (The role of the transketolases in xylose fermentation by *S. cerevisae*). Department of Biological and Environmental Sciences, University of Jyväskylä, Finland.

XXI. Alcohol Division, Alltech, Inc., 3031 Catnip Hill Pike, Nicholasville, KY 40356 USA. Communicated by D. Childs <dchilds@alltech,com>.

The fourth edition of the following book is now available. To purchase a copy (UDS$200.00), please contact Deborah Childs, Tel.: 1-859-887-3246 <dchilds@alltech.com>.

1. K.A. Jacques, T.P. Lyons, and D.R. Kelsall. 2003. The Alcohol Textbook, a reference for the beverage, fuel and industrial alcohol industries, 4th Edition. Nottingham University Press, Nottingham. ISBN 1-897676-13-1.

Contents

Foreword - T.P. Lyons.

Ethanol industry today

1. Ethanol around the world: rapid growth in policies, technology and production - T.P. Lyons.

Raw material handling and processing

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3. Enzymatic conversion of starch to fermentable sugars - R.F. Power
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yeast: now and in the future - C.A. Abbas

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14. Production of Scotch and Irish whiskies: their history and evolution - T.P. Lyons
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27. Ethanol production and the modern livestock feed industry: a relationship continuing to grow - K.A. Jacques
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The Alcohol Alphabet - A glossary of terms used in the ethanol-producing industries

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

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

1. Rosa C.A., M.A. Lachance, J.O.C. Silva, A. C.P. Teixeira, M.M. Marini, Y. Antonini & R.P. Martins. 2003. Yeast communities associated with stingless bees. FEMS Yeast Res. 4:271-275.

The yeast communities associated with the stingless bees *Tetragonisca angustula, Melipona quadrifasciata* and *Frieseomelitta varia* were studied. The bees *T. angustula* and *F. varia* showed a strong association with the yeast *Starmerella meliponinorum*. *Melipona quadrifasciata* more frequently carried a species related to *Candida apicola*, but also vectored low numbers of *S. meliponinorum*. Some of the yeasts isolated from adult bees were typical of species known to occur in flowers. Other yeast species found in adult bees were more typical of those found in the phylloplane. *Starmerella meliponinorum* and the species in the *C. apicola* complex, also part of the *Starmerella* clade, may have a mutualistic relationship with the bees studied. Many yeasts in that group are often found in bees or substrates visited by bees, suggesting that a mutually beneficial interaction exists between them.

2. Oliveira, E. S., C.A. Rosa, M.A. Morgano & G.E. Serra. 2003. Fermentation characteristics as criteria for selection of cachaça yeast. World J. Microbiol. Biotechn. 19: (in press).

The fermentation characteristics of 24 strains of *Saccharomyces cerevisiae* and one strain of *Candida apicola, C. famata, C. guilliermondii, Hanseniaspora occidentalis*, *Pichia subpelicullosa* and *Schizosaccharomyces pombe* were evaluated for the production of cachaça. They were isolated from small cachaça distilleries (27), industrial cachaça distilleries (2) and one sugarcane alcohol distillery. The yeasts showed significant differences in ethanol yield, substrate conversion, efficiency, conversion factors of substrate into ethanol ($Y_{p/s}$), cells ($Y_{x/s}$), organic acids ($Y_{ac/s}$) and glycerol ($Y_{g/s}$), and maximum specific growth rate (μ_{max}). In general the *S. cerevisiae* strains showed better fermentation potential, with yields between 83 and 91%

and μ_{max} between 0.450 and 0.640 h^{-1}, several of them being comparable with the high performance yeast used in the industrial production of ethanol, which was adopted as a reference. The non-*Saccharomyces* strains showed high efficiency, very low ethanol yield and very high $Y_{ac/s}$ and $Y_{g/s}$ values, except *Pichia subpelliculosa*, which behaved very similarly to the *S. cerevisiae* strains. Hierachical Cluster Analysis and Principal Component Analysis showed the fermentation yield (or substrate conversion) as being the variable which contributed most to the separation of the strains into different groups.

3. Rosa L.H., K.M.G. Machado, C.C. Jacob, M. Capelari, C.A. Rosa & C.L. Zani. 2003. Screening of Brazilian basidiomycetes for antimicrobial activity. Mem. Int. Oswaldo Cruz 98: 967-974.

A total of 103 isolates of basidiomycetes, representing 84 species from different Brazilian ecosystems, were evaluated for their antifungal and antibacterial activity in a panel of pathogenic and non-pathogenic microorganisms. Tissue plugs of the fruiting bodies were cultivated in liquid media and the whole culture extracted with ethyl acetate. Crude extracts from *Agaricus* cf. *nigrecentulus, Agrocybe perfecta, Climacodon pulcherrimus, Gloeoporus thelephoroides, Hexagonia hydnoides, Irpex lacteus, Leucoagaricus* cf. *cinereus, Marasmius* cf. *bellus, Marasmius* sp., *Nothopanus hygrophanus, Oudemansiella canarii, Pycnoporus sanguineus, Phellinus* sp., and *Tyromyces duracinus* presented significant activity against one or more of the target microorganisms. Eight isolates were active only against bacteria while three inhibited exclusively the growth of fungi. Two extracts presented wide antimicrobial spectrum and were active against both fungi and bacteria. Differences in the bioactivity of extracts obtained from isolates from the same species were observed.

4. Trindade, R.C., M.A. Resende, R.S. Pimenta, M.A. Lachance & C.A. Rosa. 2003. *Candida sergipensis*, a new asexual yeast species isolated from frozen pulps of tropical fruits. Antonie van Leeuwenhoek (in press).

Sixteen strains of the new yeast species *Candida sergipensis* have been isolated from frozen pulps of the tropical fruits umbú (*Spondias tuberosa* Avr. Cam.) and mangaba (*Hancornia speciosa* Gom.). *Candida sergipensis* was one of the prevalent species in the yeast community of these substrates. The new asexual ascomycetous yeast is phylogenetically related to *C.spandovensis* and *C. sorbophila*, species belonging to the *Wickerhamiella* clade, as evidenced by the sequences of the D1/D2 domains of their large subunit ribosomal DNAs. The species *C.sergipensis* and *C. spandovensis* can be separated on the basis of growth on 50% glucose agar, xylose and succinate, negative for the first species and positive for the second. The type culture is strain UFMG- R188 (CBS 9567).

5. Resende J. C.P., G.R. Franco, C.A. Rosa, R.C. Hahn & J.S. Hamdan. 2003. Phenotypic and genotypic identification for *Candida* spp. isolated from hospitalized patients. Rev. Iberoamer. Micol. 20(4): (in press).

XXIII. Department of Biology, University of Western Ontario, London, Ontario, Canada N6A 5B7. Communicated by M.A. Lachance <lachance@uwo.ca>.

Some remarks on Wickerham's culture media for yeasts.

We owe a great deal to Dr. L.J. Wickerham for having formulated many of the important media used by yeast researchers. These include **Yeast Nitrogen Base** (available traditionally in three formulations), **Yeast Carbon Base**, **Yeast Morphology (Agar) Medium**, **Vitamin-free Yeast Base**, **D20**, and **YM** agar. In particular, the family of synthetic media (**YNB**, **YCB**, etc.) has yet to be improved upon. These media allow the determination of nutritional requirements of yeasts with minimal background interference. I have often wondered how Wickerham arrived at the precise formulation of those media, and found it difficult to obtain relevant information. Wickerham was, of all evidence, a modest man, and the exact rationale for each medium cannot be found explicitly in the literature; passing allusions are hidden in various articles dealing with taxonomy in general or specific taxonomic studies.

The reason for adding two of the three amino acids found in the synthetic media is given in part in a paper on the development of **YNB** (Wickerham and Burton 1948 J Bacteriol 56:363). Methionine obviated the sulfate transport deficiency of some species currently classified in the genus *Saccharomycopsis*. This property was later found in all species of *Starmera* (Phaff et al. 1992 Int J Syst Barteriol 42:459) and *Saccharomycopsis* (Lachance et al. 2000 Can J Microbiol 46:495). Tryptophan was required by two species in the *Saccharomyces sensu lato* group. I have not come across any justification for the addition of histidine. Some users have noted that the inclusion of 0.5% ammonium sulfate in **YNB** is excessive, at it provides many times the amount of ammonium nitrogen required for the growth of any yeast. However, ammonium sulfate at that concentration is not toxic and provides a certain amount of bulk that makes the dried powder stable and conveniently weighed. I have found that agar media containing **YCB**, neat or supplemented with a small amount (*e.g.*, 0.01%) ammonium sulfate, are excellent substitutes for **Corn Meal Agar** or other media designed to stimulate filamentous growth or sporulation. Clearly, the active principle here is nitrogen limitation.

The isolation, maintenance, and sporulation medium **YM** is described in Wickerham's seminal article describing the use of an expanded array of carbon and nitrogen sources in yeast systematics (Wickerham 1951 USDA Technical Bulletin 1029). 'YM' simply stands for Yeast-Malt medium (based on the presence of yeast extract and malt extract), although some have variously interpreted the acronym as meaning 'Yeast Medium' or 'Yeast and Moulds' medium. Why are three complex ingredients included, and why are they added at the specified concentrations? Dr. J.P. van der Walt once told me that Wickerham thought it important to provide hydrolysates of tissues from all three 'kingdoms', animal, plant, and microbial, given that yeasts are found in association with all three groups of organisms. One can only speculate that the concentrations were inspired on those used for bacteriological media - *v.g.*, 0.5% beef peptone and 0.3% beef extract are used in **Nutrient Agar**. Is each of the ingredients in **YM** essential or even useful? Geneticists normally use **YPD**, which lacks malt extract. Beef peptone and yeast extract are added at higher concentrations, presumably causing yeasts (mostly *Saccharomyces cerevisiae*) to form larger colonies, more rapidly. Some workers use a medium containing only glucose and soy peptone. In Prof. H.J. Phaff's laboratory, 5% malt extract agar was used for many years

for the isolation and maintenance of yeasts. However, when Phaff and colleagues began to investigate cactophilic yeasts, they observed that several species grew poorly and slowly on malt

agar, and turned to **YM** instead (W.T. Starmer, personal communication). **YAG** (0.5% yeast autolysate 5% glucose) broth was used in Phaff's laboratory to grow yeasts for DNA extraction or enzyme isolation. Phaff indicated that the medium provides a good biomass yield and naturally resists the changes in pH observed with many other media, specially those containing ammonium salts.

I have often wondered about the use of **YM** agar for the isolation of yeasts from natural habitats. Recently, out of curiosity, I added nine media to the series normally used for the characterization of yeasts by replica plating, and compared their efficacy on 160 isolates from the nectar of a tropical plant. In addition to normal **YM** (control), I included **YM** with 5% glucose (instead of 1%), to mirror **YAG** medium; **YM** plus 0.5% NaCl, based on bacteriological custom and the observation that pulcherrimin-producing yeasts (*Kluyveromyces* and *Metschnikowia* spp.) exhibit this characteristic more intensely on high salt media; media containing glucose and either yeast extract, malt extract, or beef peptone, each at the concentration used in **YM** (0.3, 0.3, or 0.5%, respectively); and three **YM** formulations in which either yeast extract, malt extract, or peptone was deleted. An increased glucose concentration had little effect, although there were no osmophilic yeasts among the isolates. The addition of salt, even at the low concentration of 0.5%, caused some species to grow much more slowly and some not at all! The medium containing malt extract alone resulted in substantially weaker growth for all species. This is not surprising as a large proportion of the extract is occupied by carbohydrates. The use of peptone alone caused a less dramatic reduction in growth, but in some cases there was no growth at all. Yeast extract alone supported abundant growth in all species, and in most cases the yield was comparable to that obtained on complete **YM**. The 'drop-out' media demonstrated that malt extract and, to a lesser extent, peptone enhance the distinctiveness of colonies, which is an important element in the isolation of yeasts from natural habitats. The complete **YM**, as formulated by Wickerham, provided the best growth and colony differentiation.

The following papers, whose abstracts were given in the previous issue, are now in print.

1. Lachance M.A., Daniel H.M., Meyer W., Prasad G.S., Gautam S.P., Boundy-Mills K. 2003. The D1/D2 domain of the large-subunit rDNA of the yeast species *Clavispora lusitaniae* is unusually polymorphic. FEMS Yeast Res. **4**:253-8.
2. Thanh, V.N., D.A. Haia, M.A. Lachance. *Issatchenkia hanoiensis*, a new yeast species isolated from frass of the litchi fruit borer *Conopomorpha cramerella* Snellen. FEMS Yeast Res. **4**:113-117.
3. Lachance, M.A. J.M. Bowles, and W.T. Starmer. 2003. Geography and niche occupancy as determinants of yeast biodiversity: the yeast-insect-morning glory ecosystem of Kipuka Puaulu, Hawai'i. FEMS Yeast Res. **4**:104-111.

The following lectures were given at the International Course on Molecular Ecology, Taxonomy, and Identification of Yeasts, Universidade Nova de Lisboa, Caparica, Portugal.

4. Lachance, M.A. 2003. Fundamentals of molecular systematics.
5. Lachance, M.A. 2003. Molecular Ecology of Yeasts: principles and cases.
6. Lachance, M.A. 2003. Yeast Ecology and Systematics in the 21st Century.

Other lectures.

7. Lachance, M.A., J.M. Bowles, and W.T. Starmer. 2003. Geography and niche occupancy as determinants of yeast biodiversity. 23rd International Specialised Symposium on Yeasts, Budapest, Hungary.
8. Lachance, M.A. Endemic and introduced yeasts and beetles in Hawai'i. Department of Entomology, Cornell University, Ithaca, NY.
9. Lachance, M.A. 2003. The Phaff School of Yeast Ecology. International Symposium: "Learning From Yeast", Santiago de Compostela, Spain. The proceedings of this symposium have appeared in a special issue of Int. Microbiol. Vol 6.

Herman Jan Phaff's legacy includes pioneering work on the yeast cell envelope and the application of molecular approaches to yeast systematics. Clearly, his interest and knowledge spanned the whole gamut of yeast biology. Yet, his most original and most heartfelt contribution was to our understanding of the position occupied by yeasts in nature. This view developed through the juxtaposition of his childhood exposure to industrial fermentations and his training in the tradition of Beijerinck's Delft School of Microbiology. Through some of Phaff's recent writings, I have attempted to formulate the themes or principles that were implicit to his ecological thinking. Six focal points emerge. (1) Yeasts in themselves are a sufficient object of study. (2) A clear idea of a yeast community cannot be obtained unless the yeast species are correctly identified. (3) Ecologically meaningful conclusions require an adequate sample size. (4) The bacteriological dictum "everything is everywhere" is a poor account of yeast distributions. (5) The habitat is the cornerstone of yeast ecology. (6) Ecology is the most exciting aspect of yeast biology.

10. Lachance, M.A. 2003. Ecology and systematics of yeasts associated with floricolous beetles. Instituto de Investigaciones Biomédicas Alberto Sols, Madrid, Spain.

Recently accepted papers - see abstracts under Dr. Rosa's communication.

11. Rosa C. A., M. A. Lachance, J. O. C. Silva, A. C. P. Teixeira, M. M. Marini, Y. Antonini & R. P. Martins. 2003. Yeast communities associated with stingless bees. FEMS Yeast Res. 4:271-275.

12. Trindade, R.C., M.A. Resende, R.S. Pimenta, M.A. Lachance & C.A. Rosa. 2003. *Candida sergipensis*, a new asexual yeast species isolated from frozen pulps of tropical fruits. Antonie van Leeuwenhoek (in press).

The following is the abstract of a recently accepted paper.

13. Marinoni, G. and M.A. Lachance. Speciation in the large-spored *Metschnikowia* clade and establishment of a new species, *Metschnikowia borealis* comb. nov. FEMS Yeast Res. (in press).

The reproductive boundaries among species in the large-spored *Metschnikowia* clade were studied by prototrophic recombinant selection, electrophoretic karyotyping, mitochondrial DNA restriction analysis, and DNA sequence analysis. Inviable ascospores arose from crosses between the two varieties of *Metschnikowia continentalis*, indicating that they should be recognized as separate species. Prototrophic recombinants were recovered from crosses between auxotrophic mutants of *M. borealis*, *M. continentalis*, *M. lochheadii*, *Metschnikowia* sp. UWO(PS)00-154.1, and *Candida ipomoeae*, showing that some genetic exchange is possible in spite of the sterility of the asci formed in interspecific crosses. *M. hawaiiensis*, although capable of ascus formation when its h^- mating type is crossed with the h^+ mating type of the other species, did not give rise to recombinants. In the other species, some recombinants acquired the ability to form asci directly from single cells. These often contained the chromosomes of both parents, suggesting formation of allodiploid hybrids. Other recombinants behaved as haploids and were similar to one parent except for having inherited the selectable wild type allele from the other parent. In most, but not all cases, inheritance of the mitochondrial genome was uniparental and correlated with the inheritance of the nuclear chromosome complement. In some cases, what appeared to be a recombinant mitrochondrial genome was observed. Phylogenies derived from the sequences of various DNA regions were not congruent, indicating that hybridization may have taken place in nature as the large-spored species diverged from their common ancestor. Further evidence that *C. ipomoeae* arose from a natural recombination event was obtained, but a pair of *Metschnikowia* species that might represent derived forms of the parents could not be identified conclusively. *C. ipomoeae* and most of its closely related *Metschnikowia* species contained a group II intron in the mitochondrial small subunit ribosomal gene. The intron was absent in *M. borealis*, *M. hawaiiensis*, and other species in the genus *Metschnikowia*.

Gaëlle Marinoni successfully defended her doctoral thesis. She has now joined Lee Johnston's laboratory at the National Institute for Medical Research, Mill Hill, London, England, where she will be conducting postdoctoral research on the genetics of mitosis in yeast.

14. Marinoni, G. 2003. Sexual cycle and speciation in the giant-spored *Metschnikowia* species. Ph.D Thesis, Department of Biology, University of Western Ontario.

At the time this study began, the giant-spored *Metschnikowia* species consisted of *M. hawaiiensis*, *M. lochheadii*, *M. continentalis*, *M. borealis*, and the undescribed species "*M. merremiae*". The reproductive barriers between these yeasts were studied by selection and analyses of prototrophic progenies for ploidy, sporulation, as well as inheritance of the nuclear and mitichondrial genomes. The fertility patters of artificial hybrids supported the view that these yeasts are biological species separated on the basis if postzygotic isolation mechanisms and demonstrated that the varieties of *M. continentalis* are in fact two distinct species. Hopwever, the occurrence of prototrophic recombinants showed that some genetic exchange could occur between these species. Preliminary data also suggested that hybridization events between species of this clade could be a source of new species. Secondly, the asymmetrical mating phenomenon observed in *M. hawaiiensis* was investigated by isolating the mating pheromone of *M. continentalis* mating type h^-. This pheromone, termed *Minus*-factor could induce the formation of conjugation tubes in all giant-spored *Metschnikowia* species with the single exception of *M. hawaiiensis*, suggesting that failure to recognize an interspecific pheromone may be the cause of the asymmetrical mating phenomenon observed in this yeast. In addition, the *Minus*-factor of *M. continentalis* was partly characterized as a hydrophobic, possibly farnesylated, molecule of less than 1710 in molecular weight. Lastly, the presence of a maximum of two spores in asci of *Metschnikowia* species raised the question of whether these were true meiotic products. This was investigated by following the segregation patterns of varius genetic markers. Early on, both mating types were consistently recovered in pairs of sister spores, casting further uncertainty as to whether normal meiosis takes place. However, the segregation patterns for cycloheximide resistance and several auxotrophic markers were random, suggesting that normal meiosis indeed occurs. To explain the lack of second division segregation of mating types, the mating type locus was hypothesized to be centromere-linked and meiosis I tied to spore formation. The latter assumption was supported by fluorescence microscopy, which demonstrated that the second meiotic division takes place inside the spores and is followed by the resorption of two nuclei, one in each spore.

Network: Yeasts in Food and beverages

Publications regarding WINE YEASTS: FERMENTATION, SPOILAGE, METHODS FOR ISOLATION, ENUMERATION, IDENTIFICATION AND CHARACTERIZATION.

Communicated by P. Romano <pot2930@iperbole.bologna.it>.

France: UMR-Sciences pour l'Oenologie, Microbiologie et Technologie des Fermentations, Institut National de la Recherche Agronomique 2, Place Viala, 34060 Montpellier, France. Communicated by C. Camarasa <camarasa@ensam.inra.fr>

1. Camarasa C, Grivet JP, Dequin S. 2003. Investigation by 13C- NMR and TCA deletion mutant analysis of Pathways for succinate formation in *S. cerevisiae* during anaerobic fermentation. Microbiology. 149:2669-2678.

NMR isotopic filiation of 13C-labelled aspartate and glutamate was used to explore the tricarboxylic acid (TCA) pathway in Saccharomyces cerevisiae during anaerobic glucose fermentation. The assimilation of [3-13C]aspartate led to the formation of [2,3-13C]malate and [2,3-13C]succinate, with equal levels of 13C incorporation, whereas site-specific enrichment on C-2 and C-3 of succinate was detected only with [3-13C]glutamate. The non-random distribution of 13C labelling in malate and succinate demonstrates that the TCA pathway operates during yeast fermentation as both an oxidative and a reductive branch. The observed 13C distribution suggests that the succinate dehydrogenase (SDH) complex is not active during glucose fermentation. This hypothesis was tested by deleting the *SDH1* gene encoding the flavoprotein subunit of the SDH complex. The growth, fermentation rate andmetabolite profile of the *sdh1* mutant were similar to those of the parental strain, demonstrating that SDH was indeed not active. Filiation experiments indicated the reductive branch of the TCA pathway was the main pathway for succinate production if aspartate was used as the nitrogen source, and that a surplus of succinate was produced by oxidative decarboxylation of 2-oxoglutarate if glutamate was the sole nitrogen source. Consistent with this finding, a kgd1 mutant displayed lower levels of succinate production on glutamate than on other nitrogen sources, and higher levels of oxoglutarate dehydrogenase activity were observed on glutamate. Thus, the reductive branch generating succinate via fumarate reductase operates independently of the nitrogen source. This pathway is the main source of succinate during fermentation, unless glutamate is the sole nitrogen source, in which case the oxidative decarboxylation of 2-oxoglutarate generates additional succinate.

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1. Remize F, Cambon B, Barnavon L, Dequin S. 2003. Glycerol formation during wine fermentation is mainly linked to Gpd1p and is only partially controlled by the HOG pathway. Yeast, 20:1243-1253.

Glycerol 3-phosphate dehydrogenase, a key enzyme in the production of glycerol, is encoded by *GPD1* and *GPD2*. The isoforms encoded by these genes have different functions, in osmoregulation and redox balance, respectively. We investigated the roles of *GPD1*, *GPD2* and *HOG1* - the kinase involved in the response to osmotic stress - in glycerol production during wine fermentation. We found that the deletion of *GPD2* in a wine yeast-derived strain did not affect growth or fermentation performance and reduced glycerol production by only 20%. In contrast, a *gpd1*Δ mutant displayed a prolonged lag phase, and produced 40% less glycerol than the wild-type strain. The deletion of *HOG1* resulted in a slight decrease in growth rate and a 20% decrease in glycerol production, indicating that the HOG pathway operates under wine fermentation conditions. However, the hog1Δ mutant was not as severely affected as the *gpd1*Δ mutant during the first few hours of fermentation, and continued to express *GPD1* strongly. The hog1Δ_?mutant was able to increase glycerol production in response to high sugar concentration (15 to 28% glucose), to almost the same extent as the wild type, whereas this response was totally abolished in the *gpd1*Δ_?mutant. These data show that Gpd1p plays a major role in glycerol formation, particularly during the first few hours of exposure to high sugar concentration, and that *GPD2* is only of little significance in anaerobic fermentation by wine yeast. The results also demonstrate that the HOG pathway exerts only limited control over *GPD1* expression and glycerol production during wine fermentation.

2. Schuller, D., Valero, E., Dequin, S., Casal, M. 2003. Survey of molecular methods for the typing of industrial yeast strains. FEMS Microb. Lett.. in press.

A survey of the polymorphisms generated by distinct methods was performed in 23 commercial winery yeast strains. The microsatellite typing, using 6 different loci, an optimized interdelta sequence analysis and RFLP of mitochondrial DNA generated by the enzyme Hinf I had the same discriminatory power: among the 23 commercial yeast strains, 21 distinct patterns were obtained. Karyotype analysis generated 22 patterns, thereby allowing the discrimination of one of the three strains that were not distinguished by the other methods. Due to the equivalence of the results obtained in this survey, any of the methods can be applied at the industrial scale.

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1. Colombié, S., Dequin, S., Sablayrolles, J.M. 2003. Control of lactate production by *Saccharomyces cerevisiae* expressing a bacterial LDH gene. Enz Microb. Technol. 33:38-46.

Potential industrial applications for lactate, such as the production of chemicals, has led to interest in producing this organic acid by metabolically engineered yeast such as *Saccharomyces cerevisiae*. Such microorganisms are more acid tolerant than lactic acid bacteria. This paper deals with the potential of the genetically modified *Saccharomyces cerevisiae* strain K1-LDH (the Lactate Dehydrogenase gene of *Lactobacillus plantarum* has been integrated in the genome of the commercial wine yeast strain K1) to produce lactate and the ways to control this production. The importance of the pH control during fermentation is showed not only for preventing medium acidification but also enabling on-line lactate estimation. Fermentation behaviour of K1-LDH strain is compared to K1 (control strain): K1-LDH produces up to 40 $g.l^{-1}$ of lactate mainly during the stationary phase. Influences of the main medium nutrients on the lactate production were studied by varying their initial concentration. Whilst increasing glucose concentration (So) until So=200 $g.l^{-1}$ provides higher lactate yields, higher lactate productivity are achieved with high nitrogen concentration. Finally, continuous and resting cells culture experiments were performed and confirmed a higher lactate yield in non-growing than in growing conditions.

2. Roger, J.M., Sablayrolles, J.M., Steyer J.P., Bellon-Maurel V. 2002. Pattern analysis techniques to process fermentation curves. Application to descrimination of enological alcoholic fermentations. Biotechnol. Bioeng. 79:804-815.

In fermentation processes, kinetic curves are generally aimed at control purposes. However, these curves could also contain information about inherent features of the product (such as origin, quality…). This paper presents several pattern analysis techniques used to classify fermentation curves. An application to alcoholic fermentation is presented as an illustration : it aims at retrieving the origin of a must from its fermentation curve. The fermentation kinetics of 5 vineyard musts, harvested over 9 years on the same parcels have been recorded. From these curves, two sets of variables have been generated : The first one (p_1) gathers all the kinetic curve points. The second one (p_2) contains a restrained number of variables, generated by the expert knowledge of the oenologist. The set p_2 has been processed by two very different techniques : a linear one (Factorial Discriminant Analysis) and a non linear one (Artificial Neural Networks). The set p_1 has been processed by a new chemometric technique, the Discriminant Partial Least Squares Regression. For all the sets and the techniques used, the selection of the variables has been studied. The interest of the latter is largely demonstrated both by theoretical and practical discussions. The discrimination results (up to 94% of good predictions) enhance the interest of the on-line measurements and of their use in such pattern analysis tools.

3. Roustan, J.L., Sablayrolles, J.M. 2002. Impact of the addition of electron acceptors on the by-products of alcoholic fermentation. Enz. Microb. Technol. 31:142-152.

In this study, we investigated the consequences of adding electron acceptors on the production of compounds involved in the maintenance of redox equilibrium in yeast during alcoholic fermentation: glycerol, succinate, acetate, malate, acetoin and butanediol. The various mechanisms involved are only fully functional in the stationary phase. They demonstrate in particular: (i) that in these conditions, the synthesis of glycerol serves more as a means of eliminating a surplus of reducing power than as a means of protection against osmotic pressure; (ii) the importance of the synthesis of acyloins, such as acetoin, followed by reduction to diols. The results obtained also provide information concerning the synthetic pathways for succinate and acetate. These reactions are important in the production of alcoholic drinks (particularly in wine production), because they concern compounds that affect the organoleptic qualities of the products. They may also be of value for the stereospecific synthesis of certain diol-like compounds.

4. Roustan, J.L., Sablayrolles, J.M. 2002. Trehalose and glycogen in wine-making yeasts: methodological aspects and variability. Biotechnol. Lett. 24:1059-1064.

Trehalose and glycogen, which can represent up to 30 % of wine yeasts, was evaluated by different methods in (i) yeasts during fermentation of musts (200 g sugar/l) and (ii) active dry yeasts. Fermentation trials demonstrated the potential value of monitoring changes in trehalose concentration during the rehydration step so that the performance of the yeasts can be evaluated.

5. Roustan, J.L., Sablayrolles, J.M. 2002. Modification of the acetaldehyde concentration during alcoholic fermentation and effects on fermentation kinetics. J. Biosc. Bioeng. 93:367-375.

We studied the kinetic effects of increasing the residual acetaldehyde concentration during alcoholic fermentation, especially during the stationary phase. We added this compound via pulse or continuous injections. The yeast response depended on the extent of acetaldehyde addition : high additions inhibited fermentation while low ones led to stimulation. When the addition was regular enough, up to 100 mM of acetaldehyde could be added. This caused a very significant drop in the fermentation duration. We also modulated the acetaldehyde concentration by modifying the ADH-catalyzed reaction. Two approaches were tested (i) adding aldehydes (propanal and furfural) that competitively inhibited the reduction of acetaldehyde and (ii) electron acceptors that reduced the quantity of NADH available.

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1. Salmon J.M., Fornairon-Bonnefond C., Mazauric J.P. 2002. Interactions between wine lees and polyphenols : influence on oxygen consumption capacity during simulation of wine ageing. J. Food Sci. 67:1604-1609.

During wine aging on lees, some membrane lipids of yeast lees, in contact with dissolved oxygen at low concentration, may undergo mild oxidation explaining the capacity of yeast lees to consume oxygen. We studied the cross-reactivity of complex polyphenols and tannins from wine and yeast lees towards oxygen during simulation of wine aging. We observed a total decrease of oxygen consumption capacity of mixed yeast lees and wine polyphenol by comparison with the reactivity of each component studied alone. A strong loss of reactivity of yeast lees towards oxygen was observed when separated from soluble polyphenols, although only a fraction of the total polyphenols remained adsorbed on lees.

2. Fornairon-Bonnefond C., Desmaretz V., Rosenfeld E., Salmon J.M. 2002. Oxygen Addition And Sterol Synthesis In *Saccharomyces cerevisiae* during enological fermentation. J. Biosci. Bioeng. 93:176-182.

Under anaerobic conditions, yeast growth normally requires oxygen in order to favour the synthesis of sterols and unsaturated fatty acids. However, in such conditions, superfluous oxygen consumption by yeast cells is observed. The superfluous oxygen consumed by the yeast cells appears to be not related to classical respiration, but mainly to the operation of several alternative oxygen consumption pathways. In this study, the potential relationship between this superfluous oxygen consumption and the yeast sterol synthesis pathway was investigated during enological fermentation. Additions of small (7 mg L^{-1}) and excess (37 mg L^{-1}) amounts of oxygen at the end of cell growth phase were used as a method of comparing oxygen consumption by normal synthetic pathways with that by alternative respiration pathways. The superfluous oxygen consumption by yeast cells during fermentation seemed not to alter and strongly favoured fermentation kinetics and cell biomass formation. However, a marked decrease of the orderliness of the membrane phospholipids is observed, which is not related to the drop of cell viability. After oxygen additions, squalene contents of the cells decreased, while the relative proportions of ergosterol or its precursors in the total sterol fraction did not correlatively increase. It was further found that an oxygen-dependent sterol degradation occurred when oxygen was added in excess amounts with respect to the cellular requirements for sterol synthesis. At present, this modification of the sterol contents of yeast membranes has not been related to any physiological parameters.

3. Fornairon-Bonnefond C., Camarasa C., Moutounet M., Salmon J.M. 2002. New trends on yeast autolysis and wine aging on lees: a bibliographic review. J. Int. Sci. Vigne Vin 36:49-69.

In enology, “grands crus” white wines are traditionally aged by the “sur lies” method, which consists of keeping the aging wine in contact with the lees (yeasts and organic residues). The lees can come either from the first or second fermentation and can be used for both white and red wines. This practice is still in the experimental stage. We reviewed scientific studies carried out on wine lees to determine the current situation in enology. We also provide some technological information relevant to such a practice. The first part of this paper provides a clear definition of wine lees from a legal and technological point of view. The second part describes the mechanisms of autolysis and focuses on each class of autolysis product. Many scientific studies have discussed the phenomenon of yeast autolysis during wine ageing. Most of these studies simply identified the yeast macromolecules released into the wine during autolysis. However, the experimental methods used vary and it is difficult to extrapolate most of results to the process of wine ageing on lees. Only a few studies have dealt with the physicochemical properties of lees during autolysis, especially concerning oxygen, polyphenols and other wine compounds. We then summarize the recent data obtained on these topics. Finally, we discuss the technical effects of aging wine on lees.

4. Rosenfeld E., Schaeffer J., Beauvoit B., Salmon J.M. 2003. Study of yeast promitochondria isolated during anaerobic stationary phase. Antonie van Leuwenhoek J. Microbiol. 85: in press.

Under anaerobiosis, the mitochondrion of *Saccharomyces cerevisiae* is restricted to unstructured promitochondria. These promitochondria provide unknown metabolic functions that are required for growth. Since high glucose concentrations are mainly fermented by *S. cerevisiae* during stationary phase (due to nitrogen starvation), an optimized promitochondria isolation procedure was investigated. Firstly, the unusual promitochondria ultrastructure was checked in intact cells by electron microscopy using a cryo-fixation and freeze-substitution method. The rapid response of anaerobic cells toward oxygen justified the adoption of several critical steps, especially during spheroplasting. Control of spheroplasting was accompanied by a systematic analysis of spheroplast integrity, which greatly influence the final quality of promitochondria. Despite the presence of remnant respiratory chain components under anaerobiosis, characterization of isolated promitochondria by high-resolution respirometry did not reveal any antimycin A- and myxothiazol-sensitive NADH and NADPH oxidase activities. Moreover, the existence of a cyanide-sensitive and non-phosphorylating NADH-dependent oxygen consumption in promitochondria was demonstrated. Nevertheless, promitochondria only slightly contribute to the overall oxygen consumption capacity observed in highly glucose-repressed anaerobic cells.

5. Rosenfeld E., Beauvoit B., Rigoulet M., Salmon J.M. 2002. Non-respiratory oxygen consumption pathways in anaerobically-grown *Saccharomyces cerevisiae*: evidence and partial characterization. Yeast 19:1299-1322.

Despite the absence of an alternative mitochondrial ubiquinol oxidase, *Saccharomyces cerevisiae* consumes oxygen in an antimycin A- and cyanide-resistant manner. Cyanide-resistant respiration is typically used when the classical respiratory chain is impaired or absent (i.e in anaerobically-grown cells shifted to normoxia or in respiratory-deficient cells). We

characterized the non-respiratory oxygen consumption pathways operating during anoxic–normoxic transitions in glucose-repressed resting cells. High-resolution oxygraphy confirmed that the cellular non-respiratory oxygen consumption pathway is sensitive to high concentrations of cyanide, azide, SHAM and TTFA, and revealed several new characteristics. First, the use of sterol biosynthesis inhibitors showed that this pathway makes a considerable contribution (about 25%) to both endogenous and glucose-dependent oxygen consumption. Anaerobically grown glucose-repressed cells exhibited high apparent oxygen affinities (*K*m for oxygen = 0.5–1 μM), even in mutants deficient in respiration or sterol synthesis. Exogeneously added glucose and endogenous stored carbohydrates were the only substrates that were efficient for cellular oxygen consumption (apparent *K*m for exogenous glucose = 2–3 mM). On the other hand, fluorimetric measurements of the cellular NAD(P)H pool showed that the cellular oxygen consumption (sterol biosynthesis and unknown pathways) was dependent more on the intracellular level of NADPH than of NADH. High oxygen affinity NADPH-dependent oxygen consumption systems were thought to be mainly localized in microsomal membranes, and several data indicated a significant contribution made by uncoupled P450 systems, together with still uncharacterized systems. Such activities are associated *in vitro* with a massive production of $O2^{-}$ and, to a lower extent, H_2O_2 and a likely concomitant production of H_2O.

6. Rosenfeld E., Beauvoit B., Blondin B., Salmon J.M. 2003. Oxygen consumption by anaerobic *Saccharomyces cerevisiae* in enological conditions: effect on fermentation kinetics. Appl. Environ. Microbiol. 69:113-121.

The anaerobic growth of the yeast *Saccharomyces cerevisiae* normally requires the addition of molecular oxygen, which is used to synthesize sterols and unsaturated fatty acids (UFAs). A single oxygen pulse can stimulate enological fermentation, but the biochemical pathways involved in this phenomenon remain to be elucidated. We showed that the addition of oxygen (0.3 to 1.5 mg/g [dry mass] of yeast) to a lipid-depleted medium mainly resulted in the synthesis of the sterols and UFAs required for cell growth. However, the addition of oxygen during the stationary phase in a medium containing excess ergosterol and oleic acid increased the specific fermentation rate, increased cell viability, and shortened the fermentation period. Neither the respiratory chain nor *de novo* protein synthesis was required for these medium- and long-term effects. As *de novo* lipid synthesis may be involved in ethanol tolerance, we studied the effect of oxygen addition on sterol and UFA auxotrophs (*erg1* and *ole1* mutants, respectively). Both mutants exhibited normal anaerobic fermentation kinetics. However, only the *ole1* mutant strain responded to the oxygen pulse during the stationary phase, suggesting that *de no*vo sterol synthesis is required for the oxygen-induced increase of the specific fermentation rate. In conclusion, the sterol pathway appears to contribute significantly to the oxygen consumption capacities of cells under anaerobic conditions. Nevertheless, we demonstrated the existence of alternative oxygen consumption pathways that are neither linked to the respiratory chain nor linked to heme, sterol, or UFA synthesis. These pathways dissipate the oxygen added during the stationary phase, without affecting the fermentation kinetics.

7. Fornairon-Bonnefond C., Salmon J.M. 2003. Impact of oxygen consumption by yeast lees on the autolysis phenomenon during simulation of wine aging on lees. J. Agric. Food Chem. 51:2584-2590.

Potential oxygen consumption by lees, more precisely by nonviable yeasts, during wine aging was recently described. Additionally, yeast autolysis is described as the main mechanism of degradation of lees during wine aging. Thus, to understand the effect of oxygen consumption by yeast lees during wine aging, an accelerated wine aging methodology was tested. Wine aging in the presence of yeast lees was studied both in the presence and in the absence of oxygen. Different markers of yeast autolysis were followed to find a relationship between oxygen consumption by yeast lees and changes in the final wine composition after aging. No differences for compounds tested were found in the wine and in the lees except among sterol compounds in lees: in the presence of oxygen, the concentration of ergosterol in lees was significantly lower than that in the absence of oxygen. It was hypothesized that ergosterol could be oxidized under the influence of oxygen, but none of the known products of ergosterol oxidation were recovered in the corresponding yeast lees. In addition, the decrease of ergosterol content in yeast lees cannot account for the total amount of oxygen consumed by yeast lees during such wine aging.

8. Fornairon-Bonnefond C, Aguerra E., Deytieux C., Sablayrolles J.M., Salmon J.M. 2003. Impact of oxygen addition during enological fermentations on yeast lees reactivity towards oxygen during wine aging. J. Biosci. Bioeng. 95:496-503.

During enological fermentations, superfluous oxygen consumption by yeast cells is observed. The superfluous oxygen consumed by the yeast cells is mainly related to the operation of non-respiratory oxygen consumption pathways resulting in an overall decrease in the total sterol fraction in yeast. On the other hand, yeast lees remaining at the end of alcoholic fermentations exhibit specific oxygen utilization rates ranging from 1 to 4 pmol $O_2\ h^{-1}\ 10^{-10}$ cells from the second to the thirteenth month of wine aging. This oxygen consumption capacity of yeast lees was independent of residual cell viability. In this study, we investigated the potential relationship between the oxygen added to commercial yeast strains during enological fermentation and the capacity of the corresponding yeast lees to interact with oxygen. Additions of low (7 mg L^{-1}) and excess (37 mg L^{-1}) amounts of oxygen at the end of the cell growth phase were compared in terms of repercussions on the oxygen consumption activity of the corresponding yeast lees. As expected, the superfluous oxygen consumption by yeast cells during fermentation had a positive influence on the fermentation kinetics and increased cell biomass formation. Oxygen consumption rates and the total capacity of oxygen consumption by the corresponding yeast lees clearly decreased when oxygen was added during fermentation. This marked decrease in yeast lees reactivity towards oxygen was concomitantly related to an increase in ergosterol synthesis and to oxygen-dependent sterol degradation. Such degradation occurred when oxygen was added in excess. Therefore, oxygenation control during fermentation appears to be a potential way to optimize both the fermentation kinetics and control yeast lees reactivity towards oxygen. For practical applications, oxygenation control during alcoholic fermentation may be considered as a general tool for decreasing the highly reductive effect of yeast lees during wine aging.

9. Salmon J.M., Vuchot P., Doco T., Moutounet M. 2003. Maintenance and protection of yeast morphology by contact with wine polyphenols during simulation of wine aging on lees. J. Food Sci. 68:1782-1787.

Scanning electron microscopy was used to monitor yeast cell morphology during simulated wine aging. Although yeast cells in lees in the absence of wine polyphenols rapidly reach a flat shape after the end of alcoholic fermentation, they keep a spherical (and almost intact) shape in the presence of wine polyphenols. We confirmed these visual observations by calculating the dynamic viscosity of the corresponding lees. Because yeast autolysis is not affected by the contact with wine polyphenols, such maintenance of yeast morphology may be the consequence of protection of some constitutive parts of yeast cell walls by wine polyphe- polyphenols toward the action of hydrolytic enzymes.

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1. Luyten, K., Riou, C., Blondin, B. 2002. The hexose transporters of *Saccharomyces cerevisiae* play different roles during enological fermentation. Yeast 19, 713-726.

We investigated the role of hexose transporters in a *Saccharomyces cerevisiae* strain derived from an industrial wine strain by carrying out a functional analysis of *HXT* genes 1 to 7 under enological conditions. A strain in which the sugar carrier genes *HXT1* to *HXT7* were deleted was constructed and the *HXT* genes were expressed individually or in combination to evaluate their role under wine alcoholic fermentation conditions. No growth or fermentation was observed in winemaking conditions for the *hxt1-7Δ* strain. The low-affinity carriers Hxt1 and Hxt3 were the only carriers giving complete fermentation of sugars when expressed alone, indicating that these carriers play a predominant role in wine fermentation. However, these two carriers have different functions. The Hxt3 transporter is thought to play a major role as it was the only carrier that gave an almost normal fermentation profile when produced alone. The hxt1 carrier was much less effective during the stationary phase and its role is thought to be restricted to the beginning of fermentation. The high-affinity carriers Hxt2, Hxt6 and/or Hxt7 were also required for normal fermentation. These high-affinity transporters have different functions: hxt2 is involved in growth initiation whereas Hxt6 and/or Hxt7 are required at the end of alcoholic fermentation. This work shows that the successful alcoholic fermentation of wine involves at least 4 or 5 hexose carriers playing different roles at various stages in the fermentation cycle.

2. Rossignol T., Dulau L., Julien A., Blondin B. 2003. Genome-wide monitoring of wine yeast gene expression during alcoholic fermentation. Yeast, in press.

The transcriptome of a wine yeast was monitored throughout an alcoholic fermentation under conditions mimicking an enological environment. Major changes in gene expression occurred during fermentation, affecting more than 2000 genes, as the yeast adapted to changing nutritional, environmental and physiological conditions. The genes of many pathways are regulated in a highly co-ordinated manner, and genes involved in the key metabolic pathways of fermentation are strongly expressed. We showed that, during fermentation of a synthetic medium mimicking a natural must in which growth arrest was caused by nitrogen exhaustion, entry into the stationary phase triggered major transcriptional reprogramming. Many TOR target genes involved in nitrogen utilisation or other functions are induced at this stage, suggesting that this signalling pathway plays a critical role in changes in gene expression in response to nitrogen depletion. Entry into stationary phase is a key physiological event and is followed by a general stress response. The superimposition of multiple stresses, including starvation and ethanol stress, gives rise to a unique stress response, involving hundreds of genes encoding proteins involved in various cellular processes, many of unknown function.

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1. M. Sipiczki 2002. Taxonomic and physiological diversity of *Saccharomyces bayanus*. In Biodiversity and Biotechnology of Wine Yeasts, Ed. M. Ciani, Research Signpost, Kerala, pp. 53-69.

Spontaneous fermentations occur as a succession of yeasts, beinning with relatively weak (but numerically superior) fermentative species arising form the grapes and vineyard environs. As fermentation progresses, these yeasts are gradually overgrown by *Saccharomyces* species that are metabolically equipped to grow in enviroments where sugar and alcohol are present in relatively high concentrations. *S. cerevisiae* is undoubtedly the most important yeast in winemaking. Related strains usually classified into *S. bayanus* can also conduct equally effective alcoholic fermentation, particularly when fermentation occurs at very high concentrations of sugar and/or temperatures below 20 ^{0}C. *S. bayanus* is a heterogeneous taxon comprising various hybrids and non-hybrid wine strains showing characteristic features of *S. uvarum*, a species that has fallen victim to recent taxonomic revisions. This chapter provides a summary of their taxonomy and a critical review of the conventional and molecular methods used for their identification and discrimination.

2. G.I. Naumov, E.S. Naumova, Z. Antunovics, M. Sipiczki: *Saccharomyces bayanus* var. *uvarum* in Tokaj wine-making of Slovakia and Hungary. 2002. Appl. Microbiol. Biotechnol. 59:727-730.

Using genetic hybridisation analysis and molecular karyotyping we revealed an association of S*accharomyces bayanus* var. *uvarum* species with Tokaj wine-making. Along with identification of *Saccharomyces* strains isolated by E. Minárik in Slovakia the composuition of Tokaj populations in Hungary was studied. Twenty-eight Hungarian *Saccharomyces* strains were analysed in terms of karyotype. The majority of strains belong to *S. bayanus* var. *uvarum*. Two non-identified *Saccharomyces* strains were found to be polyploid according to their complex karyotype patterns.

3. Z. Antunovics, H.Csoma, M. Sipiczki 2003. Molecular and genetic analysis of the yeast flora of botrytized Tokaj wines. Bulletin de L'O.I.V. 76:380-397.

Tokaj wines come from an anacient volcanic region in the north-east corner of Hungary, across from eastern Slovakia. The most characteristic products of the region are sweet and dessert wines made from botrytized grapes by fermentation at low temperatures. The yeast flora, that has colonised the grapes during botrytization in the vineyard, dominates the first stage of fermentation. Many of these yeasts are sensitive to ethanol. Therefore, the more ethanol-tolerant strains of *Saccharomyces cerevisiae* and *S. bayanus* gradually overgrow them and complete the fementation producing a wine with high ethanol and residual sugar content. Occasionally, cryotolerant and osmotolerant strains of *S. bayanus* are also abundant int he botrytized grapes. These strains appear to be well-suited to growth in musts made from botrytized grapes and frequently become dominating by the end of fermentation. Genetic and molecular analysis of strains isolated from various wineries revealed a high degree of karyotype stability. All *S. bayanus* isolated analysed belonged to the *uvarum* sub-group (on the basis of sugar utilization tests, electrophoretic karyotypes and PCR-amplified MET2 fragments), showed low optium growth temperature and grew faster than *S. cerevisiae* at low temperatures and high sugar concentrations.

4. M. Sipiczki 2003. *Candida zemplinina* sp. nov., an osmotolerant and psychrotolerant yeast that ferments sweet botrytized wines Int. J. System. Evol. Microbiol. 53:

Four yeast strains isolated from fermenting botrytized grape musts in the Tokaj wine region of Hungary are shown to represented a new osmotolerant and psychrotolerant species. The new species, C*andida zemplinina* (type strain 10-372^T=CBS 9494^T=NCAIM Y016667^T), is closely related to *Candida stellata*, a yeast common on overripe grapes and in sweet fementing wines. The sequence of the D1/D2 domain of the *C. zemplinina* 10-372^T 26S rDNA shows 8.1% sequence difference when compared to its counterpart in *C. stellata* CBS 157^T. In the conversed 5.8S gene of the ITS1-5.8S-ITS2 region the difference is 8%. The D1/D2 domain differs only at two nucleotides from the homologous sequence of a yeast strain isolated from botrytized grapes in California, suggesting that *C. zemplinina* is a wine yeast that occurs in geographically distant localities.

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1. Caruso M., Capece A., Salzano G., Romano P. 2002. Typing of *Saccharomyces cerevisiae* and *Kloeckera apiculata* strains from Aglianico wine. Lett. Appl. Microbiol. 34:323-328.

Kloeckera apiculata and *Saccharomyces cerevisiae* yeast species are dominant, respectively, at the early and at the following stages of wine fermentation. With the aim to monitoring yeast population performing Aglianico of Vulture grape must fermentation, 30 *S.cerevisiae* and 30 *K.apiculata* strains were typed by PCR fingerprinting with $(GAC)_5$ and $(GTG)_5$ primers and by complete NTS region amplification followed by restriction with *Hae*III and *Msp*I enzymes. The results showed that the techniques used are suitable to discriminate at the strain level *S.cerevisiae* that perform the fermentative process and to recognize and follow the presence of apiculate yeasts during the fermentation.

2. Brandolini V., Tedeschi P., Capece A., Maietti A., Mazzotta D., Salzano G., Paparella A., Romano P. 2002. *Saccharomyces cerevisiae* wine strains differing in copper resistance exhibit different capability to reduce copper content in wine. World J. Microbiol. Biotech. 18:499-503.

Two wine strains of *Saccharomyces cerevisiae*, characterized by a different degree of copper resistance, were tested in grape must fermentation in the presence of different copper concentrations. The sensitive strain SN9 was strongly affected by copper concentration (32 ppm,, (32mg/l)9, whereas the resistant strain SN41 exhibited a good growth activity in presence of 32 ppm of copper and only a reduced activity in presence of 320 ppm. The different strain fermentation performance in response to the copper addition corresponded to a different capability to accumulate copper inside the cells. Both strains exhibited the capacity to reduce the copper content in the final product, eventhough a significantly greater reducing activity was exerted by the resistant strain SN41, which was able to reduce by 90% the copper concentration in the final product and to accumulate the metal in great concentrations in the cell. As high concentrations of copper can be responsible for wine alterations, the selection of *S. cerevisiae* strains possessing high copper resistance and the ability to reduce the copper content of wine has a great technological interest, in particular for the fermentation of biological products. From the results obtained, the technique proposed is not only suitable for the assay of copper residues in must, wine and yeast cells, but it also offer the advantage of easy sample preparation and low detection limit in ppb (?g/l) range.

3. Romano P., Caruso M., Capece A., Lipani G., Paraggio M., Fiore C. 2002. Metabolic diversity of *Saccharomyces cerevisiae* strains from spontaneously fermented grape musts. World J. Microbiol. Biotech. 19:311-315.

One hundred and fifteen *Saccharomyces cerevisiae* strains from Aglianico of Vulture, a red wine produced in South Italy, were characterized for the production of some secondary compounds involved in the aroma and taste of alcoholic beverages. The strains exhibited a uniform behaviour in the production levels of *n*-propanol, active amyl alcohol and ethyl acetate, whereas isobutanol, isoamyl alcohol and acetaldehyde were formed with a wide variability. Only five strains produced wines close to the reference Aglianico of Vulture wine for the traits considered. Of these, two strains were selected, underwent to tetrad analysis and the single spore cultures were tested in grape must fermentation. The progeny of one strain showed a significant metabolic variability, confirming the necessity to test starter cultures for the segregation of traits of technological interest. Our findings suggest to select specific strains for specific fermentations in function of the vine variety characteristics in order to take the major advantage from the combination grape must/*S.cerevisiae* strain.

4. Paraggio M., Caruso M., Fiore C., Capace A., Lipani G., Romano P. 2002. Production of secondary compounds by *Saccharomyces cerevisiae* wine yeasts: genetic analysis as selective tool. Industrie delle Bevande 31:116-118.

Wine quality is always dependent on the activity and growth of yeasts performing fermentation. Uncontrolled yeast strain growth can significantly alter the wine sensory properties, aroma and flavour, whereas the use of pure yeast cultures results in more predictable control of fermentation and quality. In fact, collections strains from natural fermentations have demonstrated the existence of a strong technological variability within this species. Different strains contribute to the chemical composition and sensory qualities of the resulting wine. It must be underlined that different *S.cerevisiae* strains, producing differing amounts of secondary compounds, impart desirable or undesirable flavour-determinants to the wine. This has recalled the attention of wine-researchers and wine-makers to the autochthonous strains with the aim of selecting starter cultures, which, in addition to the desirable technological characteristics, are potentially better adapted to grow in that specific grape must than any other inoculated strain.

5. Brandolini V., Salzano G., Maietti A., Caruso M., Tedeschi P., Mazzotta D., Romano P. 2002. Automated multiple development method for determination of glycerol produced by wine yeasts. World J. Microbiol. Biotech. 18:481-485.

A rapid and efficient analytical method for the determination of glycerol in wines is described. This method utilises HPTLC plates coupled with Automated Multiple Development system with an elution gradient based on acetonitrile-acetone-hexane on silica gel layers. The absence of clean-up procedures, sometimes only centrifugation, makes this method suitable also for the large scale control of alcoholic beverages. In particular the capacity of different wine yeast species (*Saccharomyces cerevisiae, Zygosaccharomyces bailii, Kloeckera apiculata* and *Saccharomycodes ludwigii*) to produce glycerol was determined. Generally, the strains of *S. cerevisiae* produced elevated amounts of glycerol together with *Z. bailii,* whereas *K. apiculata* strains formed the lowest amounts of glycerol, exhibiting also a great strain variability.

6. Capece A., Salzano G., Romano P. 2003. Molecular typing techniques as a tool to differentiate non-*Saccharomyces* wine species. Int. J. Food Microbiol. 84:33-39.

A total of thirty-two yeast strains belonging to four non-*Saccharomyces* species associated with wine making were characterized by different molecular techniques. The PCR amplification of 18S rRNA-coding DNA and nontranscribed spacer, followed by restriction analysis with the endonucleases *Hae* III and *Msp* I, and PCR fingerprinting with microsatellite primers $(GAC)_5$ and $(GTG)_5$ were used. The methods used provided species-specific profiles and proved to be fast and reliable for monitoring the evolution of the four non-*Saccharomyces* yeast populations throughout wine fermentation.

7. Romano P., Fiore C., Paraggio M., Caruso M., Capece 2003. Function of yeast species and strains in wine flavour Int. J. Food Microbiol. 86:169-180.

The diversity and the composition of the yeast microflora significantly contribute to the sensory characteristics of wine. The growth of each wine yeast species is characterized by a specific metabolic activity, which determines high/low concentrations of the flavour compounds in the final wine. It must be underlined that, however, within each species, significant strain variability has been recorded. The wide use of starter cultures, mainly applied to reduce the risk of spoilage and unpredictable changes of wine flavour, can ensure a balanced production of wine flavour, but also cause a loss of characteristic aroma and flavour determinants of the wine. Thus, the beneficial contribution of yeast becomes more significant when starter cultures for winemaking are selected on the basis of scientifically verified characteristics and are able to complement and optimise grape quality and individual characteristics. Here we report the characterization of a large number of strains of different wine yeast species, isolated throughout the spontaneous fermentation of grapes, collected from the vine plants and crushed in the laboratory. The strain selection criteria should take account of quality expectations and varietal grape characteristics with the aim to obtain evidence of occurrence of specific yeast/wine interactions.

8. Romano P., Granchi L., Caruso M., Borra G., Palla P., Fiore C., Ganucci D., Caligini A., Brandolini V. 2003.The species-specific ratios of 2,3-butanediol and acetoin isomers as a tool to evacuate wine yeast performance. Int. J. Food Microbiol. 86:163-168.

The isomers of 2,3-butanediol [*R,R; S,S; R,S* (meso-form)] and of acetoin (*R,S*) were determined in laboratory wine fermentations carried out by 50 yeast strains, l0 for each of the following species, *Saccharomyces cerevisiae, Kloeckera apiculata, Candida stellata, Metschnikowia pulcherrima* and *Brettanomyces bruxellensis,* in order to evaluate if such parameters might be used to differentiate wines obtained with different yeast species. According to analysis of variance (ANOVA), the strains of the same species behaved similarly, whereas the five yeast species behaved differently so that species specific profiles were recognized. Moreover, the discriminant analysis grouped the wines into five groups, each including the 10 wines obtained by the 10 yeast strains of the same species. Trials were also included where musts partially fermented by non-*Saccharomyces* species were inoculated with a selected strain of *S. cerevisiae* to complete fermentation, and the content in 2,3-butanediol and acetoin isomers was again determined and statistical analysis was performed. Although the final values of these parameters resembled those obtained in pure fermentation with *S. cerevisiae,* statistical analysis discriminated wines according to the yeast species performing the first fermentation phase.

9. Romano P., Paraggio M., Capece A. 2003. Wine *Saccharomyces cerevisiae* improved by using traditional approaches. Proc. 83rd General Assembly of the International Office on vine and wine (O.I.V). Paris 16-19 June.

Numerous studies in these last years have demonstrated the existence of a considerable variability in the expression of technological traits among wine strains of the species *Saccharomyces cerevisiae*. By using these differences as source of genetic variability, strain improvement can be achieved by breeding program. This technique doesn't modify the natural genetic complement, but it facilitates natural breeding by crossing strains selected and chosen from the environment. By applying this method we obtained wine strains possessing specific and stable technological characteristics, suitable for the fermentation of Aglianico of Vulture wine. These recombinant strains don't represent a hazard for the human health because they are not genetically modified, but are the product of a programmed combination of selected traits of the parental strains.

10. Capece A 2003. Application of molecular techniques to profile wine yeast species during fermentation. Proc. 23rd Int. Spec. Symp. Yeasts ISSY-23: *Interactions between yeasts and other organisms*, Budapest (Hungary), 26-29 August, p.145.

The composition of yeast microflora significantly influence wine organoleptic characteristics. Yeast diversity may also be the source of wine spoilage, due to the growth of yeasts producing unfavourable activities. The development of powerful tools, such as molecular techniques, to study yeast population evolution during wine fermentation can be applied in order to control the correct course of the process. In this research some molecular techniques were employed for the characterization of wine strains belonging to different species (*Candida stellata*, *Metschnikowia pulcherrima*, *Kloeckera apiculata* and *Schizosaccharomyces pombe*). The methods used were: ARDRA, restriction analysis of NTS region amplified, and PCR fingerprinting with the primers $(GAC)_5$ and $(GTG)_5$. All the techniques tested allowed the differentiation of the strains according to their species: each species exhibited a species-specific molecular profile. Mixed cultures of *C. stellata*, *K. apiculata* and *M. pulcherrima* were submitted to the amplification of NTS region, followed by restriction analysis. The enzyme *Hae* III allowed to differentiate all the species, yielding a composite molecular pattern derived from the overlapping of the profiles typical of each species. The monitoring of wine fermentation by using this typing techniques allows the achievement of microbiological stability and, consequently, a control of wine production.

11. Capece A., Romano P., Tedeschi P., Maietti A., Brandolini V. 2003. Genetic polymorphism in *Saccharomyces cerevisiae* wine strains differing in copper resistance. Proc. 23rd Int. Spec. Symp. Yeasts ISSY-23: *Interactions between yeasts and other organisms*, Budapest (Hungary), 26-29 August, p.137.

Low concentrations of copper ions are essential to *Saccharomyces cerevisiae* growth, while higher concentrations function as potent fungicid. To balance the stimulatory and inhibitory properties of copper, organisms are equipped with homeostatic factors. One of this relates sequestration of copper by copper-binding proteins, the metallothioneins, that are encoded by different genes, such as CUP-1, CRS-5, ACE-1, CUP-9 and CUP-2. In this research, seven *S. cerevisiae* wine strains, yet characterized for copper resistance in must fermentation, were submitted to PCR amplification of these copper-genes in order to find a correlation between phenotypic and genetic expression. A correspondence was found only in the case of the genes ACE-1 and CUP-9. The amplification of the genes CUP-1 and CUP-2 didn't allow to distinguish between the different phenotypes. The amplification of the gene CRS-5 yielded the band in all the resistant strains, whereas the sensitive ones behaved differently. This molecular approach could be applied to individuate copper-resistant strains. Taking into account the increasing level of copper residues on the grapes in biological vineyards, the selection of *S. cerevisiae* strains possessing high copper resistance, and, as a consequence, the ability to reduce the copper content in wine, assumes a great technological interest.

12. De Fina M., Vernucci S., Paraggio M., Romano P. 2003. Metabolic activity by free and immobilized cells of *Kloeckera apiculata* and *Saccharomyces cerevisiae* in mixed, sequential and pure fermentation. Proc. 23rd Int. Spec. Symp. Yeasts ISSY-23: *Interactions between yeasts and other organisms*, Budapest (Hungary), 26-29 August, p.202.

The distinct aroma and taste of wine are obtained through the activity not only of the principal wine yeast species *Saccharomyces cerevisiae*, but also of the so-called non-*Saccharomyces* yeasts. Of these, *Kloeckera apiculata* is more frequently the principal species, which survives longer both in spontaneous and inoculated fermentation, producing some important reactions in grape must. The combined use of these species may ensure the completion of the fermentation process, conferring also suitable aromatic characteristics to wines. In this study we used the immobilized cell-system to study fermentation performance in mixed, sequential and pure cultures of *S. cerevisiae* and *K. apiculata*. Fermentation tests were performed in "Aglianico" grape must with calcium alginate-entrapped cells and free ones in differing combinations. At the end of the process, wine samples were collected and analyzed for ethanol and by-products conferring organoleptic properties to wine. Consistent differences were determined in the composition of the wines obtained. The best combination resulted *K. apiculata* immobilised cells + *S. cerevisiae* free cells in sequential fermentation. When *S. cerevisiae* free cells were added to musts partially fermented (2 days) by immobilized cells of *K. apiculata*, the highest ethanol concentration was obtained (12.7 %) together with a balance in by-products.

13. Paraggio M. 2003. Interaction between *Saccharomyces cerevisiae* strain and grape must in the production of acetic acid. Proc. 23rd Int. Spec. Symp. Yeasts ISSY-23: *Interactions between yeasts and other organisms*, Budapest (Hungary), 26-29 August, p.201.

Due to its negative sensory attributes, acetic acid in appreciable amounts during grape must fermentation is highly undesirable. Acetic acid appear to be formed early in the fermentation, coming from bacterial infections or from the activity mainly of non-*Saccharomyces* yeasts of the first fermentation phase. In this study we tested one hundred strains of *Saccharomyces cerevisiae* for the production of acetic acid in wine, with the aim to individuate strains low producers and homozygous for this character. A great variability was determined among strains, which produced from a few mg/l until more than 1 g/l of acetic acid. Although the majority of the strains formed less than 600 mg/l of acetic acid (threshold value), some strains formed high amounts. The strains tested showed a great variability in function of the grape must. In Cannonau, Aglianico Puglia and Fiano wines the strains exhibited a uniform behaviour, whereas a significant variability was found in the other wines. A considerable variability was recorded especially in Vermentino wine. Three low acetic acid producing strains were tested for genetic analysis. The progeny of two strains corresponded to the parental strains resulting homozygous for the character considered and more suitable as starter culture.

14. Gavino J.P.A., Fiore C., Andreotti G., Romano P. 2003. Comparison between wine and agave yeast strains for traits of technological interest. Proc. 23rd Int. Spec. Symp. Yeasts ISSY-23: *Interactions between yeasts and other organisms*, Budapest (Hungary), 26-29 August, p.174.

In Mexico there are different alcoholic beverages obtained from agave juice, which is cooked, fermented and distilled. For the production of tequila beverage (West of Mexico) only *Agave tequilana* Weber blue variety is allowed. Most of the studies regarding alcoholic beverages have been performed in wine, beer and whisky but little is known about agave beverages, and what could be the differences between yeast strains from agave and from wine. In this study we compared yeast strains of different species (*Saccharomyces cerevisiae, Kloeckera apiculata, Candida magnolia* and *cresci*) and different origin (agave and grape juice) for parameters of technological interest, such as SO_2 and copper resistance, ethanol tolerance and enzymatic activities. In general agave strains resulted more resistant to SO_2 and ethanol, whereas wine strains exhibited positive results for β-galactosidase and β-xylosidase activity. All agave strains exhibited a good pectinolytic activity on fructose, while wine strains of *S. cerevisiae* were positive both on fructose and glucose and *K. apiculata* wine strains were negative on both sugars. As regards fermentations of *agave tequilana* juice (8 °Brix), inoculated with 10^6 cells/ml and added with ethanol at different concentrations, the growth of agave strains was generally higher than that of wine strains.

15. Fiore C. 2003. Enzymatic activities in various yeast species and strains of wine origin. Proc. 23rd Int. Spec. Symp. Yeasts ISSY-23: *Interactions between yeasts and other organisms*, Budapest (Hungary), 26-29 August, p.175.

Different wine yeast species are involved positively or negatively in the transformation of grape must in wine. Non-*Saccharomyces* yeasts, such as species of the genera *Hanseniaspora* and *Candida*, predominate in the early stages both of the spontaneous and inoculated fermentations. Among the traits of the technological interest yeast enzymatic activities play an important role. Proteases, xylosidases and glucosidases are some of the enzymes secreted by yeasts, that can considerably affect aroma formation. In this study about 300 wine strains of different species (*Saccharomyces cerevisiae*, *Kloeckera apiculata, Metschinikowia pulcherrima, Candida stellata*) were tested for the production of extracellular enzymes. All the strains of *S. cerevisiae* did not exhibit β-glucosidase activity and only a few ones were able to hydrolize all the proteins at an acceptable level. Conversely, a general high proteolytic activity was recovered in *K. apiculata* and *M. pulcherrima* strains, also at low pH. Strains of these two species exhibited the highest levels of β-glucosidase and β-xylosidase activity. Some strains of *K. apiculata* showed a general good activity of the enzymes tested, suggesting a potential use in mixed fermentation with selected *S. cerevisiae* strains.

16. Caruso M. 2003. Biogenic amine formation from strains of different wine yeast species. Proc. 23rd Int. Spec. Symp. Yeasts ISSY-23: *Interactions between yeasts and other organisms*, Budapest (Hungary), 26-29 August, p.87.

It is still not clear whether amines present in wine are the result of the yeast or lactic acid bacteria fermentative activity or both. In the present study, a total of 50 yeast strains of different wine species were inoculated in sterilized must to test their capability to produce biogenic amines. When the fermentation was completed, biogenic amines were derivatized with dansyl-chloride, extracted with diethyl ether, dried under N_2 flow, solubilized in acetonitrile and separed and quantified by HPLC using a C_{18} column, a gradient acetonitrile-water and an UV detector running at 254 nm. The strains of *Brettanomyces bruxellensis* and *Saccharomyces cerevisiae* produced in average more than 10 mg/l of amines, while the strains of *Candida stellata*, *Kloeckera apiculata* and *Metschnikowia pulcherrima* produced less than 10 mg/l. All the species formed significant amounts of agmatine, with variability among the strains. The most fermentative species, *S. cerevisiae,* exhibited the characteristic to produce the highest amount of ethanolamine. These results emphasized the importance to insert in the selection program of wine starter cultures the parameter "biogenic amine production".

17. Galgano F., Favati F., De Giorgio A., Caruso M., Lacertosa G. 2003. Health and consumption of wine: study of polyphenols and biogenic amines in wines of south Italy. VI Italian Congress on Food Science and Technology, Cernobbio (Italy) 18-19 September.

Polyphenols play an essential role in the definition of the organoleptic characteristics of wines, and among them resveratrol, present in the vine and in the skin of the berries, has a noteworthy biological activity. Several studies have pointed out how this compound, found in the *cis* or *trans* configuration as a free or glucose bound moiety, can increases the antioxidant potential of wine and may play an important role in the prevention of several pathologies in humans. However, in wine it is also possible to find potentially toxic moieties, such as biogenic amines, precursors of nitrosoamines. This presence can be a consequence of the malolactic fermentation or be due to the activity of the yeasts during the primary fermentation. In this his research 112 wines produced in Southern Italy, and commercially classified as DOC or IGT, have been characterized and classified according to their content in total polyphenols, resveratrol, as well as in biogenic amines, using multivariate statistical analysis.

18. Galgano F., Caruso M., Favati F., Romano P. 2003. HPLC determination of agmatine and other amines in wine. Int. J. Vine Wine Sci., in press.

An optimised HPLC analysis is described for the determination by dansylation of the following 11 biogenic amines in wine: agmatine, cadaverine, ethanolamine, histamine, methylamine, 2-phenylethylamine, spermine, spermidine, putrescine, tryptamine and tyramine. Seven amines were found in red and white wines produced in Southern Italy, being present at level ranging from not detectable to 10.97 mg/L. The most abundant amine resulted ethanolamine, while the polyamine present at the highest concentration was agmatine with maximum levels of 9.92 mg/L. Total biogenic amines content was higher in red wines.

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1. Granchi L., Ganucci D., Messini A., Vincenzini M.. 2002. Oenological properties of *Hanseniaspora osmophila* and *Kloeckera corticis* from wines produced by spontaneous fermentations of normal and dried grapes. FEMS Yeast Res., 2:403-407.

Strains of *Hanseniaspora osmophila* and *Kloeckera corticis*, isolated from wines produced by spontaneous fermentations of normal and dried grapes, were characterized for their fermentation behavior with and without SO_2 at 25°C. All isolates behaved as glucophilic yeasts and yielded ethanol at concentrations of about 9% (v/v); acetic acid, acetaldehyde, ethyl acetate and acetoin were always produced to high concentrations. SO_2 addition had no significant effect on growth yield and fermentation rate. These metabolic features were maintained in the presence of 400 g l31 of sugars and at 15°C, and were quite similar to those shown by *Saccharomycodes ludwigii*. Therefore, *H. osmophila* and *K. corticis* should be considered detrimental yeast species, particularly in fermentations of musts from dried grapes.

2. Granchi L., Ganucci D., Viti C., Vincenzini M. 2002. Assimilable-nitrogen content of grape must and *Saccharomyces cerevisiae* biodiversity in natural wine fermentations. Proceedings of 22nd ISSY, 25-28 March 2002, Pilanesberg National Park, South Africa, p. 84.

3. Granchi L., D. Ganucci, C. Viti, L. Giovannetti, M. Vincenzini 2003. *Saccharomyces cerevisiae* biodiversity in spontaneous commercial fermentations of grape musts with "adequate" and "inadequate" assimilable-nitrogen content. Lett. Appl. Microbiol., 36:54-58

In spontaneous wine fermentations, *Saccharomyces cerevisiae,* after an initial development of various non-*Saccharomyces* populations, commonly becomes the dominant yeast and it is responsible for the completion of the alcoholic fermentation. However, different strains of *S. cerevisiae* are generally involved in the fermentative process determining possible differences in organoleptic wine properties. Since it is known that strains of *S. cerevisiae* may differ in their nitrogen demand and that the initial nitrogen content in musts may largely vary, nitrogen-limited conditions could select strains of *S. cerevisiae* with low-nitrogen requirements and, possibly, affect *S. cerevisiae* biodiversity. With the aim to evaluate whether intraspecific diversity of *S. cerevisiae* is affected by initial assimilable-nitrogen content, 52 *S. cerevisiae* isolates from two spontaneous commercial wine fermentations started with adequate and inadequate nitrogen amounts were characterized by mithocondrial DNA restriction analysis. Several strains occurred in each fermentation, two strains, but not the same ones, being predominant at frequencies of about 30%. No significant differences were detected by comparing the biodiversity indices of the two fermentations. Cluster analysis demonstrated that the strain distribution was independent of nitrogen content, the two pairs of closely related dominant strains grouping into clusters at low similarity. According to the findings, the nitrogen availabilty in musts did not affect the genetic diversity of *S. cerevisiae* but may have induced a "selection effect" on strains dominating wine fermentations, with possible consequences on wine properties.

4. Mangani S., Guerrini S., Granchi L., Vincenzini M. 2003. Autolysis of wine yeasts and biogenic amine production by *Oenococcus oeni*. Book of Abstracts 23rd ISSY, 26-29 August 2003, Budapest, Hungary p.194.

The abundance of biogenic amines (BAs) in wine depends on the presence of both BA-producing microorganisms and concentration of precursor amino acids. As concerns the first requisite, *Oenococcus oeni*, the species of lactic acid bacteria most frequently associated with malolactic fermentation in wines, has been recently demonstrated capable to produce various BAs, depending on the strain.[1] On the other hand, amino acid concentration after the completion of alcoholic fermentation could increase as a consequence of yeast autolysis. In order to investigate on the potential contribution of this phenomenon on

BA accumulation in wines, autolysis of several species of wine yeasts was induced in a model wine medium,[2] the resulting media being thereafter inoculated with *O. oeni* strains differing in their BA producing capability. Analytical determinations on spent media demonstrated that different yeast species released different amounts of both proteins and α-amino acids and that the *O. oeni* strains produced different quantities of BAs. However the relative abundance of each produced BA was found to depend not only on the bacterial strain used but also on the autolysed yeast species. These findings highlight the importance of the yeast species involved in grape must fermentations in determining the BA abundance in wines.

[1]Guerrini, S., Mangani, S., Granchi, L., Vincenzini, M.: Current Microbiol. 44: 374-378. 2002.
[2]Martìnez-Rodriguez A. J., Carrascosa A. V., Polo M. C.: Int. J. Food Microbiol. 68: 155-160. 2001.

5. Granchi L., Guerrini S., Bastianini A., Mangani S., Vincenzini M. 2003 Response of *Oenococcus oeni* to inhibitory metabolites from wine yeasts. Book of Abstracts 23rd ISSY, 26-29 August 2003, Budapest, Hungary p.36.

Oenococcus oeni, the bacterial species usually responsible for the malolactic fermentation (MLF) of wines, was demonstrated to include strains significantly different in their fatty acid (FA) composition, as a consequence of their different capability to assimilate oleic acid.[1] Since strains possessing higher amounts of oleic acid and its methylated derivate, dihydrosterculic acid, carried out more efficiently MLF in wine, it was investigated whether differences in FA composition may be related to different responses to inhibitors produced by wine yeasts and acting on the bacterial membrane. Thus, strains with different FA profiles were exposed to the action of ethanol, higher alcohols and medium-chain fatty acids in synthetic media at low pH, and cell viability, malolactic activity and FA composition were determined. Ethanol induced changes in membrane composition but these were not related to ethanol resistance, in terms of both cell survival and malolactic activity. No effect was induced by higher alcohols; on the contrary, independently of the FA composition of the strains, medium-chain fatty acids caused a marked loss of cell viability, and MLF did not occur. Hence, different FA compositions of *O. oeni* strains do not seem to induce differences in bacterial sensitivity to inhibitory metabolites from wine yeasts.

[1]Guerrini, S., Bastianini, A., Granchi, L., Vincenzini, M.: Current Microbiol. 44: 5-9. 2002.

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1. Domizio P., Rosi I. 2003. Control of *Brettanomyces/Dekkera* presence during fermentation/maceration and ageing (Controllo della presenza di lieviti *Brettanomyces/Dekkera* durante il processo di vinificazione e affinamento del vino). Vigne Vini, 7/8:69-72.

The occurrence of *Brettanomyces* yeasts was evaluated in two different stages of wine making process: fermentation/maceration and ageing. The results obtained indicate that different factors such as the kind of recipient, its level of filling and the integrity of internal surface greatly affect the level of *Brettanomyces* contamination which, after two days of fermentation/maceration, varies from a minimum of 2 x 10 to a maximum of 1.6 x 10^4 ufc/ml. During wine ageing, besides the kind and the general condition of the recipient, also other factors had a great influence on *Brettanomyces* spoilage: availability of oxygen and initial contamination level of this spoiling yeast. In particular it was observed that wine presenting about 10^2 ufc/ml of *Brettanomyces* at the beginning of ageing showed an increase of contamination varying from 3 to 15 times after 135 days of ageing.

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1. Fia G., Paronetto L., Rosi I. 2001. Evaluation of ten *Saccharomyces cerevisiae* strains in the production of sparkling wines by "metodo classico" (Valutazione del comportamento di 10 ceppi di *Saccharomyces cerevisiae* nella produzione di spumante ottenuto con il "metodo classico") Industria delle Bevande 30:607-615.

The study was carried out to evaluate some enological traits of ten *Saccharomyces cerevisiae* commercial strains, selected for primary alcoholic fermentations, in the production of sparkling wines by "metodo classico". The results showed that only six out of ten strains have suitable biochemical and physiological characteristics for the production of sparkling wine. The fermentation rate of some strains was lower than the other strains during inoculum preparation, showing that the ethanol sensitivity is a variable property among strains of *S. cerevisiae*. The second fermentation length varied between 16 and 118 days depending on the yeast strain. After six months of second fermentation, strains with elevated fermentative activity showed lower cell viability. The results of sensory analysis of sparkling wines showed that strains with problematic fermentative activity produced wines with off-flavour (lees and hydrogen sulfide/mercaptan aroma).

2. Rosi I., Giovani G. 2003. Effect of some winemaking variables on the production of esopolysaccharids by *Saccharomyces cerevisiae* Actualités Œnologiques 2003"-VII^{ème} Symposium International d'Œnologie-Bordeaux 19-21 June 2003 (in press)

Saccharomyces cerevisiae can release parietal polysaccarides, particularly mannoproteins, during alcoholic fermentation of grape juice. The amount of parietal polysaccharides released is highly dependent on yeast strain, on metabolic phase of cells, as well as on fermentation conditions. Various positive effects on wine quality of these yeast-produced macromolecules have been proposed: increase of colour stability and decrease in astringency of red wines, regulation of volatility of the substances responsible for odour, protective effect of the tartaric and protein precipitation of wine, stimulation of malolactic fermentation. The aim of this study is to optimise the production of parietal polysaccharides by a strain of *S. cerevisiae* taking into account some of winemaking process variables. In the first stage, based on Fractional Factorial Design, seven variables

of winemaking process (i.e. pH, temperature, assimilable nitrogen concentration, sugar concentration, amount of cells at inoculum, initial macromolecule content, duration of lee contact at the end of alcoholic fermentation) were screened to allow the identification of the factors with the largest effects on the polysaccharide excretion by yeasts. The second stage, based on response surface modeling (RSM), aims to optimize and predict the response (exopolysaccharide production) using the factors derived from the previous screening investigation. As substrate of fermentation was used a polysaccharide-free synthetic medium. The evolution of fermentation was followed by CO_2 loss. At the end of fermentation cellular dry weight, ethanol and total polysaccharide concentration were determined.The results of screening indicated that fermentation temperature and initial concentration of macromolecules present in the synthetic medium were the major factors influencing polysaccharide release by yeast cells during fermentation. The results of RSM permitted to identify a region of operability for temperature and initial concentration of macromolecules (temperature around 27-28 °C, the macromolecule concentration between 75 and 250 mg/l), in order to have in the fermentation medium the major amount of exopolysaccharides. Predicted values were in good agreement with experimental values.

3. Fia G., Bertuccioli M., Rosi I. 2003. Influence of a mixture of *Saccharomyces cerevisiae* strains on wine sensory profile (Influenza dell'inoculo di una miscela di ceppi di *Saccharomyces cerevisiae* sul profilo sensoriale del vino) Proc. National Congress Italian Society of "Scienze Sensoriali", Roma 13-14 November 2003 (in press)

Several studies have demonstrated that different *Saccharomyces cerevisiae* yeast strains are involved in the course of alcoholic fermentation of grape juice. This situation is generally present during the whole fermentation process, and consequently, several strains are active simultaneously. However, the relative percentage of each strain can vary considerably, depending on its different physiological characteristics. With regards to the impact of a particular strain of the fermentation process, several authors have described the correlation between the type of wine yeast strain, and, consequently its enzymatic activities, with the quality of the final product, particularly in terms of aroma compound formation. With the aim to investigate the effect of mixed strain cultures of *S. cerevisiae* on the chemical composition and sensory quality of wine, we inoculated Sangiovese grape must with a mixture of three commercial yeast strains, in a 1:1:1 ratio for the number of viable cells. Control experiments were run by setting up individual fermentation's with each single yeast strain. Duplicate fermentation experiments were carried at 25 °C, following the technological scheme used in the Chianti area. During the fermentation process, several samples were collected and analyzed to assess different parameters, such as the fermentation rate, the dynamics of growth of the yeast population, as well as the dominance of the inoculated strain(s). This latter aspect was followed by using CHEF gel electrophoresis of yeast chromosomes, followed by analysis with an appropriate fingerprinting software. Wines were analyzed for the content of different fractions of phenols, volatile compounds as well as for other standard parameters. Sensory differences among wines were characterized by flavor profile analysis. The results showed that all inoculated strains were found to dominate the entire fermentation. In the case of fermentation with the yeast mixture, the inoculated strains were present at the end of the experiment, though in a different relative proportion when compared to the initial 1:1:1 ratio.The sensory profiles of the wines obtaines by yeast mixture were characterized by a more intense aroma of berry and ethyl acetate and a more intense sweet taste and mouthfeel when compared to wines obtained by a single yeast.

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1. Mannazzu I., Simonetti E., Guerra E., Budroni M, Thangavelu M., Clementi F. 2002. SED1 Gene Length and Sequence Polymorphisms in Feral Strains of *Saccharomyces cerevisiae*. Appl. Environ. Microbiol., 68:5437-5444.

The *SED1* gene (YDR077W), coding for the major cell wall glycoprotein of *Saccharomyces cerevisiae* stationary-phase cells, contains two blocks of tandem repeat units located within two distinct regions of the nucleotide sequence. A PCR survey of the *SED1* open reading frames (ORFs) of 186 previously uncharacterized grape must isolates of *S. cerevisiae* yielded 13 PCR profiles arising from different combinations of seven *SED1* length variants in individuals homozygous or heterozygous for the gene. Comparison of the nucleotide sequences of a group of representatives of each of the seven length variants with those of S288C and the type strain, CBS1171, unequivocally identified them as *SED1* alleles and provided evidence for the presence of two minisatellite-like sequences, variable in length, within the ORF of an *S. cerevisiae* gene. The segregation analyses of the *SED1* length variants and other genetic markers in 13 isolates representative of each PCR profile suggested that molecular mechanisms involved in minisatellite expansion and contraction may be responsible for *SED1* heterozygosities within a population of homothallic must isolates of *S. cerevisiae.*

2. Mannazzu I., Clementi F., Ciani M. 2002. Strategies and criteria for the isolation and selection of autochthonous starters. In: Biodiversity and Biotechnology of Wine Yeast. M. Ciani ed., Research Signpost Trivandrum, India

In the past few years there has been a noticeable increase in the demand for autochthonous wine yeasts to be used as fermentation starters. The requirements for these yeasts are the ability to dominate during the fermentation process, and enhance, at the same time, the typical sensorial characteristics of the wines originating from different grapevine cultivars.The first step towards the obtainment of these strains is the isolation of the wild yeasts from the natural environments associated to the winemaking area of interest. The isolation step should be followed by a thorough analysis of the enological aptitude of the isolates and the selection of the isolates presenting biotechnological properties applicable in winemaking. In this chapter, in order to promote the exploitation of wine yeast biodiversity, the criteria for the isolation and selection of autochthonous starters are discussed.

3. Ciani M., Fatichenti F., Mannazzu I. 2002. Yeasts in winemacking Biotechnology. In: Biodiversity and Biotechnology of Wine Yeast. M. Ciani ed., Research Signpost Trivandrum, India.

The seasonal and traditional characters of wine industry have been, for a long time, an obstacle to the introduction of innovations in the winemaking process. However, in the past forty years, the need to control onset and duration of the fermentation process and to obtain final product with appreciable and reproducible characteristics, have lead several researchers to propose new and alternative winemaking technologies, some of which concerning the use of wine yeasts. The first important step toward the development of wine yeast biotechnology in the history of winemaking was the introduction of selected starter cultures which occurred in ninety-sixty-two. In the following years the utilization of immobilized yeasts and of multi-starter were proposed in order improve the fermentation process and the quality of wines. More recently, the use of antimicrobial compounds in winemaking have been investigated with the aim to reduce the concentration of chemical antiseptic agents in wine. In addition, several researchers investigated on the potential use of wine yeasts enzymes to enhance stability, aroma and flavor of wines. In the present chapter all these aspects of wine yeast biotechnology are reviewed.

4. Ciani M., Pepe V. 2002. The influence of prefermentative practices on the dominance of inoculated yeast starter under industrial conditions J. Sci Food Agric. 82:573-578

The influence of prefermentative practices on the growth dynamics of a "natural" starter culture with specific phenotype (H_2S^-) concurrently to wild yeast populations was evaluated under winery conditions. Different clarification procedures and added SO_2 strongly influenced species and cell numbers isolable at the prefermentation stage. Independent treatments of must with sulphite addition or vacuum filtering clarification caused over 30-fold reduction of viable cells. Clarification procedures, enhanced by selective effect of SO_2 addition, induced the appearance of *Saccharomyces cerevisiae* "wild" yeasts. A correct application of the inoculum generally garantees the dominance of fermentation by starter cultures. However, inoculated fermentations by using unclarified white and red musts exhibited a consistent presence and persistence of non-*Saccharomyces* and/or *Saccharomyces* "wild" yeasts during fermentation. The extent and composition of the initial wild microflora at the start of fermentation may affect the presence and the persistence of wild *Saccharomyces* and non-*Saccharomyces* yeasts during guided fermentations under commercial conditions. The above findings confirm the results of previous works carried out at laboratory or pilot scale level. Furthermore, they suggest a clear correlation between modality of prefermentative practices and presence and persistence of "wild" yeasts during the fermentation.

5. Ciani M., Maccarelli F., Fatichenti F. 2003. Growth and fermentation behaviour of *Brettanomyces/Dekkera* yeasts under different conditions of aerobiosis Word J. Microbiol. Biotechnol. 19:419-422.

Brettanomyces /Dekkera yeasts grow in wine and their presence is often associated with spoiling activity. In this report, we investigated on the influence of different conditions of aerobiosis on growth and fermentation behaviour of these spoilage yeasts in wine. Results showed that in all conditions tested *Brettanomyces* strain consumed all sugars taking wine fermentation to completion. Strict anaerobic conditions influenced growth of *Brettanomyces*. Both anaerobiosis and strict anaerobiosis did not negatively affect the principal by-products of fermentation while semi-anaerobiosis caused an increase of acetic acid, acetaldehyde and ethyl acetate that negatively affect the fermentation profile of resulting products.

6. Selvi S., Cardinali G., Ciani M. 2003.Variability of *HXT2* at the protein and gene level among the *Saccharomyces sensu stricto* group. In press on FEMS Yeast Research.

Variability of *HXT2* at the protein and gene level was investigated among the *Saccharomyces sensu stricto* and in other yeast species Results showed that the *HXT2* gene is probably present in yeast genera other than *Saccharomyces*, suggesting that this gene is widely spread out in the yeast world. Chromosomal analyses indicated the stable location of *HXT2* on the same chromosome with the same copy number throughout the entire *sensu stricto* group. Results of the immunoblotting assay showed that all strains tested (with the exception of *S. cerevisiae* DBVPG 6042) exhibited a lower level of Hxt2p expression than that showed by laboratory wild-type. Moreover, Hxt2p expression seems to reinforce the taxonomical differences between the two pairs of species (*S. cerevisiae and S. paradoxus* vs. *S. pastorianus and S. bayanus*) within the group of *sensu stricto* of the genus of *Saccharomyces* that also reflect the different ecological niche of growth.

7. Marinangeli P., Angelozzi D., Ciani M., Clementi F., Mannazzu I. 2003 Minisatellites in *Saccharomyces cerevisiae* genes encoding cell wall proteins: a new way towards wine strain characterisation. In press on FEMS Yeast Research.

With the aim of developing new tools for the characterisation of wine yeasts, by means of databases available on-line we scanned the genome of *Saccharomyces cerevisiae* in search of potentially polymorphic targets. As we have previously observed for *SED1*, we found that other genes coding for cell wall proteins contain minisatellite-like sequences. A PCR survey of *SED1* and three of these others, namely *AGA1, DAN4* and *HSP150*, in a population of wild *S. cerevisiae* demonstrated that these genes are highly polymorphic in length and represent a sink of unexplored genetic variability. The primer pairs designed on the gene ORFs yield stable and repeatable amplification profiles that show a level of resolution that allows the clear discriminate between different strains. These can therefore be utilised for PCR-based typing of *S. cerevisiae.*

8. Ciani M., Mannazzu I., Marinangeli P., Clementi F., Martini A. 2003. Contribution of winery-resident *Saccharomyces cerevisiae* strains to spontaneous grape must fermentation. In press on Antonie van Leewenhoeck

The origin of the *Saccharomyces cerevisiae* strains that are responsible for spontaneous grape must fermentation was investigated in a long-established industrial winery by means of two different approaches. First, seven selected components of the analytical profiles of the wines produced by fifty-eight strains of *S. cerevisiae* isolated from different sites and phases of the production cycle of a Grechetto wine were subjected to Principal Components Analysis. Secondly, the same *S. cerevisiae* isolates underwent PCR fingerprinting by means of ? primers. The results obtained by both methods demonstrate unequivocally that under real vinification conditions, the *S. cerevisiae* strains colonising the winery surfaces are the ones that carry out the natural must fermentation.

9. Comitini F., Clementi F., Mannazzu I., Ciani M. 2003. Yeast-bacteria interactions: the case of *Saccharomyces cerevisiae* and *Oenococcus oeni*. 23rd International Specialised Symposium on Yeasts *Interactions between Yeasts and other Organisms*, Budapest, Hungary, August 26 – 29.

The wine strains which carry out must fermentation represent one of the numerous factors affecting growth and activity of malolactic bacteria (MLB). The evaluation of the interactions between *Saccharomyces cerevisiae* and *Oenococcus oeni* is therefore a topic of increasing interest for a better management of secondary fermentation. In this context, we evaluated the effect of seventy-seven *S. cerevisiae* on three *O. oeni* strains and observed that each of the wine yeasts analysed exerts either inhibitory, null or stimulatory activity depending on the MLB tested. However, three of the *S. cerevisiae* strains under study were able to strongly inhibit growth of all three MLB. We therefore investigated on the nature of the inhibitory compound/s produced by the strain F63 of *S. cerevisiae*, the one showing the strongest inhibitory activity on MLB. Our results indicate that F63 produces a heat and protease sensitive antimicrobial factor which reduces *O. oeni* growth rate with a typical saturation kinetics. The partial purification and characterization of this antimicrobial factor lead us to identify a protein, with apparent molecular weight of 15.6 kDa, as the responsible for the inhibitory activity of *S. cerevisiae* F63 against *O. oeni*.

10. Comitini F., Di Pietro N., Mannazzu I., Ciani M. 2003. The killer toxin of *Kluyveromyces phaffii*: characterisation of a new biopreservative agent. 23rd International Specialised Symposium on Yeasts *Interactions between Yeasts and other Organisms*, Budapest, Hungary, August 26 – 29.

Killer yeasts belonging to the species *S. cerevisiae* are currently used in winemaking as fermentation starters to improve the management of the process and wine quality. However, the main limit of the killer toxin of *S. cerevisiae* wine yeast (K2 type) resides in its narrow anti-yeast spectrum, which is restricted to *Saccharomyces* sensitive strains. On the contrary, the killer toxin of *Kluyveromyces phaffii* shows a wide spectrum of intergeneric activity and kills "wild" wine yeasts such as those belonging to the genera *Hanseniaspora*/*Kloeckera*. The undesired activity of these yeasts in non-sterile environment such as grape must is generally controlled by SO_2. In order to reduce or eliminate the amount of SO_2 added at the pre-fermentative stage and during the fermentation of grape must, the use of *K. phaffii* killer toxin (Kpkt) has been investigated and its application as a biopreservative agent in wine has already been proposed [1]. In this work we will report on the results of the purification and characterisation of *K. phaffii* killer toxin and show that Kpkt is a glycosilated protein of 33 kDa whose first receptorial site is represented by cell wall glucans.

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1. Ranalli G., Iorizzo M., Lustrato G., Zanardini E., Grazia L. 2002. Effects of low electric treatment on yeast microflora: J. Appl. Microbiol. 93:1-7.

The objective of the research was to contribute to the understanding of phenomena related to different electrical current intensity treatments on the growth and metabolism of selected wine yeast, *Saccharomyces cerevisiae* 404 strain and *Hanseniaspora guilliermondii* 465 strain. Low electric current (LEC) (10-30-50-100 mA) was applied to using laboratory samples of pure and co-cultures yeast. Parameters such as polarity, treatment duration (18 – 48 h) and type of inoculum yeast were varied one at a time to highlight their cause-effect relationships. The effects on cell activity as well as microflora viability were assessed. Bioindicators capable of describing the phenomena caused by the electrical current on the microflora were identified. Results demonstrated that a low voltage treatment using graphite electrodes has a greater effect on the viable *S.cerevisiae* 404 strain microflora. There was less bactericidal activity in the *S.cerevisiae* 404 strain than in the *H.guilliermondii* 465 strain. These results may be of significant importance in the development of new technological processes in the agricultural and food fields, particularly new fermenting processes controls.

2. Lustrato G., Alfano G., Belli C., Grazia L., Iorizzo M., Maiuro L., Massarella F., Zanardini E., Ranalli G. 2003. Controlling grape must fermentation in early wine-making phases: The role of electrochemical treatment. J. Appl. Microbiol. 95:1087-1095.

Traditional white winemaking presupposes, apart from skin and juice contact, several pre-fermentative treatments during which wild microflora are controlled through the addition of SO_2. Therefore how systems or techniques alternative to the addition of SO_2, which are able to selectively inhibit apiculate yeasts, are a sought-after goal. The objectives of the research were: i) to investigate the effects of Low electric current (LEC) at different intensities in grape must, through the use of graphite electrodes; ii) to evaluate, in laboratory tests, the selectivity of the electrochemical effect on the microflora in grape must; iii) to

verify, in a pilot plant, the applicability (efficiency) of low electric current in grape must, allowing its proposal as an alternative to the addition of SO_2 to control the first fermentation phases. LEC treatment had a positive effect on grape juice fermentation (yeast microflora) during the early stages of wine-making. LEC decreased the survival time and increased the death rate of *H. guilliermondii* strain 465 in co-cultures, whereas it did not affect the growth and survival of *S. cerevisiae* strain 404). From the viewpoint of practical application, the overall results appear of great interest for processes aimed at obtaining biological wines, offering a new point of reference to the production of such wines.

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1. Carraro A., Targhetta C., Duregon C., Romanato R., Corich V., Lante A., Giacomini A., Casella S. 2003. Variation in yeast population and dynamics of physico-chemical parameters during storage of grape marcs for Grappa production. 23rd International Specialised Symposium on Yeasts, Budapest, Hungary, 26-29 August, p.180.

Grappa is a typical alcoholic beverage made in Italy, obtained through the distillation of grape marcs, a by-product of the winemaking process. Before distillation, marc is stored from a few days up to several months under anaerobic conditions. During the conservation, microbial activities can induce either positive (increasing ethanol concentration and aromatic compounds) or negative effects (off-flavour formation) on the distillate that heavily influence the Grappa quality. Therefore marc conservation is a crucial step and the lack of data in the literature about microbial presence and behaviour in this complex matrix represents an obstacle towards the control of the production process. This study aims at collecting information about microflora composition, particularly yeast population, and its fluctuation during marc storage. Pinot grape marcs were obtained from an important distillery in the Veneto region (Italy), stored for 70 days into large plastic containers (60 m x 2.5 m diameter), each one filled with ca. 200 tons. A time course sampling was carried out analyzing chemical, physical (pH, temperature, sugar, alcohol content, methanol, fermentation products) and microbiological (yeast population) parameters. Moreover a phenotypical and molecular characterization of yeast isolates collected at the initial time of storage and six days later is also reported. The results show quantitative and qualitative fluctuations of yeast microflora, along with variations of the chemical parameters examined.

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1. Zara S., Farris G.A., Budroni M., Bakalinsky A. T. 2002. *HSP12* is essential for biofilm formation by a Sardinian wine strain of *Saccharomyces cerevisiae.* Yeast 19:269-276.

Sardinian sherry strains of *S. cerevisiae* form a biofilm on the surface of wine at the end of the ethanolic fermentation, when grape sugar is depleted and when further growth becomes dependent on access to oxygen. A point mutation in *HSP*12 or deletion of the entire gene results in inability to form this film. *HSP*12 encodes a heat-shock protein previously found by others to be active during stationary phase, in cells depleted for glucose, and in cells metabolizing ethanol and fatty acids, all conditions associated with sherry biofilms. The DNA sequence of *HSP*12 allele of strain Ar5-H12 has GenBank Accession No. AY046957.

2. Mannazzu I., Simonetti E., Guerra E., Budroni M., Thangavelu M., Clementi F. 2002. SED1 Gene Length and Sequence Polymorphisms in Feral Strains of *Saccharomyces cerevisiae*. Appl. Environ. Microbiol., 68:5437-5444.

The *SED1* gene (YDR077W), coding for the major cell wall glycoprotein of *Saccharomyces cerevisiae* stationary-phase cells, contains two blocks of tandem repeat units located within two distinct regions of the nucleotide sequence. A PCR survey of the *SED1* open reading frames (ORFs) of 186 previously uncharacterized grape must isolates of *S. cerevisiae* yielded 13 PCR profiles arising from different combinations of seven *SED1* length variants in individuals homozygous or heterozygous for the gene. Comparison of the nucleotide sequences of a group of representatives of each of the seven length variants with those of S288C and the type strain, CBS1171, unequivocally identified them as *SED1* alleles and provided evidence for the presence of two minisatellite-like sequences, variable in length, within the ORF of an *S. cerevisiae* gene. The segregation analyses of the *SED1* length variants and other genetic markers in 13 isolates representative of each PCR profile suggested that molecular mechanisms involved in minisatellite expansion and contraction may be responsible for *SED1* heterozygosities within a population of homothallic must isolates of *S. cerevisiae.*

3. Farris G. A., Zara S., Pinna G., Budroni M. (2002) Genetic aspect of flor yeasts. Sardinian strains, a case of study. Biodiversity and Biotechnology of Wine Yeast. *Ed. Research Signpost*

The aging of Vernaccia-type wines in Sardinia and traditional fino sherry in Spain is dependent on the presence of yeast strains that develop a biofilm on the wine surface at the end of alcoholic fermentation. The ability to form a biofilm or *flor* on the surface of wine is an unusual, but essential and poorly understood characteristic of «flor» strains of *S. cerevisiae*. The Sardinian strains not only mediate the primary fermentation (anaerobic conversion of grape sugar to alcohol), but are also responsible for the subsequent oxidative transformation of a small amount of the alcohol to acetaldehyde accompanied by other reactions that produce flavors and aromas characteristic of fino-type sherries. Only strains able to form a film on the wine surface can mediate this obligatory aerobic oxidation of alcohol because only the wine surface is aerobic. Just as in grape juice, initial metabolism of the sugar is not accompanied by formation of a biofilm as the cells ferment the sugar to produce ethanol while at the bottom of the growth vessel. However, when the sugar is depleted, cells begin to form a visible biofilm that eventually

covers the entire liquid surface. Adherence of cells to the inner wall of the growth vessel at the liquid-air interface is often observed as well. The film of cells increases in thickness over time, but eventually falls, presumably because essential nutrients have been depleted. In contrast to other microbial biofilms, this film appears to consist of a layer of buoyant cells without a suspending extracellular matrix, as no evidence for a polysaccharide or protein matrix has been reported. The buoyancy of the cells appears to be due to an elevated and/or altered lipid content. We seek to describe the biology of biofilm formation, which in this system, can be viewed as an adaptive mechanism that maintains access to oxygen, and thus permits yeast growth on a non-fermentable carbon source in an otherwise anaerobic environment. We report here on the biodiversity of flor strains and, in particular: the identification of a yeast genes found to be essential for the process and recent knowledges about flor yeast life cycles.

4. Zara S., Zara G., Budroni M., Farris G.A., Martin O., Bakalinsky A.T. 2002. *PHO23* and *SWH 1* play roles in biofilm formation by a Sardinian wine strain of *Saccharomyces cerevisiae*. Yeast Genetics and Molecular Biology Meeting, University of Wisconsin, Madison, USA, July 30-August 4

Sardinian sherry-like strains of *S. cerevisiae* form a biofilm on the surface of wine at the end of the ethanolic fermentation, when grape sugar is depleted and when further growth becomes dependent on access to oxygen. To identify genes required for biofilm (flor) formation, an auxotrophic, biofilm-forming derivative strain was subjected to transposon mutagenesis with a mini-Tn3::*URA3* transposon. Among 15,000 *Ura+* transformants screened for biofilm formation, 20 biofilm-negative mutants were found. Two mutant loci were identified by inverse PCR as *PHO23* and *SWH1*. *PHO23* is a transcriptional regulator of *PHO5* which encodes an acid phosphatase, and was previously shown by others to be associated with histone acetyltransferase activity. *SWH1* has similarity to mammalian oxysterol-binding proteins. Neither gene is essential for growth and their unknown roles in biofilm development are currently under investigation.

5. Franco M.A., Coloru G.C, Del Caro A., Emonti G., Farris G.A., Manca G., Massa T.G., Pinna G. 2002. Variability of resveratrol (3,5,4'-trihydroxystilbene) content in relation to the fermentation process by *Saccharomyces cerevisiae* strains. Eur. Food Res. Technol., 214:221-225.

The variability of the four monomeric forms of resveratrol (3,5,4'- trihydroxystilbene) caused by 14 strains of fermentative yeasts belonging to the *Saccharomyces cerevisiae* species in Cannonau grape musts during three different stages of fermentation was assesed by HPLC. During the process a decrease in the glucoside forms (cis and trans-beta-D-glucopyranoside) was observed in all the musts independently of the yeast strain used. As regards the activity of the free forms (cis and trans- resveratrol), the greatest differences were found in the first two stages of fermentation when some strains appeared to cause and increase in either one or both of the free forms while others produced a decrease in the two compounds.

6. Cabras P, Farris GA, Fiori MG, Pusino A. 2003. Interaction between fenhexamid and yeasts during the alcoholic fermentation of *Saccharomyces cerevisiae*. J Agric Food Chem., 51:5012-5015.

The behavior of the fungicide fenhexamid, N-(2,3-dichloro-4-hydroxyphenyl)-1-methyl-cyclohexanecarboxamide, has been studied at concentrations corresponding to the limits fixed for grapes (3 mg kg(-1)), or higher, during the alcoholic fermentation. The presence of the fungicide did not affect the amount of alcohol produced. The amount of fenhexamid in the liquid phase decreased by ca. 15%, but the missing fenhexamid was recovered unchanged from yeasts. This suggests that the fungicide is not degraded during the fermentation process, but adsorbed by yeasts. Two constituents of Saccharomyces cerevisiae cell wall, chitin and glucan, tested as potential adsorbents, exhibited affinity for fenhexamid.

7. Pirino G., Zara S., Pinna G., Farris G. A. and Budroni M. 2003. Diversity of Y region at *HML* locus in a *Saccharomyces cerevisiae* strain isolated from a sardinian wine. Antonie van Leeuwenhoek (in press).

Several mutations in genes involved in *Saccharomyces* mating type switching may affect the homothallic behaviour in wine yeasts. In this study the semi-homothallic (Hq) segregation of a flor wine yeast strain was analysed. We aimed understand the molecular basis of this behaviour in a flor autoctonous strain, verifying the MAT locus status by a PCR-based *HO* gene disruption and sequencing of the Y region of the *HML*, *HMR* and *MAT* loci, after nested PCR. Presence of ORFs a1 and a2 in the Y region of the *HML* locus was found. At the ORF a2 at *HML* locus, a mutation in the stop codon was found, so the a2 ORF contains 33 bases more.

8. Zara S., Zara G., Bakalinsky A.T., Pirino G., Budroni M. 2003. *FLO11* is required for biofilm formation on a liquid surface by *Saccharomyces cerevisiae*. XXI International Conference on Yeast Genetics and Molecular Biology Göteborg, Sweden - July 7-12.

Microorganisms grow as biofilms in a wide variety of terrestrial and aquatic ecosystems. The yeast *S. cerevisiae* is able to grow as a biofilm on both solid and liquid surfaces while maintaining a normal yeast cell morphology. While the mechanisms involved in biofilm formation by *S. cerevisiae* are not understood, this phenomenon continues to be exploited industrially in winemaking for the production of sherries and the sherry-like wines of Sardinia, such as Vernaccia of Oristano. *FLO11* encodes a cell surface flocculin with a structure similar to that of serine/threonine-rich GPI-anchored cell wall proteins, and is required for nitrogen starvation-induced pseudohyphal and invasive growth, and was recently shown to be required for biofilm formation on a polystyrene surface. Here, we show that *FLO11* is also required for biofilm formation on a liquid surface.

9. Demontis M.A., Budroni M., Farris G. A., Fink G. R. 2003. Genomic analysis of biofilm formation in *Saccharomyces cerevisiae*. XXI International Conference on Yeast Genetics and Molecular Biology Göteborg, Sweden - July 7-12

'Flor' strains of *Saccharomyces cerevisiae* are able to form a biofilm (flor) on the surface of the wine at the end of the alcoholic fermentation, while switching from a fermentative to an oxidative metabolism. We utilized genomic analysis in order to identify candidate genes involved in biofilm formation by flor strains of *S. cerevisiae*. We used DNA microarrays to determine the expression level in a four-day course experiment, using a diploid flor strain. The experiment was performed in triplicate. Analysis of gene expression was performed with Affymetrix S98 Yeast cDNA chips. Data were analysed with the software GeneSpring 5.0 (after appropriate normalizations), and with Cytoscape 0.9. Clustering with self-organizing maps was also performed, and then preorganized gene ontology lists were examined in search of significant associations. Many of the genes that display interesting behaviour (a greatly increased or decreased expression, or a high level of expression with subsequent increase or decrease greater than 3-fold) were genes related to the metabolism of carbohydrates and/or ethanol. Of greater interest were genes related to the cell wall structure and metabolism, a group of genes that code for proteins involved in ion transport mechanisms, and a few genes of unknown function. Northern blot analysis was performed and showed a good correlation with microarray data.

10. Zara G., Zara S., Demontis M.A., Pirino G., Budroni M. 2003. Sardinian flor yeast strains: a perspective. 23rd International Specialised Symposium on Yeasts *Interactions between Yeasts and other Organisms*, Budapest, Hungary, August 26-29.

The flor strains of *Saccharomyces cerevisiae* are responsible for the aging of sherry-type wines. These strains at the end of alcoholic fermentation, rise to surface of the wine and form a biofilm while switching from a fermentative to an oxidative metabolism. The ability to form a biofilm on the surface of wine is an unusual, but essential and poorly understood characteristic of «flor» strains of *S. cerevisiae*. We found that Sardinian flor strains showed a considerable chromosomal polymorphism. In areas where the florization is an estabilished practice the biofilm consist of strains with a "dominant" karyotype, with others less representative. Genetic studies of life cycle indicated that these strains may generate tetrads with two sporulating and two *MAT*a cultures per ascus leading a semi-homothallic behaviour. With these studies we found presence of ORFs a1 and a2 in the Y region of the *HML* locus. We utilized transposon mutagenesis and functional genomic analysis to study the phenomenon of biofilm formation. With the former technique two genes, HSP12 and RAS2, were found to be involved in biofilm formation. Finally, analysis of microarray data allow us to cluster 126 genes that display an interesting behaviour during a time course experiment.

11. Pirino G., Budroni M. 2003. Mating type interconversion in flor *Saccharomyces cerevisiae strains*. 8th Workshop on the Developments in the Italian PhD Research in Food Science and Technology.Viterbo (Italy) 24-26 September.

Wine yeasts usually show a homothallic life cycle: they are able to switch their mating type by a gene conversion event. Flor yeasts are found to have no canonic life cycles, comparing with other wine yeasts. The aim of this research is focused on understanding the mechanisms involved in mating type interconversion of flor *Saccharomyces cerevisiae* strains.

12. Demontis M.A., Budroni M. 2003. Genomic Analysis of Biofilm Formation In a *S. cerevisiae* Flor Strain. 8th Workshop on the Developments in the Italian PhD Research in Food Science and Technology. Viterbo (Italy) 24-26 September.

Biofilms occur when microbes develop into a multicellular community at an interface. In order to identify candidate genes involved in biofilm formation, we used DNA microarrays to follow the temporal changes in gene expression, that occur as yeast cells transition from a free suspension to a biofilm state. We used cDNA chips to compare the expression level in a three-day time course experiment, using a diploid biofilm-forming strain. Three main groups of genes were highly upregulated: 1) genes related to the metabolism of carbohydrates and/or ethanol, 2) genes related to cell wall structure and metabolism, 3) genes which encode proteins involved in ion transport mechanisms.

Italy: Università degli Studi di Verona, Dipartimento Scientifico e Tecnologico, Strada Le Grazie, 15, 37134 Verona, Italy Communicated by S. Torriani <sandra.torriani@univr.it>.

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1. Zapparoli G., Torriani S., Malacrinò P., Suzzi G., Dellaglio F. 2003. Interactions between *Saccharomyces* and *Oenococcus oeni* strains from Amarone wine affect malolactic fermentation and changes in wine composition. Vitis 42:107-108.

In the present study, the ability of *Saccharomyces* strains to induce malolactic fermentation (MLF) was evaluated in model fermentations. Nineteen *Saccharomyces* strains (10 *S. bayanus* var. *uvarum* and 9 *S. cerevisiae*) and two *Oenococcus oeni* strains isolated from Amarone wine were considered. Significant differences in MLF performances of the *O. oeni* cultures were found; in fact L-malic acid consumption in wines fermented by *S. cerevisiae* strains was favoured respect to wines produced by *S. bayanus* strains. Further, the percentage of L-malic acid converted differed depending upon the *O. oeni* strains and the yeast species used for fermentations. A comparison of the concentration of some secondary compounds of fermentation before and after MLF showed a significant increase in the content of n-propanol, amylic alcohols, acetoin and acetic acid after MLF. Moreover the level of isobutanol was higher in the wine fermented by *S. cerevisiae*. This study indicates that the interactions between different yeast and malolactic bacteria strains can influence MLF and the aromatic traits of wine. Therefore the selection of new indigenous yeast strains could be conducted on the basis of their interactivity with malolactic bacteria to overcome the difficulties in inducing MLF in Amarone wine.

2. Dellaglio F., Zapparoli G., Malacrinò P., Suzzi G., Torriani S. 2003. *Saccharomyces bayanus* var. *uvarum* and *Saccharomyces cerevisiae* succession during spontaneous fermentations of Amarone and Recioto wines. Ann. Microbiol. 53: (in press).

This study was undertaken to evaluate the biodiversity of the indigenous *Saccharomyces sensu stricto* population during traditional vinification processes of Recioto and Amarone wines using molecular typing techniques. In total 109 isolates, collected from eight wineries during spontaneous fermentations, were identified and characterised by conventional tests and by molecular methods, i.e. PCR fingerprinting using the primer $(GTG)_5$, mtDNA restriction and karyotype analyses. Sixty per cent of the isolates were assigned to *Saccharomyces bayanus* var. *uvarum* and 40% to *S. cerevisiae*. A succession between *S. bayanus* var. *uvarum*, which dominated the first fermentation, and *S. cerevisiae*, which appeared after the wine drawing-off operation, was observed during a traditional Amarone winemaking. An extensive polymorphism was found between the isolates, however a few specific genetic biotypes prevailed in the different wineries. This ecotaxonomic survey constitutes a basic step to safeguard and exploit the oenological potential of the yeast biodiversity in the Recioto and Amarone wine ecosystems. Such biodiversity could be further explored to correlate the genetic patterns of the isolates with oenologically useful characteristics, with the ultimate goal to carry out selection programmes of typical strains of the Valpolicella area.

3. Zapparoli G., Malacrinò P., Suzzi G., Dellaglio F., Torriani S. 2003. Rapid identification of the sibling species *Saccharomyces cerevisiae* and *S. bayanus* and their hybrid by a multiplex PCR assay. "Actualités Œnologiques 2003" VIIth International Oenology Symposium. Bordeaux, 19-21 June 2003.

The certain identification of the sibling yeast species *Saccharomyces cerevisiae*, *S. bayanus* and *S. pastorianus* (natural hybrid of *S. cerevisiae* and *S. bayanus*) is considered a tricky task in routine work. In fact, the conventional taxonomic methods based on phenotypic characteristics are time-consuming and can often lead to uncertain results. The development of PCR-based methods provides new means for examining a large number of strains in a short time. In the present research, we established a multiplex PCR assay to rapidly differentiate *S. cerevisiae*, *S. bayanus* and their hybrids. The method uses two sets of primers with sequences complementary to the YBR033W genomic region. A large number of authentic strains belonging to the *Saccharomyces sensu lato* and *sensu stricto* groups were used to optimise the assay. A single amplicon of about 1710 or 329 bp was obtained with, respectively, the species *S. cerevisiae* and *S. bayanus* only, while the presence of both bands was observed in *S. pastorianus*, according to its hybrid nature. No amplification products were obtained when DNA from other yeast species was tested. In a subsequent step, the optimised protocol was applied to a panel of industrial, laboratory and wild strains of different origin, as well as to hybrids of *S. cerevisiae* and *S. bayanus* and to intraspecific hybrids of *S. cerevisiae*. All of the strains previously assigned to the species *S. cerevisiae* and *S. bayanus* were easily distinguished due to the generation of the respective species-specific bands. Moreover, all the interspecific hybrids, as well as the *S. pastorianus* strains gave the two expected bands. Accordingly, the intraspecific hybrids of *S. cerevisiae* generated the 1710 bp amplicon only. The reliable identification of the authentic and other collection strains tested without discrepancies stresses the effectiveness of the multiplex PCR assay here developed. The capability of this approach to rapidly recognize the natural and laboratory hybrids of *S. cerevisiae* and *S. bayanus* appears of particular value. In conclusion, this method revealed to be an effective tool for a fast differentiation of the most important *Saccharomyces* sensu stricto yeasts involved in industrial fermentation processes.

4. Torriani S., Dalai I., Bovo E., Suzzi G., Dellaglio F. 2003. DGGE: an useful tool for discriminating microbial species of oenological interest. "Actualités Œnologiques 2003" VIIth International Oenology Symposium". Bordeaux, 19-21 June 2003.

In this study, Polymerase Chain Reaction Denaturant Gradient Gel Electrophoresis (PCR-DGGE) was used to detect and characterise different populations of wine yeasts and malolactic bacteria, also with the purpose of distinguishing contaminating species. In the case of yeasts, a good PCR sensitivity was obtained using a nested-PCR protocol. An already described universal primer pair, targeted to the eukaryotic 28S rDNA, was used in re-amplification of PCR fragments obtained with a newly designed yeast-specific primer in combination with the P2 primer, firstly applied in the detection of infectious fungi. The method was tested on reference strains commonly associated with wine to verify the ability of the method to discriminate among homologous amplicons. Hence, a reference ladder, comprising all the PCR fragments from the species considered, was assembled. Nested-PCR was then applied to single strains isolated from must and to DNA directly extracted from natural and synthetic fermenting must samples. These were inoculated with a mixture of selected wine yeast strains. The identification of the species was made referring DGGE bands to the ladder. Sequencing of the different amplicons was carried out for confirmation. A further application to yeasts was aimed at the distinction of species belonging to *S. cerevisiae sensu stricto* group from *S. cerevisiae sensu lato* and other yeasts. This was accomplished using a new primer pair targeted to 5S rDNA and 18S rDNA. PCR-DGGE was applied, as well, to oenological bacteria. Universal prokariotic primers, HDA1GC and HDA2, targeted to 16S rDNA, were used to obtain amplification products from bacteria implicated in malolactic fermentation and wine spoilage, i.e. lactic acid bacteria and acetic acid bacteria. The homologous amplicons from *Acetobacter aceti*, *A. pasteurianus*, *Lactobacillus plantarum*, *L. brevis*, *Pediococcus pentosaceus* and *Oenococcus oeni* could be easily discriminated by the different DGGE mobilities.

5. Bocca E, Zapparoli G., Suzzi G., Torriani S., Dellaglio F. 2003. Selezione di ceppi di *Saccharomyces cerevisiae* autoctoni per la produzione di vino Bardolino DOCG. Vignevini (in press).

In the present research, *Saccharomyces cerevisiae* strains autochthonous of the Bardolino wine-producing area (Italy) were isolated and studied in order to select specific yeasts to be used as starter. Ninety samples of grapes from the varieties Corvina, Rondinella and Molinara were collected aseptically, separately pressed and allowed to ferment. Yeast strains were isolated at the end of alcoholic fermentation from samples that showed the most vigorous fermentation kinetics. Seventy-four out of the 130 isolates were identified as *S. cerevisiae* by a novel PCR-based assay recently developed in our laboratories. The oenological potential of the isolates was evaluated by microvinification trials in must. The following properties were tested: resistance to different concentrations of sulphur dioxide, fermentative vigour, actions on malic acid, production of foam, ethanol, volatile acidity

and hydrogen sulphide during fermentation. In general, the isolates had similar fermentation curves, gave high ethanol yields and showed low production of volatile acidity. A wide variability among the isolates was observed as regards the resistance to sulphur dioxide, the production of hydrogen sulphide and foam. The sensory analysis of the wines produced by the strains selected for their good oenological traits allowed the detection of some strains able to maintain the typical aromatic profiles of Bardolino wine. The selected strains of *S. cerevisiae* with different mitochondrial DNA restriction patterns will be tested at industrial wineries in view of possible future use as starter.

6. Zapparoli G., Malacrinò P. Suzzi G., Dellaglio F. 2003. Influenza delle caratteristiche enologiche sulla successione di *Saccharomyces bayanus* e *S. cerevisiae* nei vini Recioto e Amarone della Valpolicella. Rivista di Viticoltura ed Enologia (in press).

In the present research, the oenological features of *Saccharomyces bayanus* and *S. cerevisiae* strains, autochthonous of Recioto and Amarone wines, were studied and correlated to the progressive growth pattern of these species during the spontaneous fermentation. The high fermentation vigor at low temperature of *S. bayanus* strains is the main factor determining the start of the fermentation during the first phases of vinification, while the alcohol tolerance could favor the subsequent colonization of *S. cerevisiae*. No competitive advantage for the strains could be associated to the killer factor presence. The two considered species differed for some oenological properties as ethanol yield, H_2S and glycerol production, which can influence the quality of wine.

International Commission on Yeasts

Meeting of Commissioners, 27 August 2003, International Specialised Symposium on Yeasts 23 (ISSY23), Budapest, Hungary Minutes of Meeting

Present: Lex Scheffers (Chair); Graham Fleet (Vice-Chair), Peter Biely, Tibor Deák, Matti Korhola, Cletus Kurtzman, Marc-André Lachance, Anna Maráz, Leda Mendonça-Hagler, Sally Meyer, José Peinado, Bernard Prior, Peter Raspor, Patrizia Romano, Mogens Jakobsen, Gennadi Naumov.

Apologies: I. Spencer-Martins (Portugal), B. Johnson (Canada), A. Rappaport

Report of Chair

Lex Scheffers welcomed the 16 delegates to the meeting and gave apologies for those who could not attend. He presented the agenda, requested any additional items and asked Graham Fleet to record the minutes.

ISSY23, Budapest (2003)

The Commissioners proposed a toast to Tibor Deák and thanked him for organising and hosting an excellent symposium. Tibor reported 200 participants at ISSY23, representing 29 countries.

ICY11, Rio de Janeiro (2004, August 15-20)

The next International Congress on Yeasts will be held in Rio de Janeiro, with Leda Mendonça-Hagler as Chair of the organising committee. Leda reported on progress with the organisation. A poster announcing the Congress had already been sent out to Commissioners. The Congress will be held in a five star hotel-convention centre in Rio. She had obtained feedback from Commissioners on aspects of the scientific program which was being developed around nine plenary lectures, and eight symposium sessions. She expects the next announcement to be distributed late 2003 or early 2004.

ISSY24, Valencia (2005)

Rafael Sentandreu is organising this specialised symposium in Valencia, Spain, on the topic of "Yeast cell walls, morphogenesis and related topics". It was suggested that Lex Scheffers correspond with Dr Sentandreu to remind him not to schedule this symposium at dates which clash with the next IUMS Congress to be held in San Francisco 24-29 July 2005.

IUMS Congress, San Francisco (2005)

As Vice-Chair of the Mycology Division of IUMS, Graham Fleet reported on the organisation of the next IUMS Congress in San Francisco. It is usual for the IUMS COMCOFS such as the ICY, to organise a symposium at the Congress. The meeting was asked for possible topics and the following suggestions were proposed. Yeast nucleus; apoptosis in yeasts, interaction between yeasts and other microorganisms; yeast nutrition; yeast dimorphism. Graham Fleet was to convey these suggestions to the organising committee of the IUMS San Francisco.

ISSY25, Finland (2006)

This specialised symposium will be organised by Merja Pentilla. Matti Korhola reported on her behalf. While the theme of "Metabolic Engineering in Yeasts" is being considered, it is too early to make firm decisions. It was noted that there is an International Mycology Congress in 2006 and that the dates of ISSY should not clash with this.

ISSY26, Italy (2007)

Patrizia Romano is the organiser for this specialised symposium. The symposium theme will be decided next year. It is proposed to hold the symposium at the end of May, possibly at Sorrento.

ICY12 (2008)

This topic will be considered at ICY11 in Rio de Janeiro next year. Lex Scheffers has received a proposal from Andrei Sibirny (Ukraine) but ISSY21 was recently held there. There was a view within the meeting that an ICY should be held somewhere in South East Asia (e.g. Japan, Thailand) in order to stimulate interest in ICY from that region.

Yeast Newsletter

Lex Scheffers thanked André Lachance for his efforts in editing and distributing the YEAST NEWSLETTER. It is an excellent means of communicating between yeast researchers. It was suggested that Tibor Deák prepare a report on ISSY23, for inclusion in the Yeast Newsletter.

Commissioners

Bernard Prior reported that Sakkie Pretorius had now left South Africa to take up a new position in Australia. He could no longer be a Commissioner for South Africa. Professor L. Kock, University of the Free State, was proposed and accepted as a new Commissioner for South Africa. Dr Charoen Charoenchai, Rajamangala Institute of Technology, was nominated by Graham Fleet and André Lachance to be the first Commissioner for Thailand. Dr Charoenchai's commitment to yeast research in Thailand and his attendance at recent ISSY and ISY was noted.

Other Business

Lex Scheffers highlighted the need for recruitment of new Commissioners and for current commissioners to be active in promoting ICY searching for new representatives. He noted the need for more visibility of ICY and a specific website. Graham Fleet noted that it is listed in the website of IUMS. Peter Raspor undertook to see if it could also be listed on the FEMS website. It was suggested that the first page of the Yeast Newsletter also be put on these websites. Lex Scheffers requested that an historical account of ICY be written and properly archived. Graham Fleet agreed to coordinate this activity in 2004. It was suggested that commissioners with historical information about ICY send copies of such information to Graham Fleet. A complete listing of previous ISY and ISSY symposia was requested.

Editor's note: See list, next page.

Meeting close

The meeting closed with a vote of thanks to Tibor Deák for his hospitality in providing the facilities, food and wine for the meeting.

Graham H. Fleet

List of symposia organized under the auspices of the International Commission on Yeasts

Year	Symposium		Location	Organizer	Topic
1964	ISY	1	Smolenice, Slovakia	Kocková-Kratochvílová	
1966	ISY	2	Bratislava, Slovakia	Kocková-Kratochvílová	
1969	ISY	3	The Hague/Delft, The Netherlands	Wikén	
1971	ISSY	1	Smolenice, Slovakia	Kocková-Kratochvílová	Yeasts as models in science
1972	ISSY	2	Kyoto, Japan	Terui	Technological, medical, ecological aspects
1973	ISSY	3	Otaniemi, Finland	Suomalainen	Metabolism and regulation of cellular processes
1974	ISY	4	Vienna, Austria	Klaushofer	
1976	ISSY	4	Berlin, Germany	Windisch	Yeasts in industrial use
1977	ISSY	5	Keszthely, Hungary	Novak	Yeast systematics and related problems
1978	ISSY	6	Montpellier, France	Galzy	Metabolism and regulation of cellular processes
1980	ISY	5	London, Ontario, Canada	Stewart	
1981	ISSY	7	Valencia, Spain	Sentandreu	Yeast cell surface
1983	ISSY	8	Bombay, India	Subbaiah	Yeast technology
1983	ISSY	9	Smolenice, Slovakia	Kocková-Kratochvílová	Yeasts in the human environment
1984	ISY	6	Montpellier, France	Galzy	
1985	ISSY	10	Plovdiv, Bulgaria	Venkov	Molecular genetics
1986	ISSY	11	Lisbon, Portugal	van Uden	Regulation, transport, and metabolism in yeasts
1987	ISSY	12	Weimar, Germany	Weber	Genetics of non-conventional yeasts
1988	ISY	7	Perugia, Italy	Martini	
1989	ISSY	13	Louvain, Belgium	Verachtert	Production of ethanol and fermented beverages
1990	ISSY	14	Smolenice, Slovakia	Minárik	Yeast taxonomy: theoretical and practical aspects
1991	ISSY	15	Jürmala (Riga), Latvia	Rapoport	Regulation of metabolism and biotechnology
1992	ISY	8	Atlanta, Georgia, U.S.A.	Meyer	
1993	ISSY	16	Arnhem, The Netherlands	Scheffers	Metabolic compartmentation in yeasts
1995	ISSY	17	Edinburgh, United Kingdom	Berry	Yeast growth and differentiation, biochemical and genetic aspects
1996	ISY	9	Sydney, Australia	Fleet	
1997	ISSY	18	Bled, Slovenia	Raspor	Yeast nutrition and natural habitats
1998	ISSY	19	Braga, Portugal	Leão	Yeasts in the production and spoilage of food and beverages
1999	ISSY	20	Smolenice, Slovakia	Biely	Surface structure and membrane phenomena
2000	ISY	10	Papendal (Arnhem), The Netherlands	van Dijken/Scheffers	
2001	ISSY	21	Lviv, Ukraine	Sibirny	Biochemistry, genetics, biotechnology, and ecology of non-conventional yeasts
2002	ISSY	22	Pilanesberg, South Africa	Prior	Yeast fermentations and other processes
2003	ISSY	23	Budapest, Hungary	Deák	Interactions between yeasts and other organisms

This list was compiled from Yeast Newsletter archives. I would appreciate receiving any corrections or comments.

M.A. Lachance <lachance@uwo.ca>

Recent Events

31st Annual Conference on Yeasts of the Czech and Slovak Commission for Yeasts Smolenice, Slovakia, May 19-21, 2003

The 31st Annual Conference on Yeasts, part of the series organized regularly by the Czech and Slovak Commission for Yeasts and the Institute of Chemistry, Slovak Academy of Sciences, took place in the Smolenice Castle, the Congress Center of the Slovak Academy of Sciences, May 19-21, 2003. The meeting was attended by 65 participants from the Czech Republik and Slovakia and by three distinguished guests from other countries. The oral program consisted of plenary lectures in three sessions: yeast biochemistry and biotechnology, cytoskeleton, biomembranes and cell wall, and molecular biology and genetics. The lectures were complemented by 43 posters. The titles of all contributions are listed below:

Lectures in session Biochemistry - Biotechnology

1. Šajbidor J.: Influence of environmental stress on yeast lipid composition.
2. Šmogrovičová D.: Stress of free and immobilized brewing yeasts.
3. Márová I., Kočí R., Pokorná J., Koutný O.: Influence of exogenous stress factors on the production carotenoids by red yeasts.
4. Čertík M., Breierová E., Rapta P., Márová I., Omelková J., Strhanová K.:Environmental stress and carotenogenic yeasts: membrane lipids, carotenoids formation andscavenging radicals.
5. Breierová, E., Gregor T., Strhanová, K., Koubeková A.: Role of the yeast exopolymers in autoprotection of yeast against heavy metals
6. Omelková, J., Breierová, E., Stratilová, E.: The growth and morphology of some yeasts able to grow on pectin medium in physiological conditions or under stress.
7. Stratilová, E.: Polygalacturonase produced during growth of some strains of *Aureobasidium pullulans*.
8. Klobučníková V., Leber R., Fuchsbichler S.,Turnowski F., Hapala I.:Mechanism of terbinafine resistance in the yeast *Saccharomyces cerevisiae*.
9. Vojteková G., Vajcziková I.: Production and sensorial evaluation of the different Slovak wines.

Lectures in session Cytoskeleton – Biomembranes – Cell Wall

1. Grabinska K., Janik A., Kuranda K., Orlowski J., Palamarczyk G.: Unravelling the link between dolichol biosynthesis and cell wall integrity in the yeast *Saccharomyces cerevisiae*.
2. Gabriel M., Kopecká M., Takeo K., Yoshida S.,Yamaguchi, M., Svoboda A., Nakase T., Sugita T.: Cytoskeleton and conidiogenesis in the yeasts.
3. Svoboda A.: 85 years from the discovery of sexuality in the yeasts.
4. Farkaš V., Yoshida S., Sulová Z., Kishida E., Okhusu M., Takeo K.: Accumulation of autolytic enzymes and cell lysis during spontaneous acidification of growth medium in *Cryptococcus neoformans.*
5. Novotná D., Flegelová H., Janderová B.: Killer phenomenon: the exclusive role of Kre2p.

Lectures in session Molecular Biology and Genetics

1. Hikkel I., Lucau-Danila A., Delavau T., Marc P., Devaux F., Jacq C.: A general strategy to uncover transcription factor: Properties identifies a new regulator of drug resistance in yeast.
2. Takáčová M., Gbelská Y., Sklenár P., Šubík J.: Molecular characterization of the *K. lactis* gene. Conferring resistance to cycloheximide in *S. cerevisiae*.
3. Kucejová B., Petrezselyova S., Tomáška L.: Isolation and characterization of *Saccharomyces cerevisiae* mutants selectively resistant against valinomycin.
4. Alemayehu A., Vlčková V., Lampartová Z., Marková E., Dudáš A., Brozmanová J.: Study of interaction of the *PSO3* and *RAD5*1genes of *S. cerevisiae* in the repair of oxidative DNA damage.
5. Tyčiaková S., Obernauerová, and M., Šubík J.: The *PEL1/PGS1* homologue from *Kluyveromyces lactis*.
6. Holič R., Griač P.: Sec4p homologues are involved in regulation of phospholipase D mediated phosphatidylcholine turnover in *Saccharomyce cerevisiae*.
7. Weissová P., Zeman I., Gavurníková G., Kolarov J.: 2003. Study of yeast apoptosis associated with mitochondrial functions and expression of Bcl-2 proteins.
8. Sulo P.: How to tinker new species?
9. Vlčková V., Farkašová A., Svidová S., Miadoková E.: Evaluation of the potential antimutagenic effect of glucomannan on yeast, bacteria and algae.

Posters

1. Sláviková E., Vadkertiová R.: Effects of pesticides on yeasts isolated from agricultural soil.
2. Vadkertiová R., Sláviková E.: The diversity of yeasts in the agricultural soil.
3. Sláviková E., Košíková B., Gregorová A.: Treatment of lignin-polyolefin blends with soil- inhabitant yeast species *Trichosporon pullulans*.
4. Čertík M., Breierová E., Strhanová, K, Bronišová Ž, Oláhová M.: Alterations in lipid composition of carotenogenic yeasts grown under heavy metal presence.
5. Čertík M., Breierová E., Rapta P., Žitòanová, I.: Scavenging and antioxidant properties of carotenoids generating by yeasts stressed by heavy metals.
6. Čížková H., Fiala J., Dobrý J.: HGB Technology – Effect of brewing yeast strain selection.
7. Ferzik S., Dostálek P., Enge J. , Koplík R., Čurdová E.: Brewing process as a natural biosorption process for reduction of heavy metals.
8. Fiala J., Novák J., Čížková H.: Fermentation process assessment by flow cytometry.
9. Fialová A., Čejková A. , Masák J., Jirku V., Šnjadr J.: Ways of increasing phenol hydroxylase activity and phenol-like compounds biodegradation potential by the yeast *Candida maltosa*.

10. Mrózová Z., Valachovič M., Hapala I.: Possible roles of sterol esterification in anaerobic yeast.
11. Magdolen P., Rosenberg M., Krištofíková L.: Possibilities for production of trehalose by yeasts.
12. Pokorná J., Kočí R., Márová I., Drábková M., Knoppová M.: Is production of carotenoids involved in general stress response of red yeasts?
13. Kočí R., Márová I., Koutný O., Pokorná J.: Application of mild oxidative and salt stress to higher production of carotenoids by industrial red yeasts.
14. Gregor T., Strhanová K., Fišera M., Breierová E.: Heavy metal uptake of red yeasts.
15. Vajcziková I., Sláviková E., Vojteková G., Breierová E.: Risk of contamination in soft drinks industry.
16. Pribylová, L., Sychrová, H.: Osmoresistant Yeast *Zygosaccharomyces rouxii* – Comparison of Two Mostly Studied Wild-type Strains, CBS 732 and ATCC 42981.
17. Novák J., Fiala J., Škach J., Čepicka J.: New possibilities to the optimisation of yeast propagation at the breweries.
18. Siglová M., Šnajdr J., Masák J., Čejková A., Jirku V.: Influence of humic acids on reproduction activity of yeasts.
19. Šmogrovičová D., Dömény Z., Navrátil M.: Non-Alcoholic beer production.
20. Stratilová E., Čigašová H., Dzúrová M., Breierová E., Omelková J.: The partial characterization of extracellular polygalacturonases produced in first phases of growth of *Aureobasidium pullulans* from forest soil.
21. Tomšíková A.: The problem of lipophilic yeasts.
22. Ürgeová E., Horváthová V., Pšenáková I., Šturdík E.: Contamination of the cheeses by yeast *Aureobasidium pullulans* and a possibility of their delay.
22. Dudíková J., Kolarova N.: Glycosidase activities in the yeast *Cryptococcus laurentii*.
23. Maceková D,. Farkaš V.: Cell-associated glycosidase activities in *Cryptococcus neoformans*.
24. Gabriel M., Yoshida S., Kopecká M., Takeo K., Yamaguchi M.: Perinuclear actin rings in the yeasts.
25. Kotrba D., Masák J., Čejková A., Siglová M., Jirku V.: *Candida maltosa* adhesion on polyester and modified glass surfaces.
26. Machová E., Bystrický S., Paulovičová E., Kolarova N.: Synthesis and immunogenicity of polysaccharide-protein conjugate composed of galactogluco-xylomannan of *Cryptococcus laurentii*.
27. Pichová A., Hlavatá L., Vondráková-Pelíšková D., Laun P., Jarolim S., Sauerová E., Fiala J., Heeren G., Breitenbach M.: Mother cell-specific aging in *Saccharomyces cerevisiae*.
28. Dostál J., Hrušková-Heidingsfeldová O., Hamal P., Pichová I.: Aspartic proteinases as virulence factors of *Candida* spp.
29. Fekete V., Babjaková L., Sulo P.: The sense of yeasts altruistic suicide in the nature. ("The apple heaven story").
30. Gazdag Z., Farkas N., Belágyi J., Papp G. Pesti M.: Alterations in the glutathione defence system of respiratory-deficient S*chizosaccharomyces* p*ombe* mutants.
31. Poljšak B., Gazdag Z., Pesti M., Farkas N., Plesničar S., Raspor P.: The role of antioxidant protection against chromium(VI) toxicity on model organism *Saccharomyces cerevisiae*.
32. Fujs Š., Gazdag Z., Pesti M., Belágyi J., Raspor P., Batič M.: Effect of copper and zinc on reactive oxygen species generation in yeast *Candida intermedia*.
33. Takáčová M., Gbelská Y., Sklenár P., Šubík J.: Molecular characterization of the *K. lactis* gene conferring resistance to cycloheximide in *S. cerevisiae.*
34. Horvathová V., Šlajsová K.: Hydrolysis of the corn starch by glucoamylase *S. fibuligera* IFO 0111.
35. Imrichová D., Takáčová M., Gbelská Y., Šubík J.: Chromosomal mapping of the *KlPDR5* and *KlRPL28* genes.
36. Kremnický L., Vršanská M., Biely P.: Differences in the production of acetylesterases by *Aureobasidium pullulans* during growth on xylan and galactomannan.
37. Kutejová E., Ondrovičová G., Slezáková K., Perečko D., Parkhomenko N., Janata J.: ATP-dependent proteases and their endogenous substrates.
38. Križková L., Masárová J., Mislovičová D., Šandula J., Krajčovič J.: Antimutagenic activity of yeast mannan and mannan-protein conjugates.
39. Poláková S., Slamka T., Špírek M., Bartóková J., Sulo P.: The use of mitochondrial transplacement in taxonomy.
40. Fekete V., Poláková S., Lencz P., Sulo P.: Distribution of petit positive yeasts among the species closely related to *S. cerevisiae.*
41. Vasilevova M., Váchová L., Sigler K.: Effect of cultivation conditions on detoxification function of mdr pumps in *Saccharomyces cerevisiae*.

During the conference meeting of the Committee of the Czech and Slovak Yeast Commission took place. It was agreed that the 32nd Annual Yeast Conference will be organized again in the Smolenice Castle during May 12-14, 2004. The program will be focused on molecular biology and genetics, membranes, cytoskeleton and cell walls, and generally on biochemistry and biotechnology of yeasts.

Communicated by Peter Biely

van Uden International Advanced Course: Molecular Ecology, Taxonomy and Identification of Yeasts

This course, organized by 'CREM - Centro de Recursos Microbiológicos', was held at the Faculty of Sciences and Technology of 'Universidade Nova de Lisboa', Caparica, Portugal, from July 21st to August 1st 2003. Attended by 24 students from 15 countries, from as far as the US and Australia, it included 20 hours of lectures and 35 hours of lab practicals. The following topics were covered in the lectures: classical *vs.* molecular approaches in the classification of ascomycetous (C.P. Kurtzman, ARS, Peoria, IL, USA) and of basidiomycetous yeasts (J.W. Fell, Univ. Miami, FL, USA); theoretical aspects of taxonomic analysis (M.-A. Lachance, UWO, Ontario, Canada); case studies in the systematics of ascomycetous and basidiomycetous yeasts (A. Fonseca & J. P. Sampaio, CREM/UNL, Caparica, Portugal); phylogenetic inference using DNA sequence data (M. Weiss, Univ. Tübingen, Germany); molecular detection and identification of yeasts (J.W. Fell); molecular and genomic approaches in species delineation and strain typing of food-related and clinical yeasts (T. Boekhout,

CBS, The Netherlands); molecular methods for assessment of yeast diversity in unexplored habitats (J.P. Sampaio); future directions in yeast ecology and systematics (M.-A. Lachance). Two seminar sessions comprised contributions by the lab assistants (graduate students in CREM) and the students. Practical work focused on the following methodologies: DNA extraction; PCR fingerprinting; DNA sequencing; molecular detection and identification and DNA/DNA reassociation. An additional session was devoted to the computational tools currently used for phylogenetic analyses. The different scientific and practical subjects were eagerly discussed during the two weeks. These discussions continued throughout the social programme, which included a Saturday afternoon excursion to the 'Arrábida Natural Park', a visit to a wine cellar and a farewell dinner. Everyone felt that the course was a great success and this was largely the result of the commitment from lecturers, lab assistants and local organizers and the high level of motivation and enthusiasm of the students.

Álvaro Fonseca, José Paulo Sampaio, and Isabel Spencer-Martins

The 23rd International Specialized Symposium on Yeasts

The 23rd International Specialized Symposium on Yeasts (ISSY-23) was held in Budapest, from 26 to 29 August, 2003, under the auspices of the International Commission on Yeasts (ICY) of the IUMS, as well as of the Hungarian Academy of Sciences, the Hungarian Society for Microbiology, and the Hungarian Scientific Society for Food Industry.

In addition to the above bodies, the Organizing Committee of ISSY-23 received generous support from the FEMS and a number of companies home and abroad, among which BioMerieux, Merck, Elsevier, Coca-Cola, BioRad, Springer, and others (a full list of sponsors is included in the Program).

The arrangement of the scientific program of ISSY-23 was assisted by the International Scientific Committee comprising 17 members. The program consisted of a plenary session and seven sessions for oral presentations, one evening workshop, as well as two poster sessions. In all, there were 57 oral and 134 poster presentations. ISSY-23 attracted more than 200 participants from close to 30 countries throughout the world.

The opening plenary lecture was given by Steven Oliver (University of Manchester, UK). Professor Oliver, one of the leader of the team sequenced the complete genome of the first eukaryote, *Saccharomyces cerevisiae*, in 1996, presented a personal overview on the impact of genomic approaches to yeast evolution and ecology. He noted some conflicts between the classical genetic and molecular techniques to define biological species and to classify organisms, and showed examples on the lack of correlation between phylogenetic arrangement based on single genes (mostly portions of ribosomal RNA genes) and complete genome sequences. Finally, he addressed the prospects for molecular studies on yeast ecology and its manifold application. His talk was an excellent introduction and appropriate setting up for the sessions to follow.

Thereafter the symposium was continued in sessions on the following subjects: biodiversity, taxonomy, phylogeny and identification, food yeasts, dairy yeasts, wine yeasts, clinical yeasts, and methods. Each session was introduced with a keynote lecture presented by an invited leading expert of the respective fields, followed by 6 to 10 contributing papers in each session.

André Lachance (University of Western Ontario, Canada), keynote speaker for Session 1 on Biodiversity, used a yeast-insect-flower ecosystem to demonstrate that both the intrinsic properties of the species and their interaction with each other and their inanimate habitat, as determined by biogeography, would account for the biodiversity and distribution of species. This paper introduced the main topic of the symposium: interaction between yeast and other organisms, which was further elaborated upon by the other contributors and 9 posters to the session describing yeast diversity in both natural and man-made habitats, including grapes used in winemaking.

Taxonomy, phylogeny and identification were discussed in 10 oral and 15 poster presentations. Clete Kurtzman (NCAUR/USDA, USA) outlined the use of molecular systematic as the basis for rapid detection and identification of spoilage yeast in foods and beverages. A variety of sequence-based and PCR techniques can be applied and chosen according to the purpose of the investigation. Detecting undescribed species requires combined approaches. Several contributors to this session showed that in addition to molecular techniques, traditional growth-based methods can be improved for the rapid detection and diagnosis of yeasts both in natural, clinical and food settings.

Further developing the theme of interaction, Johan Schnurer of Uppsala University, Sweden, focused on the promising field of the use of yeast as biocontrol agents. Interactions between yeasts and bacteria or yeasts and molds were exemplified in more than 20 additional presentations and posters, elaborating various other aspects of the broad field of ecology, e.g. the role of metabolites, nutrient competition, production of killer factors and bacteriocins.

Highlights of the symposium were the three sessions concerning food yeasts in relation to the production of wine, beer, dairy products as well as to spoilage agents. Virgilio Loureiro (Agricultural Research Institute, Lisbon, Portugal) keynote speaker of Session 4, Food and yeasts, based the presentation on his extensive experience of wine industry as a model. He developed points of general significance to future research in food microbiology, suggesting that research should be directed to (i) providing better knowledge of yeasts in damaged raw materials; (ii) elucidating the role of insect as vectors of spoilage yeasts; (iii) transferring molecular typing techniques from research laboratory to industry laboratories; and (iv) improving predictive models of food spoilage.

In subsequent oral presentations, Larry Beuchat (University of Georgia, USA) pointed out the possibility that yeasts are increasing the risk of human pathogenic bacteria by creating a more favorable environment; José Peinado (University of Complutense, Madrid, Spain) proposed a new concept of osmotolerance and Bernard Prior (University of Stellenbosch, South Africa) addressed the molecular aspects of osmoregulation.

Special sessions were devoted to wine yeasts and dairy yeasts, respectively. Graham Fleet (University of New South Wales, Australia), keynote speaker, discussed the complexity of yeast contribution to wine flavor, the numerous metabolites yeast produce that impart wine flavor, and their enzymatic mechanisms. This knowledge can be exploited to develop and select yeast strains for wine fermentation.

"Yeasts in dairy products: interactions and their functional impacts" was the title of the keynote lecture Mogens Jakobsen (Royal Agricultural University, Denmark) delivered in the next session. In this and other contributed papers information was presented on the role of yeast species other than the traditionally used *S. cerevisiae* in cheese ripening. These species are though to interact with lactic acid bacteria. A comment on this session suggested that yeasts in cheese and in fermented meat products should be considered simultaneously and in comparison.

Nearly half of the posters dealt with various subjects on food yeasts. Growth, population dynamics, interactions, fermentative activity, technological properties, spoilage, treatments, detection, differentiation and typing, as well as fruits, wine, beer, sourdough were the most frequent keywords in the title of these posters.

Session 6 was devoted to clinical yeasts. Teun Boekhout (CBS, the Netherlands), the keynote speaker, pointed out the functional diversity based on molecular differences within the human pathogenic *Cryptococcus neoformans*. In addition to this and other long recognized yeast pathogens (viz. *Candida albicans*), the number of opportunistic pathogen yeast species is increasing, in particular with organ transplant patients, who are predisposed to yeast infections. In the future, instead of describing case studies, more emphasis should be given to developments in molecular diagnosis. Although 9 oral and 9 poster presentations were delivered, clinical yeasts did not seem to be the main interest of symposium participants, in comparison with food yeasts. Nevertheless, it was important to have a session on this aspect of interaction between yeast and humans.

The last session concerned methodology. In his keynote paper, entitled "Molecular identification of yeasts in the 21st century" Wieland Meyer (University of Sydney, Australia) set the stage on this subject. With the exception of two papers (one on NMR spectroscopy, the other dealing with computerized identification) all other oral presentations and the majority of posters were focused on molecular techniques. This clearly proved to be a leading edge of methodological development directed towards DNA based molecular methods. Surprisingly, however, functional genomics, transcriptomics, proteomics have not yet come to the limelight as new methods applicable in those fields represented in the symposium.

Chairs of the sessions were asked to give an evaluation of the meeting. Many of them expressed not only personal views but talked with other participants about the symposium. The results of the questionnaire showed that the participants rated the symposium highly. The general opinion was that the overall scientific value was excellent. A well balanced program across all aspects of yeast biodiversity and ecology was evident. Interaction, the main topic of the program was addressed in many of the oral and poster presentations. There were excellent keynote lectures followed by questions on the subject raised. Discussions were lively and suggested further themes or at least opened some questions. An important feature of the meeting was that it provided excellent opportunities not only to learn the newest results but also to express views and exchange ideas with peers on many different subjects of common interest.

The clear message of the symposium was that the ecology of yeasts in terms of interaction with other organisms (in particular with molds and lactobacilli, as well as enteric pathogens, plant pathogenic fungi, man and even viruses) is an area in need of intense research attention. The directions to which future research should be addressed were also delineated. The following information is needed to manage foodborne microorganisms: (i) Reliable data about diversity and identity of microbes colonizing foods; (ii) Quanitative data on growth and population dynamics; (iii) Spatial distribution of populations throughout the products; (iv) Characterization of colonization process; (v) impact of environmental / ecological factors on growth and survival; (vi) correlation between activity, growth and product quality/safety/stability.

The other take-home-message was, from methodological point of view, that the relevance of genomics to food microbiology for both basic and applied research and practice is evident; more molecular updates will be needed on the applicability of these techniques.

In addition to the booklet of the Program and the Book of Abstracts (230 pages), the materials made available to all participants included a special issue of the International Journal of Food Microbiology, with 18 full papers and reviews about yeasts, published courtesy of Elsevier by the start of the symposium. Some papers presented in the symposium will also be published in FEMS Yeast Research (Elsevier), although there were not enough manuscripts submitted to justify a thematic issue.

In conclusion, all activities of the symposium were in line with the objectives of ICY, namely to increase the recognition and significance to humankind of the small group of organisms, the yeasts.

Financial resources were used in preparation and running costs, to cover expenses of invited lecturers (excluding traveling), and to sponsor young scientists and students. In all, 15 guests were invited, including the plenary lecturer and keynote speakers. In addition, 15 grants were distributed to PhD students with preference to those coming from overseas. From the generosity of sponsoring organizations and companies, nine awards were given for the best poster presentations as judged by an *ad-hoc* international evaluation committee. The awardees were from Spain (2), Hungary (2), South Africa (1), Sweden (1), Italy (1), France (1) and Japan (1).

Finally, the social parts of the program added to the participants' enjoyment and contributed to the success of the symposium.

Tibor Deák, Chair, Organizing Committee

Symposium: "Herman Phaff: Learning from Yeast" Santiago de Compostela, Spain - 23 & 24 September 2003

During the last week of September I had the pleasure of hosting the Symposium "Learning from Yeast" in memory of our beloved Herman Jan Phaff, Professor Emeritus at the University of California at Davis. The seminars were presented by former Phaff students and postdocs covering various aspects of yeast research originated and associated with their stay in Davis; Other Spanish yeast scientists presented investigations not directly linked to the Davis group but indirectly inspired by Phaff´s vision of the yeast world. The Symposium was a part of the 29th Annual Meeting of the Spanish Society of Microbiology and was sponsored by the Ramon Areces Foundation of Spain.

After introductory remarks by Prof. Villa, Prof. Carlos Hardisson, and Prof. Julio Villanueva, Dr. A.L. Demain started with a review of Phaff's career: "Herman Jan Phaff: professor, mentor, friend and colleague". This seminar, together with that of Dr. Sally Ann Meyer, "Beer, bread and beyond", offered a very human picture of a great yeast scientist. Dr. André Lachance reviewed: "The Phaff school of yeast ecology" pointing out that although Phaff's work spanned the whole gamut of yeast biology, his most original contribution was to the understanding of the position of yeasts in nature. Dr. Eric Johnson in "*Phaffia rhodozyma*: a colorful odyssey" discussed the interesting biotechnological aspects of a yeast isolated by Phaff and associates in the 1960s as pigment sources in acquaculture of salmonids and lobsters, as well as for the coloration of chicken and quail egg yolks, is one the most successful industrial applications of yeasts. Dr. Ann Vaughan-Martini discussed another Phaff passion: correct yeast identification in "Reflections on what Herman Phaff taught us about taxonomy and the classification of yeast for different end users in biotechnology, ecological studies or medicine". Dr. Enrique Herrero offered an overview of recent progress following the complete sequencing of the genomes of *Saccharomyces cerevisiae, Candida albicans* and *Schizosaccharomyces pombe* with: "Comparative genomics of yeast species: new insights into their biology".

The second day started with Dr. A.L. Demain with the talk "Fungal biotechnology". Even though the biotechnological application of fungi and yeasts surely goes back to prehistoric

times, the best is yet to come as genomes of additional fungal species are sequenced; and gene and protein arrays become available. Dr. Amparo Querol, with "Molecular evolution of yeast of biotechnological interest" offered an intriguing prospective on the "domesticated" species of *Saccharomyces* and *Kluyveromyces* which, due to millennial use in various food fermentations, have undergone an accelerated evolution as opposed to strains living in natural environments. Dr. Alessandro Martini discussed recent studies confuting the long maintained Pasteur hypothesis regarding the natural (vineyard) origin of wine strains of *Saccharomyces cerevisiae* with "Biotechnology of natural and winery-associated strains of *Saccharomyces cerevisiae"*. In Martini´s presentation it was quite clear that the domestication of these strains has led to the formation of particularly favorable characteristics useful for special biotechnological transformations, and offers an interesting prospective as to the utility of isolating autochthonous strains to be used as locality-specific starters for the wine industry. The symposium was closed with a final seminar by Dr. Mariano Gacto dealing with research done in collaboration with me and entitled: "Learning from yeasts: intracellular sensing of stress conditions". This investigation sought to determine how cells respond to stress and distinguish between different stressing stimuli.

Full manuscripts of most of these seminars have been recently published in a special edition of International Microbiology (volume 6(3):155-219. September 2003), the official journal of the Spanish Society for Microbiology (http://www.IM.microbios.org).

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Forthcoming Meetings

Physiology of Yeasts and Filamentous Fungi - PYFF2
March 24-28 2004, Anglet, France

On behalf of the organizing committee we have the pleasure to invite you to attend the PYFF2 meeting, the 2nd meeting on Physiology of Yeasts and Filamentous Fungi, to be held in Anglet (near Biarritz, Basque Coast, on the Atlantic ocean), France from March 24th to 28th, 2004. This event is co-sponsored by FEMS, (Federation European Microbiology Society) and EFB (European Federation of Biotechnology). This PYFF meeting aims to stimulate relationships between specialists, post-doc and post-graduate students active in academic and industrial researches of integrated physiology of yeasts and fungi for fundamental and applications purposes in Biotechnology.

The pre-registration is open now on our WEB-site:

http://pyff2.insa-tlse.fr

The website is now fully operational with electronic paper submission activated. All instructions for abstract submission, registration fees and other activities can be found on the WEB site. Other technical information can also be obtained at

PROGEP

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Eleventh International Congress on Yeasts, ICY 11
Rio de Janeiro, August 15-20 2004

On behalf of the International Committee on Yeasts (ICY) and the Federal University of Rio de Janeiro (UFRJ), I have the honor and pleasure to announce the Eleventh International Yeast Congress (formerly ISY) to be held during 15-20th of August, 2004 in Rio de Janeiro, Brazil.

The Conference venue is Hotel Gloria Convention Center, a traditional five star hotel, located in the south zone of Rio, close to the city center and with a panoramic view over Guanabara Bay. The first announcement information is available at the homepage http://www.icy2004.com.br. Further information can be obtained by e-mail:
<congress@icy2004.com.br>
or
<leda@icy2004.com.br>

The theme of the symposium will be "Yeasts in Science and Technology: the quest for sustainable development." The scientific program is under development and we would welcome your suggestions on the topics to be presented. Please, send your address to receive ICY2004 folder and poster. Welcome to Rio!

Leda Mendonça-Hagler, ICY2004 Chair

Brief News Items

BIOPREMIER - A New Start-Up Biotechnology Company

We are pleased to announce the formation of the start-up company, BIOPREMIER, by Manuel José Gomes Rodrigues and Mário João Gadanho. Manuel is a graduate in Biology from the University of Aveiro (1996) and Mário graduated in Microbial Biology and Genetics at the University of Lisbon (1996). Most recently they were researchers at the Centro de Recursos Microbiológicos (CREM), Faculty of Sciences and Technology, the New University of Lisbon. Together, they decided to create BIOPREMIER a start-up company with the mission to develop and provide society with the most advanced Molecular Biology technologies.

With highly qualified professionals in the field of Microbiology/Biotechnology, BIOPREMIER has secured the collaboration of researchers from important scientific areas. Through partnerships with university research centers, BIOPREMIER has available the appropriate resources and equipment. The company performs biotechnological and molecular biological studies as consultants. The services offered include detection and identification of microorganisms, strain typing, detection of GMOs, search for and selection of strains for the production of new enzymes and medicines, research and development (R&D). Various forms of microbiological or chemical analyses and DNA sequencing are contracted out. For more information, visit our web site:

www.biopremier.com

Change of Employment - A New Yeast Lab in Iowa - Dr. Martin Schmidt

Martin Schmidt has been appointed Assistant Professor for Biochemistry at Des Moines University, Des Moines, Iowa, USA. Dr. Schmidt was a Postdoctoral Fellow in the laboratory if Enrico Cabib at NIH, Bethesda, Maryland, USA. In his new lab, Dr. Schmidt will continue to examine the synthesis of chitin in yeast.

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Progress report - Frank Spencer

We are making progress towards getting the volume on "Methods in Environmental Microbiology" on the market. There are 3 or 4 chapters on yeasts in it, including one on yeasts resistant to heavy metals. The volume on "Methods in Public Health Microbiology" is also reaching the publication date. Although the book does not address issues directly connected with yeasts, some chapters might be valuable for yeast researchers.

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Change of address - Prof. Pencho Venkov

I have left the Institute of Molecular Biology and taken up the responsibility of building a new Department of Molecular Ecology in the Institute of Cryobiology and Food Technology. I shall continue to live in Sofia and can be reached at the following address:

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Department Molecular Ecology
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+359.2/6330261
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e-mail: venkov@tradel.net

Vitamin-free Yeast Base is again available

http://formedium.com/yeast_nitrogen_base_media.htm

FORMEDIUM *Saccharomyces cerevisiae* culture media range includes: complex media based upon a formulation of yeast extract, peptone and glucose, such as YPD broth and YPD agar; **Yeast Nitrogen Base** type media in many different formulations, including**without vitamins**, without amino acids, without ammonium sulphate or potassium dihydrogen phosphate.; SD media based upon Yeast Nitrogen Base formulations with a carbon source included, like glucose or galactose; drop-out supplements according to five different basic amino acid formulations. Single, double, triple, quadruple and many other formulations are readily available from stock.

FORMEDIUM *Schizosaccharomyces pombe* culture media range includes: complex media based upon a formulation of yeast extract, peptone, casamino acids and glucose, like YE, YES YSOPD Broth or Agar; synthetic defined media like EMM (Edinburgh Minimal medium), MB and MMA media; basic supplements mixture of Ade, His, Leu, Lys and Ura in different formulations.

FORMEDIUM is well equipped to make customized formulations with competitive prices. Their main marketing instrument is a website where all media formulations, including prices, are listed. Monthly news bulletin will be mailed to clients with technical details about the products and new items to be added to the product list. Orders can be made direct to FORMEDIUM UK. In the near future a network of distributors is planned.

Dr. Jack Hamer, Science director

<sales@formedium.com>

For orders, please include: purchase order number, product reference number, quantity required, delivery date required, full delivery address, invoice address.

Accounts are payable in £ Sterling and Euros. A website is currently being developed to accommodate USD. Ordering by credit card is being evaluated. Postage, packaging, and insurance are free with a standard delivery time of 5–7 working days. Urgent orders can be dispatched via courier at an extra cost to the customer. Samples are available. Discounts are available on bulk ordering.

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Editor's Note

The material in this entry is included for the information of Yeast Newsletter readers and is not meant to imply support for FORMEDIUM or approval of its products by the Yeast Newsletter or its Editor.

Postdoctoral Position - Microbial Ecological Genetics and Functional Genomics

A postdoctoral position is available immediately in the Department of Biology, Syracuse University, to investigate molecular mechanisms responsible for microbial adaptation to membrane disturbing chemical natural products present in host plants and the nature of mycological speciation mechanisms and population diversity in microbial communities. Studies will employ functional genomic methods both in wild populations of the cactophilic yeast species of the clade *Pichia amethionina* and the model baker's yeast, *S. cerevisiae*. Funds are available for up to three years of support. Interested applicants should directly contact either of the following:

W.T. Starmer <wstarmer@syr.edu>

S.E. Erdman <seerdman@syr.edu>