

Yeast

A Newsletter for Persons Interested in Yeast

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Editorials

Joshua Lederberg (1925-2008)

Although Dr. Lederberg is best known for his Nobel-winning research in bacterial genetics, he had a keen interest in many other aspects of science, including space exploration and artificial intelligence. He was instrumental in demonstrating the cytoplasmic inheritance of certain components of respiration in yeast. Dr. Lederberg was an avid reader and supporter of the Yeast Newsletter. Most recently, he expressed concern regarding the preservation of archives of the newsletter and was reassured to know that the United States Library of Congress is on our list of subscribers.

Fifty Years Ago

Thanks to the efforts of Drs. Jack Fell and Kyria Boundy-Mills, we now have a complete archive of all issues of the Yeast Newsletter. This has allowed the creation of a new section titled “Fifty Years Ago in the Yeast Newsletter”, in which material of special interest from relevant issues will be mentioned.

M. A. Lachance, Editor

I. Russian Collection of Microorganisms (VKM), Institute for Biochemistry and Physiology of Microorganisms, Pushchino, 142290, Russia. Communicated by W.I. Golubev
<wig@ibpm.pushchino.ru> <http://www.vkm.ru>

The following papers have appeared recently.

1. Golubev WI, Golubev NW 2007 Comparison of sodium selenite and sodium selenate toxicity for *Saccharomyces* complex. *Probl Med Mykol* 9:34-36.

Saccharomyces species and related organisms (125 strains) were examined for tolerance to selenate and selenite. Sodium selenite is more toxic for yeasts: almost all strains can not grow on the medium with selenite content 10^{-2} M and most of them grow weakly with 10^{-3} M, whereas the last concentration of

selenate had not effect on growth of almost all strains and the presence of 10^{-2} M of it resulted mainly in their delayed growth. Enhanced toxicity of selenite is evidently caused by fast production of highly toxic selenide in cells.

2. Golubev WI, Kulakovskaya TV, Shashkov AS, Kulakovskaya EV, Golubev NW 2008 Antifungal cellobiose lipid secreted by the epiphytic yeast *Pseudozyma graminicola*. *Mikrobiologiya* 77:201-206.

The yeast *Pseudozyma graminicola*, isolated from plants, inhibited a growth of almost all ascomycetes and basidiomycetes tested (over 270 species of about 100 genera) including pathogenic species. It secreted fungicidal agent that was identified as glycolipid, a cellobioside-containing 2,15,16-

trihydroxypalmitic acid as aglycon, 3-hydrocaproic acid and acetic acid as O-acyclic substituents. ATP leakage from cells treated by this glycolipid was indicative of its membrane-damaging activity. Basidiomycetous fungi were more cellobiose lipid-sensitive than ascomycetous ones.

Recent conference presentations.

3. Golubev WI 2008 Epiphytic yeasts from pteridophyte plants. Abstr. 2nd Congress of Russian Mycologists (16-18th April 2008, Moscow), 124-125.

The yeast populations on *Athyrium filix-femina* and *Equisetum hiemale* varied between 17,000 and 131,000 per gramme (wet weight). They represent 13-46% of total micromycetes. Up to 70-90% of the yeast communities consisted

of *Cryptococcus* species. Single isolates belong to species of the genera *Cystofilobasidium*, *Rhodotorula*, *Sporobolomyces* and *Tilletiopsis*.

4. Golubev WI 2008 Ethanol production from starch by amylolytic yeasts. Abstr. 2nd Congress of Russian Mycologists (16-18th April 2008, Moscow), 272-273.

Some species of the genera *Candida*, *Debaryomyces*, *Filobasidium*, *Saccharomyces*, *Saccharomycopsis*, *Schizosaccharomyces* and *Stephanoascus* can ferment soluble starch. The most active strains were found in the species *C. lodderae*, *Deb. occidentalis*, *Sacch. cerevisiae*, *S. capsularis*,

and *S. fibuligera*. The maximum ethanol yield with monoculture was 2°- 4°GL. Ethanol production by a coculture *Sacch. cerevisiae* (= *Sacch. diastaticus*) VKM Y-416 and *Deb.* (= *Schwan-niomyces*) *occidentalis* BKM Y-1639 was 9.7° GL using potato unhydrolysed starch in a single-step fermentation.

II. 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. Eddy AA & Barnett JA 2007 A history of research on yeasts 11. The study of solute transport: the first 90 years, simple and facilitated diffusion. *Yeast* 24:1023-1059.
2. Barnett JA 2008 A history of research on yeasts 12: medical yeasts part 1, *Candida albicans*. *Yeast* 25: in the press.
3. Barnett JA 2008 A history of research on yeasts 13: Active transport and the uptake of various metabolites (in preparation).
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III. Phaff Yeast Culture Collection, University of California, Davis. Communicated by by Kyria Boundy-Mills <KLBMILLS@ucdavis.edu>.

The Phaff Yeast Culture Collection at the University of California Davis is one of the oldest and largest public collections of wild yeasts in the world, with over 7,000 strains belonging to over 500 species. This collection is available to academic and industrial researchers around the world. The collection offers strain distribution and contract screening services. Some recent developments include:

We launched a new website this spring, with streamlined catalog searching and ordering. Please visit www.phaffcollection.org.

This is one of the few collections in the world that provide contract screening service. Hundreds or thousands of strains can be cultured and tested for useful properties. Recent projects include testing for tolerance of industrial conditions such as solvents or high

temperatures, and production of commercially important enzymes or metabolites. Recent discoveries include a yeast that produces lycopene, and yeasts that tolerate high alcohol concentrations.

A 9-page profile of the Phaff collection will be featured in the US Federation of Culture Collections column in an upcoming issue of the Society for Industrial Microbiology newsletter "SIM News".

The collection will move to a new building in August 2008, the Robert Mondavi Institute for Wine and Food Science. This new teaching and research facility was funded in part through a donation from winemaker Robert Mondavi.

For more information, contact curator Kyria Boundy-Mills by email (see above) or by phone: 530-754-5575.

IV. Dipartimento di Biologia Applicata - Microbiologia, University of Perugia, Borgo XX Giugno 74, I 06121 Perugia, Italy. Communicated by P. Buzzini <pbuzzini@unipg.it>.

Recent publications.

1. Cardinali G, Antonielli L, Rellini P and Fatichenti F 2007 ESTHER: A "R Package" implementing a novel approach to bidimensional display of multidimensional binary data. *The Open Applied Informatics Journal* 1:20-27.
 2. Cardinali G, Rellini P, Pelliccia C, Antonielli L and Fatichenti F 2008 MMS: a R package for metabolomic markers search in stress response studies. *Open Appl Informatics J* 2.
 3. Brizzio S, Turchetti B, de García V, Libkind D, Buzzini P and van Broock M 2007 Extracellular enzymatic activities (EEA) of basidiomycetous yeasts isolated from glacial and subglacial waters of northwest Patagonia Argentina. *Can J Microbiol* 53:519-525.
 4. Buzzini P, Turchetti B and Vaughan-Martini A 2007 The use of killer sensitivity patterns (KSPs) for biotyping yeast strains: the state of the art, potentialities and limitations. *FEMS Yeast Res*, 7:749-760.
 5. Buzzini P, Innocenti M, Turchetti B, Libkind D, van Broock M and Mulinacci N 2007 Carotenoid profiles of yeasts belonging to the genera *Rhodotorula*, *Rhodospiridium*, *Sporobolomyces* and *Sporidiobolus*. *Can J Microbiol* 53:1024-1031.
 6. Buzzini P, Turchetti B, Ieri F, Goretti M, Branda E, Mulinacci N and Romani A 2007 Proantocyanidins: naturally occurring O-heterocycles with antimicrobial activity. In: *Topics in Heterocyclic Chemistry: Bioactive Heterocycles*, Vol. 10, (Khan D. ed.), Springer-Verlag, Berlin, Germany, 239-263.
 7. Turchetti B, Buzzini P, Goretti M, Branda E, Diolaiuti G, D'Agata C, Smiraglia C and Vaughan-Martini A 2008 Psychrophilic yeasts in glacial environments of Alpine glaciers. *FEMS Microbiol Ecol* 63:73-83.
 8. Ponzoni C, Gasparetti C, Goretti M, Turchetti B, Pagnoni U. M, Cramarossa M. R, Forti L and Buzzini P 2008 Biotransformation of acyclic monoterpenoids by *Debaryomyces* sp, *Kluyveromyces* sp. and *Pichia* sp. strains of environmental origin. *Chem Biodiv* 5:471-483.
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V. State Scientific-Research Institute for Genetics and Selection of Industrial Microorganisms (GosNIIGenetika), I-Dorozhnyi 1, Moscow 117545, Russia. Communicated by G.I. Naumov and E.S. Naumova <gnaumov@yahoo.com>.

We are grateful to I. Masneuf-Pomarède (Bordeaux) for fruitful collaboration during our stay in her lab in April-May 2008.

The following are papers for 2007-2008.

1. Ivannikova YuV, Naumova ES, Naumov GI 2007 Viral dsRNA in the wine yeast *Saccharomyces bayanus* var. *uvarum*. Res Microbiol 158:638-643.
 2. Naumov GI, Naumova ES 2008 Chromosomal differentiation of sibling species *Pichia membranifaciens* and *Pichia manshurica*. Microbiology (Moscow) (in press).
 3. Naumov GI, Ivannikova YuV, Chernov IYu, Naumova ES 2008 Natural polymorphism of double-stranded RNA of yeast *Saccharomyces*. Microbiology (Moscow) (submitted).
 4. Naumov GI, Naumova ES, Masneuf-Pomarède I 2008 Natural introgression between *Saccharomyces cerevisiae* and so-called “*Saccharomyces uvarum*”. (in preparation).
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VI. Institut für Angewandte Mikrobiologie, Univ.f.Bodenkultur Wien. Communicated by Hansjörg Prillinger <hansjoerg.prillinger@boku.ac.at> <http://www.boku.ac.at/iam>.

The following paper is in press.

1. K Lopandic, W Tiefenbrunner, H Gangl, K Mandl, S Berger, G Leitner, G A Abd-Ellah, A Querol, R C Gardner, K Sterflinger H Prillinger 2008 Molecular profiling of yeasts isolated during spontaneous fermentations of Austrian wines. FEMS Yeast Res. (in press).

The aim of this study was to evaluate the autochthonous yeast population during spontaneous fermentations of grape musts in Austrian wine producing areas. Investigation of genomic and genetic variations among wine yeasts was a first step towards a long-term goal of selecting strains with valuable enological properties typical for this geographical region. An approach, combining sequences of D1/D2 domain of 26S rDNA and RAPD fingerprinting, was used for characterizing yeasts at species level, whereas the differentiation of *Saccharomyces* strains was accomplished by AFLP fingerprinting. At the beginning of fermentation, representatives of nine genera were identified, with

Hanseniaspora and *Metschnikowia* species characterized most frequently. *S. cerevisiae* and *S. bayanus* var. *uvarum* strains, which were identified throughout entire fermentation process, showed a high level of genetic diversity. A number of *S. cerevisiae* strains were common at multiple wineries, but a wide range of strains with characteristic profiles were characterized at individual locations. This biodiversity survey represents a contribution to the investigation and preservation of genetic diversity of biotechnologically relevant yeasts in Austrian winemaking areas.

VII. Department of Microbiology, Institute of Applied Molecular Biology, Saarland University, Postfach 151150, Building A 1.5, D-66041 Saarbrücken, Germany. Communicated by M.J. Schmitt <mjs@microbiol.uni-sb.de>.

The following is a summary of an paper in press.

1. Schmitt MJ & Reiter J 2008 Viral induced yeast apoptosis. Biochim Biophys Acta doi:10.1016/j.bbamcr.2008.01.017.

In an analogous system to mammals, induction of an apoptotic cell death programme (PCD) in yeast is not only restricted to various exogenous factors and stimuli, but can also be triggered by viral killer toxins and viral pathogens. In yeast, toxin secreting killer strains are frequently infected with double-stranded (ds)RNA viruses that are responsible for killer phenotype expression and toxin secretion in the infected host. In most cases, the viral toxins are either pore-forming proteins (such

as K1, K2, and zygocin) that kill non-infected and sensitive yeast cells by disrupting cytoplasmic membrane function, or protein toxins (such as K28) that act in the nucleus by blocking DNA synthesis and subsequently causing a G1/S cell cycle arrest. Interestingly, while all these virus toxins cause necrotic cell death at high concentration, they trigger caspase- and ROS-mediated apoptosis at low-to-moderate concentration, indicating that even low toxin doses are deadly by triggering PCD in enemy cells.

Remarkably, viral toxins are not solely responsible for cell death induction in vivo, as killer viruses themselves were shown to trigger apoptosis in non-infected yeast. Thus, as killer virus-

infected and toxin secreting yeasts are effectively protected and immune to their own toxin, killer yeasts bear the intrinsic potential to dominate over time in their natural habitat.

VIII. Department of Genetics and Applied Microbiology, University of Debrecen, POBox 56, 4010 Debrecen, Hungary. Communicated by M. Sipiczki <lipovy@tigris.unideb.hu> <http://genetics.unideb.hu>.

Recent publications.

1. Enczi K, Yamaguchi M, Sipiczki M 2007 Morphology transition genes in the dimorphic fission yeast *Schizosaccharomyces japonicus*. *Antonie van Leeuwenhoek* **92**:143-154.
2. Ragni E, Sipiczki M, Strahl, S 2007 Characterization of Ccw12p, a major key player in cell wall stability of *Saccharomyces cerevisiae*. *Yeast* **24**:309-319.
3. Sipiczki M 2007 Splitting of the fission yeast septum. (Review) *FEMS Yeast Res.* **7**:761-770.
4. Miklos I, Szilagyi Z, Watt S, Batta G, Antunovics Z, Enczi K, Bahler J, Sipiczki M 2008 Genomic expression patterns in cell separation mutants of *Schizosaccharomyces pombe* defective in the genes *sep10⁺* and *sep15⁺* coding for the Mediator subunits Med31 and Med8. *Mol Genet Genomics* **279**:225-238.
5. Csoma H, Sipiczki M 2008 Taxonomic reclassification of *Candida stellata* strains reveals frequent occurrence of *Candida zemplinina* in wine fermentation. *FEMS Yeast Res* **8**:328-336.
6. Sipiczki M Interspecies hybridisation in *Saccharomyces* wine yeasts. (Review) *FEMS Yeast Res* (in press).

Presentations at meetings

7. Sipiczki M, Kajdacs E 2007 Postharvest bioprotection of fruits by yeasts with antimicrobial activities. 35th Annual Conference on Yeasts. Czechoslovak Society for Microbiology, Smolenice, Slovakia, p 39.
8. Sipiczki M 2007 Trends in genetic improvement of wine yeasts. 26th International Specialized Symposium on Yeasts: From alcoholic beverages to bioethanol for transportation: a new challenge for fermenting yeasts. Sorrento, Italy, Book of Abstracts, p 67
9. Csoma H, Sipiczki M 2007 Taxonomic investigation of the yeast biota of botrytized grapes and "Essence" in the Tokaj wine region. 8th International Enology Symposium, Bordeaux, France, Book of Abstracts, p 174
10. Kajdacs E, Antunovics Z, Sipiczki M 2007 Selection of antagonistic yeasts from apple surface microbiota. 15th Internat. Cong. Hung. Soc. Microbiol. *Acta Microbiol Immunol. Hung.* **54**:54-55.
11. Miklos I, Szilagyi Z, Watt S, Zilahi E, Batta G, Antunovics Z, Enczi K, Nagy R, Szaszi E, Bahler J, Sipiczki M 2007 Transcriptional regulation in *Schizosaccharomyces pombe* cells. 15th Internat. Cong. Hung. Soc. Microbiol. *Acta Microbiol Immunol. Hung.* **54**:83-84.
12. Szilagyi Z, Linder T, Miklos I, Batta G, Sipiczki M, Gustafsson CM 2007 The role of the Mediator complex in the regulation of cell separation. Fourth International Fission Yeast Meeting, Copenhagen, p 257.
13. Kajdacs E, Sipiczki M, Antunovics Z 2007 Postharvest bioprotection of fruits by antifungal antagonism of *Metschnikowia* yeast. Power of Microbes in Industry and Environment. Central European Symposium on Industrial Microbiology and Microbial Ecology. Zadar, Croatia. Programme and Abstracts, p 43.

14. Sipiczki M 2007 The end is the beginning: cell separation after cytokinesis. Power of Microbes in Industry and Environment. Central European Symposium on Industrial Microbiology and Microbial Ecology. Zadar, Croatia. Programme and Abstracts, p 22.
15. Sipiczki M, Kajdacs E, Antunovics Z 2007 Postharvest bioprotection of fruits by antagonistic yeasts. 2nd Asian Congress of Mycology and Plant Pathology. Hyderabad, India. Abstracts, p 290.

IX. Department of Soil Biology, Faculty of Soil Science, Moscow State University, Vorobyovy Hills, Moscow 119992, Russia. Communicated by I.Yu. Chernov <yes@soil.msu.ru>.

The following papers have been published recently

1. Glushakova AM, Yurkov AM, Chernov Iyu 2007 Massive isolation of anamorphous ascomycete yeasts *Candida oleophila* from plant phyllosphere. Microbiology 76:799-803.

Many years of research has confirmed a wide distribution of anamorphous ascomycete yeasts in the phyllosphere of diverse plants of Moscow and the Moscow oblast. Based on the standard morphological and physiological criteria, the results of restriction analysis of the 5.8S-ITS rDNA region, and the sequencing of the D1D2 region of 26S rDNA, these yeasts were identified as *Candida oleophila* Montrocher. Previous isolation of this species has been rare, possibly due to

its incorrect identification. This species, together with phytobiotic basidiomycete yeasts, was shown to be dominant in the yeast epiphytic communities on the surface parts of plants. The relative abundance of *C. oleophila* is highest on plant fruits and increases significantly by the end of the vegetation period. Wide occurrence of this yeast species on fruits and in the phyllosphere may be related to its ability to compete with rapidly growing phytopathogenic fungi.

2. Glushakova AM, Chernov Iyu 2007 Seasonal dynamic of the numbers of epiphytic yeasts. Microbiology 76:590-595.

The numbers of epiphytic yeasts on the leaves and flowers of 25 plant species throughout their vegetation period was determined. The numbers of yeasts on the leaves were found to change regularly throughout the year. The average dynamics for all of the plant species investigated included an increase in yeast numbers during spring and summer with the maximum in late autumn and early winter. The character of the yeasts'

dynamics depends on the ecological characteristics of the plants and the duration of the ontogenesis of their leaves and flowers. Three types of dynamics of epiphytic yeasts were revealed: year-round with an increase in autumn-winter, year-round without visible changes, and seasonal with a terminal increase for annual plants.

3. Golubtsova YuV, Glushakova AM, Chernov Iyu 2007 The seasonal dynamics of yeast communities in the rhizosphere of soddy-podzolic soils. Eurasian Soil Sci 40:875-879.

The annual dynamics of the number and taxonomic composition of yeast was studied in the rhizosphere of two plant species (*Ajuga reptans* L. and *Taraxacum officinale* Wigg.) in a forb-birch forest on soddy-podzolic soil. Eurybiont phyllobasidial cryptococci and red-pigmented phytobionts *Rhodotorula glutinis* were found to predominate in the phyllosphere of these plants, whereas the typical pedobionts *Cryptococcus terricola* and *Cr. podzolicus* occurred on the surface of roots and in the rhizosphere. The seasonal changes in the number and species

composition of the yeast communities in the rhizosphere were more smooth as compared to those in the phyllosphere. In the period of active vegetation of the plants, the phytobiont yeasts develop over their whole surface, including the rhizoplane. Their number on the aboveground parts of the plants was significantly lower than that of the pedobiont forms. Thus, the above- and underground parts of the plants significantly differed in the composition of the dominant species of epiphytic yeasts.

4. Glushakova AM, Ivannikova YuV, Naumova ES, Chernov IYu, Naumov GI 2007 Massive isolation and identification of *Saccharomyces paradoxus* yeasts from plant phyllosphere. Microbiology 76:205-210.

Year-round studies of epiphytic yeast communities revealed that the number of ascosporeogenous yeasts of the genus *Saccharomyces* inhabiting living and decaying leaves of some plants increased considerably in certain short periods (at the beginning of summer and in winter). Massive isolation of saccharomycetes was performed from 11 plant species; earlier,

these yeasts had been revealed mainly in sugar-rich substrates. The isolates were identified as *Saccharomyces paradoxus* based on their physiological properties and RELP analysis of 5.8S-ITS. Possible reasons for short-term increases in the number of saccharomycetes in plant phyllosphere are discussed.

5. Yurkov AM, Vustin MM, Tyaglov BV, Maksimova IA, Sineokiy SP 2008 Pigmented basidiomycetous yeasts are a promising source of carotenoids and ubiquinone Q10. *Microbiology* 77:5-10.

Strains of basidiomycetous yeasts isolated from different sources were studied in order to determine the content of carotenoid pigments and ubiquinone Q 10 for subsequent selection work to obtain producers of these substances. The high specific productivity of carotenoids (600–700 mg/g) was revealed in the representatives of the following species: *Cystofilobasidium capitatum*, *Rhodospiridium diobovatum*, *R. sphaerocarpum*, *Rhodotorula glutinis*, *Rh. minuta*, and *Sporobolomyces roseus*. The ratio of the major pigments (torulene, torularhodine, and β -carotene) in the representatives of different species was studied.

Certain specific features of pigment formation in relation to the taxonomic position of the yeasts were determined. Eurybiont species with substantial ecological lability are the most active producers of carotenoids and ubiquinone Q10 among the epiphytes. It is the first time a comparative analysis of the coenzyme Q10 content in different taxa has been performed using several strains of the same species. The maximal coenzyme Q10 production (1.84 mg/g of dry biomass) was found in the yeast species *R. sphaerocarpum*.

- Yurkov AM, Chernov IYu, Tiunov AV 2008 Influence of *Lumbricus terrestris* earthworms on the structure of the yeast community of forest litter. *Microbiology* 77:107-111.

The taxonomic structure of yeast communities was studied in forest litter and soil, as well as in substrates transformed by the activity of *Lumbricus terrestris* earthworms (leaves in heaps, the gut contents, and coproliths). The activity of *L. terrestris* has a weak effect on the total yeast abundance but results in substantial changes in the community taxonomic

composition. The share of ascomycetous yeasts is significantly higher in the substrates associated with the activity of earthworms. The teleomorphic ascomycetes *Williopsis saturnus* were isolated from the gut contents. The effect of earthworms on the composition of the yeast community in the process of forest litter destruction is more pronounced than seasonal changes.

6. Lisichkina GA, Chernov IYu 2007 Yeasts in Kulunda steppe soda solonchaks. *Mikologia i fitopatologia (Mycology and Phytopathology)* 41:331-336 (in Russian).

The investigations carried out confirmed the isolation of everybiont yeast species on traditional medium (wort-agar neutral or acidified) from soda solonchaks. Most probably their natural ecotope is plant surface. At that time when alkalic wort-agar was used new teleomorphic species was isolated from alkali

soils – *Leucosporidium sp.* Unlike other yeast species it is not isolated when acidified agar is used. Evidently the specificity of alkali soil should be taken into account when yeasts are researched there. Its unique characteristics should be considered to elaborate and use isolation methods.

X. Instituto de Microbiologia Prof. Paulo de Góes, Laboratório de Ecologia Microbiana e Taxonomia e Coleção de Culturas de Leveduras, Universidade Federal do Rio de Janeiro (UFRJ). CCS-CP 68028-CEP 21944-590. Communicated by Leda C. Mendonça-Hagler <leda@mls.com.br>.

Current publications and theses.

1. Cabral AS, Carvalho PMB, Pinotti T, Hagler AN, Mendonça-Hagler LC, Macrae A 2008 Killer yeasts inhibit the growth of the phytopathogen *Monillioophthora pernicioso*, the causal agent of Witches Broom disease. *Brazilian J. Microbiol.* In press.
2. Grosch R, Rehn VN, Rehn K, Mendonça-Hagler LC, Smalla K, Lottmann J, Berg G. 2007. Analysis of antagonistic interactions between *Trichoderma* isolates from Brazilian weeds and the soil-borne pathogen *Rhizoctonia solani*. *J. of Plant Diseases and Protection* 114:167-175.
3. Gomes NC, Borges LR, Pinto FN, Krogerrecklenfort E, Mendonça-Hagler LC, Smalla K 2007 Diversity of ndo genes in mangrove sediments exposed to different sources of polycyclic aromatic hydrocarbon pollution. *Appl Environm. Microbiol.* 73: 7392-7399.
4. Mendonça-Hagler LC 2007 Yeast diversity in tropical environments. In: *Micologia, avanços do conhecimento*. Maia LC, Malosso E., Yano-Melo AM (eds). SBM Recife, Brasil. p 54.
5. Gotz, M., Gomes NC, Costa R, Peixoto RS, Berg G, Mendonça-Hagler LC, Smalla K 2006 Survival of GFP-tagged antagonistic bacteria in the rhizosphere of tomato plants and their effects on the indigenous community. *FEMS Microb Ecol* 56:207-218.

6. Azeredo LA, Cunha CD, Rosado A, Moura A, Freire DMG, Mendonça-Hagler LC, Sant'Anna GL 2006 New group specific 16S rDNA primers for monitoring foaming mycolata during saline waste-water treatment. *Biotechnology Letters*. 28: 447-453.
7. Maciel-Souza MC, Macrae A, Volpon AG, Ferreira PS, Mendonça-Hagler LC 2006 Chemical evaluation and microbial response from an oil-spill contaminated mangrove, Brazilian. *J. Microbiol.* 37:262-268.
8. Costa, R, Gomes NC, Peixoto R, Rumjaneck N, Berg G, Mendonça-Hagler LC, Smalla K 2006 Diversity and antagonistic potential of *Pseudomonas spp.* associated to the rhizosphere of maize grown in a sub-tropical organic farm. *Soil Biol. Biochemistry*. 38:2434-2447.

Book Chapters.

9. Mendonça-Hagler, LC, Melo IS, Inglis MC, Aniango B, Siqueira JO, Wheatley RE 2006 Non-target and biodiversity impacts in soil. 25-260 pp In: *Environmental Risk Assessment of Genetically Modified Organisms: Vol II. Methodologies for assessing Bt Cotton in Brazil*. A. Hilbeck, D. A. Andow and E. M. G. Fontes (eds). CABI Publishing, Wallingford, UK. 400 pp.
10. Mendonça-Hagler, LC, Minaré R, Langenbach T 2006 A Biodiversidade e os Marcos Legais de Biosegurança para a Biotecnologia Molecular. 135-155 pp. In: *Dimensões Humanas da Biodiversidade*. I. Garay and B. Becker. UFRJ. Ed. Vozes. Petrópolis, Brasil. 484 pp.

Theses.

11. Gonzalez A 2007 Distribuição da abundancia, diversidade e atividades microbianas ao longo de uma baía tropical poluída. Univ. Federal Rio de Janeiro. Brasil.
12. Batista S 2007 Impactos do estresse hídrico sobre as atividades e comunidades microbianas da Floresta Amazônica. Univ. Federal Rio de Janeiro, Brasil.
13. Sousa O 2006 Avaliação do perfil da comunidade microbiana de ecossistemas de manguezal receptores de efluentes de cultivo de camarão. Univ. Federal Rio de Janeiro, Brasil.

Conferences.

14. Mendonca-Hagler LC 2007 Yeast diversity in tropical environments. 5 Cong. Brasileiro de Micologia, Recife, Brasil.
15. Mendonça-Hagler LC 2007 Detecção da diversidade funcional de genes envolvidos no catabolismo de hidrocarbonetos em manguezais: aplicação em processo de fitorremediação. 24 Cong. Brasileiro de Microbiologia. Brasília, Brasil.

XI. Institute of Fermentation Technology and Microbiology, Technical University of Lodz, 90-924 Lodz, Wolczanska 171/173, Poland. Communicated by Dorota Kregiel <dkregiel@p.lodz.pl>.

Article in press.

1. Szubzda B, Kregiel D, Berłowska J, Mazurek B, Adamowska M, Ambroziak W 2008 Dielectric properties of biological structures exemplified on yeast cells. *Material Science*, 2008:230.

The paper presents the results of research which was an attempt to make use of natural biological structures occurring in yeast cells to elaborate a material characterized by high permittivity for various electrotechnical applications. Electric properties of living cells were tested in order to link the differences of the structure of cell walls and surface charge created on these walls with values of permittivity showed by

them. Three yeast strains of *Saccharomyces* and *Debaryomyces* types were selected which had different values of a surface charge, determined with use of Alcian blue method and a value of permittivity coefficient determined with use of capacitative method. A considerable interdependence was found of both electrical values measured.

XII. Laboratorio de Microbiología Aplicada y Biotecnología (Applied Microbiology and Biotechnology Laboratory), Centro Regional Universitario Bariloche, Universidad Nacional del Comahue. Quintral 1250, (8400), Bariloche, Argentina. Communicated by Diego Libkind <libkind@crub.uncoma.edu.ar>.

Recent Publications.

1. Libkind D, Moliné M, de García V, Fontenla, S, van Broock M 2008. Characterization of a novel South American population of the astaxanthin producing yeast *Xanthophyllomyces dendrorhous* (*Phaffia rhodozyma*). J Indust Microbiol Biotechnol 35:151-158.
2. Libkind D, Gadanho M, van Broock M, Sampaio JP 2008 Studies on the heterogeneity of the carotenogenic yeast *Rhodotorula mucilaginosa* from Patagonia, Argentina. J Basic Microbiol 48:93-98.
3. Russo G, Libkind D, Sampaio JP, van Broock 2008. Yeast diversity at the Volcanic acidic environment of the Lake Caviahue and Rio Agrio (Patagonia, Argentina). FEMS Microbiol Ecol FEMSEC-07-09-0385. In press.

Submitted.

4. Moliné M, Libkind, D, Diéguez, M.C, van Broock, M. Evidence of photoprotection by carotenoids in yeasts: response to UVB of naturally-occurring red and albino strains. *Photochemical and Photobiological Sciences*.
5. Libkind, D, Gadanho, M, van Broock, M, Sampaio, JP *Cystofilobasidium lacus-mascardii* sp. nov., a new basidiomycetous yeast species isolated from aquatic environments of the Patagonian Andes and *Cystofilobasidium macerans* sp. nov., the sexual stage of *Cryptococcus macerans*. Int J Syst Evol Microbiol.

XIII. CREM – Centro de Recursos Microbiológicos, Departamento de Ciências da Vida, Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, 2829-516 Caparica, Portugal. Communicated by Á. Fonseca <amrf@fct.unl.pt> and J. P. Sampaio <jss@fct.unl.pt>.

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

1. Valério E, Gadanho M and Sampaio JP 2008 *Sporidiobolus johnsonii* and *Sporidiobolus salmonicolor* revisited. Mycol Progr 7:125-131.
2. Valério E, Gadanho M and Sampaio JP 2007 A reappraisal of the *Sporobolomyces roseus* species complex and description of *Sporidiobolus metaroseus* sp. nov. Int J Syst Evol Microbiol 58:736-741.
3. Sampaio JP and Gonçalves P 2008 Natural populations of *Saccharomyces kudriavzevii* in Portugal are associated with oak bark and sympatric with *S. cerevisiae* and *S. paradoxus*. Appl Environ Microbiol 74:2144-2152.

Here we report the isolation of four *Saccharomyces* species (former *Saccharomyces sensu stricto* group) from tree bark. The employment of two temperatures (10 °C in addition to the more commonly used 30 °C) resulted in the isolation of *S. kudriavzevii* and *S. uvarum*, two species that grow at low temperatures, in addition to *S. cerevisiae* and *S. paradoxus*. A clear bias was found towards the bark of certain trees, in particular certain oak species. Very often, more than one *Saccharomyces* species was found in one locality and occasionally even in the same bark sample. Our evidence

strongly suggests that (markedly) different growth temperature preferences play a fundamental role in the sympatric associations of *Saccharomyces* species uncovered in this survey. *S. kudriavzevii* was isolated at most of the sites sampled in Portugal, indicating that the geographic distribution of this species is wider than assumed thus far. However, the Portuguese *S. kudriavzevii* population exhibited important genetic differences when compared to the type strain of the species that represents a Japanese population. In this study, *S. kudriavzevii* stands out as the species that copes better with low temperatures.

- Inácio J, Landell MF, Valente P, Wang PH, Wang YT, Yang SH, Manson JS, Lachance MA, Rosa CA and Fonseca Á 2008 *Farysizyma* gen. nov., an anamorphic genus in the Ustilaginales to accommodate three novel epiphytic basidiomycetous yeast species from America, Europe and Asia. *FEMS Yeast Res* 8:499-508.

Among many isolates that resulted from four independent surveys of yeasts associated with plants in Brazil, the USA, Portugal and Taiwan, we have characterized eighteen basidiomycetous strains, two of which were conspecific with the type strain of *Rhodotorula acheniorum*, whereas the remaining sixteen isolates appeared not to correspond to any previously described species. Microsatellite-PCR fingerprinting with primers M13 and (GTG)₅ confirmed that the latter strains formed three genetically distinct groups. Each group was considered to represent a distinct species based on nucleotide sequences of the

D1/D2 domains of the 26S rRNA gene and the internal transcribed spacer (ITS) region. Phylogenetic analyses of sequence data placed the putative novel species in a clade with *R. acheniorum* and the dimorphic smut fungus *Farysia chardoniana*. A novel anamorphic genus, *Farysizyma*, is created to accommodate the three undescribed species, which were named *Farysizyma itapuensis*, *Farysizyma setubalensis* and *Farysizyma taiwaniana*. A new combination, *Farysizyma acheniorum*, is proposed for *R. acheniorum*, which may represent the yeast-phase anamorph of *Farysia thuemennii*.

- Coelho MA, Rosa A, Rodrigues N, Fonseca Á and Gonçalves P 2008 Identification of mating type genes in the bipolar basidiomycetous yeast *Rhodospiridium toruloides*: first insight into the MAT locus structure of the Sporidiobolales. *Eukaryotic Cell*. Published ahead of print on 11 April 2008, doi:10.1128/EC.00025-08.

Rhodospiridium toruloides is a heterothallic, bipolar, red yeast that belongs to the Sporidiobolales, an order within a major lineage of basidiomycetes, the Pucciniomycotina. In contrast to other basidiomycetes, considerably less is known about the nature of the mating type (*MAT*) loci that control sexual reproduction in this lineage. Three genes (*RHA1*, *RHA2* and *RHA3*) encoding precursors of the *MAT A1* pheromone (rhodotorucine *A*) were previously identified and formed the basis for a genome walking approach that led to the identification of additional *MAT* genes in complementary mating strains of *R. toruloides*. Two mating type-specific alleles encoding a PAK kinase (Ste20 homolog) were found between the *RHA2* and *RHA3* genes, and identification in *MAT A2* strains of a gene encoding a presumptive pheromone precursor enabled prediction of the structure of rhodotorucine *a*. In addition, a putative

pheromone receptor gene (*STE3* homolog) was identified upstream of *RHA1*. Analyses of genomic data from two closely related species, *Sporobolomyces roseus* and *Sporidiobolus salmonicolor*, identified syntenic regions that contain homologs of all the above-mentioned genes. Notably, six novel pheromone precursor genes were uncovered containing, similarly to the *RHA* genes, multiple tandem copies of the peptide moiety. This suggests that this structure, which is unique among fungal lipopeptide pheromones, seems to be prevalent in red yeasts. Species comparisons provided evidence for a large, multigenic *MAT* locus structure in the Sporidiobolales, but no putative homeodomain transcription factor genes, which are present in all basidiomycetous *MAT* loci characterized thus far, could be found in any of the three species close to the *MAT* genes identified.

XIV. Food Science Program, Dalhousie University, Halifax, NS, Canada. Communicated by R. Alex Speers <Alex.Speers@Dal.Ca>.

Forthcoming presentations.

- Lake JC and Speers RA 2008 Quantitatively identifying PYF malt: Statistical modeling of yeast in suspension in small scale fermentations. Accepted for presentation at the World Brewing Congress, Honolulu, HI, August 2-6, 2008

When analysing small scale and tall tube fermentations, yeast in suspension is conventionally estimated by taking spectrophotometric measurements (600-800 nm) at specific time intervals. The collected data was then presented graphically and examined visually. Often, differences in visual observations between 'good' and PYF malts are the determining factor in identifying problematic malts. Due to natural variation in these pilot or lab-scale fermentations, qualitative identification of PYF malt can be subjective. Secondly, qualitative observations are difficult to translate from lab to lab. In this study, a statistical method of comparing the dependence of yeast in suspension with time is presented. Wort from a known PYF malt and a control malt were fermented in 15 mL test tubes with 4% glucose (w/v)

at 21°C (method accepted J. ASBC). The fermentation was analyzed optically every 5.0 min for 72 hr without disturbance using an inexpensive and easily constructed photometer/laser/data logger system. The resulting 864 data points yielded curves that were subsequently modelled using a piecewise regression technique. The continuous absorbance data exhibited different (curvilinear) behaviour before and after the maximum absorbance 'breakpoint'. Piecewise regressions were undertaken using the non-linear regression module of the Systat statistics package. The software determines the best fit of two functions, (one before and one after the breakpoint) by minimizing the sum of squares of the regression. We will report on the suitability of various functions (i.e., exponential,

Gompertz, normal, logistic and Verhulst) to describe the absorbance data. The modelling technique permitted quantitative and definitive comparisons between PYF and control fermentations by providing the best fit parameters of the two functions, the breakpoint values and related asymptotic standard errors. The qualitative differences between the PYF and control

absorbance data obtained by this modelling technique will be presented. The techniques discussed here allow improved criteria to be utilized when identifying a PYF malt. The technique may also add to our ability to track and optimize fermentation performance.

2. Lake JC and Speers RA 2008 Continuing investigations on malt causing premature yeast flocculation. Accepted for presentation at the World Brewing Congress, Honolulu, HI, August 2-6, 2008.

Premature yeast flocculation (PYF) is a reoccurring problem in breweries worldwide. There are many negative fermentation effects attributed to PYF factors, which ultimately lead to beers of low or unacceptable quality. However, due to its sporadic nature, much needed research concerning PYF (with the exception of Japanese and South African researchers) has either: not been undertaken or has remained proprietary. Consequently, many questions still abound with regards to the causes and mechanisms of PYF. It is suspected that PYF is induced by compound(s) originating in the malt, surviving through the brewing process and interacting with brewing yeast, resulting in their early removal from the fermenting medium. The nature of this compound (or compounds) is still debated and many different factors (such as arabinoxylan, beta-glucan and ferulic acid) have been described in the literature as being PYF inducers. Previously, the authors have presented (ASBC Ann. Meet., Victoria, 2007) experimental data of a series of filtrations of wort mashed with PYF positive malt. The PYF wort was filtered through both a 0.45 μ m membrane and a 100 kDa membrane prior to fermentation with a small volume (15 mL) test

tube fermentation. It was found that filtration of PYF wort through a 100 kDa membrane reduced PYF activity (as evidenced by absorbance and Plato measurements) compared with the 0.45 μ m filtered and unfiltered PYF wort. In continuation of this research, retentate from the 100 kDa PYF wort filtration was collected and inoculated back into 'control' wort for analysis via small volume test tube fermentations. It was confirmed that PYF was induced in an otherwise normal wort through the addition of the 100 kDa PYF retentate. Conversely, retentate (100 kDa) from 'normal' fermenting wort did not induce PYF when reintroduced to a 'control' wort prior to fermentation. In order to determine potential active components of the 100 kDa retentate several pure suspect compounds were added to 'control' wort and fermented. The addition of pure arabinoxylan (medium molecular weight) did not induce PYF. Additions of Ferulic acid and β -glucan (medium molecular weight) had variable influence upon addition to 'control' wort. We will report on the screening of this isolated factor and tests conducted to determine the nature of the active component(s) in the 100 kDa retentate.

XV. VTT Technical Research Centre of Finland, P.O.Box 1000, FI-02044 VTT, Finland.
Communicated by John Londesborough <john.londesborough@vtt.fi>.

Publications since our last communication include the following.

1. Guimarães PMR and J Londesborough 2007 The adenylate energy charge and specific fermentation rate of brewer's yeasts fermenting high- and very high-gravity worts. Yeast 25:47-58.

Intracellular and extracellular ATP, ADP and AMP (i.e., 5'-AMP) were measured during fermentations of high (15 °P) and very high gravity (VHG, 25 °P) worts by two lager yeasts. Little extracellular ATP and ADP but substantial amounts of extracellular AMP were found. Extracellular AMP increased during fermentation and reached higher values (3 μ M) in 25 °P than 15 °P worts (1 μ M). More AMP (13 μ M at 25 °P) was released during fermentation with industrially cropped yeast than with the same strain grown in the laboratory. ATP was the dominant intracellular adenine nucleotide and the adenylate energy charge ($EC = ([ATP] + 0.5*[ADP])/([ATP] + [ADP] + [AMP])$) remained high (> 0.8) until residual sugar concentrations were low and specific rates of ethanol production

were $< 5\%$ of the maximum values in early fermentation. The high ethanol concentrations ($> 85 \text{ g}\cdot\text{l}^{-1}$) reached in VHG fermentations did not decrease the EC below values that permit synthesis of new proteins. The results suggest that, during wort fermentations, the ethanol tolerance of brewer's strains is high so long as fermentation continues. Under these conditions, maintenance of the EC seems to depend upon active transport of α -glucosides, which in turn depends upon maintenance of the EC. Therefore, the collapse of the EC and cell viability when residual α -glucoside concentrations no longer support adequate rates of fermentation can be very abrupt. This emphasises the importance of early cropping of yeast for recycling.

2. Guimarães PMR, JP Multanen, L Domingues, JA Teixeira and J Londesborough 2008 Stimulation of zero-*trans* rates of lactose and maltose uptake into yeasts by pre-incubation with hexose to increase the adenylate energy charge. Appl Environ Microbiol 74:3076-3084.

Initial rates of sugar uptake (zero-*trans* rates) are often measured by incubating yeast cells with radiolabelled sugars for 5-30 s and determining the radioactivity entering the cells. The

yeast cells used are usually harvested from growth media, washed, suspended in nutrient-free buffer and stored on ice before assay. With this method, the specific rates of zero-*trans*

lactose uptake by *Kluyveromyces lactis* or recombinant *Saccharomyces cerevisiae* harvested from lactose fermentations were 3- to 8-fold smaller than the specific rates of lactose consumption during fermentation. No extracellular α -galactosidase was detected. The ATP content and adenylate energy charge (EC) of the yeasts were relatively low before starting the ^{14}C -lactose uptake reactions. Short (1-7 min) pre-incubation of the yeasts with 10 - 30 mM glucose caused 1.5- to 5-fold increases in the specific rates of lactose uptake. These increases correlated with increases in EC (from 0.6 to 0.9) and ATP (from 4 to 8 $\mu\text{mol}\cdot\text{g}$ dry yeast $^{-1}$). Stimulation by glucose

affected the transport V_{max} , with smaller increases in K_m . Similar observations were made for maltose transport by a brewer's yeast. These findings suggest that the electrochemical proton potential that drives transport through sugar/ H^+ symports is significantly smaller in the starved yeast suspensions used for zero-*trans* assays than in actively metabolising cells. Zero-*trans* assays with such starved yeast preparations can seriously underestimate the capacity of sugar/ H^+ symports. Short exposure to glucose allows a closer approach to the sugar/ H^+ symport capacity of actively metabolising cells.

3. Koivistoinen OM, S Hilditch, SP Voutilainen, H Boer, M Penttilä and P Richard 2008 Identification in the yeast *Pichia stipitis* of the first L-rhamnose 1-dehydrogenase gene. FEBS J Published online: 8 April 2008.

There are two distinctly different pathways for the catabolism of L-rhamnose in microorganisms. One pathway with phosphorylated intermediates was described in bacteria; here the enzymes and the corresponding gene sequences are known. The other pathway has no phosphorylated intermediates and has only been described in eukaryotic microorganisms. For this pathway the enzyme activities have been described but not the corresponding gene sequences. The first enzyme in this catabolic pathway is the NAD-utilizing L-rhamnose 1-dehydrogenase. The enzyme was purified from the yeast *Pichia stipitis* and the mass of its tryptic peptides determined using MALDI-TOF MS. This enabled the identification of the corresponding gene, *RHA1*. It

codes for a protein with 258 amino acids belonging to the protein family of short chain alcohol dehydrogenases. The open reading frame was expressed in *S. cerevisiae*. Since the gene contained a CUG codon which codes for serine in *P. stipitis* but for leucine in *S. cerevisiae* these codons were changed so that the same amino acid was expressed in *S. cerevisiae*. The heterologous protein showed the highest activity and affinity with L-rhamnose and a lower activity and affinity with L-mannose and L-lyxose. The enzyme was specific for NAD. A Northern analysis revealed that transcription in *P. stipitis* is induced during growth on L-rhamnose but not on other carbon sources.

4. Rautio JJ, A Huuskonen, H Vuokko, V Vidgren and J Londesborough 2007 Monitoring yeast physiology during very high gravity wort fermentations by frequent analysis of gene expression. Yeast 24:741-760.

Brewer's yeast experiences constantly changing environmental conditions during wort fermentation. Cells can rapidly adapt to changing surroundings by transcriptional regulation. Changes in genomic expression can indicate the physiological condition of yeast in the brewing process. We monitored by the TRAC (TRanscript analysis with aid of Affinity Capture) method the expression of some 70 selected genes relevant to wort fermentation at high frequency through 9 to 10 day fermentations of very high gravity wort (25 °P) by an industrial lager strain. Rapid changes in expression occurred during the first hours of fermentations for several genes, e.g. genes involved in maltose metabolism, glycolysis and ergosterol synthesis were strongly up-regulated between 2 to 6 h after pitching. By the time (72 h) yeast growth had stopped and total

sugars had dropped by about 50 %, most selected genes had passed their highest expression levels and total mRNA was less than half the levels during growth. There was an unexpected up-regulation of some genes of oxygen-requiring pathways during the final fermentation stages. For 5 genes, expression of both the *Saccharomyces cerevisiae* and *S. bayanus* components of the hybrid lager strain were determined. Expression profiles were either markedly different (*ADH1*, *ERG3*) or very similar (*MALx1*, *ILV5*, *ATF1*) between these two components. By frequent analysis of a chosen set of genes, TRAC provided a detailed and dynamic picture of the physiological state of the fermenting yeast. This approach offers a possible way to monitor and optimise the performance of yeast in a complex process environment.

5. Rintala E, MG Wiebe, A Tamminen, L Ruohonen and M Penttila 2008 Transcription of hexose transporters of *Saccharomyces cerevisiae* is affected by change in oxygen provision. BMC Microbiol. 8:53.

BACKGROUND: The gene family of hexose transporters in *Saccharomyces cerevisiae* consists of 20 members; 18 genes encoding transporters (HXT1-HXT17, GAL2) and two genes encoding sensors (SNF3, RGT2). The effect of oxygen provision on the expression of these genes was studied in glucose-limited chemostat cultivations (D = 0.10 h $^{-1}$, pH 5, 30C degrees). Transcript levels were measured from cells grown in five steady state oxygen levels (0, 0.5, 1, 2.8 and 20.9%

oxygen), and from cells under conditions in which oxygen was introduced to anaerobic cultures or removed from cultures receiving oxygen. RESULTS: The expression pattern of the HXT gene family was distinct in cells grown under aerobic, hypoxic and anaerobic conditions. The transcription of HXT2, HXT4 and HXT5 was low when the oxygen concentration in the cultures was low, both under steady state and non-steady state conditions, whereas the expression of HXT6, HXT13 and HXT15/16 was

higher in hypoxic than in fully aerobic or anaerobic conditions. None of the HXT genes showed higher transcript levels in strictly anaerobic conditions. Expression of HXT9, HXT14 and GAL2 was not detected under the culture conditions studied. CONCLUSIONS: When oxygen becomes limiting in a glucose-limited chemostat cultivation, the glucose uptake rate per cell increases. However, the expression of none of the hexose

transporter encoding genes was increased in anaerobic conditions. It thus seems that the decrease in the moderately low affinity uptake and consequently the relative increase of high affinity uptake may itself allow the higher specific glucose consumption rate to occur in anaerobic compared to aerobic conditions.

6. Ruohonen L, A Aristidou, AD Frey, M Penttilä and PT Kallio 2006 Expression of *Vitreoscilla* hemoglobin improves the metabolism of xylose in recombinant yeast *Saccharomyces cerevisiae* under low oxygen conditions. *Enzyme Microbiol Technol.* 39:6-14.

The yeast *Saccharomyces cerevisiae* efficiently ferments hexose sugars to ethanol, but it is unable to utilize xylose, a pentose sugar abundant in lignocellulosic materials. Recombinant yeast strains capable of xylose metabolism have been reported, however, such strains ferment xylose to ethanol poorly, with significantly lower yields and/or production rates compared to glucose. Our previous studies have indicated that oxygen is one of the key parameters in improving xylose consumption and ethanol yields. In addition, it has been shown that expression of the *Vitreoscilla* hemoglobin gene (*vhb*) in

Escherichia coli leads to a more oxidized state of the cells under low oxygen concentrations. Therefore, we wanted to investigate the possible benefits expression of Vhb encoding gene could bring to xylose fermentation in the recombinant *S. cerevisiae* yeast strain. Our results indicate that under low hypoxic fermentation conditions Vhb improves the ethanol yield from the xylose metabolized. Less carbon was lost in xylitol, which is the major unwanted side product of the xylose-to-ethanol fermentation process.

7. Saloheimo A, J Rauta, OV Stasyk, AA Sibirny, M Penttilä and L Ruohonen 2007 Xylose transport studies with xylose-utilizing *Saccharomyces cerevisiae* strains expressing heterologous and homologous permeases. *Appl Microbiol Biotechnol* 74:1041-1052.

In the present study, we modified xylose uptake properties of a recombinant xylose-utilizing yeast *Saccharomyces cerevisiae* by expression of heterologous and homologous permease-encoding genes. In a mutant yeast strain with the main seven hexose transporter genes deleted, and engineered for xylose utilization, we screened an expression cDNA library of the filamentous fungus *Trichoderma reesei* (*Hypocrea jecorina*) for enhanced growth on xylose plates. One cDNA clone with significant homology to fungal sugar transporters was obtained but when the clone was retransformed into the host, it did not support significant growth on xylose. However, during a long liquid culture of the strain carrying the cDNA clone, adaptive

mutations apparently occurred in the host, which led to growth on xylose but not on glucose. The new transporter homologue, *Trxlt1* thus appears to code for a protein specific for xylose uptake. In addition, xylose-transporting properties of some homologous hexose transporters were studied. All of them, i.e. Hxt1, Hxt2, Hxt4 and Hxt7 were capable of xylose uptake. Their affinities for xylose varied, K_m values between 130 and 900 mM were observed. The single-Hxt strains showed a biphasic growth mode on xylose, alike the *Trxlt1* harbouring strain. The initial, slow growth was followed by a long lag and finally by exponential growth.

The following theses have been successfully presented.

8. Mervi Toivari 2007 Engineering the pentose phosphate pathway of *Saccharomyces cerevisiae* for production of ethanol and xylitol. Doctoral thesis, University of Helsinki.
 9. Jari Rautio 2007 Development of rapid gene expression analysis and its application to bioprocess monitoring. Donctoral thesis, University of Oulu.
 10. Jyri-Pekka Multanen 2008 Genetic and environmental factors affecting α -glucoside uptake by lager yeasts. Diploma engineer's thesis, Helsinki University of Technology.
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The following publications have appeared in the last three years.

2006

1. Diaz MR, Boekhout T, Theelen B, Bovers M, Cabañes FJ & Fell, JW 2006 Barcoding and flow cytometry as a high-throughput identification system for *Malassezia* species. *J Med Microbiol* 55:1197-1209.
2. Hagen F. & Boekhout T. (2006). Epidemiologische trends in cryptokokkose. *Infectieziektenbulletin* 17: 220-224.
3. Gaitanis G, V Robert, A Velegraki 2006 Verifiable single nucleotide polymorphisms of the Internal Transcribed Spacer 2 region for the identification of 11 *Malassezia* species. *J Dermatol Sci* 43:214-217.
4. Gildemacher PR, Heijne, B, Silvestri, M, Houbraken, J, Hoekstra, E, Theelen, B. & Boekhout, T. 2006 Interactions between yeasts, fungicides and apple fruit russetting. *FEMS Yeast Research* 6:1149-1156.
5. Bovers M, Hagen F, Kuramae EE, Diaz MR, Spanjaard L, Dromer F, Hoogveld HL & Boekhout T 2006 Unique hybrids between the fungal pathogens *Cryptococcus neoformans* and *Cryptococcus gattii*. *FEMS Yeast Research* 6:599-607.
6. Knutsen AK, Robert V, Poot GA, Epping W, Figge M, Holst-Jensen A, Skaar I, Smith MT 2007 Polyphasic re-examination of *Yarrowia lipolytica* strains and the description of three novel *Candida* species: *Candida oslonensis* sp. nov., *Candida alimentaria* sp. nov. and *Candida hollandica* sp. nov. *Int J Syst Evol Microbiol.* 57:2426-35.
7. Kuramae, E, Robert, V, Snel, B, Weiss, M. & Boekhout T 2006 Conflicting phylogenetic position of the fission yeast, *Schizosaccharomyces pombe*. *Genomics* 88:387-393.
8. Kuramae, E, Robert, V, Snel, B, Weiss, M & Boekhout T 2006 Phylogenomics reveal a robust fungal tree of life. *FEMS Yeast Research* 6:1213-1220.
9. Liu J, van der Putten P, Hagen F, Chen X, Boekhout T & Verbeek FJ 2006 Detecting virulent cells of *Cryptococcus neoformans* yeast: clustering experiments. *IEEE* 1112 - 1115.
10. Passel MWJ van, Kuramae EE Luyf ACM, Bart A & Boekhout T 2006 The reach of the genome signature. *BMC Evol. Biol.* 6:84.
11. Robert V, Stalpers J, Verkley G 2006 Culture collections, genomics and bioinformatics, the golden triangle. *Phytopathology* 96:S133.
12. Wu, ZW, Robert V, Bai FY 2006 Genetic diversity of the *Pichia membranifaciens* strains revealed from rRNA gene sequencing and electrophoretic karyotyping, and the proposal of *Candida californica* comb. nov. *FEMS Yeast Res* 6:305-311.

2007

13. Adler A, Hidalgo-Grass C, Boekhout T, Theelen B, Sionov E & Polacheck I 2007 *Pichia farinosa* blood-stream infection in a lymphoma patient. *J Clin Microbiol* 45:3456-3458.
14. Bovers M, Diaz MR, Hagen F, Spanjaard L, Duim B, Visser CE, Hoogveld HL, Scharringa J, Hoepelman IM, Fell JW & Boekhout T 2007 Identification of genotypically diverse *Cryptococcus neoformans* and *Cryptococcus gattii* isolates using Luminex xMAP technology. *J Clin Microbiol* 45:1874-1883.

15. Cabañes FJ, Theelen B, Castellá G & Boekhout T 2007 Two new lipid-dependent *Malassezia* species from domestic animals. FEMS Yeast Res. 7:1064-1076.
 16. Driel KGA van, Boekhout T, Peer A. van, Wösten HAB, Verkleij AJ & Müller WH 2007 Laser microdissection of fungal septa as visualised by scanning electron microscopy. Fung Genet Biol 44:466-473.
 17. Driel KGA van, van Peer AF, Wösten HAB, Verkleij AJ, Boekhout T & Müller WH 2007 A method to enrich perforate septal pore caps from the Basidiomycete *Rhizoctonia solani*. J Microb Meth 71:298-304.
 18. Dunlap CA, Evans KO, Theelen B, Boekhout T & Schisler DA 2007 Osmotic shock tolerance and membrane fluidity of cold-adapted *Cryptococcus nodaensis* OH 182.9; a biocontrol agent of *Fusarium* head blight. FEMS Yeast Res 7: 449-458.
 19. Dutilh B, Noort V van, Heijden RTJM, Boekhout T, Snel B. & Huynen MA 2007 From phylogenetics to phylogenomics at successive levels: super- and orthology methods tested on the fungi. Bioinformatics 23:815-824.
 20. Kantarcioglu AS, Boekhout T, de Hoog GS, Theelen B, Yücel A, Ekmekci TR, Fries BC, Ikeda R, Koslu A & Altas K 2007 Subcutaneous cryptococcosis due to *Cryptococcus diffluens* in an apparently healthy young patient with sporotrichoid lesions. Med Mycol 45:173-181.
 21. Kuramae EE, Robert V, Echevarrii-Erasun C & Boekhout T 2007 Cophenetic correlation analysis as a strategy to select phylogenetically informative proteins: an example from the fungal kingdom. BMC Evol. Biol. 7:134.
 22. Lindberg J, Hagen F, Lauersen A, Stenderup J & Boekhout T 2007. *Cryptococcus gattii* risk for tourists visiting Vancouver Island, Canada. Emerg Infect Dis 13:178-179.
 23. Nyanga LK, Nout MJR, Gadaga TH, Boekhout T, Zwietering M 2007 Yeasts and lactic acid bacteria microbiota from masau (*Ziziphus mauritania*) fruits and their fermented fruit pulp in Zimbabwe. Int J Food Microbiol 120:159-166.
 24. Okoli I, Oyeka, CA, Kwon-Chung KJ, Theelen B, Robert V, Groenewald JZ, McFadden DC, Casadevall A & Boekhout T 2007 *Cryptotrichosporon anacardii* gen. nov, sp. nov, a new trichosporonoid capsulate basidiomycetous yeast from Nigeria that is able to form melanin on niger seed agar. FEMS Yeast Res. 7:339-350.
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PhD theses

42. Marjan Bovers: *Cryptococcus neoformans* and *Cryptococcus gattii*: speciation in progress. Utrecht University. The Netherlands, November 29, 2007.
43. Kenneth van Driel: Spetal pre caps in Basidiomycetes: ultrastructure and composition. Utrecht University. The Netherlands, December 17, 2007.

The following papers have now appeared in print.

1. Lachance MA & Starmer WT 2008 The yeast genus *Kurtzmaniella* gen. nov. and description of the heterothallic, haplontic species *Kurtzmaniella cleridarum* sp. nov., the teleomorph of *Candida cleridarum*. *Int J Syst Evol Microbiol* 58:520-524.
2. Inácio J, Landell M, Valente P, Wang PH, Manson J, Lachance MA & Fonseca A 2008 *Farysizyma* gen. nov., an anamorphic genus in the Ustilaginales to accommodate three novel epiphytic basidiomycetous yeast species from America, Europe and Asia. *FEMS Yeast Res* 8:499-508.

The abstract is given under Drs. Fonseca and Sampaio's entry.

The following were accepted recently.

3. Lachance MA, Bowles JM, Anderson TM & Starmer WT 2008 *Metschnikowia shivogae* sp. nov., a yeast species associated with insects of morning glory flowers in East Africa. *Int J Syst Evol Microbiol* (Accepted February 2008).

The new species *Metschnikowia shivogae* is described to accommodate three isolates recovered from insects of morning glory flowers at two localities in East Africa. The isolates differ slightly in rDNA ITS and D1/D2 LSU sequences and one isolate featured a two-base heterogeneity that might be the result of recombination between two variant rDNAs. *M. shivogae* is a

sister species to *Metschnikowia aberdeeniae* and shares the same habitat. The reproductive boundaries of *M. aberdeeniae*, which were not clear in the past, have now been elucidated further. The type strain of *M. shivogae* is strain SUB 04-310.1^T (*h*⁺, CBS 10292^T, NRRL Y-27924^T), and the allotype is strain UWOPS 07-203.2 (*h*, CBS 10770, NRRL Y-48447).

4. Fidalgo-Jiménez A, Daniel HM, Evrard P, Decock C & Lachance MA 2008 *Metschnikowia cubensis* sp. nov., a new yeast species isolated from flowers in Cuba. *Int J Syst Evol Microbiol* (Accepted March 2008).

A novel yeast species is described from nine strains isolated from flowers in four different localities in Cuba. The species is so far known only from Cuba. Characteristic asci and ascospores as well as the phylogenetic analysis of the rDNA place the new species in the genus *Metschnikowia*. The new species belongs to the New World subclade of large-spored *Metschnikowia*. Mating tests with other members of the subclade resulted in the formation of sterile asci without ascospores, showing that the Cuban strains represent a distinct biological

species. Intra-species matings lead to the production of fertile asci containing large needle-shaped ascospores. The new species was further distinguished from its close relatives by rDNA sequences, as well as mini- and microsatellite based PCR-fingerprinting. We propose the name *Metschnikowia cubensis* and designate MUCL 45753^T (= CRGF 279^T = CBS 10832^T, *h*⁺) as the type strain and MUCL 45751 (= CRGF 278 = CBS 10833, *h*⁻) as the allotype.

Conference presentations.

5. Berkers T 2008 Great Lakes-St.Lawrence Mycology Workshop, April 2008, University of Toronto.

Yeast identification relies more and more on DNA sequence comparisons. A clear identification, however, is frustrated by the presence in public databases of sequences of questionable quality. We investigated various means of solving this problem. The easiest would have been to erect a filter-list of accession numbers corresponding to sequences of authentic strains originating from reliable laboratories. The user would

conduct a normal BLAST search, but would limit the search by invoking a filter ("Authentic yeast strains") that would exclude unreliable data. As this approach was not acceptable to NCBI staff, we investigated the use of standalone BLAST where the database consists of only a list of sequences obtained from reliable sources. Implementation on a web-based site would accomplish the desired objective.

6. Dobson J 2008 Great Lakes-St.Lawrence Mycology Workshop, April 2008, University of Toronto.

In the course of a study of the genetic structure of *Candida azyma*, it became clear that strains assigned to that species in fact did not belong to a single species. Sequences of the ITS/5.8S and D1/D2 LSU rDNA regions were therefore determined for all of the 65 strains available in our collection.

Parsimony haplotype network analysis suggested that strains recovered from ephemeral flowers or their insects around the world and assigned to *C. azyma* in fact belong to at least five species, one of which is most prevalent and highly divergent from the type in rDNA sequences.

7. Wardlaw A, Berkers T, Man K, Lachance MA 2008 Genetic structure of three yeast species: no sex, some sex, and lots of sex. Canadian Society for Ecology and Evolution, May 11-14, University of British Columbia, Vancouver BC.

We have examined the genetic structure of three species of yeast that are associated with nitidulid beetles found in ephemeral flowers. *Metschnikowia borealis* is Nearctic and *Metschnikowia lochheadii* is endemic to Central America. Both species are capable of sexual reproduction but grow mostly as haploid clones in nature (but essentially all their growth is in the form of asexual budding of haploid strains). A related species, *Candida ipomoeae* has a broader geographic range across the New World and is strictly asexual. There is evidence that the last two species were introduced into Hawai'i in the early part of the twentieth century. We compared the three species using polymorphic DNA markers generated by SWAPP screening (Burt *et al.* 1994). We studied 10-13 loci in each species using SSCP electrophoresis and/or sequencing to identify alleles. We determined F_{ST} and linkage disequilibrium, performed Mantel tests, and constructed parsimony haplotype networks using TCS1.21 (Clement *et al.* 2000). As expected for an asexual

species the alleles of *C. ipomoeae* were in strong linkage disequilibrium and their parsimony network was strictly divergent. One haplotype was sufficiently divergent to suggest the possibility of a separate species. A somewhat similar pattern was found in *M. lochheadii* although some loci were in equilibrium, suggesting the occurrence of recombination. The observation of reticulate and hybrid patterns in the haplotype network provided further evidence for recombination. Hawaiian strains of both species were genetically homogenous, confirming the hypothesis that they were introduced by way of adventive beetles. Although *M. borealis* was collected over a wide geographic range, the loci were generally in equilibrium and F_{ST} values were negligible. The haplotype network had a high degree of reticulation that cannot be explained by homoplasy, and the distribution of alleles followed a latitudinal gradient. Both of these observations may be the result of seasonal movement of the yeasts' vectors over thousands of years.

Obituary

Professor Joshua Lederberg (1925-2008)

Joshua Lederberg, University Professor and president emeritus of The Rockefeller University, died from pneumonia Saturday, February 2, at NewYork-Presbyterian Hospital. An adviser to nine United States presidential administrations, he was a distinguished molecular geneticist whose achievements helped lay the foundation for the current revolution in molecular biology and biotechnology.

Lederberg was a recipient of the 1958 Nobel Prize in Physiology or Medicine, at the age of 33, for his work on the organization of genetic material in bacteria.

The son of a rabbi, Lederberg was born in Montclair, New Jersey, in 1925, and graduated from Stuyvesant High School in New York City at the age of 15. He received his bachelor's degree from Columbia College in 1944 and his Ph.D. from Yale University in 1947. He held appointments at the University of Wisconsin and Stanford University School of Medicine before coming to the Rockefeller University as its fifth president in 1978. During his presidency, the university recruited several world-class faculty, created the University Fellows Program, which brought outstanding young scientists to campus, and constructed a major new research building. On his retirement as president in 1990, he returned to research as University Professor Emeritus, the Raymond and Beverly Sackler Foundation Scholar, and head of the Laboratory of Molecular Genetics and Informatics.

Lederberg was a pioneer in the field of bacterial genetics. While at Yale, he made the seminal discovery that a form of sexual reproduction occurs in bacteria, demonstrating that bacteria possess a genetic mechanism, called recombination, similar to that of higher organisms, including humans. He later showed that bacterial genetic material is exchanged not only by conjugation, when the entire complement of chromosomes is transferred from one bacterial cell to another, but also by transduction, when only fragments are transferred. More recently, his work addressed how the activation of genes alters their vulnerability to mutagenesis. In addition, he had interests in genetics, chemistry, evolution and the origin of life; the use of computer models for scientific reasoning; and the application of scientific understanding to direction of research, public health and policy.

Lederberg served in the U.S. Navy and worked on many government advisory committees and boards dealing with research on physical and mental health. He played an active role in the Mariner and Viking missions to Mars sponsored by the U.S. National Aeronautics and Space Administration. He was a consultant to the Arms Control and Disarmament Agency during the negotiation of the biological weapons disarmament treaty, and he continued to advise on national security problems in a variety of capacities including membership on the U.S. Defense Science Board and the Secretary of Energy Advisory Board.

In addition to the Nobel Prize, Lederberg was honored with many awards and prizes, including the National Medal of Science and the Presidential Medal of Freedom. He was also a member of the boards of several foundations, including the Carnegie Corporation, the Revson Foundation and the Camille and Henry Dreyfus Foundation, and he served as chairman of the scientific advisory board of the Ellison Medical Foundation.

His interest in improving communications among scientists, the general public and government policymakers led Lederberg to write extensively for lay audiences, at one time including a weekly column syndicated for several years by The Washington Post on the social impact of scientific progress.

Lederberg is survived by his wife, Dr. Marguerite S. Lederberg of New York City; his children, Anne Lederberg of New York City and David Kirsch of Chevy Chase, Maryland; and two grandchildren.

Rockefeller University Newswire



Forthcoming Meetings

12th International Congress on Yeasts, Kyiv, Ukraine, August 11-15, 2008

I am pleased to inform that, according to the decision of the International Commission on Yeasts, adopted at 11th International Congress on Yeasts (Rio-de-Janeiro, Brazil, August 2004), the 12th International Congress on Yeasts will be held in 2008 in Kyiv (Kiev), the capital of Ukraine. At the moment, the Local Organizing Committee, the Secretariat and the International Scientific Committee have been established. The Congress venues will be the Kyiv National Convention Centre and the Kyiv National University. The dates of the Congress are fixed for August 11-15, just after finishing the IUMS 2008, Istanbul, Turkey, i.e. after 12th International Congress of Bacteriology and Applied Microbiology and 12th International Congress of Mycology. In such a way, people attending IUMS in Istanbul could participate also in our Congress in Kyiv, which is especially convenient for people outside of Europe. Total number of participants will be limited to 500. The regular registration fee will be EUR 400 and the student registration fee will be EUR 350. The fee will include the bag with abstract and program, get-together party, 4 lunches, coffee breaks, Kyiv city tour and the opening concert. Organizers plan to collect sponsor money which will be used in part to promote participation of the students from developing countries.

Recently, the organizers have posted the web page of

Prof. Andriy A. Sibirny
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Drahomanov Street
14/16, Lviv 79005
Ukraine

or to:

the Congress (see: www.icy2008.org.ua). Besides, the preliminary scientific program of the Congress is available. The list of the oral and poster sessions is as follows:

1. Yeast Systematics and Ecology
2. Food and Beverage Yeasts
3. Medically Important Yeasts
4. New Tools in Yeast Research
5. Systems Biology
6. Genomics and Proteomics
7. Transcriptional and Translational Regulation
8. Cell Cycle
9. Sensing and Signaling
10. Membrane Structure and Functions
11. Traffic and Secretion
12. Autophagy and Stress Response
13. Organelles
14. Yeast as Model of Human Diseases and Drug Testing
15. Production of Heterologous Proteins
16. Metabolic Engineering
17. Yeasts for Fuel Ethanol Production and other Biorefineries
18. Yeast Biochemical Engineering

All correspondence and inquiries should be sent to:

Dr. Andriy Y. Voronovsky (same address)

Phone: 380 322 740363

FAX: 380 322 721648.

The most convenient is to send inquiries to the special e-mail address: icy2008@cellbiol.lviv.ua

Glutathione and related thiols in microorganisms Nancy, France, August 27-29 2008

A symposium concerning the multiple facets of Glutathione and related thiols in microorganisms will be held in Nancy (France) in August 2008 (27-29). The research effort on microbial thiols, in particular (but not only) glutathione, increased in the last ten years, in part because thiols were identified as important components of cell defence against oxidative, metals, xenobiotics, nutritional, osmotic, acid, etc. stresses, but also because these derivatives are now recognized as involved in signal transduction mechanisms. Thiols were also identified as playing major roles in cell cycle, from differentiation to apoptosis, and cell organisation as exemplified by aggregation, biofilm formation and also in interaction between microorganisms and plants. Moreover, glutathione is of interest for applications in food, pharmaceutical, cosmetic industry and environmental technology, especially waste water treatment. Different investigation fields will be thus covered in the planned symposium. We think that so it will be a nice opportunity to create a platform for exchanging ideas, and assembling investigators coming from different horizons. The three day meeting will include invited plenary lectures, oral

communications and poster sessions. Arrangement will be taken with a scientific editor to publish communications in an international peer-reviewed journal.

In the last ten years, research effort on microbial thiols, in particular (but not only) glutathione, increased strongly. This was because thiols were identified as important components of cell defence against oxidative, metals, xenobiotics, nutritional, osmotic, acid, etc... stresses, but also considering that these derivatives are now recognized as involved in signal transduction mechanisms. Thiols were also identified as playing major roles in cell cycle, from differentiation to apoptosis, and cell organisation as exemplified by aggregation, biofilm formation and also in interaction between microorganisms and plants. Microorganisms, in particular yeast, are also considered as useful eukaryotic cell models, for example in studies on the oxidative stress and signal transduction mechanisms. Moreover, glutathione is of interest for applications in food, pharmaceutical, cosmetic industry and environmental technology, especially waste water treatment. Different investigation fields will be thus covered in the planned symposium. We think that so it will be a

nice opportunity to create a platform for exchanging ideas, and assembling investigators coming from different horizons.

The organizing and scientific committee has already planned a programme in order to cover all the considered items.

Docteur J. Coulon, Maître de Conférences
LCPME UMR 7564, Faculté de Pharmacie
5 rue Albert Lebrun
BP 80403
F-54001 NANCY Cedex

Full informations concerning the symposium and registration, abstract submission are available on the following web site:
<http://www.thiolmicrob.uhp-nancy.fr>.

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<Joel.coulon@pharma.uhp-nancy.fr>

Cancellation: Biology of *Kluyveromyces lactis*

Due to the insufficient number of interested participants, we regret to announce that the 21th meeting on the Biology of *Kluyveromyces lactis*, to be held in Lyon (France) on the 6-7th of September 2008, is cancelled.

Micheline Wésolowski-Louvel
Marc Lemaire
Michel Aigle

Fifty Years Ago in the Yeast Newsletter (Volume VII Number 1, May 1958)

Dr. JP van der Walt reported on the isolation from larval feed of bumble bee, of a haplontic, strongly fermentative, unnamed yeast that forms asci by heterogamy or isogamy and whose 1-4 ascospores are oblate-ellipsoidal and brownish in colour.

Dr. M di Menna disclosed the presence of large densities of *Cryptococcus laurentii* in Antarctic moss samples. She also mentioned the isolation of psychrophilic yeasts in Antarctic soil.

Dr. J Boidin described the new genus *Pachysolen* with two species isolated from tanning liquor.

Dr JM Carpenter communicated the isolation of numerous yeast species from *Drosophila* spp. and suggested that the yeasts and the flies may engage in a specific interaction.

Dr N van Uden described the isolation of a number of yeast species from bovine caeca.

Dr HJ Phaff announced the publication of a paper on the ecology and taxonomy of *Saccharomycopsis* (syn. *Cyniclomyces*) *guttulata*.

Dr TD Brock deplored the confusion that existed in the nomenclature of mating types and proposed the uniform use of “+” and “-”, as originally suggested by Blakeslee.
