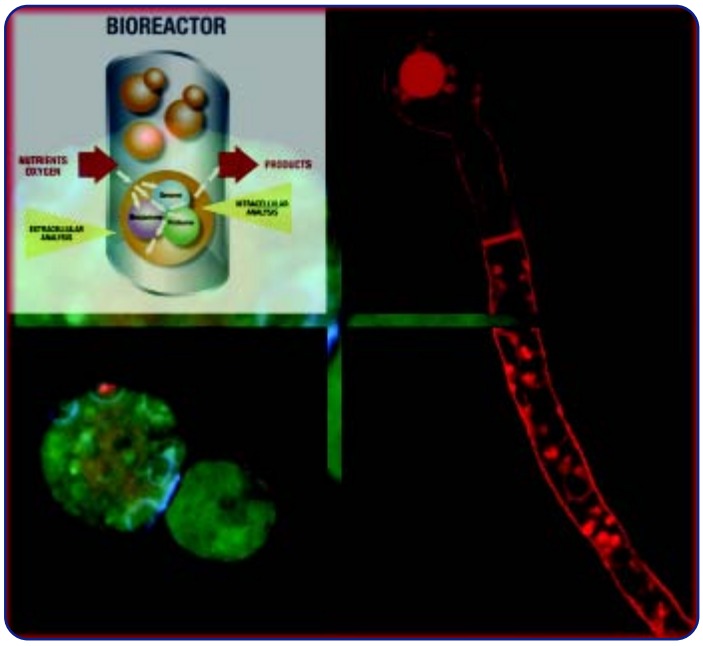
ESPOO 2007 VTT SYMPOSIUM 245



3rd European Federation of Biotechnology Conference

Physiology of Yeasts and Filamentous Fungi

PYFF3

VTT SYMPOSIUM 245

Keywords: yeast, filamentous fungi, microbial physiology, systems biology, genomics, proteomics, metabolomics, modelling, stress responses, metabolic engineering, protein production

3rd European Federation of Biotechnology Conference

Physiology of Yeasts and Filamentous Fungi PYFF3

June 13–16, 2007

Marina Congress Centre, Helsinki, Finland

Edited by

Annemari Kuokka-Ihalainen, Markku Saloheimo & Tiina Pakula

Organised by

VTT Technical Research Centre of Finland

Finland

ISBN 978-951-38-6313-5 (soft back ed.)
ISSN 0357–9387 (soft back ed.)

ISBN 978-951-38-6314-2 (URL: http://www.vtt.fi/publications/index.jsp)
ISSN 1455–0873 (URL: http://www.vtt.fi/publications/index.jsp)

JULKAISIJA – UTGIVARE – PUBLISHER

VTT, Vuorimiehentie 3, PL 1000, 02044 VTT
puh. vaihde 020 722 111, faksi 020 722 4374

VTT, Bergsmansvägen 3, PB 1000, 02044 VTT
tel. växel 020 722 111, fax 020 722 4374

VTT Technical Research Centre of Finland
Vuorimiehentie 3, P.O.Box 1000, FI-02044 VTT, Finland
phone internat. +358 20 722 111, fax + 358 20 722 4374

VTT, Tietotie 2, PL 1000, 02044 VTT
puh. vaihde 020 722 111, faksi 020 722 1000

VTT, Datavägen 2, PB 1000, 02044 VTT
tel. växel 020 722 111, fax 020 722 1000

VTT Technical Research Centre of Finland, Tietotie 2, P.O. Box 1000, FI-02044 VTT, Finland
phone internat. +358 20 722 111, fax +358 20 722 1000

Edita Prima Oy, Helsinki 2007

Preface

Dear Colleagues,

On behalf of the Microbial Physiology Section of the European Federation of Biotechnology and the local organising committee, we are delighted to welcome you to Helsinki to the 3rd Conference on Physiology of Yeasts and Filamentous Fungi (PYFF3).

The two previous conferences in this series have been organised in Denmark by Lisbeth Olsson and in France by Jean-Marie Francois. The conferences have been successful and appreciated by audience from both academic institutions and industrial laboratories. This time there are approximately 200 participants from 32 countries in the conference. We have received 139 abstracts from participants, and 27 of them were selected by the session chairpersons to be presented orally in the sessions. We feel that the abstracts are of excellent quality and many more of them deserved to be selected for oral presentations than we have time for. We anticipate high quality lectures and poster sessions with interesting and inspiring discussions during the meeting!

The interest which the PYFF3 meeting has received and the quality of the science to be presented shows the importance of eukaryotic microbes both as model organisms in fundamental studies on physiology and as production organisms in the industrial bioprocesses. The current trend aiming at production of fuels and chemicals from renewable resources has placed both yeasts and filamentous fungi into the centre of biotechnological research.

We thank the local organising committee members, the international scientific committee and our colleagues at VTT for their help. We are very grateful to our industrial and organisational sponsors for their financial contributions; without their support this meeting would not have taken place. Our special thanks for administrative and secretarial help go to Ms. Anita Tienhaara (University of Helsinki) and Ms. Annemari Kuokka-Ihalainen (VTT).

Our warmest welcome to PYFF3!

Merja Penttilä Markku Saloheimo

Local organising committee

Merja Penttilä, VTT
Markku Saloheimo, VTT
Tiina Pakula, VTT
Laura Ruohonen, VTT
Niklas von Weymarn, VTT
Kalervo Hiltunen, University of Oulu
Jussi Jäntti, University of Helsinki
Matti Korhola, University of Helsinki
Kaisa Tapani, Sinebrychoff Brewery
Jari Vehmaanperä, Roal Oy
Annemari Kuokka-Ihalainen, VTT (secretary)

Scientific committee

David Archer (UK)
Eckhard Boles (DE)
Jean Marie Francois (FR)
Lisbeth Olsson (DK)
Danilo Porro (IT)
Jack Pronk (NL)
Juan Martin (ES)
Diethard Mattanovich (AT)
Joost Teixeira de Mattos (NL)
Merja Penttilä (FI)
Markku Saloheimo (FI)

Contents

Programme

Wednesday 13.6.

17.00–20.30 Registration, Marina Congress Centre

19.00–20.30 Welcome and Get-together party, Marina Congress Centre

Thursday 14.6.

Session 1: Genomics and functional analysis
Chairs: Diego Martinez, Joint Genome Institute, Los Alamos (US) and Han de Winde, Kluyver Centre Delft (NL)

8.40–9.20 **Masayuki Machida**, National Institute of Advanced Industrial Science and Technology (JP)
Transcriptional regulation of *Aspergillus oryzae* genes and fermentation

9.20–10.00 **Charles Boone**, University of Toronto (CA)
Synthetic Genetic Array Analysis: Mapping genetic networks and systematic studies of filamentous growth in yeast

10.00–10.20 **Mikko Arvas**, VTT Technical Research Centre of Finland (FI)
Comparison of protein coding gene contents of fungal phyla *Pezizomycotina* and *Saccharomycotina*

10.20–10.40 Coffee break

10.40–11.00 **Mikael Rørdam Andersen,** Centre for Microbial Biotechnology, Technical University of Denmark (DK)
Elucidating acid and enzyme production by *Aspergillus niger* with systems biology

11.00–11.20 **Olivier Guais**, Ingénierie des Systèmes Biologiques et des Procédés, INSA & CinaBio (FR)
Use of proteomic strategies for an extensive coverage of the secreted proteins from the filamentous fungus *Penicillium funiculosum*

11.20–11.40 **Gertien Smits,** Swammerdam Institute for Life sciences, University of Amsterdam (NL)
Meta-analysis of global fitness experiments reveals a novel non-transcriptionally regulated general stress response

11.40–12.00 **Harri Savilahti,** Institute of Biotechnology, University of Helsinki & University of Turku (FI)
Novel genetic tool: Transpososome-mediated insertional mutagenesis yields exhaustive mutant libraries for genomics studies in yeast

12.00–13.15 Lunch

13.15–14.45 **Poster session 1**

Session 2: Fundamental cell functions and stress
Chairs: Joost Teixeira de Mattos, University of Amsterdam (NL) and
Jack Pronk, Kluyver Centre, Leiden (NL)

14.45–15.25 **Miguel Penalva**, Centro de Investigaciones Biologicas (ES)
On how fungi and yeasts thrive under changing ambient pH conditions

15.25–16.05 **Vladimir Titorenko**, Concordia University, Montreal (CA)
The life cycle of the peroxisome

16.05–16.30 **Nuno Mira,** IBB Institute for Biotechnology and Bioengineering, Instituto Superior Técnico (PT)
Adaptive response and resistance to acetic and propionic acids: Involvement of Haa1p- and Rim101p- dependent regulons

16.30–16.50 Coffee break

16.50–17.15 **Laura Dato,** Università degli Studi di Milano-Bicocca (IT)
Overexpression of the yeast plasma membrane proton pump Pma1p can improve growth in acidic environments

17.15–17.40 **Yujiro Higuchi**, The University of Tokyo (JP)
Analysis of the endosomal compartments in the filamentous fungus *Aspergillus oryzae*

17.40–18.05 **Ana Kitanovic,** Institute for Pharmacy and Molecular Biotechnology, Ruperto-Carola University of Heidelberg (DE)
Analysis of FBPase funtional domains in *S. cerevisiae* and their role in cellular response to MMS-induced DNA damage and aging

Friday 15.6.

Session 3: Nutrition, signalling and transport
Chairs: Jean-Marie Francois, Laboratoire de Biotechnologie et Bioprocedes (FR) and Eckhard Boles, University of Frankfurt (DE)

8.40–9.20 **Johan Thevelein**, University of Leuven (BE)
Molecular genetics and physiology of nutrient regulation in yeast

9.20–10.00 **Ronald de Vries**, University of Utrecht (NL)
Regulation of genes encoding extracellular and intracellular enzymes of fungi involved in the consumption of plant polysaccharides

10.00–10.17 **Florian Freimoser**, Institute of Plant Sciences, ETH Zurich (CH)
Systematic analysis of polyphosphate in yeast reveals novel functions of phosphate transporters in polyphosphate metabolism

10.17–10.40 Coffee break

10.40–10.57 **Eckhard Boles**, Institut fuer Molekulare Biowissenschaften, Goethe-Universitaet Frankfurt (DE)
Cloning of an L-arabinose transporter from the yeast pichia stipitis and its functional expression in recombinant Saccharomyces cerevisiae

10.57–11.14 **Karen van Eunen**, Department of Molecular Cell Physiology, Vrije Universiteit (NL)
Changes in fermentative capacity under nitrogen starvation and glucose excess conditions in baker's yeast are regulated by altered V_{max} levels

11.14–11.31 **Carmen-Lisset Flores**, Instituto de Investigaciones Biomédicas (ES)
Differential repression by glucose of genes encoding gluconeogenic enzymes in *Yarrowia lipolytica*

11.31– 11.48 **Ruben Ghillebert**, Katholieke Universiteit Leuven (BE)
Regulation of phosphate signalling through the Pho84 high-affinity and the Pho87 low-affinty phosphate transporters

11.48–12.05 **Isabel Soares-Silva**, University of Minho (PT)
Structural analysis by comparative modelling of the *Saccharomyces cerevisiae* lactate permease Jen1p

12.05–13.15 Lunch

Session 4: Metabolic pathways and energetics
Chairs: Lisbeth Olsson, Technical University of Denmark (DK) and Hannu Maaheimo, VTT Technical Research Centre of Finland (FI)

13.15–13.55 **Günter Daum**, Graz University of Technology (AT)
Formation and mobilization of neutral lipid depots in the yeast

13.55–14.35 **Lisbeth Olsson**, Technical University of Denmark (DK)
Monitoring novel metabolic pathways in *Aspergillus nidulans* using metabolomics

14.35–15.00 **Pascale Daran-Lapujade**, Kluyver Lab. for the Genomics of Industrial Fermentations, Delft University of Technology (NL)
Quantitative proteomics and transcriptomics of anaerobic and aerobic yeast cultures reveals post-transcriptional regulation of key cellular processes

15.00–15.20 Coffee break

15.20–15.45 **Thomas Walther,** INSA Toulouse, Laboratoire d'Ingénierie des Systèmes Biologiques et Procédés (FR)
Response of yeast metabolism to sudden changes in glucose concentration – implication of the purine salvage pathway in purine base homeostasis

16.45–16.10 **Wolfgang Burgstaller,** Institute of Microbiology, Innsbruck (AT)
Uncoupling of catabolism and anabolism in the anamorphic fungus *Penicillium simplicissimum*

16.10–16.35 **Qiang Li**, Strathclyde Fermentation Centre, University of Strathclyde (UK)
Effect of H_2O_2 and metal catalyzed oxidation on carbohydrate and ammonia metabolism in a filamentous fungus, *Aspergillus niger B1-D*

16.40–18.00 **Poster session 2**

18.15–19.15 Reception of the City of Helsinki, Helsinki City Hall

19.15– Optional Social Program

Saturday 16.6.

Session 5: Protein production
Chairs: David Archer, University of Nottingham (UK) and Diethard Mattanovich, University of Natural Resources and Applied Life Sciences (AT)

8.40–9.20 **Stefan Wildt**, Glycofi, Lebanon, (US)
Glycoengineered *Pichia pastoris* as a novel discovery and protein expression platform for therapeutic glycoproteins

9.20–10.00 **Tiina Pakula**, VTT Technical Research Centre of Finland (FI)
Cellular responses to protein production in the fungus Trichoderma reesei

10.00–10.25 **Peter Dekker**, DSM Food-Specialties, Delft (NL)
Engineering of a novel protein secretion pathway in *Aspergillus niger*

10.25–10.45 Coffee break

10.45–11.10 **Brigitte Gasser**, University of Natural Resources and Applied Life Sciences, Vienna (AT)
Novel helper factors for protein secretion in yeasts

11.10–11.35 **Philippe Joyet**, Agroparistech, Microbiologie et Génétique Moléculaire, CBAI (FR)
Non-conventional export of the yeast enolase

11.35–12.00 **Peter Punt**, TNO Quality of Life, Zeist (NL)
New screening approaches for fungal strain development

12.00–13.15 Lunch

Session 6: Metabolite production
Chairs: Juan Martin, University of Leon (ES) and Danilo Porro, University of Milan (IT)

13.15–13.55 **Jack Pronk**, Kluyver Centre for Genomics of Industrial Fermentation (NL)
Use of auxiliary substrates to enhance product formation: stoichiometry versus kinetics

13.55–14.35 **Walter van Gulik,** Technical University of Delft (NL)
Relation between central metabolism and beta-lactam production in *Penicillium chrysogenum*: a metabolomics study

14.35–15.00 **Chris Paddon,** Amyris Biotechnologies (US)
Production of artemisinic acid, precursor to artemisinin and of the potent antimalarial combination therapies, by yeast

15.00–15.20 Coffee break

15.20–15.45 **Kari Koivuranta,** VTT Technical Research Centre of Finland (FI)
Lactic acid production by *Candida sonorensis*

15.45–16.10 **Zeynep Petek Cakar**, Istanbul Technical University (TR)
Riboflavin-producing cobalt-resistant *Saccharomyces cerevisiae* obtained by evolutionary engineering

16.10–16.35 **Gerald F. Bills**, Centro de Investigación Básica CIBE (ES)
A miniaturized media-intensive screening system for antibiotic and secondary metabolite detection from filamentous fungi

16.40– Biorefinery session

19.30– Farewell party
Restaurant ‘Katajanokan Kasino’

ABSTRACTS OF
ORAL PRESENTATIONS
T1 – T39

Transcriptional regulation of *Aspergillus oryzae* genes and fermentation

M. Machida[1,2], M. Sano[2], K. Tamano[1], Y. Terabayashi[1], N. Yamane[1], O. Hatamoto[3], G. Umitsuki[3], T. Takahashi[3], T. Toda[1], M. Sunagawa[1], H. Koike[1], K. Abe[4], Y. Koyama[3] and S. Ohashi[2]

[1] National Institute of Advanced Industrial Science and Technologies (AIST)
[2] Kanazawa Institute of Technology
[3] Noda Institute for Scientific Research
[4] Tohoku University

Aspergillus oryzae has been widely used in Japanese fermentation industries, *sake* (Japanese alcohol beverage), *miso* (soy bean paste), *shoyu* (soy sauce) and *su* (rice vinegar) for longer than a thousand of years. The high potential of secretory production of proteins has led *A. oryzae* to the application to modern biotechnology. The *A. oryzae* genome size (37.5 Mb) (Machida et al., 2005) is very close to those of *A. flavus* and *A. niger* (Archer and Dyer, 2004), and 20–30% bigger than those of *A. nidulans* (Galagan et al., 2005) and *A. fumigatus* (Nierman et al., 2005). Comparison of *A. oryzae* genome with those of the two species of smaller genome size revealed non-syntenic blocks (NSBs) specifically existing in the *A. oryzae* genome in a mosaic manner. The NSBs are highly enriched with the genes concerning secondary metabolism and the *A. oryzae*-specific genes including so called extra homologs, which are supposed to be acquired by horizontal gene transfer.

The transcriptional expression levels of the genes on NSBs were significantly lower than those on syntenic blocks (SBs) by the analysis of redundancy of ESTs (Machida et al., 2005) and by absolute gene expression levels on DNA microarray. The gene expression profiles of the two blocks, NSBs and SBs, showed marked difference. The genes on NSBs and SBs were globally down- and up-regulated, respectively, at heat shock. In contrast, the genes on NSBs appeared globally up-regulated in solid-state cultivation (SSC), which is widely used in Japanese fermentation industries. The induction of the NSB-genes in SSC suggests that the genes specifically expanded in the *A. oryzae* genome may play an important role on SSC. Considering that most of the extra homologs are involved in metabolism, the enzymes encoded on the NSB-genes might enhance degradation of the raw materials. Interestingly, most of the extra homologs repressed at heat-shock in submerged culture were not repressed in membrane cultivation condition, which was a mimic of SSC (Ishida et al., 1998). Further, we found two groups of heat-shock response genes, one induced in submerged cultivation and the other induced in membrane cultivation. This mechanism might allow efficient expression of the NSB genes in SSC. These results suggest that SSC is an ideal culture condition to take the advantage of utilizing the genes uniquely existing in the *A. oryzae* genome.

Synthetic Genetic Array Analysis: Mapping genetic networks and systematic studies of filamentous growth in yeast

C. Boone

University of Toronto, Canada

Synthetic Genetic Array (SGA) analysis automates yeast genetics, enabling a number of different large-scale/systematic studies. In particular, we are attempting to generate the complete synthetic lethal genetic interaction map for yeast cells. This map can be used to define complexes and pathways in the cell, but perhaps more importantly, it adds functional information to the protein-protein interaction map, identifying complexes and pathways that buffer one another and somehow work together as backup systems. One of our major challenges has been to generate a quantitative model for scoring genetic interactions based upon plate images and the predicted fitness of the double mutant relative to each single mutant. Another challenge has been to develop robotic platforms for our own high-throughput analysis and individual users in other labs. Another large-scale study that is relevant to this conference is our deletion mutant project in the yeast strain Σ1278b, which undergoes filamentous growth upon nutrient deprivation. We've generated a complete deletion mutant collection in Σ1278b and we are scoring haploid invasive growth, biofilm formation, and pseudohyphal growth systematically.

Comparison of protein coding gene contents of fungal phyla *Pezizomycotina* and *Saccharomycotina*

M. Arvas[1], T. Kivioja[2], A. Mitchell[3], M. Saloheimo[1], D. Ussery[4], M. Penttilä[1] and S. Oliver [5]

[1] VTT Technical Research Centre of Finland, Finland
[2] University of Helsinki, Finland
[3] European Bioinformatics Institute, UK
[4] Center for Biological Sequence Analysis Denmark
[5] University of Manchester, UK

Background. Several dozens of fungi encompassing traditional model organisms, industrial production organisms and human and plant pathogens have been sequenced recently and their particular genomic features analysed in detail. In addition comparative genomics has been applied to make analyses within sub groups of fungi. Notably, analysis of the phylum *Saccharomycotina* has revealed major events of evolution such as the recent genome duplication and subsequent gene loss. However, little has been done to gain a comprehensive comparative view to the fungal kingdom. We have carried out a computational genome wide comparison of protein coding gene content of the phyla *Saccharomycotina* and *Pezizomycotina*, which include industrially important yeasts and filamentous fungi, respectively.

Results. Our analysis shows that based on genome redundancy the traditional model organisms *Saccharomyces cerevisiae* and *Neurospora crassa* are exceptional among fungi. This can be explained by the recent genome duplication in *S. cerevisiae* and the repeat induced point mutation mechanism (RIP) in *N. crassa*. Interestingly, in *Pezizomycotina* a subset of protein families related to plant biomass degradation and secondary metabolism are the only ones showing signs of recent expansion. In addition, *Pezizomycotina* have a wealth of phylum-specific poorly characterised genes with a wide variety of predicted functions. These genes are well conserved in *Pezizomycotina*, but show no signs of recent expansion. The genes found in all fungi except *Saccharomycotina* are slightly better characterised and predicted to encode mainly enzymes. The gene classes specific to *Saccharomycotina* are enriched in transcription and mitochondrion related functions. Especially mitochondrial ribosomal proteins seem to have diverged from those of *Pezizomycotina*.

Conclusions. Our analysis predicts that all *Pezizomycotina* unlike *Saccharomycotina* can potentially produce a wide variety of secondary metabolites and secreted enzymes and that the respective genetic systems are likely to evolve fast. Both types of fungal products can be of commercial value, but on the other hand can cause harm to humans. In addition, a great number of novel predicted and known enzymes are found from all fungi except *Saccharomycotina*. Therefore further studies and exploitation of fungal metabolism appears very promising.

Elucidating acid and enzyme production by *Aspergillus niger* with systems biology

M. R. Andersen, M. G. Jakobsen, L. Lehmann and J. Nielsen

Centre for Microbial Biotechnology, BioCentrum-DTU, Technical University of Denmark

The ubiquitous and saprophytic filamentous fungus *Aspergillus niger* is of great industrial impor-tance due to its capabilities of producing organic acids and extracellular biomass-degrading proteins. Especially citric acid, gluconic acid and a multitude of hydrolases and enzyme-mixtures are commercially important *A. niger* metabolites with billion-dollar markets.

To better understand the processes governing the metabolism of these compounds, we have made several initiatives to explain the make-up of *A. niger* metabolism on a system-wide scale. Through a thorough study of the available literature, a graphical map of all reported pathways of the metabolism has been made. Together with customly designed Affymetrix DNA arrays (usable for three different *Aspergillus* species), this is being used interpret transcription data in the context of metabolic pathways. Software has been made to plot results of metabolism-scale experiments such as transcription data onto the above-mentioned graphical map, a method of analysis capable of easing the interpretation of data greatly.

Batch-cultivations of *A. niger* at different controlled pH-values and on several defined complex carbonsources have been analysed using transcription analysis and is showing the physiological response of the fungus to these conditions. New knowledge has been generated on the pH-controlled metabolism and on the carbon-source induced complex mixtures of enzymes secreted from the fungus.

Use of proteomic strategies for an extensive coverage of the secreted proteins from the filamentous fungus *Penicillium funiculosum*

O. Guais[1,3], G. Borderies[2], M. Maestracci[3], O. Tourrasse[3], J. L. Parrou[1], J. M. François[1] and M. Rossignol[2]

[1] Ingénierie des Systèmes Biologiques et des Procédés, INSA, France
[2] Plateforme protéomique, Castanet-Tolosan, France
[3] CinaBio, Adisseo France S.A.S, France

The fermentation of the filamentous fungus *Penicillium funiculosum* (Rovabio™ Excel, patent n°WO0068401) produces a large variety of hydrolytic enzymes. Nineteen different enzymes activities, mainly carbohydrolases (xylanases, β-glucanases, mannanases, pectinases, …) as well as proteases are currently followed by enzymatic dosages. This wide enzymatic profile allows an efficient action on a large variety of feedstuffs as well as on numerous animal species. In order to better know the potential of this *Penicillium funiculosum* natural cocktail, a proteomic approach has been used to characterize its protein content, taking into account that the genome of this industrial fungus has not been sequenced. The peptide sequences worked out by mass spectrometry were compared to those from recently sequenced fungal genomes including *Aspergillus niger, Aspergillus fumigatus, Aspergillus nidulans,….* A classical bi-dimensional electrophoresis performed on the enzymatic cocktail yielded at least 103 well-separated spots. The proteins within these spots were trypsin-digested and analysed by mass spectrometry. This resulted in the identification of about 100 proteins. A second method was to run the enzymatic cocktail on a one dimensional SDS-gel electrophoresis. A dozen of pieces were cut from the gel digested with trypsin and analysed by mass spectrometry. Within the 112 proteins identified by this method, only 27 were common with the previous proteomic analysis. Therefore, we carried out a third method called the 'Peptidic ShotGun'. Briefly, the crude sample of Rovabio™ Excel was hydrolysed by trypsin, the released peptides were separated by ionic chromatography and the eluates were then injected into a mass spectrometer. This method yielded 29 identifiable proteins, from which 17 were not found using the two previous techniques. This work illustrates the need to combine three proteomic strategies to identify ~ 200 proteins in the enzymatic cocktail secreted by *Penicillium funiculosum* under industrial process, among which 55 different glycosidic activities were uncovered. Furthermore, this work demonstrates the efficacy of proteome analysis to explore the genome expression of a non-sequenced organism by taking advantage of genome sequences of phylogenetically related filamentous fungi.

Meta-analysis of global fitness experiments reveals a novel non-transcriptionally regulated general stress response

A. Zakrzewska, A. Boorsma, F. M. Klis, S. Brul and G. J. Smits

Swammerdam Institute for Life sciences, University of Amsterdam, The Netherlands

An enigmatic finding in genome wide responses of *Saccharomyces cerevisiae* is the apparent absence of overlap in genes whose transcription is upregulated in response to a change in environment, and whose function is required upon that same change. To obtain insight into functional requirements of yeast in response to environmental changes, we have performed a meta-analysis of almost thousand expression and 159 genome wide mutant analyses in yeast. To compare experiments performed in different laboratories and using different methods, we have re-analyzed all data using T-profiler, an algorithm that determines the combined deviation of all genes or mutants belonging to a pre-defined group within the context of any one experiment. We find no clear co-functionality of gene groups defined by common transcriptional regulators, with the marked exception of genes controlled by Fhl1p, Rap1p and Sfp1p, transcription factors that together control ribosomal protein gene expression. Interestingly, mutants in these gene groups are generally less sensitive to environmental change than the rest of the population. We do find extensive co-functionality of gene groups derived from GO-ontology or MIPS, which share functions, localization, morphology etc. Most remarkable is the multi-environment sensitivity of mutant groups functioning in intracellular vesicular transport. Interestingly, these functional groups are not transcriptionally co-regulated and thus can be regarded as a non-transcriptionally regulated general stress response in yeast. We continue to analyze the variation of functional group requirement in relation to variation in transcriptional regulation, in order to draw conclusions about the level on which yeast regulates its responses to adapt to its ever-changing environment.

Novel genetic tool: Transpososome-mediated insertional mutagenesis yields exhaustive mutant libraries for genomics studies in yeast

H. Savilahti[1,2]

[1] Institute of Biotechnology, Viikki Biocenter, University of Helsinki, Finland
[2] Division of Genetics and Physiology, University of Turku, Finland

Insertional mutagenesis approaches to generate genomic mutant libraries constitute powerful means for the dissection of a variety of biological processes in cells. Our earlier studies have shown that bacteriophage Mu DNA transposition system can be exploited for efficient genomic insertional mutagenesis in both Gram-negative and Gram-positive bacteria. Similar approach has now been established for the yeast *Saccharomyces cerevisiae*. The procedure involves *in vitro* assembly of active DNA transposition complexes (transpososomes) with custom-designed yeast-compatible transposons, and their delivery via electroporation into cells where they faithfully insert the transposon DNA into the genome. The system proved to be highly effective also in *S. cerevisiae*, and it can be used to generate genome-wide exhaustive single-gene knockout mutant libraries for genomics studies. The insertion spectrum of transposons shows no evident bias towards any particular chromosome, and a good control over insertion copy number guarantees insertion libraries of high quality. The results indicate that the Mu transposition technology, with all its advantages, can be exploited in yeast and expands the applicability of the Mu transpososome delivery strategy into eukaryotes. As the system is intrinsically species non-specific, it should be readily modifiable for other yeast and fungal species as well.

On how fungi and yeasts thrive under changing ambient pH conditions

M. A. Peñalva

Centro de Investigaciones Biológicas CSIC, Spain

Regulation of gene expression by ambient pH involves a zinc-finger transcription factor (PacC/Rim101) and a signal transduction pathway (the *pal/RIM* pathway) involving six dedicated proteins, all of which are conserved in *Saccharomyces cerevisiae*. The *pal* pathway responds to ambient pH alkalinisation, leading to the proteolytic activation of the transcription factor and involves membrane compartments at two different levels. The Bro1-domain proteins PalA (Rim20) and PalC and the cysteine protease PalB (Rim13) are interactors of ESCRT-III components located on endosomes. With PacC/Rim101, itself a PalA/Rim20 interactor through two YPXL motifs, these proteins form an endosomal receptor module. At a second level, the 7-TMD protein PalH/Rim21 (almost certainly the receptor of the pathway) and its accessory factor PalI/Rim9 act at the plasma membrane, where the cytosolic tail of PalH interacts with PalF/Rim8. These three proteins form a plasma membrane sensor module. PalF is the first demonstrated example of a β-arrestin in the fungal lineage[1]. Arrestins typically down-regulate G-protein coupled 7-TMD receptors, leading to their 'desensitization', a paradigm recently challenged by the finding that, like PalF, metazoan β-arrestins may have a positive, rather than a negative role in signal transduction[2]. β-arrestins are demonstrated endocytic adaptors, strongly indicating that they mediate their positive role in signalling by promoting endocytosis of their cognate receptors[3]. Two recent examples of for this positive role of β-arrestin involve endocytosis of the 7-TMD protein smoothened[3,4]. Smoothened mediates activation of the Hedgehog/Sonic Hedgehog pathway, a pathway which is likely to share ancestry with the fungal pH signalling pathway[5]. In the fungal pH signalling pathway, signalling from endosomes may help bringing together two spatially separated 'sensor' and 'receptor' modules of Pal proteins.

1. Herranz, S. *et al.* Arrestin-related proteins mediate pH signaling in fungi. Proc. Natl. Acad. Sci. U. S. A 102, 12141–12146 (2005).

2. Lefkowitz, R.J. & Shenoy, S.K. Transduction of receptor signals by beta-arrestins. Science. 308, 512–517 (2005).

3. Chen,W. *et al.* Activity-dependent internalization of smoothened mediated by beta-arrestin 2 and GRK2. Science. 306, 2257–2260 (2004).

4. Wilbanks, A.M. *et al.* Beta-arrestin 2 regulates zebrafish development through the hedgehog signaling pathway. Science. 306, 2264–2267 (2004).

5. Arst, H.N., Jr. & Peñalva, M.A. pH regulation in *Aspergillus* and parallels with higher eukaryotic regulatory systems. Trends Genet. 19, 224–231 (2003).

The life cycle of the peroxisome

V. I. Titorenko

Department of Biology, Concordia University, Montreal, Quebec, Canada

Recent advances in our understanding of the strategies and molecular mechanisms that evolutionarily diverse organisms have evolved for assembling, maintaining, propagating and inheriting the peroxisome, an organelle known for its essential role in lipid metabolism, marked a Renaissance period in the field of peroxisome biogenesis. One of the hallmarks of this period is the evolution of our view on the peroxisome as a subcellular compartment that originates from the endoplasmic reticulum. Moreover, it appears that, analogous to some organelles of the secretory endomembrane system, peroxisomes found in the yeast *Yarrowia lipolytica* constitute a multi-compartmental endomembrane system. This peroxisomal endomembrane system exists as a dynamic organelle population consisting of several structurally distinct compartments that differ in their import competency for various proteins. The individual compartments of the peroxisomal endomembrane system undergo a multi-step conversion to mature peroxisomes in a time-ordered manner. Our recent studies in *Y. lipolytica* have suggested a model for the multistep process of peroxisome assembly and maturation. Despite the substantial progress achieved recently in developing a new paradigm of peroxisome biogenesis, several fundamental issues remain unresolved. The scope of my presentation is to summarize the growing evidence in support of the essential role that individual lipid species and lipid domains in the peroxisomal membrane of the yeast *Y. lipolytica* play at the checkpoints of the multistep processes of peroxisome assembly, maintenance and propagation. In addition, I will outline the most important unanswered questions related to the molecular mechanisms through which membrane lipids regulate the assembly, remodeling and functioning of protein complexes in the peroxisomal membrane at each of these checkpoints.

Adaptive response and resistance to acetic and propionic acids: Involvement of Haa1p- and Rim101p- dependent regulons

N. P. Mira[1], A. B. Lourenço[1], A. R. Fernandes[1], J. D Becker[2] and I. Sá-Correia[1]

[1] IBB- Institute for Biotechnology and Bioengineering, Centre for Biological and Chemical Engineering, Instituto Superior Técnico, Portugal
[2] Affymetrix Core Facility, Gulbenkian Institute of Science, Portugal

The understanding of the molecular mechanisms that may contribute to counteract the deleterious effects of weak acids as fungistatic agents is essential to guide suitable preservation strategies. The involvement of the transcription factor Haa1p in yeast adaptation to weak acids used as food preservatives, especially to the more hydrophilic acetic and propionic acids was recently demonstrated [1]. Genome-wide transcription analysis revealed that Haa1p is required for the activation of 65% of the acetic acid-responsive genes, mainly encoding plasma membrane and cell wall proteins of poorly characterised or unknown function, and also proteins of lipid metabolism, protein kinases and other kinases and transcription factors. The role of all the Haa1p-dependent up-regulated genes in yeast resistance to acetic acid was examined. The more evident susceptibility phenotype was registered for a mutant with the *HRK1* gene deleted. *HRK1* encodes a protein kinase involved in the activation of plasma membrane H^+-ATPase by glucose. Based on the higher accumulation of labelled acetic acid registered in the delta*hrk1* mutant, compared with the parental strain, we are examining the hypothesis of the involvement of the protein kinase Hrk1p in the modulation of the activity of plasma membrane transporters that may affect the active export of acetate.

Results from a screening for propionic acid susceptibility of the yeast disruptome indicated that *RIM101* and other members of the *RIM* pathway are determinants of resistance to propionic acid. The comparison of the transcriptomes of wild type and delta*rim101* strains indicated that *RIM101* deletion leads to the reduction of the transcript levels from several genes in unstressed cells (at pH 4.0), in particular from genes encoding cell wall proteins or proteins associated with cell wall synthesis. However, genome-wide transcription activation induced by propionic acid is apparently independent of the presence of Rim101p. *RIM101* expression was found to play a prominent role in yeast resistance to 1,3-beta-glucanase, consistent with its association with cell wall assembly. However, as suggested by the level of cellular resistance to lyticase, the presence of Rim101p had no detectable effect on cell wall remodelling registered upon weak-acid stress [2].

[1] Fernandes et al., Biochem Biophys Res Comm, 337, 95–103, 2005; [2] Simões et al., Appl Environ Microbiol, 72, 7168–7175, 2006.

Overexpression of the yeast plasma membrane proton pump Pma1p can improve growth in acidic environments

L. Dato, D. Porro and P. Branduardi

Università degli Studi di Milano-Bicocca, Dipartimento di Biotecnologie e Bioscienze, Italy

Yeasts are able to survive in acidic environments by partially counteracting the toxic effects of acids. Some yeast species are in fact the most frequent food contaminants as they can tolerate low pH together with the presence of weak organic acids, commonly used as food preservatives. Besides the problems it causes to the food industry, this feature may be an advantage if exploited for biotechnological purposes. For example, yeasts could be suitable candidates for industrial weak organic acids productions: such products are broadly utilized as additives in many industrial fields.

The acid challenge determines a general growth impairment, principally due to the huge energy demand needed to counteract cytoplasmic acidification. For the setting of a successful production process, it would be ideal to further increase the host resistance capabilities in order to improve growth levels. However, the molecular mechanisms of yeast acid stress response are not fully understood.

The plasma membrane proton pump (H^+-ATPase) Pma1p is known to be strongly implicated in the acid response in *Saccharomyces cerevisiae*. Here we present our results obtained by overexpressing the *ScPMA1* gene: growth kinetics in selective conditions show that this manipulation improves *S. cerevisiae* resistance to organic acids. Cytofluorimetric analysis reveals different fractions of dead cells in the wild type and the transformed (PMA1+) strains growing under the same physiological conditions. The improved resistance of PMA1+ strain correlates with higher Pma1p protein levels and higher Pma1p specific enzymatic activity. We then evaluated the effects of oxygenation levels and found remarkable differences between the w.t. and the PMA1+ strain in the presence of organic acid. It is important to underline that we analysed a long-term, not just a short-term, exposure to acids.

We also found that the nonconventional yeast *Zygosaccharomyces bailii*, which is more acid-resistant than *S. cerevisiae*, shows higher levels of specific Pma1p activity. We cloned the respective coding gene, previously unknown, and tested the effects of its overexpression both in *S. cerevisiae* and in *Z. bailii* cells.

Analysis of the endosomal compartments in the filamentous fungus *Aspergillus oryzae*

Y. Higuchi, J. Shoji, M. Arioka and K. Kitamoto

Department of Biotechnology, The University of Tokyo, Japan

Recently, the existence of endocytosis has been made clear in filamentous fungi. We used AoUapC-EGFP, the fusion protein of a putative uric acid-xanthine permease with EGFP (enhanced green fluorescent protein), in the filamentous fungus *Aspergillus oryzae* to visualize endocytosis. Upon the addition of ammonium into the medium the fusion protein was internalized from the plasma membrane. This internalization possessed the general features of endocytosis in that it was dependent on temperature, energy, and actin cytoskeleton. By the induction of endocytosis, EGFP fluorescence appeared as moving particulate structures within the cells, suggesting that these structures are endosomal compartments. The endosomal compartments displayed intermittent and bidirectional movement longitudinally along the hyphae, and moved more than 50 micro meters in hyphae and at the average velocity of 5 micro meters/sec in a microtubule-dependent manner. Furthermore, we examined the correlation between the dynamics of endosomal compartments and dynein, a microtubule-dependent motor. In single deletion strains of either *arpA* (actin-related protein) and *dhcA* (dynein heavy chain) encoding components of dynein motor protein complex, the endosomal compartments moving along the hyphae disappeared, suggesting that their movement is dependent on the dynein motor. At present we are further analyzing the endosomal compartments using FM4-64, an endocytic marker dye.

Analysis of FBPase funtional domains in *S. cerevisiae* and their role in cellular response to MMS-induced DNA damage and aging

A. Kitanovic and S. Wölfl

Institute for Pharmacy and Molecular Biotechnology, Ruperto-Carola University of Heidelberg, Germany

Alterations in energy metabolism can influence cellular sensitivity to oxidative stress or DNA damaging agents, aging process or apoptotic program. Our previous investigations revealed that overexpression of the key enzyme of gluconeogenesis, fructose-1,6-bisphosphatase (*FBP1*), significantly increases cellular sensitivity to DNA alkylating agent methyl methanesulfonate (MMS) and shortens chronological life span (Kitanovic and Wölfl, 2006). Here we present that the function of *S.cerevisiae* fructose-1,6-bisphosphatase in determination of cellular response to MMS is, at least partially, independent from its catalytic activity in gluconeogenesis. Site directed mutagenesis of Asn75→Ala75, a highly conserved residue in the loop 63-83 (porcine loop 52-72; Choe *et al.*, 2000) that associates to the active site of the enzyme and coordinates binding of metal ions, abolished the increased sensitivity of *FBP1* overexpressing cells to MMS. The catalytic activity of enzyme was not disrupted with this mutation. In addition, the His324→Ala324 mutation slightly reduced enzymatic activity, but the overexpression of this mutated form did not influence cellular sensitivity to MMS. On the other side, mutations in highly conserved catalytic site of the enzyme, Asp-Pro-(Ile or Leu)-Asp-(Gly or Ser)-(Thr or Ser) domain (York *et al.*, 1995), or of residues that coordinate water molecule (Choe *et al.*, 2000), Asp79→Ala79 and Glu109→Ala109, dramatically decreased both enzymatic activity and ability of the enzyme to increase cellular sensitivity to MMS. Competition analysis of cells lacking *FBP1* showed selective advantage against wild-type cells only on MMS treated medium. However, these cells were unable to recover and initiate growth when the influence of MMS was removed, suggesting that FBPase may have an important role in removing the seriously damaged cells from the population. Localisation studies proved that yeast FBPase is, in addition to its cytosolic localisation, present in the yeast nucleus. Taken together, for the first time we could show that *S.cerevisiae* FBPase is a multifunctional metabolic enzyme with important role in DNA damage defence, aging and apoptosis. Preserved catalytic function of the enzyme is necessary for this role, but it seems that conformational changes, possibly in the loop 63-83, and its localisation in the nuclei compartment are involved in this function.

Choe, J.Y, Fromm, H.J., Honzatko, R.B. (2000) Crystal Structures of Fructose 1,6-Bisphosphatase: Mechanism of Catalysis and Allosteric Inhibition Revealed in Product Complexes. *Biochemistry 39*, 8565–74.

Kitanovic, A., Wölfl, S. (2006) Fructose-1,6-bisphosphatase mediates cellular responses to DNA damage and aging in Saccharomyces cerevisiae. *Mutat. Res.* 594, 135–47.

York, D., Ponder, J.W., Majerus, P.W. (1995) Definition of a metal-dependent/Li(+)-inhibited phosphomonoesterase protein family based upon a conserved three-dimensional core structure. *Proc Natl Acad Sci U S A* 92, 5149–5153.

Molecular genetics and physiology of nutrient regulation in yeast

J. M. Thevelein, B. Bonini, D. Castermans, S. Haesendonckx, J. Kriel, W. Louwet, T. Peeters, Y. Popova, W. Schepers, S. Van de Velde, P. Vandormael, G. Van Zeebroeck, P. Verhaert, M. Versele and K. Voordeckers

Laboratory of Molecular Cell Biology, Katholieke Universiteit Leuven and Department of Molecular Microbiology, VIB, Belgium

Nutrients exert dramatic regulatory effects on cells of yeast and other fungi. Although single nutrient effects have been studied most, in some cases different nutrients can affect the same signaling pathway. A striking example is found in yeast where rapidly-fermented sugars and other nutrients that are essential for growth (i.e. nitrogen source, phosphate and sulfate) activate the PKA pathway. This pathway controls many targets in metabolism, physiology and development. Glucose and sucrose activate the PKA pathway through stimulation of cAMP synthesis via a G-protein coupled receptor system and an ill-defined glucose phosphorylation dependent system. Recent work has identified a bypass pathway for adenylate cyclase, directly from the Galpha protein Gpa2 to PKA. This pathway may play a role in the integration of the glucose signal with signals generated by other nutrients. Addition of an amino acid or ammonium to nitrogen-starved cells or addition of phosphate to phosphate-starved cells in the presence of glucose triggers rapid activation of the PKA pathway. In this case it is not mediated by cAMP as second messenger. Recent work has shown that amino acids, ammonium as well as phosphate are sensed by transporter-receptor proteins, called transceptors, Gap1 in the case of amino acids, Mep2 in the case of ammonium and Pho84 in the case of phosphate. For Gap1, we have screened a collection of amino acid analogues for inhibitors of Gap1 transport and identified compounds that are not transported and act as competitive inhibitors. Some of these compounds acted as agonist of the signaling function of Gap1for activation of the PKA pathway whereas others did not. The latter indicates that mere binding of a compound to Gap1 is not sufficient to activate its amino acid sensing function. Our results suggest that Gap1 signaling requires a ligand-induced specific conformational change, which might be part of, but does not require the complete transport cycle. Using SCAM analysis (Substituted Cysteine Accessibility Method) we identified the Ser^{388} and Val^{389} residues as being exposed in the amino acid binding site of Gap1 and we found that transported and non-transported agonists use the same binding site for activation of signaling as that used in the transport process. Although the signaling pathway from Gap1 to PKA is not clear yet, we have obtained evidence for involvement of the yeast PDK1 homologues (Pkh1, Pkh2 and Pkh3) and the yeast PKB homologue Sch9.

Regulation of genes encoding extracellular and intracellular enzymes of fungi involved in the consumption of plant polysaccharides

R. P. de Vries

Microbiology, Science Faculty, Utrecht University, The Netherlands

Plant polysaccharides are the predominant carbon source for most of the fungal species in particular saprophytic fungi. As polymeric compounds can not be taken up by the fungal cell, they need to be degraded to monomeric and small oligomeric compounds. To this end, fungi produce a wide range of polysaccharide degrading enzymes, some of which are specific for one polysaccharide while others are active on several polysaccharides. To produce the most optimal mix of enzymes a complex regulatory network is required that dives expression of the corresponding genes. These systems have been studied in most detail in *Aspergillus niger* and *Trichoderma reesei*. In this presentation I will discuss the various regulatory systems of these fungi involved in polysaccharide degradation. In addition, these regulatory systems have also been shown to affect metabolic pathways related to monomeric sugars, thus linking liberation of the monomeric components to intracellular utilization. These links will be discussed and the best studied case, pentose catabolism in *A. niger*, will be presented as an example.

Systematic analysis of polyphosphate in yeast reveals novel functions of phosphate transporters in polyphosphate metabolism

H. C. Hürlimann, N. Amrhein and F. M. Freimoser

Institute of Plant Sciences, ETH Zurich, Switzerland

Inorganic polyphosphate (poly P) is a linear polymer that consists of phosphoanhydride linked phosphate residues, occurs in all organisms and cells, functions as a phosphate store and buffer and regulates the activation of enzymes or gene expression. The yeast *Saccharomyces cerevisiae* can store up to 20% of its dry weight in the form of poly P, almost entirely in the vacuole. Despite this ubiquitous occurrence and important functions it is still unclear how poly P is synthesized and how poly P metabolism is regulated in eukaryotes.

To elucidate poly P metabolism in eukaryotes we have performed a systematic screen of poly P levels in the knockout strains of all non-essential yeast genes and thereby identified 255 genes (almost 4% of the yeast genome) that are involved in the maintenance of normal poly P levels. Many of these mutants were knockouts of genes that encode vacuolar proteins or proteins functioning in intracellular transport and cell homeostasis. Besides reduced poly P content, many strains also exhibited reduced total phosphate content, showed altered ATP and glycogen levels and were disturbed in the secretion of acid phosphatase. Therefore, poly P metabolism strongly influences phosphate metabolism and the *PHO* pathway and is tightly interconnected with primary metabolism, in particular energy metabolism.

Poly P content was then used as the read-out for a thorough characterization of mutant strains affected in the *PHO* pathway and in the five phosphate transporters in yeast. Only one of the five yeast phosphate transporters, Pho84, could support normal poly P levels if present alone. Pho84 is therefore the most important phosphate transporter in yeast; both for phosphate uptake and for poly P accumulation. Surprisingly, the low-affinity transporters Pho90 and Pho91 negatively regulated poly P metabolism and caused an increase in poly P content when deleted. Detailed analysis of these deletions showed that the inhibitory effect of Pho90 or Pho91 on poly P accumulation was not due to a regulation of the *PHO* pathway or phosphate uptake. In contrast, it was concluded that these two proteins serve as intracellular phosphate transporters that function in phosphate translocation and allocation within the cell. With poly P content as a novel distinguishing mark it was thus possible to define novel functions of the low-affinity phosphate transporters in phosphate, poly P and cell metabolism.

Cloning of an L-arabinose transporter from the yeast *Pichia stipitis* and its functional expression in recombinant *Saccharomyces cerevisiae*

M. Keller and E. Boles

Institut fuer Molekulare Biowissenschaften, Goethe-Universitaet Frankfurt, Germany

Lignocellulosic biomass is considered to be an ecologically and economically ideal feedstock for the production of bioethanol. Hydrolysates hereof contain hexoses and pentoses. L-arabinose is, after D-xylose, the second most abundant pentose in lignocellulosic hydrolysates. Recombinant *S. cerevisiae* strains fermenting both pentoses to ethanol have recently been developed.

The uptake of L-arabinose across the plasma membrane is regarded as a limiting factor in the fermentation of the pentose to ethanol. In *S. cerevisiae*, a specific uptake system for L-arabinose does not exist and L-arabinose is only transported unspecifically by the galactose permease Gal2.

We have developed a screening system for L-arabinose transporter genes by using a *S. cerevisiae hxt* null mutant strain (EBY.VW4000) engineered for L-arabinose utilization. This strain is not able to take up hexose and pentose sugars, and therefore cannot grow on them. Functional expression of an L-arabinose transporter gene should enable the tester strain to grow with L-arabinose as the only carbon source. We have screened a genomic DNA library of the yeast *P. stipitis* which is able to grow on L-arabinose media.

We cloned a gene encoding a protein with homology to high-affinity sugar transporters, containing 12 predicted hydrophobic membrane spanning domains. Expression of this gene in the *S. cerevisiae* tester strain preferentially restored growth with L-arabinose but not with other sugars, indicating that the transporter is rather specific for uptake of L-arabinose. We will present physiological and biochemical data characterizing the first L-arabinose transporter that could be functionally expressed in *S. cerevisiae*. Furthermore, we will report data about its influence on L-arabinose fermentation in recombinant *S. cerevisiae* cells.

Changes in fermentative capacity under nitrogen starvation and glucose excess conditions in baker's yeast are regulated by altered V_{max} levels

K. van Eunen[1], J. Bouwman[1], A. Lindenbergh[1], H.V. Westerhoff[1,2] and B.M. Bakker[1]

[1] Department of Molecular Cell Physiology, Vrije Universiteit, The Netherlands
[2] Manchester Centre for Integrative Systems Biology, The University of Manchester, UK

Adaptation of yeast cells to changes in environment needs action of many different regulatory mechanisms, such as changes in mRNA and protein levels, post-translational modification of enzymes and feedback inhibition by intermediates of metabolism. Time-dependent hierarchical regulation analysis determines the relative importance of these regulatory mechanisms when the cell is actively adapting its physiology. Regulation can be divided into *(i)* changes of maximum enzyme activity (V_{max}, called hierarchical regulation) and *(ii)* changes in interaction of the enzyme to its substrate(s) and product(s) (called metabolic regulation). This is the first experimental study in which the regulation in time was followed for changes in fermentative capacity occurring during nitrogen starvation in the presence of a glucose excess, to prevent double starvation. Therefore, a control experiment was performed in which nitrogen and glucose was present in excess, to discriminate the effects due to nitrogen starvation and glucose excess conditions. Fermentative capacity was decreased when the yeast cells were starved for nitrogen, but increased under glucose excess conditions. In order to determine whether the change in fermentative capacity is caused by a change in V_{max} (hierarchical regulation) or by a change at metabolic level regulation analysis was performed. Results showed that a group of enzymes is completely hierarchical regulated one hour after the start of the perturbation. An explanation for this could be that the fermentative capacity is an offline measurement, while the enzyme activity is measured in samples directly from the fermentor. In the case of the nitrogen starvation, these results indicate that protein degradation plays an important role in regulation of the fermentative capacity.

Differential repression by glucose of genes encoding gluconeogenic enzymes in *Yarrowia lipolytica*

C.-L. Flores, R. Jardón and C. Gancedo

Instituto de Investigaciones Biomédicas "Alberto Sols" CSIC-UAM, Spain

The repression by glucose of the transcription of the genes *YlFBP1, YlPCK1* and *YlICL1* encoding respectively fructose-1,6-bisphosphatase, PEP carboxykinase and isocitrate lyase has been studied in *Y. lipolytica*. Levels of enzymatic activity and of mRNA were compared in cultures grown in media with different gluconeogenic carbon sources or with glucose. Levels of Pck and Icl were much lower in glucose grown cultures than in those with gluconeogenic carbon sources and the corresponding mRNAs showed a repression of the transcription of the corresponding genes although a basal level of transcription was detectable in glucose cultures. In contrast, enzyme levels of fructose-1,6-bisphosphatase remained constant in all carbon sources tested including glucose. The protein measured in western blot experiments remained also constant and the levels of mRNA followed the same pattern.

The gene *YlFBP1* has been cloned and disrupted with two different disruption cassettes. The disruption abolished growth in gluconeogenic carbon sources in short term experiments. Surprisingly when the promoter of *YlFBP1* was fused to *E.coli lacZ* a certain repression by glucose was observed. The amount of beta-galactosidase increased when the construction was expressed in an *YlFBP1* disrupted background suggesting that the lack of enzyme influenced its transcription. Initial experiments have shown that a part of YlFbp is located within the nucleus.

Strains with a disrupted *YlFBP1* grew after several days in gluconeogenic carbon sources. The growth was not due to the appearance of suppressors but to the existence of a phosphatase activity that hydrolyzed fructose-1,6-bisphosphate. Levels of this activity remained constant in different carbon sources and amounted to about 15% of the total fructose-1,6-bisphosphatase measured. This activity was not due to a classical fructose-1,6-bisphosphatase as shown by its lack of inhibition by AMP and fructose-2,-6-bisphosphate. The affinity of the enzyme for fructose-1,6-bisphosphate was low (Km 2.3 mM) as compared with that of the genuine fructose-1,6-bisphosphatase (Km 30 μM) Our results show that regulation of gluconeogenesis in *Y. lipolytica* differs substantially from that observed in the model yeast *Saccharomyces cerevisiae*.

Regulation of phosphate signalling through the Pho84 high-affinity and the Pho87 low-affinty phosphate transporters

E. Swinnen, R. Ghillebert, J. Rosseels and J. Winderickx

Functional Biology, Katholieke Universiteit Leuven, Belgium

When starved for phosphate, the main phosphate transport activity in yeast is performed by the Pho84 high-affinity permease, consistent with the induction of *PHO84* expression by the PHO pathway under these conditions. In addition, the Pho84 transporter is also required for rapid phosphate signalling after re-addition of Pi to starved cells. However, we clearly observed phosphate-induced signalling in strains with a deletion of either *PHO4* or *PHO81*, both of which lack expression of *PHO84*. We found that phosphate signalling in these strains is mediated specifically by the Pho87 low-affinity phosphate transporter. Interestingly, the negative regulation of Pho87 function by the PHO pathway occurs at a posttranscriptional level and Pho87 acts as a low-affinity phosphate sensor. We also show that Pho4 activity is modulated by glucose signalling which may offer a physiological relevance of phosphate signalling via Pho87.

Interestingly, we found that the Rim15 protein kinase, a direct target of the Pho85-Pho80 kinase, is involved in nuclear exclusion of Pho4 upon glucose removal, indicating reciprocal regulation between glucose and phosphate signalling via Rim15. Hence, a model emerges wherein Rim15 does not solely control G0-entry by functioning downstream of nutrient-regulated signalling pathways but also plays a crucial role in the dynamical cross-talk between its own upstream signalling pathways.
Finally, we demonstrate that optimal growth recovery after phosphate starvation depends on the interplay of rapid phosphate consumption and subsequent inactivation of the Pho4 transcription factor, and that an active Pho4 appears to have a general growth-inhibiting effect.

Structural analysis by comparative modelling of the *Saccharomyces cerevisiae* lactate permease Jen1p

I. Soares-Silva[1], S. Paiva[1], G. Diallinas[2] and M. Casal[1]

[1] Center/Department of Biology, University of Minho, Portugal
[2] Faculty of Biology, Department of Botany, University of Athens, Greece

In *Saccharomyces cerevisiae* the lactate/proton symporter is encoded by the *JEN1* gene, a member of the lactate/pyruvate:H^+ symporter subfamily (TC#2.A.1.12.2) of the Major Facilitator Superfamily [1]. Structure-function relationships in Jen1p were studied using a rational mutational analysis based on the identification of conserved amino acid residues. The conserved sequence ^{379}**N**XX[S/T]**H**X[S/T]**QD**XXX**T**^{391} was found to be involved in the function definition of Jen1p substrate translocation pathway [2]. Substitution of these amino acid residues, even with very similar amino acids, resulted in altered transport capacity and substrate specificity. Residues N379, H383 or D387 were involved in changes both at the transport capacity level as well as at Jen1p specificity. Substitutions of Q386 and T391 resulted in no or moderate changes in Jen1p transport capacities but presented altered binding affinities, furthermore several of the mutants showed altered inhibition constants for several carboxylic acids tested. Given the high probability of Jen1p having a common structure with the LacY and GlpT, we were able to create *in silico* a three dimensional model for Jen1p, by homology threading. In this model, the domain is predicted to be located in the 7th transmembrane segment; the amino acids N379, H383, Q386 and D387 are facing the internal pore confirming the potential to interact directly with the substrate; T391 is the only one facing outside the pore, being this the one residue that less affects binding and transport capacity. Based on the kinetic analysis and the fact that 379 is an irreplaceable residue, we proposed that N379 might bind Jen1p substrates. It is possible that, as in the case of LacY and GlpT [3, 4], the two MFS proteins with solved structures, TMS7 of Jen1p is part of the substrate translocation pathway.

This work was supported by Portuguese grant POCI/BIA-BCM/57812/2004 (Eixo 2, Medida 2.3, QCAIII - FEDER). ISS received a fellowship from the Portuguese government: SFRH/BPD/22976/2005.

[1] Casal, M., Paiva, S., Andrade, R. P., Gancedo, C. and Leão, C. (1999) Journal of Bacteriology 181, 2620–2623.
[2] Soares-Silva, I., Paiva, S., Diallinas, G. and Casal, M. (2007) Molecular Membrane Biology, in press.
[3] Huang, Y. F., Lemieux, M. J., Song, J. M., Auer, M. and Wang, D. N. (2003) Science 301, 616–620.
[4] Abramson, J., Smirnova, I., Kasho, V., Verner, G., Kaback, H. R. and Iwata, S. (2003) Science 301, 610–615.

Formation and mobilization of neutral lipid depots in the yeast

G. Daum[1], K. Athenstaedt[1], A. Wagner[1], T. Czabany[1], K. Grillitsch[1], S. Rajakumari[1], M. Spanova[1], D. Zweytick[2] and E. Ingolic[3]

[1] Institute of Biochemistry, Graz University of Technology, Austria
[2] Institute for Biophysics and Nanosystem Research, Academy of Sciences, Austria
[3] Research Institute for Electron Microscopy, Graz University of Technology, Austria

In yeast as in most other cells neutral lipids TAG (triacylglycerols) and STE (steryl esters) are stored in subcellular fractions called lipid particles. In *Saccharomyces cerevisiae,* TAG and STE serve as a depot of building blocks for the formation of membranes. Major enzymes catalyzing synthesis of these neutral lipids, namely the TAG synthases Dga1p and Lro1p, and the steryl ester synthases Are1p and Are2p, have been identified and partially characterized. Recently, we investigated the process of neutral lipid storage in more detail making use of triple mutants with only one of the gene products, Dga1p, Lro1p, Are1p or Are2p, active. All four triple mutants form lipid particles, although at different rate and different lipid composition. These experiments showed, however, that TAG or SE alone are sufficient to form lipid particles. The different types of lipid particles from the respective mutants were investigated using biochemical, cell biological and biophysical methods. Mobilization of neutral lipid depots occurs through catalysis of TAG and STE hydrolases. Three TAG lipases named Tgl3p, Tgl4p and Tgl5p, and three STE hydrolases named Tgl1p, Yeh1p and Yeh2p were identified at the molecular level. Although these hydrolases exhibit overlapping function, they are specific to some extent regarding substrate specificity. The coordinate process of neutral lipid depot mobilization is currently under investigation. In summary, our investigations demonstrated that enzymes involved in TAG and STE metabolism exist in redundancy, and enzymatic steps of neutral lipid storage and mobilization require strict regulation.

This work was financially supported by FWF projects 18857 and W901-B05 (DK Molecular Enzymology) to G.D.

Monitoring novel metabolic pathways in *Aspergillus nidulans* using metabolomics

G. Panagiotou and L. Olsson

Center for Microbial Biotechnology, Kgs Lyngby, Denmark

Metabolomics is one of the omics tools that rapidly are developing, but still are less developed and used than transcriptomics. This presentation will discuss some of the possibilities of using metabolomics for characterising metabolic pathways. As the prime example, the physiological phenotype of *Aspergillus nidulans,* which was quantified by the intracellular and extracellular metabolite pools, will be discussed. The collected data gave clear evidence for the presence of a novel fungal metabolic pathway, the phosphoketolase pathway, in *A. nidulans*. Metabolomics and machine learning tools were successfully used to monitor the alteration caused by the inhibition of glyceraldehyde-3-P dehydrogenase (G3PD) in the metabolism of *A. nidulans* grown on different carbon sources. Furthermore, the gene encoding phosphoketolase was overexpressed in order to elucidate the importance of the phosphoketolase pathway in the metabolism of *A. nidulans.* The fluxes were quantified through metabolic flux analysis using fractional enrichment data where the carbon labeling experiments were performed with a reference strain (*A. nidulans* A4) and a mutant strain overexpressing the phosphoketolase gene.

Quantitative proteomics and transcriptomics of anaerobic and aerobic yeast cultures reveals post-transcriptional regulation of key cellular processes

M. J. L. de Groot[1,4,5], P. Daran-Lapujade[2,4], B. van Breukelen[1,5], T. A., Knijnenburg[3,4], E. de Hulster[2,4], M. J. T. Reinders[3,4], J. T. Pronk[2,4], A. J. R. Heck[1,5] and M. Slijper[1,5]

[1] Department of Biomolecular Mass Spectrometry, Utrecht University, The Netherlands
[2] Department of Biotechnology, Delft University of Technology, The Netherlands
[3] Information and Communication Theory Group, Delft University of Technology, The Netherlands
[4] Kluyver Centre for Genomics of Industrial Fermentation, The Netherlands
[5] Netherlands Proteomics Centre, The Netherlands

Background: *Saccharomyces cerevisiae* is unique among yeasts for its ability to grow rapidly in the complete absence of oxygen. *S. cerevisiae* is therefore an ideal eukaryotic model to study physiological adaptation to anaerobiosis. Recent transcriptome analyses have identified hundreds of genes that are transcriptionally regulated by oxygen availability but the relevance of this cellular response has not been systematically investigated at the key control level of the proteome.

Results: The proteomic response of the *S. cerevisiae* to anaerobiosis was investigated using metabolic stable isotope labeling in aerobic and anaerobic glucose-limited chemostat cultures, followed by proteome analysis to relatively quantify protein expression. Using independent replicate cultures and stringent statistical filtering, a robust dataset of 474 quantified proteins was generated, of which 249 showed differential expression levels. While some of these changes were consistent with previous transcriptome studies, many responses of *S. cerevisiae* to oxygen availability were hitherto unreported. Comparison of transcriptome and proteome from identical cultivations yielded strong evidence for post-transcriptional regulation of key cellular processes, including glycolysis, amino-acyl tRNA synthesis, purine-nucleotide synthesis and amino-acid biosynthesis.

Conclusions: The use of chemostat cultures provided well-controlled and reproducible culture conditions, which are essential for generating robust datasets at different cellular information levels. Integration of transcriptome and proteome data led to new insights in the physiology of anaerobically growing yeast that would not have been apparent from differential analyses at either the messenger RNA or protein level alone, thus illustrating the power of multi-level studies in yeast systems biology.

Response of yeast metabolism to sudden changes in glucose concentration – implication of the purine salvage pathway in purine base homeostasis

T.H. Walther, M. Novo, M.O. Loret and J. M. Francois

INSA Toulouse, Laboratoire d'Ingénierie des Systèmes Biologiques et Procédés, France

Respiring yeasts that are exposed to a sudden increase of the glucose concentration in their growth medium respond by a dramatic reprogramming of their metabolism. This transition from respiratory to respiro-fermentative growth can be distinguished into an immediate metabolic response, and a subsequent reprogramming of the yeast transcriptome and proteome facilitating the repartitioning of carbon flux between mitochondria, reserve carbohydrate and fermentative pathways, respectively. The present study focuses on the early transition period which is characterized by the accumulation of glycolytic intermediates upstream of GAPDH and a drastic drop of ATP. These observations were earlier explained by an imbalance of the carbon flux through the upper and lower part of the EMP pathway. However, the pronounced drop of the cellular adenine nucleotide content (AXP = ATP + ADP + AMP) represented a long standing problem since neither its metabolic fate, nor the reason for this behaviour could be explained until now.

We demonstrate that AXP nucleotides are converted to inosine by the purine salvage pathway after the glucose pulse. The accumulation of inosine is transient and reaches its maximum approximately 10 minutes after the pulse. Initial AXP levels are restored 30 minutes after exposure to glucose, concurrently with the complete degradation of the inosine pool. We identified the metabolic pathway for inosine formation and recycling to AXP. Furthermore, we investigated the kinetics of inosine accumulation and its recycling to AXP in different mutants, monitored changes in the enzymatic activities of implicated pathway reactions, and identified signal transduction cascades that are involved in the regulation of the process. Our results show that the cycling of AXP nucleotides through the purine salvage pathway is a regulated process rather than a passive response of the metabolism. It involves changes of enzyme activities at a very short time scale, and can be altered by mutations in various signal transduction pathways.

Uncoupling of catabolism and anabolism in the anamorphic fungus *Penicillium simplicissimum*

W. Burgstaller, P. Vrabl, W. Mutschlechner and M. Gallmetzer

Institute of Microbiology, Innsbruck, Austria

The excretion of incompletely oxidized metabolites ("organic acids") under aerobic conditions is a frequent phenomenon with anamorphic fungi – in soil and in laboratory cultures. This property is exploited, e. g. for the industrial production of citric acid by *Aspergillus niger* or for the leaching of metals with *Penicillium simplicissimum*. In the early stages of our work we wanted to increase citrate excretion by *P. simplicissimum* to improve metal leaching efficiency. Later we became aware, that this overflow-metabolism may be an important regulatory point in energy metabolism. The most obvious physiological role of overflow-metabolism is to adjust the catabolic carbon flow to the nutrient supply and the activity of catabolic pathways. Overflow-metabolism is energy spilling, which makes it relevant at high energy need, e. g. during the expression of heterologous proteins. Despite of this general importance of overflow-metabolism, systematic studies with anamorphic fungi are rare, partly because cultivation of these fungi in a chemostat is not as simple as with single-celled bacteria or yeasts.

We studied the excretion of organic acids by *P. simplicissimum* in the chemostat at several specific growth rates (0.02–0.2 h^{-1}), at different nutrient limitations (glucose, NH_4, PO_4, NO_3, K), pH values (2–7) and osmolarities (0.2–1.5 osmol kg^{-1}). We also tested the effect of the inhibitors benzoic acid, imidazole, 2,4-dinitrophenol, gaseous nitrogen, and salicylhyroxamic acid. Additionally, we carried out transients from glucose limitation to ammonium limitation. During the transients we determined the Energy Charge to look, if the EC changes simultaneously with the onset of organic acid excretion. Our main interest with the transients was not the short time response to a glucose pulse, but the longer period from the start of the transient until a distinct excretion of organic acids could be observed (between 3 and 10 hours).

To complement the picture we are working on the following questions:

- Is glucose at high concentrations (100–400 mM) taken up by simple diffusion? Presently, the answer is no.
- Is glucose uptake at high concentrations controlled by metabolism? Presently, the answer is yes.
- Does the glucose concentration influence the extent of overflow-metabolism? Presently, the answer is yes.

Selected results will be discussed in the presentation. The important issue of the pronounced strain specificity of overflow-metabolism will be addressed too.

Effect of H_2O_2 and metal catalyzed oxidation on carbohydrate and ammonia metabolism in a filamentous fungus, *Aspergillus niger B1-D*

Q. Li, L. M Harvey and B. McNeil

Strathclyde Fermentation Centre, University of Strathclyde, Glasgow, UK

H_2O_2, is an inevitable by product of all living organisms which rely on aerobic respiration for energy production. The main site of H_2O_2 production is the respiratory chain, and it is also involved in a number of oxidase reactions, such as beta-oxidation. The cytotoxicity of H_2O_2 is due to its ability to damage cellular macromolecules, including DNA, lipids and proteins. Moreover, H_2O_2 is able to react with transition metal ions giving rise to highly toxic hydroxyl radicals in the Fenton reaction, the process of which is usually regarded as metal catalyzed oxidation (MCO). Previous studies have shown that H_2O_2 has profound effects on the physiology of filamentous fungi, including growth arrest, protein degradation and induction of antioxidant enzymes. However, little is known about the effects of H_2O_2 on the substrate metabolism. In order to close this gap, we used *Aspergillus niger* as a model, to study the responses of carbohydrate and ammonia metabolism to addition of exogenous H_2O_2. A series of H_2O_2 ranging from 0.1 to 1mM were added to batch cultures of *A. niger* in exponential phase. 1mM H_2O_2 with 0.2mM ferrous sulfate was also added to elucidate the effects of MCO. We monitored extracellular glucose and ammonia, and intracellular trehalose concentrations in these cultures by accurate enzymatic assays. Our results indicate that H_2O_2 addition has a dose-dependant, inhibitory effect on both glucose and ammonia uptake rates, and it also promotes intracellular trehalose accumulation, a key carbon reserve and stress metabolite in microorganisms, at the same time. We have found that the reduced glucose uptake could be attributed to the decreased glyceraldehydes-3-phosphate dehydrogenase activity(GAP-DH, a glycolytic enzyme) activity by H_2O_2. In addition, we showed that glucose-6-phoshpate dehydrogenase (G6P-DH) activity, which catalyzes the first step of pentose phosphate pathway (PPP), is quite resistant to H_2O_2 stress. Following H_2O_2 addition trehalose accumulated in these cultures. This may indicate that carbon flux is directed from glycolysis towards trehalose synthesis in response to H_2O_2. Interestingly, the activity of two enzymes responsible for ammonia condensation in fungi, glutamine synthetase (GS) and glutamate dehydrogenase (GDH), showed great resistance to H_2O_2 up to 1mM. We propose that the decreased ability to utilize ammonia could be due to reduced production of ATP and NADPH *in vivo* which are required by these two enzymes to function.

Glycoengineered *Pichia pastoris* as a novel discovery and protein expression platform for therapeutic glycoproteins

S. Wildt

GlycoFi Inc. Lebanon, USA

Recent findings have helped to establish a relationship between the function of therapeutic proteins, in particular antibodies, and distinct N-glycosylation structures. Mammalian production cell lines, such as CHO cells, secrete therapeutic glycoproteins as a mixture of glycoforms. Within this mixture certain glycoforms are known to confer greater therapeutic efficacy than others.

Yeast as a production system offers a number of distinct advantages: Short cycle-times from sequence to production cell-line, demonstrated bioprocess scalability, a sequenced genome as well as well developed molecular and cell-biology tools.

Over the past years we have re-engineered the glycosylation machinery of *Pichia pastoris*. Targeted yeast metabolic engineering efforts have allowed us to create biosynthetic pathways required for human-like glycosylation, such as UDP-galactose and CMP-sialic pathways. The creation of yeast strains capable of fully complex human N-linked glycosylation required the coordinated expression of close to twenty heterologous genes and a number of gene deletions. We have created a library of glycoengineered strains. Each of these strains is capable of producing proteins with one particular glycoform at near uniformity. Currently we are utilizing a library of strains to not only take advantage of a yeast based production system with specific and predetermined oligosaccharide structures but also to identify the glycoforms which impart the highest level of efficacy on therapeutic glycoproteins.

Cellular responses to protein production in the filamentous fungus *Trichoderma reesei*

T. M. Pakula

VTT Technical Research Centre of Finland, Finland

The filamentous fungus *Trichoderma reesei* is known as an efficient producer of a variety of extracellular enzymes, the major products being cellulases and hemicellulases e.g. cellobiohydrolases, endoglucanases, β-glucosidases, xylanases, and hemicellulose side-chain cleaving enzymes. Altogether, analysis of the genome sequence has revealed over 200 genes classified in glycoside hydrolase gene families. *T. reesei* has potential to produce extracellular proteins in very large quantities, and it has been used as an industrial host organism for production of both the fungal enzymes as well as for heterologous proteins.

Production of secreted proteins in large quantities or production of the heterologous proteins originating from distantly related organisms challenge the capability of the cells to fold and transport the proteins, and are known to provoke stress responses in the cell. Impaired protein folding in the endoplasmic reticulum (ER) activates the unfolded protein response pathway (UPR) which result in induction of a number of genes involved e.g. in folding, glycosylation and transport. The fungal cells have also a feed-back mechanism to reduce the load in the secretory pathway by negative transcriptional regulation of genes encoding the major secreted proteins. The availability of the genome sequence information has made it possible to apply genome-wide approaches in studies of the cellular responses to protein production under different conditions.

Specifically, we have compared the effects of production of two different heterologous proteins, human tPA and *Melanocarpus albomyces* laccase, in *T. reesei* using proteome and transcriptome data. The analysis showed a clear difference between the responses induced by the proteins, the main difference being in the induction of the UPR pathway. Furthermore, in order to obtain information on protein production at different physiological conditions we have carried out transcriptome and proteome analysis of carbon-limited chemostat cultures of *T. reesei* under different conditions, e.g. at different specific growth rates and cell density.

Engineering of a novel protein secretion pathway in *Aspergillus niger*

R. Damveld, P. ten Haaft, M. Hartog, I. Minneboo, P. Dekker, T. Wenzel and C. Sagt

DSM Food-Specialties, Delft, The Netherlands

Production of enzymes on an industrial scale is often limited to extracellular enzymes. When enzymes are produced intracellular, the downstream processing is costly and process robustness is not guaranteed.

We have developed a technology which enables the secretion of enzymes which are normally localized in the cell. The folding conditions of intracellular proteins differ from those of secreted proteins. In the cytosol proteins fold under relative reducing conditions compared to the oxidizing environment in the ER. Moreover, many secreted proteins undergo extensive N-glycosylation and disulphide bridge formation. The set of folding enzymes and chaperones in the secretory pathway and in the cytosol are different as well. Therefore the simple solution of forcing intracellular proteins through the secretory pathway does in many cases fail to result in biologically active secreted enzymes.

We have developed a method to secrete intracellular enzymes in an active form. This technology allows the intracellular enzymes to be folded in their native environment in the cytosol using cytosolic folding enzymes and chaperones. After folding the intracellular proteins are translocated to a modified cell compartment. After translocation in this modified cell compartment the content is released into the medium by a specific process. Using controlled fermentations we have established that the physiology of cells containing this novel secretory system is not dramatically different form non-modified cells. We will show the recently obtained proof of principle of this concept with the Green Fluorescent Protein (GFP). In addition we have demonstrated the concept for intracellular enzymes, which are secreted in an active form using this novel approach. Moreover we also have preliminary evidence that this technology can be used to secrete metabolites, which are normally localized intracellular.

This is the first report that describes the introduction of a completely novel secretory pathway in eukaryotic cells, which allows the production of intracellular enzymes in a secreted active form enabling industrial application on an economical feasible scale.

Novel helper factors for protein secretion in yeasts

B. Gasser[1], M. Sauer[1,2], M. Maurer[1,2], K. Benakovitsch[1] and D. Mattanovich[1,2]

[1] University of Natural Resources and Applied Life Sciences, Institute of Applied Microbiology, Vienna, Austria
[2] School of Bioengineering, University of Applied Sciences, Austria

Efficient production of heterologous proteins with yeasts and other eukaryotic hosts is often hampered by inefficient secretion of the product. Limitation of protein secretion has been attributed to a low folding rate, and a rational solution is the overexpression of proteins supporting folding, like protein disulfide isomerase (PDI), or the unfolded protein response transcription factor HAC1.

Assuming that other protein factors which are not directly involved in protein folding may also support secretion of heterologous proteins, we set out to analyze the differential transcriptome of a *Pichia pastoris* strain overexpressing human trypsinogen versus a non-expressing strain. 524 genes were identified to be significantly regulated. Excluding those genes with totally divergent functions (like e.g. core metabolism), we reduced this number to 64 genes with potential function in the secretion machinery and in stress regulation. Out of these, 13 genes which were upregulated in the expression strain, and are not part of a protein complex, were selected for further analysis.

The respective *Saccharomyces cerevisiae* homologs of these genes, plus PDI1 and HAC1 as positive controls were cloned and overexpressed in a *P. pastoris* strain expressing a human antibody Fab fragment. All genes except one showed a positive effect on Fab fragment secretion, as did the controls. The increase of secreted product concentration ranged from over 2 fold to a moderate 10 % increase.

Potential effects of these novel secretion helper factors as well as the practical application in lab scale production processes will be discussed.

Non-conventional export of the yeast enolase

P. Joyet, G. Boel, J. Deutscher and J. M. Beckerich

Agroparistech, UMR INRA-CNRS Microbiologie et Génétique Moléculaire, France

Enolases of different bacteria and the yeast *Saccharomyces cerevisiae* use 2-P-glycerate to automodify (Boel *et al.*, J Mol Biol. 2004; 337: 485-96). In the *Escherichia coli* enzyme, the modification leads to the formation of a peptide bond between the epsilon-NH_2-group of lysyl residue 341 and the carboxyl group of 2-phosphoglycerate (2-PG) and this automodification seems to be necessary for the export of the enzyme to the extracellular medium although this protein do not contain any signal sequence. In this study, the role of this modification in the export of enolase in the yeast *Saccharomyces cerevisiae* was investigated and the three enolase isoforms Eno1, Eno2 and Err were studied. All these proteins were shown to be auto-modified *in vitro* and *in vivo*. In the mutants K345A and K345E, modifying the lysine homologous to lysine 341 in *E. Coli*, this automodification is blocked. Eno1 and Eno2 were exported outside in the medium but Eno2 is exported by far more efficiently than Eno1, indicating that this export was very specific. This export was blocked in the K345A and K345E mutants showing that this export depended on the automodification. In order to understand the export mechanism, we have studied Eno2 secretion in mutants (*sec6*, *sec7*, *sec14*, *sec18*) thermosensitive for the general secretion pathway or deleted for non classical export genes (YPR149w andYGR131w). To this end, a pulse chase protocol was established. The data have shown a dependence of Eno2 export from some markers of the general secretion pathway as *SEC14* and *SEC18*, but from the others. It was shown also to be dependant from non classical export. Moreover, the intracellular association of Eno2 to lipid rafts was investigated. These results will be discussed.

New screening approaches for fungal strain development

P. J. Punt[1], X. O. Weenink[2], M. van der Maarel[1], J. Jore[1], A. Ram[2] and C. van den Hondel[2]

[1] TNO Quality of Life, Zeist, the Netherlands
[2] Leiden University, Leiden, the Netherlands

Since the development of recombinant DNA technologies for yeast and filamentous fungi, a considerable part of the strain development programs was diverted to the use of molecular genetic tools. Whereas these approaches have exerted considerable success, recent developments in our laboratory have shown that new developments in classical biological screening approaches, or a combination of both, can still be very useful. A first purely classical approach is based on the discovery of a so-called suicide (SUI) substrate, which we have successfully used for the selection of protease deficient fungal host strains. These protease deficient strains show an increased resistance to the SUI substrate allowing their selection. The advantage of this non-GMO approach is that it can be applied to new and already established production strains. A combination of a molecular and classical approach is based on the use of the so-called glucoamylase carrier approach. Combining this approach with fungal strains unable to use starch as a carbon source allowed us to select for hyper secretive fungal strains generated by classical mutagenesis (Weenink et al., 2006). Moreover, the same approach also allows for selection of the highest producers in a collection of primary transformant strains expressing a glucoamylase-fusion gene.

Use of auxiliary substrates to enhance product formation: Stoichiometry versus kinetics

J. T. Pronk

Kluyver Centre for Genomics of Industrial Fermentation & Department of Biotechnology, Delft University of Technology, The Netherlands

In the industrial production of chemicals by yeasts and filamentous fungi, sugars are generally used as the sole source of carbon, reducing power and free energy. It may be attractive to fulfil the latter two functions by other cheap auxiliary substrates (e.g. methanol or H_2) that cannot serve as a carbon source, but whose oxidation can provide the cell with NADH and, via oxidative phosphorylation, with ATP. Most yeasts and fungi are capable of oxidizing formate to carbon dioxide via NAD-dependent formate dehydrogenases, but cannot assimilate formate. This presentation focuses on two cases in which formate has been used as a model auxiliary substrate.

The first case concerns production of Penicillin-G by *Penicillium chrysogenum*. Penicillin-G production requires a large input of ATP that, in conventional processes, has to be provided by glucose dissimilation. When a high-yielding strain of *P. chrysogenum* was grown on mixtures of glucose and formate in carbon-and energy-limited chemostat cultures, coconsumption of formate and glucose occurred. Over a range of formate-to-glucose rations, this led to increased Penicillin G yields on glucose. However, at high formate-to-glucose ratios, formate accumulated in the cultures, leading to reduced biomass and Penicillin G yields.

The second case concerns conversion of glucose into glycerol by *Saccharomyces cerevisiae*. By a combination of genetic and evolutionary engineering, an *S. cerevisiae* strain was constructed in which reactions that compete for NADH with glycerol production were systematically eliminated. In contrast to earlier studies, a functional triosephosphate isomerase was retained, to enable glycerol yields exceeding 1 mol per mol glucose (the theoretical maximum yield for strains lacking triose phosphate isomerase). After initial experiments indicated kinetic constraints in the reduction of dihydroxyacetone phosphate by formate, additional engineering was performed to enhance NADH/NAD^+ cycling. Consumption of glucose-formate mixtures by the engineered strains led to glycerol yields that exceeded 1 mol glycerol per mol glucose. However, kinetic constraints – probably related to the kinetic properties of the formate dehydrogenase reaction – limited practical applicability. These studies underline the need for a thorough kinetic analysis of enzymes used in metabolic engineering strategies that are aimed at improving process stoichiometry.

Relation between central metabolism and β-lactam production in *Penicillium chrysogenum*: A metabolomics study

W.M. van Gulik[1], R.J. Kleijn[1], U. Nasution[1], R. Seifar[1], L. Wu[2], R.A.L. Bovenberg[2], W. A. van Winden[1] and J.J. Heijnen[1]

[1] Department of Biotechnology, Delft University of Technology, The Netherlands
[2] DSM Anti-Infectives, Delft, The Netherlands

Successful improvement of the production rate and yield of β-lactam antibiotics should not be limited to metabolic engineering of the product pathway alone, but should rather be applied to the product pathway in concert with the relevant parts of central metabolism. A suitable model organism to study this approach is the penicillin producing filamentous fungus *Penicillium chrysogenum*, because the product biosynthesis pathway is well known. It can be inferred from the stoichiometry of this pathway that in order to sustain a significant rate of antibiotic production, significant amounts of carbon precursors, energy (ATP) and reducing equivalents (NADPH) are required, which have to be generated in central metabolism. We have developed a number of different methods to study the impact of β-lactam antibiotic production on central metabolism. Among these are stimulus response studies combined with metabolome measurements using Isotope Dilution Mass Spectrometry (IDMS), to assess the in-vivo kinetic properties of the relevant metabolic pathways and ^{13}C metabolic flux analysis, either based on whole isotopomer modeling or on a local node flux analysis approach. Using these approaches the impact of β-lactam antibiotic production in a high producing strain of *P. chrysogenum* was studied by cultivation under producing as well as under non-producing conditions.

Production of artemisinic acid, precursor to artemisinin and of the potent antimalarial combination therapies, by yeast

C. Paddon[1], P. Westfall[1], D. Eng[1], O. Spirina[1], M. Yu[1], D. Pitera[1], J. Lenihan[1], R. Regentin[1], E. Paradise[2], D.-K. Ro[2], Y. Shiba[2], M. Ouellet[2], J. Kirby[2], K. Ho[2], N. Prasad[2], R. Chan[2], J. Keasling[2] and J. Newman[1]

[1]Amyris Biotechnologies, Emeryville, USA
[2] Berkeley Center for Synthetic Biology, University of California, USA

Malaria affects 500–600 million people leading to 1–3 million deaths annually. Traditional drug treatments have become ineffective in many areas as the *Plasmodium* parasite has developed resistance. Artemisinin-based combination therapies (ACTs) are highly effective anti-malarials, but additional supplies of artemisinin are required to supplement botanically derived artemisinin (extracted from *Artemisia annua*) at a lower cost to make ACTs available to those who need them in the developing countries of the world. We are developing a process to produce artemisinin semi-synthetically following production of the precursor artemisinic acid in yeast, *Saccharomyces cerevisiae*. Ro et al. (2006; Nature 440 940-3) described the development of a yeast strain with an engineered mevalonate pathway that produced over 100mg l^{-1} of amorphadiene, the sesquiterpene precursor to artemisinic acid following expression of amorphadiene synthase from *A. annua*. This strain was used to isolate genes encoding the final 2 enzymes for the conversion of amorphadiene to artemisinic acid, a cytochrome P450 amorphadiene monooxygenase and its cognate reductase, allowing production of over 100mg l^{-1} of artemisinic acid following induction of enzyme expression from the GAL1 promoter in shake-flask culture. Further work involved engineering of the pyruvate dehydrogenase bypass to enhance production of cytoplasmic acetyl-CoA as a precursor to the mevalonate pathway (Shiba et al., Metab. Eng. 2007, 9 160–8). This presentation will describe the further development of artemisinic acid producing yeast strains, both in terms of genetics and production in fermenters as a prelude to industrial production of artemisinic acid.

Lactic acid production by *Candida sonorensis*

K. Koivuranta[1], M. Ilmén[1], P. Suominen[2], A. Aristidou[2], V. Rajgarhia[2,3], L. Ruohonen[1] and M. Penttilä[1]

[1] VTT Technical Research Centre of Finland, Espoo, Finland
[2] NatureWorks LLC, Minnetonka, USA
[3] Present address: Mascoma, Lebanon, USA

C. sonorensis readily ferments glucose to ethanol, utilizes several carbon sources other than glucose, and is tolerant to acidic conditions. *C. sonorensis* was engineered for lactate production by individually expressing different lactate dehydrogenase genes in pyruvate decarboxylase positive and negative strain backgrounds to generate strains with distinctive characteristics. Production of lactate and ethanol from glucose was strongly affected by the deletions of the two pyruvate decarboxylase genes *PDC1* and *PDC2*, the properties of the LDH enzymes, and by LDH enzyme activity which varied with *LDH* gene copy number. *C. sonorensis LDH* strains were also shown to ferment xylose to lactic acid efficiently via the endogenous xylose pathway enzymes, e.g. via xylose reductase and xylitol dehydrogenase. An alternative xylose fermentation pathway was introduced into *C. sonorensis* by expressing the *XYLA* gene from *Piromyces sp.* encoding xylose isomerase, and xylose was also shown to be metabolized via this pathway. Co-expression of the *XKS1* gene from *Saccharomyces cerevisiae* encoding xylulokinase with the *XYLA* gene enhanced fermentation relative to expression of *XYLA* alone. Deletion of the endogenous reductase and xylitol dehydrogenase genes from a strain expressing xylose isomerase had a positive effect on lactate production from xylose.

Riboflavin-producing cobalt-resistant *Saccharomyces cerevisiae* obtained by evolutionary engineering

C. Alkim[1,2], B. Yilmaz[1,2], C. K. Vata[3], C. Tamerler[1,2,4], M. Sarikaya[2,4], J. François[5], L. Benbadis[5] and Z. P. Çakar[1,2]

[1] Department of Molecular Biology and Genetics, Istanbul Technical University, Turkey
[2] Istanbul Technical University, Biotechnology and Genetics Research Center, Turkey
[3] Department of Pathology, Duke University, Durham, USA
[4] Department of Materials Science and Engineering, University of Washington, USA
[5] Ingeniere des Systemes Biologique et des Procedes, CNRS & INRA, France

For the purpose of improving and tailoring various cellular properties, evolutionary engineering based on applying selective pressure towards a desired phenotype is becoming increasingly utilized. In this study, we have designed and employed evolutionary engineering strategies to obtain cobalt-resistant mutant yeasts. A chemically mutagenized *Saccharomyces cerevisiae* CEN.PK113-7D culture was used as the starting population to create genetic diversity. The selection of cobalt-resistant mutant populations was accomplished under pulse cobalt stress conditions, as well as at constant and increasing cobalt concentrations. During selections with increasing cobalt concentrations, 36 mutant generations were obtained that were resistant to $CoCl_2$ concentration significantly higher than the minimum inhibitory cobalt concentration of wild type *S. cerevisiae*. Individual mutants were randomly selected from final mutant populations, and their resistance, in terms of percent survival, was determined using a high-throughput most-probable number (MPN)-based method. Interestingly, most of the mutant individuals selected were changing the color of the growth medium to bright yellow, when grown in liquid yeast minimal medium (YMM). Spectrophotometric measurements of the culture supernatants revealed maximum absorption at 444 nm, the typical wavelength for riboflavin. These results were verified by HPLC measurements using commercial riboflavin standards. In shake flask cultures in YMM containing 1 mM $CoCl_2$, up to about 17 mg/l riboflavin was produced by the mutants, which was about four-fold higher than in the absence of cobalt. We are currently using DNA microarray technology to analyse gene expression profiles of these mutants in order to get insight into the transcriptomic mechanism that is responsible for this hyperproduction of riboflavin. Considering that the wild-type strain CEN.PK113-7D does not produce riboflavin at detectable levels, it is likely that conferring cobalt resistance to the mutants may have triggered riboflavin production, possibly by activating a redox pathway. Understanding the genetic mechanism of cobalt resistance and riboflavin production in yeast may be useful for basic and applied medical research such as oxidative stress damage and ageing. Additionally, understanding the development of high cobalt resistance in *S. cerevisiae* along with the metal-specific properties would be useful for designing biomimetics-based systems for practical applications such as bioremediation and biomineralization.

A miniaturized media-intensive screening system for antibiotic and secondary metabolite detection from filamentous fungi

G. F. Bills

Centro de Investigación Básica (CIBE), Madrid, Spain

The “System Duetz” is a set of tools for miniaturized parallel preservation and aerated cultivation of unicellular microorganisms and actinomycetes in 96-well format. Growth and manipulation of filamentous fungi with the System presented several challenges related to fungal growth forms. Most fungi used in our laboratory only form vegetative mycelia in vitro, and filamentous fungal growth in 2-ml vessels cannot be agitated effectively. Therefore, new protocols were needed to adapt the System to the distinctive biology of filamentous fungi. Non-sporulating fungi were effectively transferred among wells as hyphal suspensions. Fungal metabolites were efficiently extracted from growth in microwell plates, and metabolite production was adequate for detection of biological activities. Extraction directly from fungi grown in microplates streamlined downstream processing and screening manipulations because subsequent steps were carried out in a 96-well footprint compatible with automated liquid handling stations. Fungal growth and antibiotic detection in the System Duetz were compared with growth and detection in flask formats. Using reduced-scale parallel fermentations, the effects of numbers and kinds of growth media on the detection of antibiotic signals were modeled using growth inhibition of the pathogen, *Candida albicans,* as a readout of biological activity. Fungal sets were replicated in plates and tested with between eight to twelve nutritional regimes resulting in a two- to three-fold higher probability of antibiotic detection from any given strain compared to flask systems with three growth media. The System Duetz enabled a discovery strategy that more thoroughly exploited the metabolic potential of individual strains within heterogeneous fungal collections.

ABSTRACTS OF POSTER SESSION I

Genomics and functional analysis

Posters P1 – P11

Analysis of transcription regulatory associations in *Saccharomyces cerevisiae* using the YEASTRACT database

M. C. Teixeira[1], P. Monteiro[2], S. Tenreiro[1], N. P. Mira[1], A. B. Lourenço[1], A. M. Carvalho[2], N. Mendes[2], S. d'Orey[2], H. Pais[2], A. T. Freitas[2], A. L. Oliveira[2] and I. Sá-Correia[1]

[1] IBB/CEBQ, Inst Superior Tecnico, Lisbon, Portugal
[2] KDBIO/ALGOS, INESC-ID, Lisbon, Portugal

The YEASTRACT (Yeast Search for Transcriptional Regulators And Consensus Tracking; www.yeastract.com) database is a tool for the analysis of transcription regulatory associations in *Saccharomyces cerevisiae*. Last updated in January 2007, this database contains over 27800 regulatory associations between transcription factors (TF) and target genes and includes 281 specific DNA binding sites for more than 100 characterized TF. Computational tools are also provided to facilitate the exploitation of the gathered data when solving a number of biological questions. They are particularly interesting in the analysis of global gene expression results. YEASTRACT was presented and described in a paper published in the 2006 Database Issue of Nucleic Acids Research [1]. During 2006, researchers from more than 300 different groups from 60 different countries have performed over 100.000 queries per month using YEASTRACT.

YEASTRACT will shortly include DISCOVERER, a set of computational tools that can be used to identify complex motifs over-represented in the promoter regions of co-regulated genes. DISCOVERER is based on MUSA [2] and RISO [3] algorithms. These algorithms take as input a list of genes (for example those coming out from microarray experiments) and identify over-represented motifs. These motifs can be compared with known transcription factor binding sites described in the YEASTRACT database. A demonstration of the use of these new tools will be presented.

[1] Teixeira et al. (2006) *Nucleic Acids Research*, Database Issue, 34: D446–D451.

[2] Mendes *et al.* (2006) Bioinformatics, 22: 2996–3002.

[3] Carvalho et al. (2006) IEEE Transactions on Computational Biology and Bioinformatics, 3: 126–140.

Elucidating acid and enzyme production by *Aspergillus niger* with systems biology

M. R. Andersen, M. G. Jakobsen, L. Lehmann and J. Nielsen

Centre for Microbial Biotechnology, BioCentrum-DTU, Technical University of Denmark

The ubiquitous and saprophytic filamentous fungus *Aspergillus niger* is of great industrial impor-tance due to its capabilities of producing organic acids and extracellular biomass-degrading proteins. Especially citric acid, gluconic acid and a multitude of hydrolases and enzyme-mixtures are commercially important *A. niger* metabolites with billion-dollar markets.

To better understand the processes governing the metabolism of these compounds, we have made several initiatives to explain the make-up of *A. niger* metabolism on a system-wide scale. Through a thorough study of the available literature, a graphical map of all reported pathways of the metabolism has been made. Together with customly designed Affymetrix DNA arrays (usable for three different *Aspergillus* species), this is being used interpret transcription data in the context of metabolic pathways. Software has been made to plot results of metabolism-scale experiments such as transcription data onto the above-mentioned graphical map, a method of analysis capable of easing the interpretation of data greatly.

Batch-cultivations of *A. niger* at different controlled pH-values and on several defined complex carbonsources have been analysed using transcription analysis and is showing the physiological response of the fungus to these conditions. New knowledge has been generated on the pH-controlled metabolism and on the carbon-source induced complex mixtures of enzymes secreted from the fungus.

Meta-analysis of global fitness experiments reveals a novel non-transcriptionally regulated general stress response

A. Zakrzewska, A. Boorsma, F. M. Klis, S. Brul and G. J. Smits

Swammerdam Institute for Life sciences, University of Amsterdam, The Netherlands

An enigmatic finding in genome wide responses of *Saccharomyces cerevisiae* is the apparent absence of overlap in genes whose transcription is upregulated in response to a change in environment, and whose function is required upon that same change. To obtain insight into functional requirements of yeast in response to environmental changes, we have performed a meta-analysis of almost thousand expression and 159 genome wide mutant analyses in yeast. To compare experiments performed in different laboratories and using different methods, we have re-analyzed all data using T-profiler, an algorithm that determines the combined deviation of all genes or mutants belonging to a pre-defined group within the context of any one experiment. We find no clear co-functionality of gene groups defined by common transcriptional regulators, with the marked exception of genes controlled by Fhl1p, Rap1p and Sfp1p, transcription factors that together control ribosomal protein gene expression. Interestingly, mutants in these gene groups are generally less sensitive to environmental change than the rest of the population. We do find extensive co-functionality of gene groups derived from GO-ontology or MIPS, which share functions, localization, morphology etc. Most remarkable is the multi-environment sensitivity of mutant groups functioning in intracellular vesicular transport. Interestingly, these functional groups are not transcriptionally co-regulated and thus can be regarded as a non-transcriptionally regulated general stress response in yeast. We continue to analyze the variation of functional group requirement in relation to variation in transcriptional regulation, in order to draw conclusions about the level on which yeast regulates its responses to adapt to its ever-changing environment.

Conserved synteny in *Trichoderma reesei* with related filamentous ascomycetes

D. Martinez[1,2], M. Saloheimo[3], R. Berka[4], B. Henrissat[5] and M. A. Nelson[2]

[1] Los Alamos National Laboratory/Joint Genome Institute, USA
[2] Department of Biology, University of New Mexico, Albuquerque, USA
[3] VTT Technical Research Centre of Finland, Finland
[4] Novozymes Biotech Inc, USA
[5] Centre de Recherches sur les Macromolécules Végétales, Grenoble, France

The current pace of whole genome sequencing has made it almost trivial to produce nearly complete fungal sequences. This now gives us the ability to perform whole genome comparisons of fungi to investigate biological questions and genome evolution. To this end, syntenic regions between *Trichoderma reesei* and four other filamentous fungi (*Fusarium graminearum, Neurospora crassa*, *Magnaporthe grisea* and *Aspergillus nidulans*) were identified. An algorithm was designed that takes homologs from two fungi and places them side by side, optimizing homolog density, and minimizing gaps between homologs and the number of homologs in a syntenic region. As would be expected, the percent coverage of synteny between *T. reesei* and the other four ascomycetes declines with time since the last common ancestor, with *F. graminearum* having the highest synteny and *A. nidulans* the lowest. This approach enabled the identification of regions in the *T. reesei* genome that were conserved in multiple genomes as well as highlighting regions of the *T. reesei* genome that have changed dramatically. Finally, for genes within these highly conserved regions and for the gaps, GO terms and Enzyme Commission codes were assigned. These results were used to assess the possibility that there is selective pressure to force genes that are in the same biochemical pathway, have similar function or are involved in similar processes to maintain or gain proximity.

Annotation and validation through genome-scale metabolic model of *Aspergillus oryzae*

W. Vongsangnak[1], P. Olsen[2], K. Hansen[2], S. Krogsgaard[2] and J. Nielsen[1]

[1] Center for Microbial Biotechnology, Biocentrum-DTU, Denmark
[2] Novozymes A/S, Bagsvaerd, Denmark

Aspergillus oryzae is an industrially important filamentous fungi as it is used for production of industrial enzymes and fermented food products. Its safety for industrial use is well demonstrated by the long history of its use for manufacturing of fermented foods, and there is therefore much interest on exploiting *A. oryzae* widely for production of industrial enzymes and proteins of pharmaceutical interest. However, the cellular mechanisms involved during protein production remains largely unknown, and there is therefore much interest in performing detailed annotation, metabolic model simulation and validation at the whole-genome scale. The genome of *A. oryzae* RIB 40 has been sequenced by Machida [1]. The 37-megabase (Mb) genome contains 12,074 genes which have been annotated by COGs database. Nonetheless, the number of protein-coding genes remains a matter of debate. Hence, a genome re-annotation is the first step for construction of an accurate metabolic model. Then, model simulation and validation by flux balance analysis are the next step. In the present study, we therefore undertook a detailed strategy consisting of three steps. Firstly *A. oryzae* annotation was done by EST sequencing project, using several gene/protein databases, and an integrated bioinformatics tools (i.e. BLAST, HMMER, PSI-BLAST). Secondly, the metabolic network of *A. oryzae* was reconstructed. Finally, the model was simulated and validated based on literature and physiological studies. This approach resulted in that number of protein-encoding genes increased. New genes were approximately discovered 1,048. Of 13,122 protein-encoding genes, 2,025 metabolic genes were used to map to reconstruct metabolic network. Currently, our *A. oryzae* model contains 1,184 genes, 712 enzymes, 1,040 metabolites and 1,679 reactions (1060 Unique). After model simulations, it was validated by determination of growth on different carbon sources. The accuracy of model was satisfying according to our physiological data and literature of *A. oryzae*. Therefore, ultimately, this model will be used for simulation of enzyme production and analysis of transcriptome data obtained during industrial enzyme production processes.

This research is funded by Novozymes A/S and Technical University of Denmark

1. Machida, M. Genome sequencing and analysis of *Aspergillus oryzae*. Nature 438, 1157–61 (2005).

Unlocking the ale yeast genome for genetic mapping purposes

J. A.W. Vanmiddelem[1,2,3], K. Schaerlaekens[2,3], J. M. Thevelein[2,3] and F. R. Delvaux[1]

[1] Centre for Malting and Brewing science, Department of Food and Microbial Technology, Belgium
[2] Laboratory of Molecular Cell Biology, K.U. Leuven, Belgium
[3] Department of Molecular Microbiology, VIB, Belgium

Applying genetic techniques on brewing yeast has always been seen as a challenge since they are thought to be polyploid and/or aneuploid to a certain degree, homothallic, sporulation deficient, and if tetrads are formed, the spores almost never germinate. If viable spores can be isolated, many of them are expected to have poor growth or poor fermentation performance or to be mating deficient. Given these disadvantages, breeding programs with ale yeasts are very rare, and in many cases a black box approach is chosen for strain improvement, such as cell fusion or forced evolution. These approaches can be useful solutions for practical problems, but they do not reveal anything about the underlying genetic basis. By enlarging our knowledge of the polygenic basis of important commercial traits in ale yeasts and the relevant QTLs, a more direct and optimized approach can be taken for strain improvement. This can be done by mapping the QTLs of the polygenic trait, which requires a mating capable haploid segregant with the phenotype of interest. In this work, the steps taken to obtain such segregants from a commercial ale strain producing beer with a strong fruity flavour due to very high ester production will be explained.

Characterization of a mitotic recombination hotspot on chromosome III of the asexual fungus *Aspergillus niger* and the use of effectors to increase recombination

P. J. I. van de Vondervoort[1,3], S. M. J. Langeveld[1], A. F. J. Ram[1], J. Visser[2], H. Pel[3] and N. N. M. E. van Peij[3]

[1] Institute of Biology, Leiden University, The Netherlands
[2] FGT Consultancy, Wageningen, The Netherlands
[3] DSM Food Specialties, Delft, The Netherlands

Aspergillus niger has many uses in food applications, for example as a producer of organic acids and enzymes. Strain improvement has thus far mainly been achieved by subsequent rounds of mutations and selection, and in some cases by the recombination of independent mutations located on different linkage groups. Another use of the parasexual cycle is the construction of diploids with improved characteristics. *A. niger* is an asexual fungus, but using the parasexual cycle, strains can be combined into heterozygous diploids, and upon haploidization, different combinations of unlinked mutations can easily be obtained. However, the exchange of mutations positioned on the same chromosomes, requiring mitotic crossing-over or gene conversion in the diploid phase, is very rare.

In order to study crossing-over, we improved the genetic map of chromosome III using the physical map. Using 6 auxotrophic markers, linkage group III specific marker strains were constructed. In a diploid containing five of these markers, various effectors that could influence recombination of linked markers during haploidization were tested. Recombination frequencies between these markers were compared with the physical map of chromosome III. Chromosomal locations with increased mitotic cross-over frequencies were searched for sequence motifs and patterns, which are possibly involved in recombination. In addition, the effect of various effectors on (enhanced) mitotic recombination has been investigated.

Improved gene targeting in *Penicillium chrysogenum*

P. de Boer[1], H. Touw[2], J. Bastiaans[1], R. Kerkman[2], K. Dukić[1], M. van den Berg[2] and R. Offringa[1]

[1] Add2X Biosciences BV, Leiden, The Netherlands
[2] DSM Anti Infectives, Delft, The Netherlands

In most eukaryotes, including filamentous fungi, the predominant mode of DNA integration is via non-homologous recombination (NHR). For functional genomics studies and various other applications however, DNA integration via homologous recombination leading to gene targeting (GT) is the preferred pathway. The recent identification of key components of the NHR pathway, such as Ku70 and Ku80, has provided new tools for improving GT efficiencies. Knocking out the NHR pathway has resulted in efficient GT in a variety of eukaryotes, including several *Aspergillus* species. In the present study, we show that deletion of the *Ku70* or *Ku80* genes also results in significantly improved GT in the β-lactam producer *Penicillium chrysogenum*.

T-DNA insertional mutagenesis, integration analysis and expression in the model ectomycorrhizal fungus *Laccaria bicolor*

M. Kemppainen[1], S. Duplessis[2], F. Martin[2] and A.G. Pardo[1]

[1] Departamento de Ciencia y Tecnología, Universidad Nacional de Quilmes, Argentina
[2] INRA-Nancy, Champenoux, France

The usefulness of *Agrobacterium*-mediated gene transfer (AMT) in fungi is currently opening a new era for fungal genetics. As whole genome sequences of several fungi are being released studies about T-DNA integration patterns are needed in order to understand the integration mechanisms involved and to evaluate the AMT as an insertional mutagenesis tool for different fungal species. Sequencing of the genome of the first symbiotic fungus, the basidiomycete *Laccaria bicolor*, was accomplished by the Joint Genome Institute (http://genome.jgi-psf.org/Lacbi1/Lacbi1.home.html). The fact that the genome is public since July 2006 and that was transformed via AMT (Kemppainen et al. 2005) makes this fungus an excellent model for functional genomic studies in mycorrhizal research. Data about the integration patterns and conservation of the border regions of the T-DNAs in *Laccaria bicolor* genome were not available, thus a plasmid rescue approach was optimized for this fungus. The rescue element was cloned into pCAMBIA1300-based binary vector pBGgHg in a form of total pBluescript KS+. This binary vector (pHg/pBSk) allowed the selection of transformed *Laccaria* lines with hygromycin and the rescue of the right border (RB) with a unique restriction enzyme under ampicillin selection in *E. coli*. *Laccaria bicolor* dikaryotic strain S238N was transformed with *Agrobacterium* AGL-1 carrying the rescue binary vector pHg/pBks and a total of 51 fungal lines were analyzed. We demonstrate that the plasmid rescue method can be used for resolving T-DNA integration sites in *Laccaria*. With the presented protocol we were able to rescue 74% of analyzed fungal lines and 62% could be sequenced with the Post-RB primer. The RB was shown to conserve well during transformation of *Laccaria bicolor*. The integration site analysis showed no clear sequence homology between different sites or the sites and the T-strand border sequences indicating non-homologous and random integration of the transgenes. Majority (74%) of integrations were located in putative transcribed sites (interrupted ORFs or their upstream / downstream elements). Among these there were genes with proposed protein functions belonging to several cellular functional groups. Some of them were subjected to expression analysis by RT-PCR in the dikaryotic transformed strains. Our results demonstrate that AMT can be used for gene validation in *Laccaria*.

Efficient insertional mutagenesis of *Saccharomyces cerevisiae* via electroporation of Mu transposition complexes

H. Turakainen[1] and H. Savilahti[1,2]

[1] Institute of Biotechnology, Viikki Biocenter, University of Helsinki, Finland
[2] Department of Biology, University of Turku, Finland

We have shown earlier that the phage Mu DNA transposition system can be used for efficient insertional mutagenesis in both Gram-negative and Gram-positive bacterial species (Lamberg et al. 2002: Appl. Environ. Mictrobiol. 68:705–712; Pajunen et al. 2005 Microbiology 151:1209–1218). Here, we describe a similar strategy that generates random insertions in the genome of the yeast *Saccharomyces cerevisiae*. Initially, MuA transposase protein was incubated with an artificial mini-Mu transposon that contained a selectable marker for yeast (kanMX4). This resulted in functional Mu DNA transposition complexes, transpososomes. Following their delivery into yeast cells by electroporation, the transpososomes integrated the transposon DNA into yeast chromosomes with the efficiency of more than 1000 CFU/μg of input transposon DNA. No evident bias towards any particular chromosome was observed. A 5-bp target site duplication flanking the transposon DNA was generated upon integration, verifying that the insertion had been generated by genuine DNA transposition chemistry. The results indicate that Mu transposition can be exploited in *Saccharomyces cerevisiae* and expand the applicability of the Mu transpososome delivery strategy into eukaryotes.

Validation of reference genes for real-time RT-PCR assays by the use of genorm: Example of long-term yeast cultures

M. Duquenne, M. A. Teste, J. Francois and J. L. Parrou

Laboratoire Ingénierie des Systèmes Biologiques et des Procédés (LISBP), France

Real-time RT-PCR is now widely used for gene expression analysis, especially for the validation of microarray datasets. Accurate analysis requires that the samples be normalised. There are two main recognised methods for sample normalisation, a) to a standardised input amount of material (cells, total RNA, spike, etc), or b) to reference (housekeeping) genes. When available, microarray-based experiments represent a treasure trove of data and allow searching for those candidate reference genes whose expression doesn't change significantly in the dataset. Since it is difficult to identify and certify that a single gene does not vary as a function of treatment or condition, we decided to apply the method proposed by Vandesompele and colleagues (2002, GeNorm (1). In this method, the calculated normalising factor is based on the geometric mean of multiple reference genes, which have beforehand been carefully selected to display minimal variation across the treatments or strains of interest.

The aim of our study was to compare the expression level of a multigene family with high sequence identity, in WT *versus tps1* cells, during the time-course from exponential to late stationary phase in galactose medium. Using the SGD's Expression Connection tool and microarray datasets from DeRisi and Gash, we pre-selected a dozen of genes that belong to different biological processes to avoid co-regulation and whose expression appeared unchanged through exponential, post-diauxic and stationary phases. We also included in this panel classical reference genes like *ACT1*, *PDA1*, *RDN18*, etc., widely used in the bibliography dedicated to the yeast *S. cerevisiae*. All these genes where analysed, in all our samples, by real-time RT-PCR. From this crude dataset, GeNorm identified three genes (*PMT6*, protein glycosylation; *UBC6*, protein ubiquitination; and *TFC1*, RNA Pol III initiation of transcription) as the most stable ones, sufficient to get a high quality normalising factor. In contrast, when normalised to *PMT6*|*UBC6*|*TFC1*, *RDN18* significantly fluctuated up and down amongst the samples, and *ACT1* expression level dropped more than 50-fold in stationary phase for both WT and *tps1* cells. As main conclusions, we propose a set of suitable genes and emphasize the mediocrity of *ACT1*, for normalisation of gene expression during time course analysis of yeast cultures on fermentable carbon sources.

(1) Vandesompele, J, *et al.*, (2002), GenomeBiology, 3.

ABSTRACTS OF POSTER SESSION II

Fundamental cell functions and stress

Posters P12 – P44

The putative methyltransferase (*dmtA*) gene in *Aspergillus oryzae* is methylated

H. A. Lee[1], M.J. Dickinson[2] and D.B. Archer[1]

[1] School of Biology, University of Nottingham, UK
[2] School of Biosciences, University of Nottingham, UK

Methylation of carbon 5 of cytosine, an epigenetic process of many eukaryotic genomes, is involved in defence against invading DNA and in gene regulation. In filamentous fungi DNA methylation occurs in *Neurospora crassa* and *Ascobolus immersus* at levels of 2–3% of all cytosines, and in *Aspergillus flavus* it has been shown to occur at the lower level of 0.25%. Although studies in *N. crassa* and *A. immersus* have demonstrated the involvement of DNA methylation in silencing repeat sequences in filamentous fungi, no functional genes have yet been shown to be methylated. Here we report that the *dmtA* gene, which may encode a DNA methyltransferase, is itself methylated. The *dmtA* gene is a homologue of *masc1* (*A. immersus*) and *RID* (*N. crassa*) both of which are involved in the silencing mechanisms, MIP and RIP respectively. Both of these mechanisms involve DNA methylation, although the relationship between the two processes is not fully understood. Neither masc1 nor RID has been shown to display methyltransferase activity *in vitro*, and it has been suggested that RID may possibly be a deaminase. Using Southern Blotting we have shown that DNA methylation occurs in the *dmtA* gene towards the 3' end, and is stronger when *A. oryzae* is grown in minimal medium rather than rich medium. Additionally reverse-transcriptase PCR has revealed that *dmtA* is upregulated when *A. oryzae* is grown in minimal medium. This study provides the first evidence for DNA methylation in a specific gene in a filamentous fungus.

Comparison of signaling cascades in *Saccharomyces cerevisiae* and *Ashbya gossypii*

R. Rischatsch, S. Voegeli, F. Dietrich, P. Demougin, M. Primig and P. Philippsen

Applied Microbiology and Genome Biology, Biozentrum, Basel, Switzerland

A. gossypii carries a genome which resembles that of *S. cerevisiae* prior to its whole genome duplication. Despite the high degree of conservation of gene order and gene content *A. gossypii*, a subtropical ascomycete, strictly grows as fungal mycelium with constantly elongating and branching hyphae. We wanted to know whether signaling cascades are conserved in both organisms despite the differences in habitats and modes of growth. As a first step we analyzed mitogen-activated protein kinase (MAPK) pathways which are involved in the transduction of a variety of extracellular signals to intracellular targets in eukaryotes. The haploid *A. gossypii* genome encodes homologs of all major components that are required in *S. cerevisiae* to respond to mating pheromone and filamentation signals. This includes not only the MAPKs, but also the pheromone receptors (STE2, STE3), the G-protein subunits (STE18, STE4, GPA1), the scaffold protein STE5, the repressor DIG1/2, the transcription factors STE12 and TEC1, and most target genes. We will present a bioinformatics analysis of target gene promoters, experimental data on transcription levels as well as phenotypes of *A. gossypii* strains lacking DIG1/2, STE12 and TEC1. Genome-wide transcription data of *A. gossypii* was obtained with custom made gene chips from Affymetrix that cover 4435 of the 4728 genes. Preliminary results of a second project that aims to study the transcriptional program underlying the physiological events of spore germination and development will also be presented.

Saccharomyces cerevisiae adaptation to acetic acid stress involves the Haa1p-dependent response regulon

N. P Mira[1], J. D Becker[2] and I. Sá-Correia[1]

[1] IBB- Institute for Biotechnology and Bioengineering, Inst. Superior Técnico, Portugal
[2] Affymetrix Core Facility, Gulbenkian Institute of Science, Portugal

The understanding of the molecular mechanisms that may contribute to counteract the deleterious effects of weak acids as fungistatic agents is essential to guide suitable preservation strategies. We have recently demonstrated the involvement of the transcription factor Haa1p in yeast adaptation to weak acids used as food preservatives, especially to the more hydrophilic acetic acid [1]. This new weak acid sensing regulon is described in the present work. The comparison of yeast transcriptomes of wild-type and *Δhaa1* strains in the presence or absence of acetic acid stress, revealed that Haa1p is required for acetic-acid induction of 65% of the responsive genes. Many of these genes encode plasma membrane and cell wall proteins whose function is poorly characterised or unknown. They also encode proteins of lipid metabolism, protein kinases and other kinases, transcription factors and other proteins of unknown function. The role of all the genes that are up-regulated genes in response to acetic acid, in an Haa1p-dependent way, was examined. Results indicate that the more evident susceptibility phenotype was registered in a mutant with the *HRK1* gene deleted. *HRK1* encodes a protein kinase involved in the activation of plasma membrane H^+-ATPase by glucose [2]. We will show evidences indicating that the delta*hrk1* mutant accumulates a higher concentration of labelled acetic acid than the parental strain during incubation in acetic acid supplemented growth medium. Studies are in progress to elucidate the hypothesized role of the protein kinase Hrk1p in the modulation of the activity of plasma membrane transporters that may affect the active export of acetate.

[1] Fernandes *et al.*, Biochem Biophys Res Comm, 337, 95–103, 2005.
[2] Goosens *et al.*, Mol Cell Biol, 20, 7654–61, 2000.

The *RIM101* pathway is involved in *Saccharomyces cerevisiae* resistance to propionic acid

N. P. Mira[1], A. B. Lourenço[1], A. R. Fernandes[1], J. Becker[2] and I. Sá-Correia[1]

[1] IBB- Institute for Biotechnology and Bioengineering, Centre for Biological and Chemical Engineering, Instituto Superior Técnico, Portugal
[2] Affymetrix Core Facility, Gulbenkian Institute of Science, Portugal

Propionic acid is used as an antifungal agent in foods, typically in dairy and baking products. In this work, we searched for new genes and pathways that are key players in yeast response and resistance to this weak acid. Results from a screening for propionic acid susceptibility of the yeast disruptome indicated that the more evident susceptibility phenotypes are registered for deletion mutants encoding proteins involved in mitochondrial function, ergosterol metabolism, vacuolar acidification, cell wall integrity and intracellular trafficking, particularly those belonging to the vacuolar protein sorting class (VPS), also involved in the regulation of the *RIM* pathway. This pH-sensing signalling pathway is known to be involved in the transcriptional response to alkaline pH through the action of the transcription factor Rim101p. In this work we found that *RIM101*, as well as two other members of the *RIM* pathway, namely *RIM8* and *RIM13* presumably involved in the processing of Rim101p, are determinants of resistance to propionic acid. Using DNA microarrays we compared the transcriptomes of wild type and *Δrim101* cells in the presence or absence of propionic acid stress. We will present results indicating that in the absence of the weak acid (at pH 4.0), the deletion of *RIM101* leads to the reduction (from 2.0 to 6.3 fold) of the transcript levels from several genes, in particular genes encoding cell wall proteins or proteins associated with cell wall synthesis, consistent with the proposed relationship between the *RIM101* pathway and *S. cerevisiae* cell wall assembly. However, apparently, propionic acid-induced transcription activation is not dependent on the presence of Rim101p. Among the genes whose transcription levels suffer a Rim101p-dependent increase, in cells grown either in the presence or absence of the acid, the *KNH1* gene, involved in β-1,6-glucan synthesis, and of the ORF *YIL029c*, related with response to drugs, were proved to be determinants of resistance to propionic acid.

Influence of the temperature variation on the *Saccharomyces cerevisiae* and *Escherichia coli* cells

M. Geicu[1], S. Jurcoane[2], F. Radoi-Matei[1] and P.A. Marechal[3]

[1] USAMV Bucharest, Faculty of Biotechnology, Romania
[2] Center of Microbial Biotechnology, Bucharest, Romania
[3] ENSBANA-UBourgogne, Dijon, France

The control of the temperature variation kinetics can be useful in different biotechnological treatments as the food stabilization.

The objective of this work was to study the influence of the temperature variation and of the time of maintenance at low temperature on the *Saccharomyces cerevisiae and E. coli* kinetic. The applied ranges were 30°C–0°C for both microorganisms. Cellular viability according to the thermal kinetics of disturbance after various times of maintenance to 0°C was given. The behavior of the microorganisms is a function of the physiological stage, the kinetics of cooling and the duration of the time of maintenance at low temperature in growth media nutritive or stress media. Our results showed that the cells resulting from the exponential phase of growth are much more sensitive to the thermal stresses applied than those in stationary phase.

A cumulative effect of a double stress, for example temperature/pressure would be useful to be studied in order to develop so called “soft techniques” of safeguard treatments.

Correlation between cultural conditions, physiology and cell size in *Saccharomyces cerevisiae* studied in different continuous culture conditions

D. Losio, D. Porro and L. Brambilla

Università di Milano – Bicocca, Dipartimento di Biotecnologie e Bioscienze, Italy

The budding yeast *Saccharomyces cerevisiae* is able to grow on a broad range of substrates and conditions, using alternatively a respiratory or fermentative metabolism or often a mix of both. To do this it must change its growth rate to match different energetic and anabolic potential of different substrates, therefore a coordination system between growth and division is needed in order to prevent cells to become too small or too big. This system determines for each condition characteristic distributions and mean values of cell size; a correlation between metabolism and cell size had been already suggested: size is substantially independent from the growth rate until metabolism remains completely respiratory and begin to increase in a almost linear correlation with growth rate when respire-fermentative metabolism starts. Moreover it is possible to induce an increase in cell size forcing cells to adopt a fermentative metabolism.

Our study is focused on identifying and elucidating a correlation between characterizing elements of a given cultural condition, physiological aspects of cells and cell size. We operated in chemostat, so as to be able to manipulate the parameters of interest and at the same time to reduce the magnitude of variations necessary to induce changes in cell physiology. We chose cultural conditions that allow us to manipulate catabolic and anabolic parameters in the most independent way.

In the present work we present data collected using fermentable or not fermentable limiting substrate, as well as anaerobic growth or anabolic limited cells (nitrogen limitation).With this experimental set-up we could focus our attention on different aspects of cell metabolism and physiology and better distinguish the contribution of growth rate, catabolic and anabolic parameters in determining cell size. Results obtained seems indicates that more than one aspect may take part in determining cell size and in particular that growth rate and the kind of metabolism adopted can act also in independent ways.

.

The study of static and dynamic environmental stress of *Saccharomyces cerevisiae* using heat shock protein Hsp12p-Gfp2p construct

I. Nisamedtinov[1], G. Lindsey[2], R. Karreman[2], K. Orumets[1], M. Koplimaa[1], K. Kevvai[1] and T. Paalme[1]

[1] Competence Centre of Food and Fermentation Technologies, Estonia
[2] Department of Molecular and Cellular Biology, University of Cape Town, South Africa

The expression level and rate of a small heat shock protein Hsp12p in *S. cerevisiae* in response to different static and dynamic environmental stress conditions was studied using the fluorescent fusion protein construct Hsp12p-Gfp2p. Modifications of turbidostat and chemostat cultures – auxo-accelerostat and A- and D-stat respectively, were used to compare the effect of smooth changes in the stress conditions to rapid changes. The study of the glucose effect in A-stat demonstrated that the expression of Hsp12p-Gfp2p increased with decreasing dilution rate and glucose concentration. The maximum steady-state expression level and rate observed in A-stat ($a=-0.01\ h^{-1}$) was lower than that at a corresponding dilution rate in a shift-down experiment. Additional common stress factors such as osmotic pressure (high NaCl concentration), high temperature and high ethanol concentration applied in D-stat culture (smooth change) and chemostat (rapid change) caused different stress response profiles in the cells, depending on the stress factor itself, the value of stress factor, as well as on the rate of change of stress factor. In auxostat cultures in excess glucose concentrations ($20\ g\ l^{-1}$), the initial expression level as well as the Hsp12p synthesis rate observed prior to the smooth increase in the stress factor was lower than that in glucose-limited chemostat cultures. During the smooth increase in stress factors (T, NaCl and ethanol concentration) in auxo-accelerostat or D-stat cultures, the synthesis rate of Hsp12p increased but to a significantly lower extent (between 3 and 10 times lower depending on the stress factor) than observed in the corresponding shift-up experiments. The results suggest that the expression rate of small heat shock proteins such as Hsp12p determined by the fluorescence intensity of Hsp12p-Gfp2p is a good indicator of both static and dynamic environmental stresses of yeast cells.

Analysis of FBPase functional domains in *S. cerevisiae* and their role in cellular response to MMS-induced DNA damage and aging

A. Kitanovic and S. Wölfl

Institute for Pharmacy and Molecular Biotechnology, Ruperto-Carola University of Heidelberg, Germany

Alterations in energy metabolism can influence cellular sensitivity to oxidative stress or DNA damaging agents, aging process or apoptotic program. Our previous investigations revealed that overexpression of the key enzyme of gluconeogenesis, fructose-1,6-bisphosphatase (*FBP1*), significantly increases cellular sensitivity to DNA alkylating agent methyl methanesulfonate (MMS) and shortens chronological life span (Kitanovic and Wölfl, 2006). Here we present that the function of *S.cerevisiae* fructose-1,6-bisphosphatase in determination of cellular response to MMS is, at least partially, independent from its catalytic activity in gluconeogenesis. Site directed mutagenesis of Asn75→Ala75, a highly conserved residue in the loop 63-83 (porcine loop 52-72; Choe *et al.,* 2000) that associates to the active site of the enzyme and coordinates binding of metal ions, abolished the increased sensitivity of *FBP1* overexpressing cells to MMS. The catalytic activity of enzyme was not disrupted with this mutation. In addition, the His324→Ala324 mutation slightly reduced enzymatic activity, but the overexpression of this mutated form did not influence cellular sensitivity to MMS. On the other side, mutations in highly conserved catalytic site of the enzyme, Asp-Pro-(Ile or Leu)-Asp-(Gly or Ser)-(Thr or Ser) domain (York *et al.*, 1995), or of residues that coordinate water molecule (Choe *et al.,* 2000), Asp79→Ala79 and Glu109→Ala109, dramatically decreased both enzymatic activity and ability of the enzyme to increase cellular sensitivity to MMS. Competition analysis of cells lacking *FBP1* showed selective advantage against wild-type cells only on MMS treated medium. However, these cells were unable to recover and initiate growth when the influence of MMS was removed, suggesting that FBPase may have an important role in removing the seriously damaged cells from the population. Localisation studies proved that yeast FBPase is, in addition to its cytosolic localisation, present in the yeast nucleus. Taken together, for the first time we could show that *S.cerevisiae* FBPase is a multifunctional metabolic enzyme with important role in DNA damage defence, aging and apoptosis. Preserved catalytic function of the enzyme is necessary for this role, but it seems that conformational changes, possibly in the loop 63-83, and its localisation in the nuclei compartment are involved in this function.

Choe, J.Y., Fromm, H.J., Honzatko, R.B. (2000) Crystal Structures of Fructose 1,6-Bisphosphatase: Mechanism of Catalysis and Allosteric Inhibition Revealed in Product Complexes. *Biochemistry 39*, 8565–74.

Kitanovic, A., Wölfl, S. (2006) Fructose-1,6-bisphosphatase mediates cellular responses to DNA damage and aging in Saccharomyces cerevisiae. *Mutat. Res.* 594, 135–47.

York, D., Ponder, J.W., Majerus, P.W. (1995) Definition of a metal-dependent/Li(+)-inhibited phosphomonoesterase protein family based upon a conserved three-dimensional core structure. *Proc Natl Acad Sci U S A* 92, 5149–5153.

Nitrosative stress in yeast

O. Lushchak and V. Lushchak

Department of Biochemistry Precarpathian National University, Ivano-Frankivsk, Ukraine

A large body of evidence shows that the generation of nitric oxide (NO) and reactive oxygen species (ROS) and the rate of ROS/NO play an important role in the biological system. Intensification of NO-generation and/or increase of its concentration in extracellular environment usually results in nitrosative stress. In the present work different yeast *Saccharomyces cerevisiae* strains, defective in genes encoding main antioxidant enzymes and a regulatory protein Yap1, were treated with NO-donors: sodium nitroprusside (SNP) and S-nitrosoglutathione (GSNO) at concentrations of 1, 5 and 10 mM. The activities of superoxide dismutase (SOD), catalase, glutathione reductase (GR) and aconitase as well as levels of reduced and oxidized glutathione concentrations were studied in cells exposured to NO-donors for 1 h. We have found dose-dependent activation of antioxidant enzymes by both donors. The treatments of derivative strains defective in peroxisomal or cytosolic catalases showed that the only peroxisomal catalase was activated by nitrosative stress. We have also discovered that the main redox sensitive regulatory protein Yap1 is involved in upregulation of antioxidant enzymes under nitrosative stress. The activities of antioxidant were not affected in Yap1p defective strain. The using by protein synthesis inhibitor cycloheximide gave us a possibility to study the mechanism of enzyme activation. Surprisingly, the only catalase activation was blocked by inhibitor, whereas the increase in SOD activity has been defined in such experimental conditions. The difference in mechanisms of antioxidant enzymes activation gave us possibility to hypothesize that activation of catalase was a result of *de novo* synthesis of enzyme molecules and unexpected activation of SOD can be explained by intensification of post-translation modification of apoenzyme. The activity of oxidation sensitive aconitase and the level of reduced and oxidized glutathione were used as markers of stress conditions. Yap1-dependent mechanism of antioxidant enzymes activation as well as inactivation of aconitase and increase of oxidized glutathione levels show that nitrosative stress in yeast cells activates the main components of antioxidant defence.

.

An intraperoxisomal signaling cascade initiates peroxisome division by triggering the stepwise remodeling of lipid composition of the peroxisomal membrane

P. Kyryakov, C. Gregg, T. Boukh-Viner, T. Guo, A. Goldberg, S. Bourque, S. Chowdhury, F. Banu, K. Hung Yeung San, S. Milijevic, N. Ramlal, C. Sison, J. Solomon, V. Wong and V.I. Titorenko

Department of Biology, Concordia University, Montreal, Canada

We have identified an unusual mechanism regulating organelle division. Yeast peroxisomes do not grow and divide at the same time. The growth of immature peroxisomal vesicles, which is accomplished by the stepwise import of matrix proteins, and their development into mature peroxisomes occur before completely assembled mature peroxisomes undergo division. The division of immature peroxisomal vesicles is negatively regulated by Pex16p, a protein that binds lysophosphophatidic acid (LPA) in the inner membrane leaflet of these vesicles. The binding of Pex16p to LPA prevents the formation of diacylglycerol (DAG), a particularly potent inducer of negative curvature and membrane bending, in a membrane-associated biosynthetic pathway. The stepwise import of distinct subsets of matrix proteins into different immature intermediates along the peroxisome assembly pathway causes the redistribution of a peroxisomal protein, acyl-CoA oxidase (Aox), from the matrix to the membrane. A significant redistribution of Aox occurs only in mature peroxisomes. Inside mature peroxisomes, Aox interacts with Pex16p. This interaction between Aox and Pex16p greatly decreases the affinity between Pex16p and LPA, thereby allowing LPA to enter the biosynthetic pathway leading to the formation of DAG in the inner membrane leaflet. The subsequent spontaneous movement of DAG into the outer leaflet promotes the reorganization of the bilayer configuration of the membrane and recruits the dynamin-like GTPase Vps1p to the cytosolic face of the peroxisome. A complex between a distinct set of peroxisomal membrane proteins, Vps1p, and several actin cytoskeletal proteins is then assembled on the peroxisomal surface. This protein team promotes membrane fission, thereby executing the terminal step of peroxisome division.

Oxidative stress tolerance of *Candida albicans* – significance in the development of candidiasis and future anticandidal therapies

I. Pócsi, A. Fekete, Á. Gyetvai, T. Emri and B. Lenkey

Department of Microbial Biotechnology and Cell Biology, University of Debrecen, Hungary

Although antioxidative enzymes of fungal pathogens are regarded as important "persistence factors" the induction of oxidative stress responses upon contact with immune system cells may inhibit dimorphic switches, an important virulence attribute in *Candida albicans* (Fradin *et al.* 2005). For example, the development of oxidative stress tolerant *C. albicans* mutants during chronic oxidative stress caused by exposure to increasing concentrations of *tert*-butylhydroperoxide, a lipid peroxidation accelerating agent, resulted in a reduced germ tube, pseudohypha and hypha-forming capability, a decreased phospholipase secretion and, hence, a considerably decreased virulence in mice (Fekete *et al.* 2007). Such an increased oxidative stress tolerance may therefore be disadvantageous when *C. albicans* cells escape from blood vessels and invade deeper tissues. The oxidative stress tolerant phenotype of the mutants was explained with the continuous induction of the antioxidative defence system by steadily high intracellular concentrations of endogenous oxidants, *e.g.* peroxide, glutathione disulphide, lipid-hydroperoxides, conjugated dienes and thiobarbituric acid-reactive substances (TBARS), *i.e.* with adaptation to persistent oxidative stress (Fekete *et al.* 2007). Importantly, the decreased virulence of *C. albicans* cells sensing chronic oxidative stress may be an exploitable phenomenon in the treatment of different types of candidiasis. For example, combinations of corticosteroids, *e.g.* methylprednisolone, which increases the intracellular concentrations of conjugated dienes and TBARS but decreases concomitantly the specific activities of several antioxidant enzymes, with oxidants, *e.g.* menadione, or with oxidative stress-generating polyene antimycotics, *e.g.* amphotericin B, could be considered in the treatment of *C. albicans* infections in patients with prolonged topical application of corticosteroids (Gyetvai *et al.* 2007). It is important to note that corticosteroid-antimycotic interactions should be analysed with care because corticosteroid treatments may even weaken the antifungal effects of certain drugs, *e.g.* lovastatin, with a mechanism of action not including the initiation of oxidative stress in *C. albicans* cells (Gyetvai *et al.* 2006 and 2007).

Fekete, A. *et al.* (2007) FEMS Yeast Res, in press
Fradin, C. *et al.* (2005) Mol Microbiol 56: 397–415.
Gyetvai, Á. *et al.* (2006) FEMS Yeast Res 6: 1140–1148.
Gyetvai, Á. *et al.* (2007) FEMS Yeast Res 7: 265–275.

Influence of functional phenolics on cell growth and catalase/catechol oxidase production in the thermophilic fungus *Scytalidium thermophilum*

Y. Yuzugullu, Ö. Erçin and Z.B. Ögel

Food Engineering Dept., Middle East Technical University, Turkey

Oxidative stress can be defined as an imbalance between body antioxidant defence and the production of free radicals as a result of oxidative mechanisms, such as lipid peroxidation. Free radicals are known as unstable, highly reactive and low molecular weight chemical species that possess one or more unpaired electrons. These so-called reactive oxygen species (ROS) are mutagenic and thought to be important in the generation of cancer and other various diseases. The antioxidant enzymes of cells aimed at defence against ROS are catalase, superoxide dismutase and glutatione peroxidase. In addition, plant-derived phenolic compounds have shown antioxidant properties and related anti-cancer and anti-aging functions. However, in some cases, these antioxidants may turn out to be pro-oxidants causing oxidative stress, thus, becoming the cause of ROS generation. In our laboratory, a phenol oxidase enzyme of *Scytalidium thermophilum* (STEP) is shown to be a catalase with dual activity (Sutay, D., unpublished). Multiple sequence allignment studies have shown that STEP has highest homology to the monofunctional large catalases. The secretion of STEP is induced in the presence of phenolic compounds such as gallic acid. This suggests, STEP is produced for defence against toxicity of phenolics and that its oxidase activity has a role in this process. To test this hypothesis, we have exposed *S. thermophilum* cells to a number functional phenolics, selected among flavonoids, derivatives of cinnamic acid, coumarins, and phenolic acids, at different concentrations, and analysed growth and catalase/catechol oxidase activities. The biocatalysis products of these phenolics when exposed to purified STEP is also analysed. Taken together, these data are exposed to guide us in suggesting the biological role of this dual activity and may shed light on the dose-effects of phenolics on cellular functions.

.

Exposing yeasts to an acid stress: A chemostat study

L. Brambilla and D. Porro

Dept. of Biotechnology and Biociences, Università degli Studi di Milano-Bicocca, Italy

Yeasts are micro-organisms able to usually growth at low pH values, but among these organisms are species that display a peculiar attitude to growth in acid conditions. The study of the molecular and physiological mechanisms that confer resistance to the acid stress are of particular interest by an academic point of view and they appear to be intimately linked to a general mechanism of stress tolerance. The knowledges acquired on stress tolerance have also biotechnological interest, since in each industrial application the producing cell is exposed to a wide number of different stresses, which negatively affect biomass yield and production levels.

In order to study the effects of an acid stress upon the classic *Saccharomyces cerevisiae* baker's yeast we have run some chemostats experiments exposing cells to a mild pH gradient during the growth. Physiological data have been collected at different growth rates, with a particular attention to biomass yield, residual glucose and to the determination of the highest growth rate sustainable in this conditions by the cells. The same data have been obtained by growing in parallel experiments the stress tolerant yeast *Zygosaccharomyces bailii*. Our results point to a critical pH value, below which the answer of the two yeast substantially diverges. While *S. cerevisiae* cells display a sudden interruption of the growth, *Z. bailii* continues its duplication, even if with a slow rate, that is also affected by the cultural conditions.

The different behaviour of the two species, together with the synergic effects of the growing conditions upon the growth rates, raised the question of how these yeast species actually compete in the natural environment. To answer to this question, we run some mixed continuous cultures with the two yeasts, applying different combination of starting conditions and pH gradients, and we followed the effects of the experimental set-up on the permanence of the two strains in the fermenter. Although the two strain used displays very similar maximum growth rate in the standard growing conditions, we could individuate some situations leading to the complete prevalence of alternatively one on the two yeast.

Differential proteomics of the yeast plasma membrane: Analysis of the acid stress response

L. Dato, P. Paganoni, D. Porro and P. Branduardi

Università degli Studi di Milano-Bicocca, Dipartimento di Biotecnologie e Bioscienze, Italy

The yeast acid stress response has been only partially characterized to date, and still the molecular mechanisms involved are not fully understood. It is known that inorganic and organic acids exert a different effect within the cell, and hence evoke different cell responses. We are trying to better elucidate the changes that *Saccharomyces cerevisiae* cells undergo when grown in acidic environments, searching for new key factors involved in long term response and tolerance. We chose the plasma membrane as target for this study, because of its active role as selective filter and information exchange site between the cell and the environment. Our approach consists of a differential analysis of the *S. cerevisiae* plasma membrane proteome obtained from cells grown in three different conditions. A glucose minimal medium was taken as the control, and a low pH medium and low pH in the presence of lactic acid as the inorganic and organic acidic stress conditions respectively. Here we show our method, consisting of a cell fractionation to obtain enriched plasma membrane preparations followed by separation of the protein samples by two dimensional gel electrophoresis, and the results obtained for gels resolved in a pH range of 4 to 7. We obtained a list of proteins found to be overexpressed in acidic conditions. We than analysed the growth of the corresponding single deletion mutants in acids and found marked differences in their tolerance abilities with respect to the wild type.

Global analysis of the mechanisms underlying yeast resistance and response to the fungicide mancozeb

T. Simões, P. M. Santos, P. J. Dias, M. C. Teixeira and I. Sá-Correia

IBB-Institute for Biotechnology and Bioengineering, Centre for Biological and Chemical Engineering, Instituto Superior Técnico (IST), Portugal

Global genomic expression technology has the potential to identify genes that are affected by a particular chemical at the early stages of the toxicological response. Since the major metabolic and signaling pathways are conserved in eukaryotes, this new systems toxicity approach favors the establishment of parallelisms between different organisms and has an unprecedented potential to elucidate gene-environment interactions. The fungicide mancozeb, widely used in agriculture, is a probable human carcinogen and has been linked to Parkinson disease in certain individuals upon chronic exposure. The early global response to mancozeb was examined by expression proteomics using the eukaryotic model *Saccharomyces cerevisiae* and two-dimensional electrophoresis. Results from this quantitative proteomic analysis were analysed using the YEASTRACT database (www.yeastract.com; [1]), developed to provide assistance on the analysis of transcription regulatory associations in yeast. The main transcription activators and target genes of the complex proteome response to this toxicant were found to be related with the yeast response to mitochondrial dysfunction and oxidative stress, to retrograde regulation, and to disassembling protein aggregates and degradation of damaged proteins. The role of the multi-drug resistance (MDR) transporter Flr1p, involved in yeast response and resistance to mancozeb, in the observed proteome changes was also examined. Results indicate that the up-regulation of the candidate biomarkers of mancozeb action and of other responsive genes in the *Δflr1* mutant was significantly higher than in the wild type strain, consistent with the postulated role of Flr1p in mediating the active expulsion of mancozeb from the cell interior.

Results from a screening for mancozeb susceptibility of the yeast disruptome when exposed to mancozeb stress are consistent with the indications obtained from the proteomic analysis. A large number of genes involved in mitochondrial function, oxidative stress response and protein degradation and protein protection were identified as determinants of mancozeb resistance. Based on genome-wide expression and phenotypic data, the regulatory networks behind the yeast response and resistance to mancozeb are inferred. Selected genes/proteins that may provide useful information as early and sensitive biomarkers of the toxicological response to mancozeb are proposed.

[1] Teixeira et al. (2006) Nucleic Acids Research, 34: D446–D451.

Evolutionary engineering of copper-resistant yeast under pulse stress conditions

Ç. Karabulut[1,2], C. Alkım[1,2], N. Tokman[3], S. Akman[3], C. Tamerler[1,2,4], M. Sarıkaya[2,4] and Z.P. Çakar[1,2]

[1] Department of Molecular Biology and Genetics, Istanbul Technical University, Turkey
[2] Istanbul Technical University, Biotechnology and Genetics Research Center, Turkey
[3] Department of Chemistry, Istanbul Technical University, Istanbul, Turkey
[4] Department of Materials Science and Engineering, University of Washington, USA

Evolutionary engineering based on applying selective pressure towards a desired phenotype is becoming increasingly utilized in improving and tailoring various cellular properties. Improved phenotypes obtained upon evolutionary engineering can be further optimized by different metabolic engineering strategies. Here we have designed and employed evolutionary engineering strategies to obtain copper-resistant mutant yeasts. Wild-type *Saccharomyces cerevisiae* cells were chemically mutagenized, in order to obtain a genetically variant population. The selection of resistant mutant populations was accomplished under pulse copper stress conditions, and at constant and increasing copper concentrations. By applying increasing levels of copper concentrations at each generation, a final generation that could resist up to 10 mM $CuCl_2$ stress is obtained. During the selections with increasing copper concentrations, 56 mutant generations were obtained that were resistant to $CuCl_2$ concentrations significantly higher than the minimum inhibitory copper concentration of wild type *S. cerevisiae*. Individual mutants were randomly selected from final mutant populations, and their resistance, in terms of percent survival, was determined using a high-throughput most-probable number (MPN)-based method. Mutant individuals were also characterized with respect to their cellular copper contents using flame atomic absorption spectroscopy. Additionally, cross-resistance to other metal and non-metal stresses were determined. These results of the mutant characteristics were compared with those of the wild-type and the degree of their phenotypic changes was discussed. Revealing the genetic basis of copper resistant phenotypes could potentially be useful for practical applications in the fields of bioremediations and/or bionanotechnology.

mRNA turnover and gene regulation in *Aspergillus nidulans*

S. Chooluck, I. Y. Morozov, M. G. Jones and M. X. Caddick

Division of Integrative Biology, School of Biological Sciences, The University of Liverpool, UK

The stability of any given transcript plays a crucial role in determining the level of the mRNA and subsequent gene expression. Highly stable transcripts are optimal for achieving high level of gene expression. As a consequence RNA stability varies significantly among genes, and in some cases this is directly regulated as a means of controlling gene expression. We have previously demonstrated the differential rate of decay for the *areA* transcript, which encodes the major transcription factor responsible for mediating nitrogen metabolite repression. The *areA* transcript is destabilised by the presence of intracellular Gln, a signal of nitrogen sufficiency. The same mechanism is important to the expression of various structural genes involved in nitrogen metabolism, including *niiA* and *niaD*. Intriguingly, some of these genes are also subject to an additional regulatory mechanism which acts at the level of RNA stability, stabilising the transcripts in the presence of the respective protein's substrates. Bioinformatics analysis of the untranslated regions of *Aspergillus* genes reveal the presence of a number of conserved motifs suggests a large number of genes are likely to be regulated at the level of RNA stability. Moreover, many include conserved elements likely to be involved in interactions with the Pumiliohomology domain (Puf) RNA-binding proteins. In other organisms, including *Saccharomyces cerevisiae* and *Drosophila melanogaster*, these proteins have been found to coordinate expression of specific groups of genes. In our laboratory, we have identified a number of proteins whose orthologues are shown to be involved in RNA degradation. Amongst these are the five Puf proteins, PufA to PufE. Using recombinant-PCR technique, we have successfully deleted *pufA*, *pufB*, *pufC*, *pufD* and *pufE*. Deletion of *pufD* result in a dramatically reduced response to Gln with respect to destabilisation of *areA* and *meaA* transcripts, and *areA* also revealed significantly reduced basal degradation. Deletion of *pufA* appears to disrupt the sexual cycle. *PufB*, *pufC* and *pufE* deleted strains are currently being examine.

.

The role of modulation in energy metabolism in cellular response to DNA damage

S. Wölfl, A. Kitanovic and J. Holzwarth

Institute for Pharmacy and Molecular Biotechnology, Ruperto-Carola University of Heidelberg, Germany

Very important aspect of the DNA damage response is the modulation of major pathways of energy metabolism, which may be closely linked to the oxidative burst in cells exposed to stress. Induction of many key enzymes involved in glycolysis, pentose phosphate pathway, or the Krebs (citrate) cycle may be necessary for generating reducing equivalents (NADH, NADPH) that are needed for cellular antioxidant systems (Kültz, 2005). Moreover, it was shown that early increase in ATP level and mitochondrial membrane potential ($\Delta\Psi$) are required for certain steps of apoptosis (reviewed in Skulachev, 2006). Therefore, it seems that the energy metabolism and ATP level could serve as a monitoring parameter of cellular damage and by this insure proper balance between apoptotic and anti-apoptotic signal.

Here we demonstrate that the low doses of alkylating agent methanesulfonate (MMS) triggers the activation of environmental stress response (ESR), increase ROS production and mitochondrial membrane potential ($\Delta\Psi$) and induce mitochondrial biogenesis. This was accompanied with a strong increase in cellular ATP level and disintegration of tubular mitochondrial network and mitochondrial filaments into small roundish mitochondrial clusters. However, if we applied 0.1% MMS treatment during the same period, we observed rather opposite effect. Such a high MMS concentration decreased mitochondrial biogenesis, lowered $\Delta\Psi$ and ATP production, and cause complete collapse of the tubular mitochondrial network. Moreover, Cultivation of cells in acetate/low glucose medium, that was proven to induce apoptosis (Knorre *et al.*, 2005), or minimal medium could efficiently decreases sensitivity to MMS and allow more cells to survive the treatment with this toxic agent. The effect seemed to be connected with increased NADPH level that resulted in elevated NADPH/NADP+ ratio, enhanced ROS production and elevated ATP level. However, in those two media cells age faster.

In conclusion, we believe that in *S. cerevisiae* treatment with relatively low concentrations of MMS leads to important alterations in cellular bioenergetic conditions, which serve to induce repair mechanisms and removing of seriously damage cells by apoptosis. Nevertheless, it seems that the development of such defence mechanisms has as a consequence an effective DNA damage repair, but also a shortened life-span of the cells.

Kültz, D. (2005) Molecular and evolutionary basis of the cellular stress response. Annu. Rev. Physiol. 67, 225–257.

Skulachev, V.P. (2006) Bioenergetic aspects of apoptosis, necrosis and mitoptosis. Apoptosis 11, 473–85.

Knorre, D.A., Smirnova, E.A., Severin, F.F. (2005) Natural conditions inducing programmed cell death in the yeast Saccharomyces cerevisiae. Biochemistry (Mosc) 70, 264–6.

Autolysis of *Aspergillus nidulans* – a physiological approach

T. Emri, T. Pusztahelyi, Z. Molnár, É. Leiter and I. Pócsi

Department of Microbial Biotechnology and Cell Biology, University of Debrecen, Hungary

A deeper understanding of the physiology and regulation of fungal autolysis may help us to engineer the morphology of industrially important filamentous fungi in the stationary and autolytic phases of growth. Moreover, this information could also provide us with new, promising targets in future antifungal drug research. The autolysis of the filamentous model fungus *Aspergillus nidulans* is a complex physiological process depending on both energy and *de novo* protein synthesis (Emri *et al.* 2004). In submerged carbon-depleted cultures, the FluG-BrlA sporulation signalling pathway was shown to play a pivotal role in the initiation of autolysis (Emri *et al.* 2005a). Although the autolytic events hydrolase production, hyphal fragmentation and autolytic loss of biomass were tightly coupled they were disconnected by loss-of-function mutations in the FluG-BrlA regulatory network indicating the sophisticated regulation of the process. Protein and peptide (*e.g.* glutathione; GSH) reserves were degraded fast in carbon starving cultures, which was regulated separately *via* FadA-FlbA and GanB-RgsA heterotrimeric G-protein signalling (Molnár *et al.* 2004). The concentration of reactive oxygen species (ROS) increased steadily in autolysing mycelia concomitantly with the onset of glutathione/glutathione disulphide redox imbalance and declining cell vitality. The accumulation of ROS coincided with the appearance of both autolytic and apoptotic markers but these processes were regulated differently (Emri *et al.* 2005b). The production of autolytic enzymes (protease, chitinase) was controlled by the transcriptional factors BrlA, CreA and AbaA. Much less is know about cell death signalling and regulation but the involvement of FadA-FlbA and GanB-RgsA heterotrimeric G-protein signal transduction pathways seems to be likely (Leiter *et al.* 2005, Molnár *et al.* 2006). As far as the nitrogen sources are concerned, the autolysis observable in complex culture medium containing yeast extract was slower than that recorded in nitrate-minimal medium. Interestingly, the inclusion of yeast extract into the culture medium did not hinder the autolysis of a MeaB mutant strain.

Emri *et al.* (2004) Folia Microbiol 49: 277–284.
Leiter *et al.* (2005) Antimicrob Agents Chemother 49: 2445–2453.
Molnár *et al.* (2004) Appl Biochem Biotechnol 118: 349–360.
Emri *et al.* (2005a) Mycol Res 109: 757–763.
Emri *et al.* (2005b) FEMS Microbiol Lett 251: 297–303.
Molnár *et al.* (2006) J Basic Microbiol 46: 495–503.

Differentiation of cells in population of yeast colonies

Z. Palkova[1], L. Vachova[2], H. Kucerova[1,2] and M. Ricicova[1]

[1] Department of Genetics and Microbiology, Charles University, Czech Republic
[2] Institute of Microbiology, Academy of Sciences of the Czech Republic, Czech Republic

Our previous studies showed that yeast colonies periodically change the pH of their surroundings from acidic to alkali. In the acidic phase colonies grow, whereas in the alkali phase they produce ammonia as the signalling molecule and their growth is transiently inhibited (Palkova et al., *Nature* 390: 532–536, 1997). The microarray and other analyses showed that the transition of colonies from acidic to the alkali phase is connected with extensive reprogramming of cell metabolism. This includes on one side progressive repression of environmental stress response genes and parallel repression of genes for mitochondrial oxidative phosphorylation and genes for some of the enzymes of degradative steps of mitochondrial citrate cycle, and on other side activation of many genes connected with amino acid metabolism, genes important for peroxisome biogenesis and other genes for enzymes involved in predicted adaptive metabolism (Palkova et al., *Mol. Biol. Cell*., 13: 3901–3914, 2002). Alterations connected with ammonia production appear to be indispensable for long-term colony adaptation, development and survival as indicated by studies on colonies formed by strain mutated in Sok2p transcription factor (Vachova et al., *J. Biol. Chem*. 279: 37973–81). Colony population as a whole was used for the experiments which revealed changes described above. This means that they can represent either relatively moderate modifications in all cells in the colony or, alternatively, big changes in a part of the population. Our recent results proved that at least in some cases the second possibility is valid, i.e. that yeast colony behaves as a differentiated structure. For the beginning, we analysed physiology of cells located at older, chronologically aged colony areas and in relatively young areas, respectively, and we focused on monitoring of localisation of previously described metabolic and stress changes. Here, we will present data on localisation of stress and dying features, as well as on localisation of cells expressing selected transporters and metabolic genes. The significance of this differentiation for colony development will be discussed. This work is supported by GACR 204/05/0294, LC531, AV0Z50200510, MSM0021620858 and HHMI award to Z.P.

Application of image analysis in microbiology

T. Krulikovska, P. Patakova and V. Albrecht

Department of Fermentation Chemistry and Bioengineering, Institute of Chemical Technology, Czech Republic

The method of image analysis is known for a decades but its application in microbiology is still limited. However the advance in video and computer technology and price reduction has enabled expansion of the method to other application areas. In this work the image analysis will be presented as highly versatile and efficient tool for microbiologists. The application of the method during our work is summarised.

The method was used for monitoring and quantifying of yeast growth. *Rhodotorula mucilaginosa* and *Rhodotorula glutinis* were cultivated under different cultivation conditions in shaked flasks and on solid media. During cultivation in liquid media the changes in cell size, total number and number of budding yeast were measured. These parameters suitably replenished classically determined parameter-biomass concentration.

On solid media the influence of temperature and NaCl addition to cultivation media was monitored. The growth rate was expressed as diameter change in time. As *Rhodotorula* is a significant producent of carotene pigment, the content of pigments was determined by HPLC. The carotene pigment content was correlated with colony colour.

In addition to these the method of image analysis was used for determination of optimal cultivation conditions of *Trichosporon cutaneum* cultivated on cellophane, for monitoring of bacterial biofilm formation and for CFU quantifying on agar plates. In all cases the application of the method brought acceleration and increase effectiveness of determination process.

The structural organization and expression of kinesin motor molecules in the homobasidiomycete *Schizophyllum commune*

M. Raudaskoski[1], V. Salo[2] and M. Uuskallio[2]

[1] Plant Physiology and Molecular Biology, Department of Biology, University of Turku, Finland
[2] Department of Biological and Environmental Sciences, University of Helsinki, Finland

Microtubules play a central role in mitotic spindle and support the vesicle transport towards the actin cytoskeleton at the hyphal tip. Both processes require MT-based motor molecules kinesins and dyneins. We have isolated three kinesin genes from *S. commune* and analyzed their expression by qRT-PCR. Sckin1 amino acid sequence is highly homologous with conventional kinesin known to be involved in vesicle transport in filamentous fungi. Sckin2 shows high identity to kinBimC implicated in mitotic spindle function. *Sckin1* and *Sckin2* genes are separated by 1200 bp and transcribed in different directions. A similar syntenic region with two closely located kinesin genes transcribed in different direction is found in *Coprinopsis cinerea* genome. *Sckin3* is highly homologous with a not yet annotated kinesin also present in *C. cinerea*. The structural organization of Sckin3 suggests that it may play a role in nuclear division. All the three kinesin genes are constitutively transcribed during vegetative growth and mating interaction with the lowest expression observed for *Sckin2*. *Sckin3* expression is much higher than that of *Sckin2*, which suggests that *Sckin3* might have an additional role to that in nuclear division perhaps it is necessary for the extensive intracellular nuclear movements typical to homobasidiomycetes. No difference was observed in the expression levels of *Sckin* genes in relation to the carbon source in the growth medium.

Heterotrimeric G alpha protein Pga1 of *Penicillium chrysogenum* controls conidiation mainly by a cAMP-independent mechanism and its deletion leads to a sporulation microcycle in liquid cultures

R. O. García-Rico[1, 2], F. Fierro[1, 2] and J. F. Martín[1, 2]

[1] Instituto de Biotecnología de León, INBIOTEC, Spain
[2] Área de Microbiología, Universidad de León, Spain

Heterotrimeric G proteins in fungi regulate different processes related to development. Asexual sporulation is the main mechanism of propagation in filamentous fungi. To study the role of the heterotrimeric G alpha subunit Pga1 of *Penicillium chrysogenum* in conidiogenesis, a *pga1* deleted strain (delta*pga1*) and transformants with constitutively activated (*pga1*^{G42R}) and inactivated (*pga1*^{G203R}) Pga1 alpha subunits were obtained. They showed phenotypes that clearly implicate Pga1 as an important negative regulator of conidiogenesis. Pga1 affected positively the levels of intracellular cAMP, which acts as secondary messenger of Pga1 mediated signalling; although cAMP has some inhibitory effect on conidiation, regulation of asexual development by Pga1 is exerted mainly by cAMP-independent pathways. Regulation of conidiation by Pga1 is mediated by repression of the *brlA* and *wetA* genes, whose transcript levels vary in accordance to the level of Pga1. The delta*pga1* strain and transformants with the constitutively inactive Pga1^{G203R} subunit developed a sporulation microcycle in cultures. This unusual developmental process is triggered by the high expression of *brlA* and *wetA* genes that are deregulated in the absence of active Pga1. Differences between the delta*pga1* strain and the transformants with a constitutively inactive Pga1^{G203R} subunit support the implication of the beta gamma dimer in regulation of conidiogenesis.

Cloning, expression and purification of the N-terminal domain of Flo1p from *Saccharomyces cerevisiae*

K. Goossens, D. Maes, L. Wyns and R. Willaert

Dept. Ultrastructure, Vrije Universiteit Brussel, Flemish Interuniversity Institute for Biotechnology, Belgium

Yeast cells possess a remarkable capacity to adhere to other cells, which is called flocculation. Flocculation is defined as the phenomenon wherein yeast cells adhere in clumps and sediment rapidly from the medium in which they are suspended. These cell-cell-interactions are mediated by a class of special cell wall proteins, called flocculins that stick out of the cell walls of flocculent cells and selectively bind mannose residues present in the cell walls of adjacent yeast cells. For brewers, flocculation is a desirable property of industrial *Saccharomyces cerevisiae* strains as it allows the easy separation of cells from the fermentation product. Only at the end of the fermentation process, the yeast cells start adhering to each other to form macroscopic 'flocs' consisting of many thousands of cells. Flocculins consist of three domains. The first one is the mannose binding domain. The middle domain consists of a variable number of repeats and is heavily glycosylated. The protein is anchored to the cell wall by the C-terminal domain.

In this work, we studied the Flo1-protein, which is the most important flocculin responsible for flocculation of yeast cells. The final goal is the characterization of the binding mechanism of the protein to mannose. As the mannose binding site is situated in the N-terminal domain, this domain is the most interesting one to study. Therefore it was cloned in *Escherischia coli* using the pGEX-vector. In this vector, the N-terminal domain of Flo1p, was coupled to GST (Glutation-S-Transferase). After induction of the *E. coli*-cells, the fusion protein was overexpressed in the cytosol of the cell. The fusion proteins were purified by affinity chromatography, using a Glutathione-Sepharose-column. To remove the GST-tag, an enzymatic cleavage with thrombin was used. Finally the N-terminal domain was further purified by affinity chromatography, using a mannose-column.

This work was supported by the Belgian Federal Science Policy Office and European Space Agency PRODEX program, the Institute for the Promotion of Innovation by Science and Technology in Flanders (IWT) and the Research Council of the VUB.

Biofilm formation and invasive growth of *Saccharomyces cerevisiae* in microgravity

S. Van Mulders[1], C. Stassen[2], G. Toye[1], B. Devreese[2], F. Delvaux[1] and R. Willaert[3]

[1] Centre for Malting and Brewing Science, Leuven, Belgium
[2] Lab. Protein Biochemistry & Protein Engineering, Universiteit Gent, Belgium
[3] Dept. Ultrastructure, Vrije Universiteit Brussel, Belgium

The influence of microgravity on the growth of *S. cerevisiae* on solid agar (i.e. biofilm formation and invasive growth) has been studied during an experiment on board of the International Space Station (ISS).

Three yeast strains (wild-type prototrophic strain *S. cerevisiae* Sigma 1278b, *S. cerevisiae* Sigma 1278b *flo11* delta and an industrial brewer's strain) were grown as colonies on 0.8% and 2.0% (w/v) agar in microgravity during 9 days. The obtained results were compared with a similar 1g-ground experiment. Two photosessions where performed on board the ISS in order to determine the growth rate of the colonies in microgravity. The growth rate was comparable with the one on earth. The performed postflight analyses were the determination of the invasive growth, budscar and proteomics analyses. The invasive growth of the wild-type strain on 2% agar was reduced in microgravity in the center of the microcolony. Yeast cells grown in microgravity showed a higher percentage (≈14%) of random bud scars compared to the ground experiment. A protocol was set up to perform proteomic analysis (2D-gel electrophoresis followed by mass spectrometric identification) on the yeast colonies. The proteomic results for colonies grown on 2% agar will be presented.

Acknowledgements
This work was supported by the Belgian Federal Science Policy Office and European Space Agency PRODEX program.

Yeast RNA is preserved by RNA*Later* at high temperatures

R.G.E. van Eijsden[1], S.E.B. Van Mulders[2], C. Stassen[3], B. Devreese[3], F.R. Delvaux[2], R. Willaert[4] and P. Van Hummelen[1]

[1] MicroArray Facility, VIB, Leuven, Belgium
[2] Centre for Malting and Brewing Science, Department of Food and Microbial Technology, Leuven, Belgium
[3] Laboratoryof Protein Biochemistry & Protein Engineering, Ghent University, Belgium
[4] Department of Ultrastructure,Vrije Universiteit Brussel, Belgium

In our project *Yeast In No Gravity,* the influence of microgravity on cellular adhesion, biofilm formation and invasive growth in the model eukaryote *Saccharomyces cerevisiae* will be studied. For the preservation of RNA after sampling yeast cells, cooling on ice or flash freezing in liquid nitrogen followed by storage at -80°C is often recommended. Because of practical or regulatory reasons, neither of these options is acceptable for microgravity experiments in space (International Space Station). Therefore, RNA*Later* (Ambion) was selected as fixating agent because of its non-hazardous composition and its positive performance on other tissues and cell types, as has been reported in other studies. During landing, yeast cells will be exposed to temperatures up to 30°C for 24 up to 48 hours, influencing the quality and integrity of RNA and proteins, impeding postflight gene expression and proteomic analysis. As the performance of RNA*Later* has never been tested on yeast cells, at least not under the conditions mentioned above, we have set up an experiment where yeast cell cultures were fixed with RNA*Later* and stored during various time periods and temperature ranges. RNA was isolated using TRIzol Reagent (Invitrogen). RNA quality and quantity was determined spectrophotometrically using the Nanodrop ND-1000 (Nanodrop Technologies) and RNA integrity was assessed by generating RNA profiles using a Bioanalyzer 2100 (Agilent). The average RNA yield from 10^8 cells was 8.8 µg. The RNA was of good quality with A260/A280 ratio's > 2 and A260/A230 ratio's between 1.5 and 2.0. Moreover, Bioanalyzer results showed no excessive RNA breakdown. The effect of RNA*Later* on the protein content of the samples is still to be assessed. We concluded that fixation of yeast cells with RNA*Later* preserved the quality and integrity of the RNA for use in gene expression studies, even after incubation for 48 hours at 30°C.

Acknowledgements:
This work was supported by the Belgian Federal Science Policy Office and European Space Agency PRODEX program.

.

Molecular evolution of multisubunit RNA polymerases: The unity in a diversity

G. V. Shpakovski and E. K. Shematorova

Laboratory of Mechanisms of Gene Expression, Russian Academy of Sciences, Russia

Analysis of literary data on structure and function of transcription apparatuses of different members of the three Urchkingdoms of living organisms (Archaea, Bacteria, and Eucarya) and results of our own studies of structure-function conservation of the basal transcription machinery of eukaryotes (first of all, *Schizosaccharomyces pombe*, *Saccharomyces cerevisiae*, and *Homo sapiens*) [Mol. Cell. Biol., 1995, 15: 4702–4710; Bioorg Khim., 1999, 25: 791–796; J. Mol. Biol., 2000, 295: 1119–1127; Nucleic Acids Res., 2006, 34: 3615–3624] has allowed us to complete a coherent system of classification of all subunits of multicomponent (archaeal, eubacterial, and eucaryal) RNA polymerases. For the first time, structural homologue of the RNA polymerases I-III common subunit Rpb8 was detected among subunits of archaeal enzyme. This observation provides last and decisive evidence of an equal archaeal/eucaryal RNA polymerase architecture (general organization), and is a strong argument in favour of a common evolutionary origin of all known multisubunit RNA polymerases. As a result, in a classification system proposed twelve the most important for transcription and cell viability subunits of every nuclear RNA polymerases (I, II, and III) of eukaryotes are placed in twelve distinct triads (protein superfamilies). Proteins homologous to all twelve these superfamilies were found among different components of archaeal transcription apparatus. Structural domains (homologous regions) with strong or only distant, but revealing similarity to proteins of at least ten of these superfamilies could be detected in the primary structures of different subunits of transcription holoenzyme of various eubacterial species.

The origin of some small subunits of eukaryotic RNA polymerases as a result of duplications of the genetic regions encoding two largest RNA polymerase subunits of the last common ancestor of Bacteria, Archaea and Eucarya is postulated. Structure-function correspondence among the rest two and five subunits of eukaryotic RNA polymerases I (Rpa49, Rpa34) and III (Rpc51, Rpc37, Rpc31, Rpc34, Rpc82), respectively, is also established. A unified nomenclature of the subunits of all multicomponent RNA polymerases, resulted from aforementioned findings, will be presented.

This work was supported by the program "Molecular and Cellular Biology" of the Russian Academy of Sciences and by grant No. 07-04-01167 of the Russian Foundation for Basic Research (RFBR).

Functional dissection of the Rpb9 subunit of RNA polymerase II of *Saccharomyces cerevisiae*

E. K. Shematorova[1], G. V. Shpakovski[1] and P. Thuriaux[2]

[1] Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Sciences, Russia
[2] l'Institut de Biologie et de Technologies de Saclay, France

One of two small nonessential subunits of RNA polymerase II of *Saccharomyces cerevisiae* is Rpb9. The full deletion of Rpb9 leads to conditional growth defects (16°C, 37°C) and sensitivity to mycophenolate, an NTP-depleting drug. Rpb9 occupies the "upper" jaw of the yeast RNA polymerase II crystal structure, and includes two Zn-binding domains separated by a linker (Cramer et al., 2000). Both are conserved, especially the C-terminal part. However, deletion of C-terminal conserved region has no detectable growth defect, whereas N-terminal deletion behaves like null mutant. With use of coimmunoprecipitation (CoIP) we have demonstrated that the N-terminal part of Rpb9 is critical for an incorporation of this subunit into RNA polymerase II.

To understand function of the Rpb9 C-terminal domain, we were looking for genetic contexts in which this part of the protein becomes essential for cell viability. Our data suggest that C-terminal part of Rpb9 is important in cells lacking Rpb4, another nonessential subunits of yeast RNA polymerase II, *rpb1-d104* mutant with partially truncated CTD of Rpb1, the largest subunit of RNA polymerase II and a deletion of the *SOH1* gene, encoding a conserved subunit of Mediator.

Some mutations in C-terminal region of Rpb9 affects start site selection and suppresses cold sensitive mutation in TFIIB (*sua7-1*) (Sun et al., 1996). To understand the nature of these events, we have tested a set of point/deletion mutants in the C-terminal region of Rpb9. Our mutants can be divided into the three groups. A first group only changes start site selection, a second group suppresses *sua7* mutation and a third group affects both processes.

Recently, two-hybrid interactions shown that the Rpb9 C-terminal part may bind the largest subunit of TFIIE (Van Mullem et al., 2002), a general transcription factor. We found that Spt7, a structural subunit of SAGA, interacts with the same region of Rpb9. Taken together, these results suggest a possible role of the Rpb9 C-terminal domain in regulation of transcription initiation.

This work was supported by the program "Molecular and Cellular Biology" of the Russian Academy of Sciences and by grant No. 07-04-01167 of the Russian Foundation for Basic Research.

Exploring resistance to phosphinothricin in *Aspergillus niger*

M. Ahuja and N. S. Punekar

Biotechnology Group, SBB, Indian Institute of Technology-Bombay, India

Phosphinothricin (PPT) is a slow, tight binding, irreversible inhibitor of glutamine synthetase. This anti-metabolite is an established herbicide. Resistance to PPT, imparted through *bar* gene, was successfully exploited as a dominant marker to transform *Aspergillus niger*. Colonies with spontaneous PPT resistance, albeit of moderate growth, were frequently encountered during *bar* selection. Possible mechanisms of PPT tolerance, apart from that conferred by *bar* gene, were therefore explored. Glutamine synthetase activity profile of the spontaneous mutants did not indicate any over-expression of the target enzyme. Presence of a mutant form of glutamine synthetase was also unlikely since the inhibition profiles with either PPT or methionine sulfoximine were not altered. A competitive amelioration of inhibition by elevated external and/or endogenous glutamate levels was evaluated. Enhanced metabolism of PPT in the spontaneous mutants, likely to occur through the action of – transaminase, dehydrogenase or L-amino acid oxidase activities, was assessed. A decrease in PPT transport/ uptake seems responsible for the PPT resistance of the spontaneous mutants.

The utilisation of the prebiotic, neokestose, by potentially probiotic yeasts and *Saccharomyces cerevisiae* var. *boulardii*

J. R. Coangae, S. G. Kilian and J. C. du Preez

Department of Microbial, Biochemical and Food Biotechnology, University of the Free State, South Africa

Probiotic preparations are live microbes that, when ingested, beneficially modify the intestinal microbiota of the host. Prebiotics, on the other hand, stimulate beneficial intestinal microbes and are mostly oligosaccharides. Combinations of probiotics and prebiotics, known as synbiotics, are also sometimes administered. Some reports indicate that the probiotic yeast *Saccharomyces cerevisiae* var. *boulardii* can successfully be used in combination with prebiotics, meeting the concept of a synbiotic. This prompted us to investigate the ability of *S. cerevisiae* var. *boulardii,* as well as other yeasts reported to have potential probiotic properties, to utilise the prebiotic trisaccharide, neokestose, of which the potential for inclusion into a synbiotic has not been evaluated before. The yeasts were cultivated on complex medium in shake flasks under aerobic as well as oxygen limited conditions and biomass and sugars were monitored. Based on maximum specific growth rates, biomass yields, biomass yield coefficients and the rates of neokestose utilisation, *G. geotrichum* had the best overall growth properties under aerobic conditions, while *S. cerevisiae* var. *boulardii* performed best under oxygen limited conditions. *Kluyveromyces lactis* and *Pichia anomala* also had promising growth characteristics on neokestose. These results show that neokestose might be successfully combined with *S. cerevisiae* var. *boulardii* in a synbiotic mixture. In addition, other potentially probiotic yeasts also show promise for use in synbiotics and may be easier to cultivate than *S. cerevisiae* strains, which tend to divert substrate into ethanol instead of biomass, even under aerobic conditions.

Assessment of antimicrobiological properties of yeasts intended for probiotics destined for animals

K. Piasecka-Jóźwiak, J. Rozmierska, B. Chabłowska, K. M. Stecka and M. Kępa

Institute of Agricultural and Food Biotechnology, Warsaw, Poland

The aim of the work was to determine the parameters for cultivation of yeast strains, being selected for application in probiotic preparations intended for animal nutrition; the studies were directed towards preserving the antibacterial properties of the previously selected yeasts during the enlargement of the culture scale.

The research material included the following yeast strains: *Saccharomyces cerevisiae* KKP 512, *S. cerevisiae* C11, *S. boulardi* An-1, *Pichia guilliermondi* T1 and *Candida zeylanoides* T7. These strains were selected from among collection yeasts and those ones, isolated from natural environment on the ground of, *inter alias*, revealed antibacterial properties. The experiments were carried out in micro-technical conditions (fermentors Biostat B.Braun), using molasses wort, the pH of which during cultivation was equal to 3, 4, 5 and 6 units. Antimicrobiological properties of the particular yeast strains were examined in relation to panel of indicatory bacterial strains: *Escherichia coli* 1 (hemolytic), *E. coli* 2 (hemolytic), *E. coli* 3 (hemolytic), *Listeria innocua*, *L. monocytogenes* 1, *L. monocytogenes* 2, *Salmonella serotype* DO, *Salmonella serotype* CO, *Salmonella enteritidis* and *Bacillus subtilis*.

The highest antibacterial activity, irrespectively of pH value of molasses wort, in which the culture was carried out, was demonstrated by S. cerevisiae KKP 512 which inhibited completely growth *L. innocua*, *Salmonella* DO, *L. monocytogenes* 1 and limited growth of other indicatory strains. In case of the remaining examined yeasts, the effect of pH value of the environment in which the cultivations were carried out, on antimicrobiological properties was observed. *Pichia guilliermondi* T1 strain was characterized by the highest antibacterial activity after cultivation at pH 3, inhibiting partly the growth of *E. coli* 1, *E. coli* 2, *E. coli* 3, *L. monocytogenes* 2, *L. innocua* and *Salmonella* serotype DO. The strain *S. boulardi* An-1, cultivated at pH 3 showed the highest antibacterial activity at a considerable lowering of substrate's yield (44%); it limited partly growth of *E. coli* 3, *L. innocua*, *Salmonella* serotype DO and *Salmonella enteritidis*.

It was found that the examined yeast strains were characterized by antimicrobial properties in relation to the highest number of the employed strains of indicatory bacteria, after cultivations, conducted at lower pH values (that is, 3 and 4 pH units).

The ability of yeasts from Saudi Arabian soil to oxidize elemental sulphur

A. M. Al-Falih

Dept. of Biology, Teachers College in Riyadh, Saudi Arabia

Candida tropicalis, *Geotrichum capitatum*, *Geotrichum candidum and Rhodotorula minuta* were isolated from soils of Saudi Arabia. The ability of these soil yeasts to mediate sulphur oxidation *in vitro* was studied in Czapek Dox medium to thiosulphate, tetrathionate and sulphate.

The highest production of sulphate was formed by *G. candidum* (103 μg/ml) and *G. capitatum* (87 μg/ml) while moderate sulphur oxidation was exhibited by *R. minuta* (45 μg/ml). On the other hand, the soil yeast *Candida tropicalis* reached the lowest amount of sulphate with 5 μg/ml of sulphate by the end of the incubation period. Sulphur oxidation was found to be highly correlated with yeast biomass formed. These oxidation processes led to a marked reduction in the pH of the medium.

Removal of Cd^{2+}, Zn^{2+} and Ni^{2+} by modified *Saccharomyces cerevisiae*

O. Schreiberová, T. Krulikovská, J. Masák, A. Čejková and V. Jirků

Department of Fermentation Chemistry and Bioengineering, Institute of Chemical Technology, Czech Republic

Heavy metal pollution has become a serious problem today due to the increasing production by industry. In the recent years the possibility of removal by the means of biosorption onto yeast cells was examined.

In the present study the biosorption of heavy metal ions by *Saccharomyces cerevisiae* strains with modified cell wall structure was investigated. Three different heavy metal ions were examined – cadmium, nickel and zinc. Two types of experiment set-up were compared – either the cells were grown in the presence of various heavy metals or the metals were added to cells in late stationary phase. The sorption potential was assessed by the monitoring of the accumulation of heavy metals in supernatant, cell walls and cytoplasm. The content of mannose as a potentional bearer of ligands for heavy metals was estimated and the hydrophobicity and permeability of cells was determined.

It was found that the presence of metals severely inhibited the biosorption of metals by growing cells. The hydrophobicity of cells grown in the presence of heavy metals was higher than in cells grown without heavy metals, which corresponded with the detected lower content of mannose in the former.

ABSTRACTS OF POSTER SESSION III

Nutrition, signalling and transport

Posters P45 – P55

Defect in two pleiotropic drug resistance transporters causes extensive changes in physiology of *Saccharomyces cerevisiae* populations

O. Hlavacek[1], H. Kucerova[1,2], Z. Palkova[2] and L. Vachova[1]

[1] Institute of Microbiology, Academy of Sciences of the Czech Republic, Czech Republic
[2] Department of Genetics and Microbiology, Charles University, Czech Republic

The survival of yeast as well as of other microorganisms in natural environment is dependent on their ability to protect themselves against the progressive effect of long-term starvation and various stresses. These may originate either from the environment (e.g. toxic metabolites extruded by the cell population into the surroundings during culture development or toxic compounds produced by cells of competitor species) or from the cell interior as by-products of cellular metabolism. Thus, for all populations, an essential feature is the elimination of intracellular toxic substances either by their intracellular detoxification or by their expelling out of the cells. Extrusion systems, which may be involved in protection against either toxic compounds penetrating into the cells from the environment or toxic intracellular metabolites, include a large group of transporters denoted pleiotropic drug resistance (PDR) transporters (similar to mammalian multidrug resistance transporters) which localise either to the plasma membrane or to other cell membranes (e.g. vacuolar membrane). In this study, we asked question whether and how changes in PDR extrusion systems could influence physiology of yeast population growing exponentially or under starvation conditions. For the beginning, we focused on yeast cells exhibiting alterations in PDR transporters controlled by Pdr1p transcription factor. We used strain carrying modified allele *PDR1-3* encoding hyperactive form of this factor, which (among others) highly upregulates expression of genes for PDR transporters Pdr5p, Snq2p and Yor1p, as well as various isogenic mutants deleted in different combinations of genes for these transporters. We compared physiological properties (growth profile, survival, level of stress defence mechanisms, expression of genes representing particular metabolisms etc.) of parental and mutant strain populations occurring at different developmental phases. The data will be presented showing that the absence of Pdr5p and Snq2p (but not Yor1p) significantly influences the whole physiology of the respective population, supporting thus the hypothesis that PDR plasma membrane extrusion systems could be essential for proper development and survival of aged yeast population. The project is supported by GAČR (204/05/P175 and 525/05/0297), AV0Z50200510 and by LC531.

Systematic analysis of polyphosphate in yeast reveals novel functions of phosphate transporters in polyphosphate metabolism

H. C. Hürlimann, N. Amrhein and F. M. Freimoser

Institute of Plant Sciences, ETH Zurich, Switzerland

Inorganic polyphosphate (poly P) is a linear polymer that consists of phosphoanhydride linked phosphate residues, occurs in all organisms and cells, functions as a phosphate store and buffer and regulates the activation of enzymes or gene expression. The yeast *Saccharomyces cerevisiae* can store up to 20% of its dry weight in the form of poly P, almost entirely in the vacuole. Despite this ubiquitous occurrence and important functions it is still unclear how poly P is synthesized and how poly P metabolism is regulated in eukaryotes.

To elucidate poly P metabolism in eukaryotes we have performed a systematic screen of poly P levels in the knockout strains of all non-essential yeast genes and thereby identified 255 genes (almost 4% of the yeast genome) that are involved in the maintenance of normal poly P levels. Many of these mutants were knockouts of genes that encode vacuolar proteins or proteins functioning in intracellular transport and cell homeostasis. Besides reduced poly P content, many strains also exhibited reduced total phosphate content, showed altered ATP and glycogen levels and were disturbed in the secretion of acid phosphatase. Therefore, poly P metabolism strongly influences phosphate metabolism and the *PHO* pathway and is tightly interconnected with primary metabolism, in particular energy metabolism.

Poly P content was then used as the read-out for a thorough characterization of mutant strains affected in the *PHO* pathway and in the five phosphate transporters in yeast. Only one of the five yeast phosphate transporters, Pho84, could support normal poly P levels if present alone. Pho84 is therefore the most important phosphate transporter in yeast; both for phosphate uptake and for poly P accumulation. Surprisingly, the low-affinity transporters Pho90 and Pho91 negatively regulated poly P metabolism and caused an increase in poly P content when deleted. Detailed analysis of these deletions showed that the inhibitory effect of Pho90 or Pho91 on poly P accumulation was not due to a regulation of the *PHO* pathway or phosphate uptake. In contrast, it was concluded that these two proteins serve as intracellular phosphate transporters that function in phosphate translocation and allocation within the cell. With poly P content as a novel distinguishing mark it was thus possible to define novel functions of the low-affinity phosphate transporters in phosphate, poly P and cell metabolism.

Multiple signalling pathways interact to produce the diverse effects of glucose in *Saccharomyces cerevisiae*

J. M. Gancedo and M. M. Belinchón

Instituto de Investigaciones Biomédicas CSIC-UAM, Spain

The availability of glucose has a strong influence on the physiology of *S. cerevisiae*. This sugar causes the induction of a variety of genes and the repression of many others, as well as changes in the activity and stability of diverse enzymes. The different processes triggered by glucose might depend on a unique early signal targeting different regulatory pathways, or on different signals; in the latter case each signal could control a distinct process, or there could be a more complex combination of signals. To address this question, we have investigated the effects of the lack of plasma membrane glucose sensors and of glucose phosphorylating enzymes on different processes. We found that the sensors Snf3/Rgt2, required for induction of glucose transporters, affected only slightly induction of *SUC2* by low glucose, and were dispensable for induction of pyruvate decarboxylase. The G-protein-coupled receptor Gpr1 was required for full induction of *SUC2* but not for induction of *HXT1-lacZ* or of pyruvate decarboxylase. Lack of Snf3/Rgt2 or of Gpr1 did not affect glucose repression of different genes nor activation by glucose of plasma membrane ATPase, while in the absence of Gpr1 the rate of glucose-induced degradation of fructose-1,6-bisphosphatase was decreased. In an *hxk1hxk2glk1* strain, unable to phosphorylate glucose, most of the responses to this sugar were suppressed, although high concentrations of glucose caused partial induction of the transporter Hxt1 and full induction of invertase. In these conditions, however, induction of *SUC2* became completely dependent on Gpr1. In the absence of Hxk2 (or Hxk1 and Hxk2) repression of genes such as *SUC2*, *GAL1* and *GDH2* was relieved but genes *FBP1* and *ICL1*, encoding gluconeogenic enzymes, were still fully repressed. Hxk1 or Hxk2 were needed for complete induction of pyruvate decarboxylase and for activation of plasma membrane ATPase, but not for induction of *SUC2* by low glucose. These results indicate that, even for a single type of process such as induction or repression, there is not a unique pathway for glucose signalling but several ones that may, singly or in combination, initiate the diverse effects triggered by glucose.

Physiology of *Rhizopus oryzae* at limiting glucose concentrations

A. O. Büyükkileci[1], M. Yücel[1,2] and H. Hamamcı[1,3]

[1] Middle East Technical University, Department of Biotechnology, Turkey
[2] Middle East Technical University, Department of Biological Sciences, Turkey
[3] Middle East Technical University, Department of Food Engineering, Turkey

Rhizopus oryzae ATCC 9363 produces mainly lactic acid, ethanol and minor amounts of glycerol and fumaric acid in the presence of high concentrations of fermentable sugars. In an effort to better understand the pyruvate branch point of this fungus, it was immobilized in alginate beads and grown at limiting glucose concentrations in continuous culture with cell retention. Glucose concentration in the fermenter was kept at certain levels (0.03–0.5 g/l) by changing the feed glucose concentration. It was not possible to divert all of the glucose to biomass. Lactate and ethanol was detected at all runs. Generally lactate and ethanol yields decreased and biomass yield increased with increasing culture glucose concentrations. Ethanol yield was higher than lactate yield at lower concentrations; however at high glucose feed, lactate production was predominant. In accordance to these, lactate dehydrogenase (LDH) activity was considerable only at this concentration. Pyruvate decarboxylase and alcohol dehydrogenase activities were high even at very low glucose concentrations, and they increased slightly as glucose concentration was increased. Similarly, after a glucose pulse to glucose limited continuous culture, activities of three enzymes increased at similar rates, however LDH activity increased more than 10 fold after 12 hours of growth.

A TRAC study of gene expression in wort fermentations reveals novel aspects of the regulation of sugar transport and membrane synthesis

J. Rautio, V. Vidgren, J. Londesborough and A. Huuskonen

VTT Technical Research Centre of Finland, Finland

During wort fermentations yeast must efficiently use the major wort sugars, glucose, maltose and maltotriose. The first step is transport across the cell membrane. Transport is determined by the presence or absence of appropriate sugar transporters and also by the membrane's lipid composition. Under laboratory conditions, glucose represses the expression of genes for α-glucosidases (maltases) and maltose and maltotriose transporters. Further, biosynthesis of sterols and unsaturated fatty acids requires oxygen. Because oxygen is present only at the start of brewery fermentations, yeast membranes are deficient in these lipids at the end of such fermentations. To help understand how brewer's yeasts adjust their physiology to changing sugar composition and oxygen availability during fermentation of brewer's wort, we used the rapid TRAC technology to follow expression of several genes involved in sugar uptake and lipid synthesis.

Lager yeasts are hybrids containing components from both *S. cerevisiae* and *S. bayanus* genomes. We followed the expression of over 70 S. *cerevisiae* and S. *bayanus* genes at frequent intervals (up to every 2 h) through tall tube wort fermentations by industrially cropped lager yeast. Genes for maltose and maltotriose metabolism (*MALx1*, *MALx2* and the *S. bayanus MTT1*) showed strong co-ordinate increases before glucose began to be used and declined while maltose concentrations were still high. Expression of genes for ergosterol synthesis (needed for correct membrane function) peaked in the first few hours and again late in fermentation when oxygen was absent. Evidently the physiology of lager yeast fermenting wort diverges from that of laboratory strains in laboratory media.

Glucose uptake by glucose-limited and glucose-excess grown mycelia of *Penicillium simplicissimum*

P. Vrabl. and W. Burgstaller

Institute of Microbiology, Austria

Oligotrophic anamorphic fungi can grow with nanomolar to molar glucose concentrations. The characteristics of glucose uptake are relevant for growth in natural habitats, as well as for biotechnological processes using these fungi. The rate of glucose uptake is also important for the rate of overflow-metabolism, i. e. the excretion of organic acids. Glucose uptake in the organic acid excreting fungus *Penicillium simplicissimum* was studied, using glucose-limited and ammonium-limited/glucose-excess chemostats and bioreactor batch cultures (starting with 400 mM glucose) to estimate kinetic parameters. Studies with several other anamorphic fungi suggested, that glucose uptake at low (0.01 mM) to moderate (20 mM) glucose concentrations is mediated by one low affinity and one high affinity glucose uptake system. At high glucose concentrations (100 mM – 800 mM) simple diffusion was postulated as the most probable uptake mechanism[1]. Glucose-limited mycelium showed one saturable transport system (K_M = 0.01 mM; V_{max} = 1.2 mmol g^{-1} h^{-1}) at low glucose concentrations, and a first order term for moderate glucose concentrations (slope 0.025 mmol g^{-1} h^{-1}). From the first order term a permeability coefficient of P = 10^{-7} cm s^{-1} was calculated. Ammonium-limited/glucose excess grown mycelium showed also only one saturable transport system (K_M = 0.25 mM; V_{max} = 0.45 mmol g^{-1} h^{-1}) at low glucose concentrations and a first order term for moderate glucose concentrations (slope 0.01 mmol g^{-1} h^{-1}). From the first order term a permeability coefficient of P = 10^{-7} cm s^{-1} was calculated. At high glucose concentrations a linear relationship between glucose uptake rate and glucose concentration was observed. However the plot was biphasic, with two straight lines of different slopes (phase 1, 400–350 mM: 0.049 mmol g^{-1} DW h^{-1}, P = 10^{-7} cm sec^{-1}; phase 2, 350–150 mM: 0.005 mmol g^{-1} DW h^{-1}, P = 10^{-8} cm sec^{-1}).

Main conclusions. P in artificial lipid bilayers is 3 x 10^{-10} cm sec^{-1}. Accepting this, glucose uptake at high concentrations could not be due to simple diffusion, although a linear relationship between glucose uptake rate and glucose concentration was observed. Facilitated diffusion with a very high K_M value must thus be favoured. Glucose uptake is more probably regulated by metabolism than vice versa. Extracting kinetic values from biphasic Eadie Hofstee plots is prone to error and should be done very cautiously[2].

[1] Wayman, F. M. and Mattey, M. (2000). Biotechnol Bioeng 67, 451–456.
[2] Fuhrmann, G. F. and Völker, B. (1993). Biochim Biophys Acta 1145, 180–182.

Characterization of the Gap1 amino acid permease as a nutrient sensor in *Saccharomyces cerevisiae*

G. Van Zeebroeck, B. M. Bonini, M. Versele and J. M. Thevelein

VIB Department of Molecular Microbiology, K.U. Leuven, Belgium

Sensing mechanisms triggered by nutrients is a relatively recent concept. The general amino acid permease Gap1 is an example of a transporter-related nutrient receptor (called transceptor). It functions as a sensor for amino acid activation of the Fermentable-Growth-Medium induced pathway (FGM signalling pathway) that controls PKA and its targets in *Saccharomyces cerevisiae*. Addition of a nitrogen source to cells starved for nitrogen, in glucose-containing medium (FGM pathway) triggers a phosphorylation cascade which rapidly switches the cells from a low to high PKA phenotype. One of the PKA targets affected is trehalase. The enzyme is activated and trehalose mobilized. The action mechanism of transporting and non transporting transceptors is unknown. We have screened 319 amino acids analogues for inhibition of amino acid transport by yeast Gap1. Some analogues like the dipeptides L-Leu-L-Gly (LG) can trigger a signal without being transported. Using SCAM analysis (Substituted Cysteine Accessibility Method) with an allele having a Cys substitution of Ser^{388} in the TMD VIII of yeast Gap1, we show that the dipeptide L-Leu-L-Gly acts as agonist using the same binding site as amino acids to trigger signalling of Gap1.

Functional analysis of *Kluyveromyces lactis* carboxylic acids permeases: Heterologous expression of *kljen1* and *kljen2* genes

L. Pereira[1,2], O. Queirós[1,2,3], S. Paiva[1], P. Moradas-Ferreira[2,4] and M. Casal[1]

[1] Centro de Biologia, Universidade do Minho, Braga, Portugal
[2] Instituto de Biologia Molecular e Celular, Universidade do Porto, Portugal
[3] Instituto Superior de Ciências da Saúde-Norte, Portugal
[4] Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto, Portugal

The yeast *Kluyveromyces lactis* presents two homologue genes to *JEN1* of *Saccharomyces cerevisiae* (Casal *et al.* 1999): *KlJEN1* encodes a monocarboxylate permease and *KlJEN2* encodes a dicarboxylic acid permease. In the strain *K. lactis* GG1888, expression of these genes does not require an inducer and activity for both transport systems was observed in glucose-grown cells. To confirm their key role for carboxylic acids transport in *K. lactis*, *null* mutants were analyzed. Heterologous expression in *S. cerevisiae* has been performed and chimeric fusions with *GFP* showed their proper localization in the plasma membrane. *S. cerevisiae jen1*Δ cells transformed with *KlJEN1* recovered the capacity to use lactic acid, as well as to transport labeled lactic acid by a mediated mechanism. When *KlJEN2* was heterologously expressed, *S. cerevisiae* transformants gained the ability to transport labeled succinic and malic acids by a mediated mechanism, exhibiting, however, a poor growth in malic acid containing media. The results confirmed the role of KlJen1p and KlJen2p as mono and dicarboxylic acids permeases, respectively, not subjected to glucose repression, being fully functional in *S. cerevisiae*.

Financially supported by this work was supported by the portuguese grant POCI/BIO/38106/2001.

Casal, M. et al. (1999). J. Bacteriol., 181, 2620–2623.

The alpha-glucoside permease encoded by the *Hansenula polymorpha* genomic MAL locus

K. Viigand, K. Tammus and T. Alamäe

Institute of Molecular and Cell Biology, University of Tartu, Estonia

Here we give the first report on a genetically difined sugar transporter of *H. polymorpha* – the alpha glucoside permease encoded by *HpMAL2* of the genomic *MAL* locus. The HpMAL2p is most similar to the hypothetical maltose permeases of *Candida albicans*, *Debaryomyces hansenii* and to Agt1p of *S. cerevisiae.* Properties of the HpMal1p were studied using a chromogenic alpha-glucoside, PNPG. Genomic disruption of the *HpMAL2* resulted in inability of cells to grow on maltose, sucrose, trehalose, maltotriose and turanose as well as loss of PNPG transport. PNPG uptake was competitively inhibited by all these substrates with K_i values between 0.23–1.47 mM. Affinity of the permease was shown to be highest for maltose, trehalose and sucrose. Transport by HpMal2p was sensitive to pH and CCCP revealing its energization by proton gradient over cell membrane. Despite *HpMAL2* was responsible for trehalose uptake, its expression was not induced during trehalose growth. Maltase was shown to be responsible for intracellular metabolism of maltose, sucrose, maltotriose and turanose, but not needed for trehalose growth. *H. polymorpha* strains have been isolated from rotten orange juice, cacti, and from soil irrigated with distillery waste. In accordance with ecology of this yeast, our data show that with regard to disaccharide utilization, metabolic apparatus of *H. polymorpha* has been mostly adapted to sucrose that is probably the most abundant disaccharide in the habitat of this yeast.

Secretion mechanism of acid trehalase in *Saccharomyces cerevisiae*

S. He, J. Francois and J. L. Parrou

Laboratoire Ingénierie des Systèmes Biologiques et des Procédés (LISBP), France

The yeast *Saccharomyces cerevisiae* can synthesize trehalose and also use this disaccharide as a carbon source for growth. It possesses two types of trehalose hydrolases, referred to as “acid” and “neutral” trehalases in accordance with the optimal pH for activity. The “neutral” trehalase has been well studied and is known to hydrolyse trehalose in cytosol. The “acid” trehalase, initially proposed to be localized in the vacuole, is actually mostly targeted to the cell surface as for other fungi (Jules *et al.*, AEM 2004). To characterize the mechanism required for Ath1p secretion, two directions were investigated. On one hand, we screened the EUROSCARF collection of single deletion mutants for their inability to grow on trehalose as the sole carbon source. To this end, we took advantage of the obligation of secreted Ath1p in the absence of the “Agt1p transporter – Nth1p” salvage pathway for the growth on trehalose of the BY family strains (Parrou *et al.*, FEMS Yeast Res 2005). Amongst potential candidate genes, we identified *YGP1* that encodes a small, highly glycosylated and secreted protein called gp37, which co-purified with Ath1p (Destruelles *et al.*, MCB 1994). All these elements favoured the hypothesis that this protein may drive Ath1p to the cell surface. However, we found that the *ygp1::kan* clone from the collection also harboured a respiratory mutation, which actually explained the absence of growth on trehalose. Nevertheless, new *ygp1*deletion mutants created by PCR mediated deletion (BY, CEN.PK and KT1112 lab strains) exhibited 30 % reduced Ath1p activities as compared to the wild type, suggesting a role of Ygp1p/gp37in the maturation and/or secretion of the trehalase. The other aspect of the work was to search for the potential domain of Ath1p that is needed for its secretion to the cell surface. We found that Ath1p lacking the N-terminal transmembrane segment (aa 47-69) was no longer active and impaired growth on trehalose. Additional work is currently underway to verify that this domain is sufficient to export intracellular protein to the cell surface and to search whether the secretion of Ath1p is mediated by the classical secretory pathway.

Growth of cellulolytic microorganisms on cellulose substrates

J. Hrdinova, V. Jirku, A. Cejkova and J. Masak

Department of Fermentation Chemistry and Bioengineering, Institute of Chemical Technology Prague, Czech Republic

Great amounts of cellulose wastes are annually produced worldwide as residues from agricultural activities and industrial food processing or as municipal wastes. Consequently, the using of microorganisms in order to remove, reduce or ameliorate these potential polluting materials is a real environmental challenge and for that reason we should definitely deal with these problems and try to make more effective the cellulose biodegradation process.

This study was aimed at finding of optimal conditions (pH, temperature, nitrogen source etc.) for growth and cellulolytic activity of yeast *Trichosporon cutaneum* on cellulose substrates as a carboxymethylcellulose (CMC) and a cellophane which served as the sole sources of carbon. The cultivations were carried out in basal salt medium supplemented with CMC (1% w/v) in liquid shake culture. The yeast showed growth at a range of temperature from 15 °C to 32 °C and at pH values of media from 5 to 10. The highest cellulolytic activity was proved between 21–28 °C and about pH value 5.8. It appeared organic nitrogen source (peptone) did not effect the growth more significantly than inorganic compounds. Furthermore, it was found this yeast can also colonize insoluble cellulose substrate – cellophane. The growth was monitored as a diameter change in time and eveluated by the method of image analysis. This technique could be used for monitoring of growth rate in the various cultivation conditions on solid cellulose wastes.

ABSTRACTS OF POSTER SESSION IV

Metabolic pathways and energetics

Posters P56 – P75

Regulation analysis – unravelling the dynamics of metabolic and gene-expression regulation

J. Bouwman, A. Lindenbergh, S. Agbo, K. van Eunen, M. Siderius, H. V. Westerhoff and B. M. Bakker

Molecular Cell Physiology, Vrije Universiteit Amsterdam, The Netherlands

Regulation Analysis [1] is a conceptual framework to dissect regulation at the gene-expression level (quantified by $\rho_{hierarchical}$) from metabolic regulation (quantified by $\rho_{metabolic}$). It has been derived that the total regulation ($\rho_{hierarchical}$ plus $\rho_{metabolic}$) sums up to one. Recently, the theory has been expanded to dissect the regulation at the different levels within the gene-expression cascade (Daran-Lapujade, submitted). We applied Regulation Analysis to the response of glucose-limited chemostat cultures of *Saccharomyces cerevisiae* to hyperosmotic stress. In time we monitored the flux of glycerol and the expression of the glycerol-producing enzymes at the levels of mRNA, protein and V_{max}. The data were used to calculate the regulation at various levels of the gene-expression cascade and at the metabolic level. The results indicate that metabolic regulation of the glycerol flux precedes hierarchical regulation. This raises questions about the physiological function of the gene-expression regulation of the glycerol-producing enzymes by the Hog pathway.

1. Rossell, S., *et al.* (2006) Proc. Natl. Acad. Sci. 103, 2166–2171.

Differences in the PPP capacity among *S. cerevisiae* strains

A. Cadière, S. Heux and S. Dequin

UMR-Science pour l'Oenologie, Microbiologie, INRA, France

A *S. cerevisiae* phosphoglucose isomerase (*pgi1*) mutant cannot use the pentose phosphate pathway (PPP) to oxidize glucose. This phenotype has been explained by the lack of mechanism for reoxydation of the NADPH surplus. Consistent with this hypothesis, (Fiaux et *al.*[1]), have partially restored growth of the ENY.WA-1A *pgi1* mutant by overexpression of *Udha*, encoding the soluble transhydrogenase of *E. coli*, which oxidizes NADPH to $NADP^+$ while reducing NAD^+ to NADH. We show in this work that the growth of two other strains (V5 and FY1679-01B) lacking *pgi1* was not rescued by overexpression of *Udha*. The estimated split ratio between glycolysis and PPP was respectively 3 and 1.8 fold higher in ENY.WA-1A strain compared to V5 and FY1679-01B, suggesting that the capacity of the PPP is higher in ENY.WA-1A. This was recently confirmed by comparative ^{13}C flux analysis of ENY.WA-1A and V5, which showed a 20-fold higher flux through the PPP in ENY.WA-1A than in V5 (Heux et *al*., submitted). Since differences in the specific activities of the glucose–6-phosphate dehydrogenase (G-6-PDH) were observed between ENY.WA-1A and the other strains, we examined the possibility that the first step of the PPP is a limiting factor in the strain V5. Overexpression of *ZWF1* gene encoding G-6-PDH in V5 *pgi1Udha* did not rescue growth on glucose, suggesting that factors other than G-6-PDH and the NADPH reoxydation capacity limit the flux through the PP pathway in this strain.

This work shows a high diversity in the PPP capacity among *S. cerevisiae* strains. These variations can serve as a basis for understanding the regulation mechanisms of this pathway.

1. Fiaux et al., (2003). Metabolic-flux profiling of the yeasts *Saccharomyces cerevisiae* and *Pichia stipitis*. Eukaryotic Cell 2, 170–180.

Real-time HPLC measurement of extracellular metabolites

J. Ahtinen[1,2], J.-P. Pitkänen[*1], N. Tohmola[2], V. Parviainen[2] and R. Renkonen[2]

[1] Medicel Oy, Finland
[2] Department of Bacteriology and Immunology, Haartman Institute and Biomedicum, University of Helsinki, Finland * Current affiliation VTT, Finland

Cells uptake substrates such as glucose and convert them in metabolic reactions to energy and precursor metabolites to be used in growth. In some growth conditions cells produce metabolites such as acetate or glycerol that are excreted or diffuse freely across the cell membrane to extracellular medium. The real-time measurement of extracellular metabolites provides rapid information for the adjustment of growth conditions during cultivations. This may avoid the accumulation of undesired products. Another obvious advantage of the real-time measurement of extracellular metabolites is the exclusion of manual steps in the analysis procedure. Normally, the manual operation requires taking the sample, separating the cultivation medium from the cells, storing the sample for analysis, and performing the analysis. In this study we constructed automated HPLC equipment to be used in real-time measurement of extracellular metabolites. Our real-time HPLC equipment consists of a sampling pump, a cross-flow filter, an injection valve, an isocratic HPLC pump, an HPLC column, and a refractive index (RI) detector. The equipment enables automated analysis of main metabolites present in the fermentation medium without any manual work. Concentrations of glucose, glycerol, acetate and ethanol can be obtained in five-minute analysis time. We performed aerobic batch cultivations on glucose of yeast *Saccharomyces cerevisiae* strain CEN.PK113-7D with a 5-liter bench-top bioreactor, connected to our in-line HPLC system. In-line samples were taken in six-minute intervals during the whole cultivation. In addition, in-line optical density (OD) probe and exhaust gas sensors were used to obtain further real-time information of the cultivation. Manual samples were taken every hour as a reference to be compared to the in-line measurements.

Fungal metabolomics: The importance of experimental design

M. Braaksma[1], P.J. Punt[1], R.A. van den Berg[1], H. Meerman[2] and M.J. van der Werf[1]

[1] TNO Quality of Life, The Netherlands
[2] Genencor International, Palo Alto, USA

Metabolomics, the non-biased determination and analysis of the complete metabolite profile of an organism, is an emerging powerful tool to better understand microbial physiology and facilitate metabolic pathway engineering to increase the production of specific proteins and fine chemicals by industrial organisms. A very robust and inert metabolomics platform has been developed (van der Werf et al.; Koek et al.; Coulier et al.) and applied for studying different questions in fungal biotechnology. A first important step when generation biological samples for metabolomics, especially when applying it in a question-driven manner, is experimental design. This starts with a sharp definition of the biological question to be answered. Moreover, in order to improve the information content of the metabolome data sets to be generated it is important to generate samples that show large variations in the phenotype of interest. This is in particular relevant as it will improve the accuracy whereby relevant parameters can be identified by biostatistics. Here we present an approach to develop an experimental design in order to unravel the induction of protease activity by *Aspergillus niger*.

In another approach, the metabolomics platform has been applied to elucidate the regulation of cellulase production in hyper-secreting variants of the filamentous fungus, *Trichoderma reesei*. Samples of mycelia were collected under inducing and non-inducing fermentation conditions, quenched to halt cellular metabolism, processed and subsequently analyzed by comprehensive GC-MS and LC-MS techniques. The metabolomics data were analyzed with the multivariate data analysis tool PLS in order to rank the important metabolites. The data were biologically interpreted and a number of genetic targets were identified. Mutants in which genes related to these targets were deleted, were recently constructed in the group of B. Seiboth and C.P. Kubiceck (TU Vienna, Austria) and tested on the expression of one of the major cellulase genes, *cbh1*. This resulted in a decrease in the induction of *cbh1*, indicating that the selection of targets for strain and medium improvement via the metabolomics-methodology is a valid approach.

Koek, M.M., et al., *Anal. Chem.* 78, 1272–1281. 2006.
Coulier, L., et al. *Anal. Chem*. 78, 6573–6582. 2006.
van der Werf, M.J., et al., *J. Ind. Microbiol. Biotechnol*. 32, 234–252. 2005.

Responses of *Saccharomyces cerevisiae* to changing oxygen level

M. Wiebe, E. Rintala, M. Toivari, A. Tamminen, L. Salusjärvi, P. Jouhten, A. Huuskonen, H. Simolin, J. Kokkonen, J. Kiuru, H. Maaheimo, L. Ruohonen and M. Penttilä

VTT Technical Research Centre of Finland, Espoo, Finland

We studied the responses of S*accharomyces cerevisiae* in varying oxygen concentrations on metabolite, transcriptome and proteome level. The *S. cerevisiae* strain CEN.PK113-1A was grown in D-glucose-limited chemostat cultures in five different oxygen concentrations: 0%, 0.5%, 1%, 2.8% and 20.9% O_2 of the inlet gas. After reaching a steady state the oxygen was shut off from the cultures of 1% and 20.9% O_2 of the inlet gas and the metabolism followed for approximately 70 hours. From both steady state and transient culture samples 17 metabolites of central carbon metabolism were analyzed. Changes in the transcriptome were monitored with Affymetrix-arrays and with the VTT-TRAC method. In addition, alterations in the proteome were analyzed with 2-D gels.

The concentrations of metabolites of tricarboxylic acid cycle (TCA) and most glycolytic metabolites were, in general, higher in anaerobic or low aeration cultures (0%, 0.5% and 1% O_2 of the inlet gas) compared with more aerobic conditions (2.8% and 20% O_2 of the inlet gas). Similarly, after oxygen shut off from the cultures of 1% or 20.9% O_2 of the inlet gas the level of TCA metabolites and those of upper glycolysis increased. On the transcriptome level the responses varied more, some genes being upregulated and others down regulated in steady state conditions and showing varying responses during transient conditions. As evaluated from both the metabolite and transcription data approximately 30 h was required to reach a new steady state of anaerobiosis.

Adaptation of respiring *Saccharomyces cerevisiae* to fermentative conditions: A systems biology approach

J. van den Brink, A. Canelas, W. van Gulik, J. Pronk, P. Daran-Lapujade and H. de Winde

Kluyver Centre for Genomics of Industrial Fermentation, Delft University of Technology, The Netherlands

The primary role of bakers' yeast (*Saccharomyces cerevisiae*) in leavening of bread dough is the production of carbon dioxide via the dissimilation of sugars in the glycolytic pathway. Fermentative capacity is defined as the specific rate of carbon dioxide production. Fermentation critically resumes at a high rate immediately upon introduction of yeast into dough [1]. At steady state in carbon-limited chemostat, the change in flux through glycolysis and hence, in fermentative capacity was not primarily controlled at the transcriptional level [2]. We strive to investigate and quantify the contribution of transcription and all other cellular levels (*i.e.* protein synthesis and degradation, post-translational modification and metabolic regulation) in the regulation of fermentative capacity and fermentation. Induction of fermentative capacity was studied in a well-controlled set-up by shifting an aerobic glucose-limited chemostat to anaerobic glucose-excess batch growth mimicking the dough environment. Glycolytic activity was monitored for two hours, measuring *in vitro* enzyme activities (V_{max}), intracellular metabolites (LC-MS/MS) and *in vivo* carbon-flux (using a stoichiometric model). To quantify the relative contribution of changes in V_{max} (called hierarchical regulation [3]) to the changes in glycolytic flux, the multi-level dataset was analysed by time-dependent regulation analysis [4].

While *in vivo* glycolytic flux was rapidly (minutes) and strongly (*ca.* 55 fold) increased at the onset of fermentation, regulation analysis identified two major time-responses in the regulation of the glycolytic flux. Hierarchical regulation of hexokinase, phosphofructokinase and alcohol dehydrogenase did not contribute to the increase in flux. Hence, these three enzymes were purely regulated by modulation of the *in vivo* activity by metabolites (effectors), and not by changes in V_{max}. Conversely, for the nine remaining glycolytic reactions, a shift in the regulatory mode was observed 45 minutes after the onset of fermentation. Following pure metabolic regulation, the contribution of the hierarchical regulation component gradually and substantially increased during the remainder of the experiment. Concurrently, the intracellular concentrations of glycolytic intermediates and of the major glycolytic effectors were clearly changing 45 minutes after the shift to fermentative conditions. Implications of these results will be discussed.

[1] van Hoek, P., van Dijken, J.P., Pronk J.T. (2000) Enzyme Microb Technol 26:724–736.
[2] Daran-Lapujade, P., Jansen, M.L.A., Daran, J.M., van Gulik, W., de Winde, J.H., Pronk, J.T. (2004) Journal of Biological Chemistry 279: 9125–9138.
[3] ter Kuile, B.H., Westerhoff, H.V. (2001) FEBS Lett. 500(3):169–171.
[4] Bruggeman, F.J., de Haan, J., Hardin, H., Bouwman, J., Rossell, S., van Eunen, K. et al. (2006) Syst Biol (Stevenage) 153:318–322.

The response of anaerobically grown *Saccharomyces cerevisiae* to low and high oxygen in glucose-limited chemostat cultures

M. G. Wiebe, E. Rintala, A. Tamminen, H. Simolin, L. Salusjärvi, M. Toivari, P. Jouhten, A. Huuskonen, H. Maaheimo, L. Ruohonen and M. Penttilä

VTT Technical Research Centre of Finland, Finland

Saccharomyces cerevisiae was grown anaerobically in glucose-limited chemostat culture for several generations and then exposed to either low (1.0%) or high (20.9%) concentrations of oxygen. Under these conditions the cells required approximately 2 h before starting to take up oxygen or to produce more biomass. Glycerol and ethanol concentrations, began decreasing within an hour of providing oxygen, indicating that they were no longer being produced or were being produced at a slower rate. Little glycerol consumption occurred, but ethanol consumption started after approximately 3 h in cultures receiving 20.9% oxygen. Specific glucose consumption rates decreased as biomass increased in the presence of oxygen. Although the dilution rate was maintained constant at 0.10 h^{-1}, accumulation of biomass in the presence of oxygen indicated that the cells were growing at specific growth rates of 0.21 (1.0% O_2) and 0.32 h^{-1} (20.9% O_2) between 2 and 10 h after oxygen was provided to the cultures.

Although oxygen uptake did not occur until 2 h after it was provided to the cultures, most glycolytic genes showed down-regulation within 10 minutes of its presence in the culture. Regulation of genes involved in glucose, ethanol and glycerol metabolism and respiration similarly occurred within 10 minutes of the presence of oxygen. In contrast, the concentration of most of the metabolites of central carbon metabolism did not change until more than 10 minutes exposure to oxygen.

The effect of external oxygen conditions on the metabolic flux distribution of *Saccharomyces cerevisiae*

P. Jouhten[1], E. Rintala[1], A. Huuskonen[1], A. Tamminen[1], M. Toivari[1], M. Wiebe[1], A. Rantanen[2], L. Ruohonen[1], M. Penttilä[1] and H. Maaheimo[1]

[1] VTT Technical Research Centre of Finland, Finland
[2] Department of Computer Science, University of Helsinki, Finland

The yeast *Saccharomyces cerevisiae* is a facultative anaerobic organism able to switch the metabolism to fermentation when the aerobic respiration is limited or completely prevented. The availablity of oxygen is a major determinant of the metabolism of *S. cerevisiae* mainly because when the oxygen availability is restricted, cells need other acceptors to the electrons of NADH and $FADH_2$. Furthermore, when the function of the respiratory system is limited additional means for ATP generation and cross-membrane transport of metabolites and ions are required. Growth under limited respiration thus requires major redistribution of metabolic fluxes compared to the aerobic metabolism.

S. cerevisiae CEN.PK113-1A was grown in glucose-limited chemostat culture with 0%, 0.5%, 1.0%, 2.8% and 20.9% O_2 in the inlet gas (D = 0.10 h^{-1}, pH 5, 30 ˚C) and labeled with 10% of [U-^{13}C]-glucose to study the metabolic flux distribution response to oxygen. The labeled biomass was sampled for METAFoR (Metabolic Flux Ratio) analysis using ^{13}C HSQC NMR spectroscopic detection (Szyperski *et al.*, 1999). A stoichiometric model of the central carbon metabolism of *S. cerevisiae* was set up comprising of 29 reactions and transport fluxes and 20 metabolites in two intracellular compartments. Additional constraint equations for the stoichiometric model were obtained from the six metabolic flux ratios from METAFoR analysis (Fischer *et al.*, 2004). The uptake and production rates were experimentally measured and the precursor requirements for biomass synthesis were taken from literature. The metabolic net fluxes were solved using an in-house program for weighted optimization based on the matlab function *fmincon*.

A slight decrease in the oxygen uptake rate resulted in low ethanol production already in the 2.8% oxygen conditions. However, major changes in the intracellular metabolic flux distribution compared to aerobic conditions, in particular in the pyruvate branching point, were observed in the 1% oxygen conditions when the oxygen availability was severely reduced.

Fischer et al., Anal.Biochem., 325 (2004) 308.
Szyperski et al., Eur.J.Biochem., 232 (1995) 433.

The effect of oxygen availabilty on yeast glycolysis

I. Tuzun, K. Hellingwerf and J. Teixeira de Mattos

Department of Molecular Microbial Physiology, SILS-UVA, The Netherlands

The Crabtree positive yeast *S. cerevisiae* catabolizes fully oxidative only when oxygen is saturating and glucose availability is limited, while fully fermentative growth is found under strictly anaerobic conditions. Intermediate situations, characterized by a mixed respiro-fermentative growth, are found either when the oxygen availability is limited, or when well-aerated cultures are subjected to glucose excess. Whereas much quantitative data are available with respect to the latter condition, our knowledge about low oxygen conditions is scarce and qualitative only. A major cause for this is caused by the difficulties inherent to defining oxygen availability. Here, we present a quantitative method to culture *S. cerevisiae* under preset, quantified and reproducible low-oxygen regimes. Using this system, we are studying at the metabolic, the enzyme activity, the proteomic and the transcriptomic levels the contribution of regulatory events to the final catabolic performance of the organism. The physiological behaviour of these cultures is quantified in terms of specific substrate consumption rates (glucose, O_2) and product formation rates (biomass, CO_2, ethanol etc.) and related to enzyme and transcript profiles. It will be shown that whereas the physiology of the cell changes *gradually* with changing oxygen availability, transcript profiles changes *abruptly*, suggesting that major regulation events occur at the metabolic level. The data set is sufficiently complete for quantitative regulation analysis.

Further, it will be shown by calculations on the energetics of growth that the efficiency of the respiratory chain (as reflected by the P/O ratio) is dramatically decreased when oxygen supply is severely curtailed by a hitherto unknown mechanism.

Directed evolution of promoter regulation: Generation of a yeast promoter induced by switching off the oxygen supply

E. Nevoigt[1,2], C. Fischer[1], O. Mucha[2], F. Matthäus[2] and G. Stephanopoulos[1]

[1] Department of Chemical Engineering, Massachusetts Institute of Technology, USA
[2] Department of Microbiology and Genetics, Berlin University of Technology, Germany

Systems for easily-controlled, conditional induction or repression of gene expression are indispensable tools in fundamental research and industrial-scale biotechnological applications. Both native and rationally-designed inducible promoters have been widely used for this purpose. However, inherent regulation modalities or toxic, expensive or inconvenient inducers can impose limitations on their use. Tailored promoters with user-specified regulatory properties would permit sophisticated manipulations of gene expression. Here, we report a generally applicable strategy for the directed evolution of promoter regulation. Specifically, we applied random mutagenesis and a multi-stage flow cytometry screen to isolate mutants of the oxygen-responsive *Saccharomyces cerevisiae DAN1* promoter. Two mutants were isolated which were induced under less-stringent anaerobiosis than the wild-type promoter enabling induction of gene expression in yeast fermentations simply by oxygen depletion during cell growth. Moreover, the engineered promoters showed a markedly higher maximal expression than the unmutated *DAN1* promoter, under both fastidious anaerobiosis and microaerobisois.

Tackling compartmentation: Estimation of the cytosolic free NAD/NADH ratio in yeast using a heterologous indicator reaction

A. B. Canelas, W. van Gulik and S. Heijnen

T.U. Delft, Kluyver Laboratory for Biotechnology, The Netherlands

The compartmentation of metabolism in eukaryotes poses a problem for the determination of (relevant) concentrations of intracellular metabolites. Thermodynamic analysis of glycolysis shows that with published intracellular metabolite concentrations the pathway is not feasible. Clearly, the data generated so far is insufficient to understand even well-known pathways. NAD and NADH play a central role in energy metabolism and are also involved in transcriptional regulation, oxidative stress, metabolic disorders and cell ageing. They are present in the different cell compartments and their distribution is uneven. Furthermore, in addition to the free species (kinetically and thermodynamically relevant), NAD and especially NADH are partly present in protein-bound form. Therefore, measurements of total, whole-cell, concentrations of NAD and NADH provide no information on the *in vivo* redox state of the NAD-NADH couple (1).

In mammalian cells, the free NAD/NADH ratio has been determined using indicator reactions (2; 3). These studies have shown how the free NAD/NADH ratios in the cytosol and mitochondria can be calculated from the concentrations of reactants in near-equilibrium reactions. The application of this method to *S. cerevisiae* is not straightforward. The indicator reactions used successfully in mammalian cells are not present in yeast and there is no suitable alternative. Therefore, we have chosen to introduce a heterologous indicator reaction. For this purpose, we introduced the gene for mannitol-1-phosphate dehydrogenase in *S. cerevisiae* and cultivated the cells under well-defined conditions in aerobic glucose-limited chemostats. From the measured intracellular concentrations of fructose-6-phosphate and mannitol-1-phosphate we calculated a free cytosolic NAD/NADH ratio of 100. This is 5- to 100-fold higher than previously reported from whole-cell total concentrations. In addition, we could observe fast dynamic changes (time-scale of seconds) in the free cytoslic NAD/NADH in response to different perturbations. To our knowledge, this is the first study where a heterologous reaction was introduced for the estimation the free NAD/NADH ratio and constitutes the most reliable estimate of the cytosolic redox state in yeast.

1. Veech, R. L. (2006) Biochemistry and Molecular Biology Education 34, 168-179.
2. Veech, R. L., Egglesto, L. V., and Krebs, H. A. (1969) Biochemical Journal 115, 609-&.
3. Stubbs, M., Veech, R. L., and Krebs, H. A. (1972) Biochemical Journal 126, 59-&.

Avoiding post-translational modification of 6-phosphofructo-1-kinase in *Aspergillus niger*

M. Capuder, G. Tevž, T. Šolar, M. Benčina and M. Legiša

National Institute of Chemistry, Hajdrihova, Slovenia

By studying physiology of *Aspergillus niger* during the early stages of citric acid accumulation a new type of regulation of central metabolism has been described and recently published in two papers that appeared in Applied and Envirnomental Microbiology. It is based on post-translational modification of 6-phosphofructo-1-kinase (PFK1), a key regulatory enzyme of glycolysis. In a two stage process the native enzyme is first cleaved by specific protease followed by phosphorylation of initially inactive fragment by cAMP dependent protein kinase in order to regain enzyme activity. Changed kinetic parameters of the shorter fragment have been determined and no inhibition by citrate could be detected any more, while specific effectors like fructose-2,6-bisphosphate and ammonium ions increased the activity of the modified enzyme to a higher level in respect to the native enzyme. In the cells spontaneous post-translational modification results in enhanced metabolic flux through glycolysis leading to an increase in concentration of tricarboxylic acid cycle intermediates enabling citrate overflow or more rapid anabolic reactions.

Mammalian PFKs evolved by a process of tandem gene duplication and fusion to yield a protein that is more that double the size of prokaryotic enzymes. While the active site residues are located on N-terminal half of the protein, a number of allosteric ligand binding sites developed on both parts of the eukaryotic enzyme during evolution. Ligands such as citrate, ATP, fructose-2,6-phosphate, ammonium ions and AMP enable fine control of enzyme activity in the eukaryotic cells, while prokaryotic PFK1 enzymes are poorly regulated. Spontaneous posttranslational modification of PFK1 enzyme in *A. niger* cells presents a reversed evolutionary process, where less regulated enzyme is formed, yet it remained sensitive for positive regulation.

To avoid complex post-translational modification *pfk*A gene encoding native *A. niger* protein has been modified. Initially a truncated gene has been prepared carrying information for the synthesis of N-terminal part of the native enzyme, while in the second step site directed mutations were introduced to elude phosphorylation of the shorter fragment. Insertion of the mt-*pfk*A gene into the cells resulted in increased productivity of citric acid and alpha-amylases, while in growth promoting medium higher specific growth rate was recorded in respect to the parental *A. niger* strain. Transformants of *Aspergillus terreus* carrying mt-*pfk*A gene showed significantly increased productivity and yields of itaconic acid.

Screening of the probable hexokinase genes from *Rhizopus oryzae* in expressed genome

S. Acar[1], E. Alagoz[1], D. Dedeoglu[1], M. Yucel[2] and H. Hamamci[3]

[1] Middle East Technical University (METU), Department of Biotechnology, Turkey
[2] Middle East Technical University (METU), Department of Biological Sciences, Turkey
[3] Middle East Technical University (METU), Department of Food Engineering, Turkey

Rhizopus oryzae is a filamentous fungus which is used for lactic acid production and which has a potential for ethanol production. In order to find ways to increase the yield of metabolite production in this organism we are trying to find the controlling mechanisms. In the light of the studies done for the control of glycolytic flux in *S. cerevisiae* the hexokinase enzymes and corresponding genes of *R. oryzae* are being studied. It is known that there is a hexokinase and a glucokinase in *R. oryzae* which have been purified and characterised in our laboratory (in preparation for publication). However, we could not obtain N-terminal sequences of the proteins since the concentration of pure proteins were very low. Therefore, for cloning hexokinase genes we have used the information on genome database of the organism and found ten probable hexokinase genes by doing a blast search with the known hexokinase protein sequences of *S. cerevisiae*. All of these genes encode hypothetical proteins having two conserved domains called hexokinase1 and hexokinase2. Primers at 3' and 5' ends of these genes were designed based on the sequences obtained from the database. Using each primer set and cDNA first strand as template PCR screening was done. The first results have shown that at least three of these genes are found in the expressed genome. Depending on the purification studies at least two hexokinase structural genes were expected to be expressed but the results suggest that there might be another protein. Comparison of the bioinformatics data with the *S. cerevisiae* genome database implies that the third gene might be a regulatory protein or a transcription factor like the one from *S. cerevisiae* Emi2p which is required for transcriptional induction of some genes and which has conserved domains similar to hexokinases and glucokinase of the same organism. The next step of the study is to express the cloned genes in *S. cerevisiae* triple hexokinaseless mutant. These studies are in progress. Further studies to show the effect of different hexokinase proteins on the glycolytic flux and control of glycolysis are underway.

The fungal path for D-galacturonate catabolism

S. Hilditch[1], S. Berghäll[1,2], J. Liepins[1,3], M. Penttilä[1] and P. Richard[1]

[1] VTT Technical Research Centre of Finland, Finland
[2] University of Helsinki, Department of Applied Chemistry and Microbiology, Finland
[3] University of Latvia, Institute of Microbiology and Biotechnology, Latvia

D-galacturonate is the major component of pectin and consequently an important carbon source for microorganisms living on decaying plant material or for biotechnological processes where cheap raw materials such as sugar beet pulp are used. A bacterial catabolic pathway has been described while a eukaryotic pathway has remained unknown. For *E. coli* a pathway was described consisting of five enzymes converting D-galacturonate to pyruvate and D-glyceraldehyde-3-phosphate. The enzymes of this pathway are uronate isomerase, NADH-utilizing D-tagaturonate reductase, altronate dehydratase, D-erythro-3-deoxy-hexulosonate kinase and D-erythro-3-deoxy-hexulosonate-6-phosphate aldolase.

We show that the D-galacturonate pathway is different in the mold *Hypocrea jecorina* (*Trichoderma reesei*). In this fungal catabolic pathway D-galacturonate is converted to pyruvate and glycerol. The intermediates are L-galactonate, L-threo-3-deoxy-hexulosonate and L-glyceraldehyde. The pathway contains four enzymes, NADPH-utilizing D-galacturonate reductase, L-galactonate dehydratase, L-threo-3-deoxy-hexulosonate aldolase and a glycerol dehydrogenase that converts L-glyceraldehyde to glycerol in the reverse reaction. All the corresponding genes have been identified, cloned, and expressed in the heterologous host *S. cerevisiae*, and the kinetic properties of the enzymes have been determined.

A simple reaction but two enzymes: Conversion of homocitrate to homoisocitrate

F. Schöbel and M. Brock

Junior Research Group Microbial Biochemistry and Physiology; Leibniz-Institute for Natural Product Research and Infection Biology, Germany

Fungi, like *Aspergillus fumigatus*, as well as some bacteria (e.g. *Thermus thermophilus*) are able to synthesise lysine *via* the alpha-aminoadipate pathway. The pathway starts with the formation of homocitrate *via* homocitrate synthase. Homocitrate must become isomerised to homoisocitrate, which involves a dehydration to homo-*cis*-aconitate and a rehydration to homoisocitrate. Both reactions were first assumed to be catalysed by a single enzyme, the so called homoaconitase. Activity determinations with enriched homoaconitase from yeast using homoisocitrate as a substrate only yielded homo-*cis*-aconitate but not homocitrate. Therefore, a second enzyme was supposed to be involved in the conversion of homocitrate to homoisocitrate. A candidate for this reaction was Lys7p, because deletion led to lysine auxotrophy. Later studies revealed that Lys7p is involved in oxidative stress response *via* the superoxide dismutase but not directly involved in the isomerisation of homocitrate. We, therefore, aimed in the elucidation of the enzymes involved in the isomerisation of homocitrate. Due to the extreme lability of the homoaconitase the enzyme was never purified to homogeneity before. Our first approaches to overproduce and purify the homoaconitase from *A. fumigatus* in its native host also failed because of the extreme instability of the enzyme during purification. Recently, we were able to produce the enzyme heterologously in *Escherichia coli*. The enzyme was hardly active in crude lysates and completely inactive after Ni-NTA chromatography. Enzyme activity was regained after anaerobic incubation in the presence of sulphide and iron, which indicates the requirement for an iron sulphur cluster. As proposed before, the enzyme acted on homo-*cis*-aconitate, but not on homocitrate, which confirms that only the rehydration of homoaconitate is performed by this enzyme. In contrast, purified aconitase from *Aspergillus nidulans* was able to convert homocitrate to homoaconitate although the equilibrium is far on the site of homocitrate. This may explain, why a homoaconitase deletion strain shows an accumulation of homocitrate but not homoaconitate. We conclude that, the combined action of the citric acid cycle aconitase and that of the alpha-aminoadipate pathway specific homoaconitase is required for complete isomerisation. Currently we try to overproduce the homoisocitrate dehydrogenase in order to reconstitute this part of the biosynthetic pathway *in vitro*.

The role of glucosamine-6-phosphate deaminase at the early stages of growth in *Aspergillus niger* cells

T. Šolar, J. Turšič and M. Legiša

National Institute of Chemistry, Ljubljana, Slovenia

Aspergillus niger is a filamentous fungus that accumulates high levels of citric acid. Beside that is used also for the production of various homologous and heterologous proteins. In *A. niger* cells, at the early stages of growth in a high citric acid yielding medium apparently two enzymes, 6-phosphofructo-1-kinase and glucosamine-6-phosphate deaminase compete for the same substrate, fructose-6-phosphate. Since fructose-6-phosphate is the intermediate of the glycolytic pathway, consumption of this molecule by glucosamine-6-phosphate deaminase diminishes metabolic flux over the primary metabolism and retracts the flow of precursors for citric acid synthesis. Basic kinetic parameters of the enzyme were measured and will be presented in the poster. High values of specific activities for glucosamine-6-phosphate deaminase were recorded in germinating spores, while later activities gradually decreased. After 20 hours, at the time of ammonium depletion from the medium, the specific activity was reduced by more than threefold, while during the stage of glucosamine-6-phosphate re-consumption specific activity of only 6 mU/mg was recorded.

Kinetic characteristics of the enzyme were measured in both, in aminating and deaminating direction. The enzyme exhibited high affinity toward fructose-6-phosphate with K_m value of about 1 mM and the affinity increased to the value of 0,5 mM in the presence of the activator, N-Acetyl-D-glucosamine-6-phosphate. The enzyme showed strong pH dependence with highest activities detected at pH value of 6,8, while at pH = 7,7 only one third of maximal activity could be measured. In the deaminating direction affinity of the enzyme toward glucosamine-6-phosphate as a substrate was not so high, with a K_m the value of only 5 mM. N-acetylglucosamine-6-phopshate didn't change affinity of the enzyme toward the substrate but significantly increased maximal velocity. By comparing kinetic parameters of *A. niger* glucosamine-6-P deaminase with those of other organisms, a crucial difference in affinity toward fructose-6-phosphate was observed. While K_m values of all so far measured enzymes exhibit values of more than 1,7 mM (*E.coli*), 3,5, mM (*Homo sapiens*) up to 36 mM (*Musca domestica*), the *A. niger* enzyme showed extremely high affinity toward fructose-6-phosphate with K_m value of 1 mM that dropped even to 0,5 mM in the presence of N-acetylglucosamine-6-phosphate as an activator.

Re-identification of Ach1p: CoA-transferase rather than acetyl-CoA hydrolase

C. Fleck and M. Brock

Leibniz Institute for Natural Product Research and Infection Biology, Germany

Since nearly twenty years the yeast enzyme Ach1p, which is strongly induced during growth on acetate, was denoted as an acetyl-CoA hydrolase. The deletion of the gene leads to a significant impairment of growth on acetate as sole carbon and energy source. Since acetyl-CoA has to be generated from acetate by a very energy consuming reaction and the yield of total ATP is much lower than that obtained during growth on glucose, only speculations on the physiological role of such an “energy destroying” enzyme have been made. In our research on the propionate metabolism of filamentous fungi we identified a CoA-transferase, which is important for the transfer of the CoASH-moiety from toxic propionyl-CoA to acetate when grown on acetate/propionate containing media. Acceptable CoASH donors were acetyl-CoA, propionyl-CoA and succinyl-CoA and only their corresponding acids acted as CoASH acceptor molecules. Identification of the coding sequence of this CoA-transferase revealed that the amino acid sequence was highly identical to that of Ach1p from *Saccharomyces cerevisiae*. This drove us to the re-characterisation of the yeast enzyme. After overproduction of the protein in an *ach1* deletion strain, the protein was purified to homogeneity and characterised for its catalytic abilities. The purified enzyme turned out to act strongly as a CoA-transferase with only minor acetyl-CoA hydrolase activity. In addition, specificity for CoASH donors and acceptors was identical to that of the CoA-transferase from the filamentous fungus *Aspergillus nidulans*. We, furthermore, characterised the impairment of the Ach1p deletion strain during growth on acetate in more detail. When compared to the wild type the *ach1* mutant displayed an extremely prolonged lag phase till the exponential growth phase was reached. However, an acetate pre-grown culture transferred to fresh acetate containing media was hardly distinguishable in its growth rate from the wild type. We currently try to identify metabolites, which may accumulate during the adaptation phase to draw a picture on the physiological role of Ach1p.

Dynamic ergosterol- and ceramide-rich domains in the peroxisomal membrane serve as an organizing platform for peroxisome fusion

T. Boukh-Viner, P. Kyryakov, C. Gregg, T. Guo, A. Goldberg, S. Bourque, S. Chowdhury, F. Banu, K. Hung Yeung San, S. Milijevic, N. Ramlal, C. Sison, J. Solomon, V. Wong and V.I. Titorenko

Department of Biology, Concordia University, Montreal, Canada

All membrane fusion reactions inside the secretory and endocytic systems of vesicular flow operate by very similar mechanisms and are served by a similar set of core protein components. On the other hand, the fusion of mitochondria and peroxisomes do not require intracellular fusion machines that function in the secretory and endocytic pathways and may therefore involve unique, yet unknown, mechanisms. We have identified an unusual mechanism regulating fusion of yeast peroxisomes. Peroxisome fusion is an initial step in a multistep pathway that leads to the formation of mature peroxisomes, P6, carrying the complete set of matrix and membrane proteins. The immature peroxisomal vesicles P1 and P2, the earliest intermediates in the peroxisome assembly pathway, undergo fusion to generate larger vesicles, P3. We have demonstrated the existence of unusual ergosterol- and ceramide-rich (ECR) domains in the membranes of P1 and P2. Several key features of these membrane domains clearly distinguish them from lipid raft domains in the plasma membrane. ECR domains in the membranes of P1 and P2 are dynamic assemblies of a distinct set of proteins, including two ATPases, Pex1p and Pex6p, as well as phosphoinositide- and GTP-binding proteins. All of these proteins are essential for the fusion of P1 and P2. ECR domains function as an organizing platform for peroxisome fusion. Our findings suggest a model for the dynamics of temporal and spatial reorganization of the protein team that transiently resides in ECR domains and controls peroxisome fusion. The lateral movement of individual protein components of the peroxisome fusion machinery from ECR domains to ergosterol- and ceramide-poor domains initiates the peroxisome proton gradient-dependent release of Ca^{2+} from the peroxisome lumen and the concomitant recruitment of calmodulin to the organelle surface, ultimately leading to the fusion of peroxisomal vesicles P1 and P2.

Lipid metabolism in peroxisomes, endoplasmic reticulum and lipid bodies controls chronological aging in yeast

S. Bourque, A. Goldberg, T. Boukh-Viner, C. Gregg, P. Kyryakov, S. Chowdhury, Z. Aziz, A. Chang, D. Cyr, S. Kenth, S. Milijevic, M. Noei, R. Noei, N. Ramlal, J. Solomon, V. Wong and V.I. Titorenko

Department of Biology, Concordia University, Montreal, Canada

The diet known as calorie restriction (CR) extends life span and delays diseases of aging. It seems that the fundamental mechanisms of aging and the stimulatory effect of CR on life span are conserved from yeast to humans. We use yeast as a model organism for studying the molecular and cellular mechanisms of aging. Our research is aimed at understanding how defects in the biogenesis and function of the peroxisome, an organelle known for its essential role in lipid metabolism, affect the life span of yeast placed on the CR diet. We found that CR promotes the lipolysis of neutral lipids (NL) stored in lipid bodies (LB). In addition, the CR diet stimulates the synthesis of peroxisomal enzymes involved in oxidation of the LB-derived CoA esters of fatty acids (FA-CoA), thereby leading to the rapid consumption of free fatty acids (FFA) in chronologically aging cells. Using lipidomics, we monitored the dynamics of the age-related changes in the intracellular levels of NL, FFA, diacylglycerols (DAG) and cardiolipins (CL) in numerous long- and short-lived mutants. Our findings imply that the mobilization of FA-CoA from LB and their subsequent oxidation in peroxisomes play a key role in regulating chronological life span and in protecting CR yeast from caspase- and mitochondria-dependent apoptosis and various stresses. Moreover, our data provide evidence that the steady-state levels of the LB-derived FA-CoA and of the LB- and endoplasmic reticulum (ER)-derived DAG control the rate of chronological aging. Furthermore, our findings suggest that lipid metabolism in peroxisomes of CR yeast modulates the steady-state levels of CL in the inner mitochondrial membrane. This, in turn, regulates the efficiency of mitochondrial respiration, the electrochemical potential across the inner mitochondrial membrane, the level of reactive oxygen species in mitochondria, and the ability of mitochondria to undergo fusion in chronologically aging cells. Our data suggest a mechanism by which the remodeling of lipid metabolism in peroxisomes, ER and LB of CR yeast extends their life span by regulating the apoptosis- and stress response-related mitochondrial functions.

Improvement of the determination of the activity of the plasma membrane H+-ATPase in *Penicillium simplicissimum*

W. Mutschlechner, S. Kraler and W. Burgstaller

Institute of Microbiology, Innsbruck, Austria

The plasma membrane of plants and fungi contains a vanadate-inhibited H^+-ATPase. This master enzyme provides the proton motive force for many nutrient-proton-symports and it is also highly specific for the plasma membrane itself. We purified plasma membrane vesicles from *Penicillium simplicissimum*, a citrate excreting fungus, which has also been used in metal leaching processes. The mycelium was disrupted by means of a glass bead mill. Right-sight-out plasma membrane vesicles were purified from the crude extract by using a 6.1 % polyethylene glycol / 6.1 % dextran two phase system[1]. We quantified the vanadate-inhibited ATPase activity by the release of inorganic phosphate. The phosphate was determined by photometry using the formation of a phosphomolybdate complex which was reduced to a blue coloured system by hydroquinone[2, 3]. The presented work discusses the main problems which occur when applying this method.

Inorganic phosphate was still released abiotically from ATP after the reaction was stopped by adding sulphuric acid (0.5 M). The extent of this ongoing release depended on the temporal delay between introducing the acidic stop and the formation of the blue coloured complex. An increase of about 25 % of inorganic phosphate was observed after 30 min. Moreover, the absorption of the blue complex at 730 nm declined rapidly, namely 7 % after 30 min. A strict time plan, which managed all working steps from the start to the measurement, was necessary to overcome both problems. A plan which contained a minimum of idle time was only able to be maintained by working in pairs. Ignoring the mentioned time delays and its implications can give rise to two problems: Generally, data can be inconsistent and confusing. Furthermore, ATPase activity data from different working groups cannot be compared clearly. The improvement of the determination of the vanadate-inhibited ATPase activity presented here solves these difficulties as well as leads to a broader application of the assay in physiology.

1 Müller, B., Zanella, A. and Burgstaller, W. (2001) J Basic Microbiol, 41 (5), 281–288.
2 Ugalde, U. (1991) Mycol Res, 95 (11), 1303–1307.
3 Kitson, R. E. and Mellon, M. G. (1944) Ind Eng Chem. Anal Ed, 16: 466–469.

ABSTRACTS OF POSTER SESSION V

Protein production

Posters P76 – P95

Spatially segregated snare protein interactions in filamentous fungus *Trichoderma reesei*

M. Valkonen[1], M. Saloheimo[1], M. Penttilä[1], N. D. Read[2] and R. R. Duncan[3]

[1] VTT Technical Research Centre of Finland, Finland
[2] Fungal Cell Biology Group, Institute of Cell Biology, University of Edinburgh, UK
[3] Membrane Biology Group, University of Edinburgh, UK

The machinery for trafficking proteins through the secretory pathway is well conserved in eukaryotes, but remains poorly characterized in filamentous fungi. Secretion is believed to be highly polarized in fungal hyphae and to mainly occur from hyphal tips. We describe the isolation of the snc1 and sso1 genes encoding exocytic SNARE proteins from Trichoderma reesei. The encoded SNCI protein can complement Snc protein depletion in S. cerevisiae whilst the T. reesei SSOI protein was unable to complement depletion of its yeast homologues. The localization and interactions of the T. reesei SNARE proteins were studied with advanced fluorescence imaging methods using fluorescent fusions of the SNARE proteins. The SSOI and SNCI proteins co-localized in sterol-independent clusters on the plasma membrane in sub-apical but not apical hyphal regions. The v-SNARE SNCI (but not the t-SNARE SSOI) localized to the apical vesicle cluster within the Spitzenkörper of the growing hyphal tips when expressed from the homologous T. reesei cbh1 promoter. Using fluorescence lifetime imaging microscopy (FLIM) and fluorescence energy transfer (FRET) analysis, we quantified the interactions between these proteins with high spatial resolution in living cells. Our data showed that the site of SNARE complex formation between these proteins is on the plasma membrane of non-growing hyphae in old sub-peripheral regions of the colony, but that there is no interaction between the proteins in growing hyphal tips in at the colony margin. These findings suggest spatially distinct sites of exocytosis within filamentous fungi and the existence of multiple exocytic SNAREs which are functionally and spatially segregated

New screening approaches for fungal strain development

P. J. Punt[1], X. O. Weenink[2], M. van der Maarel[1], J. Jore[1], A. Ram[2] and C. van den Hondel[2]

[1] TNO Quality of Life, Zeist, the Netherlands
[2] Leiden University, Leiden, the Netherlands

Since the development of recombinant DNA technologies for yeast and filamentous fungi, a considerable part of the strain development programs was diverted to the use of molecular genetic tools. Whereas these approaches have exerted considerable success, recent developments in our laboratory have shown that new developments in classical biological screening approaches, or a combination of both, can still be very useful. A first purely classical approach is based on the discovery of a so-called suicide (SUI) substrate, which we have successfully used for the selection of protease deficient fungal host strains. These protease deficient strains show an increased resistance to the SUI substrate allowing their selection. The advantage of this non-GMO approach is that it can be applied to new and already established production strains. A combination of a molecular and classical approach is based on the use of the so-called glucoamylase carrier approach. Combining this approach with fungal strains unable to use starch as a carbon source allowed us to select for hyper secretive fungal strains generated by classical mutagenesis (Weenink et al., 2006). Moreover, the same approach also allows for selection of the highest producers in a collection of primary transformant strains expressing a glucoamylase-fusion gene.

Visualisation of the ER in the industrially-exploited filamentous fungus *Trichoderma reesei* using a Bip-Venus fusion protein

H. Yu[1,2], D. Birch[3], M. Nykänen[4], J. Te'O[1,2,5], E. Goldys[6,7] and H. Nevalainen[1,2,5]

[1] Department of Chemistry and Biomolecular Sciences, Macquarie University, Australia
[2] Macquarie University Biotechnology Institute, Macquarie University, Australia
[3] Department of Biological Sciences, Macquarie University, Sydney, Australia
[4] Medical School, University of Tampere, Finland
[5] Applimex Pty Ltd, Macquarie University, Sydney, Australia
[6] Department of Physics, Macquarie University, Sydney, Australia
[7] ARC/NHMRC Network for Fluorescence Applications in Biotechnology and Life Sciences, Australia

Tracking the progress of proteins through the secretory pathway requires reliable markers for cell organelles participating in secretion. Endoplasmic reticulum (ER) is the cytoplasmic organelle where the early steps of the protein secretory pathway are localised. Chemical markers such as $DIOC_6(3)$ and ER tracker series have an inherent problem of non-specificity and therefore are not optimal for detailed subcellular localisation studies. Fluorescent proteins on the other hand, provide better specificity by means of genetic tagging of organelles of interest. Here we report the fusion of the coding sequence for the VenusYFP to the fungal gene encoding Bip, a molecular chaperone located in the ER. Visualisation of the ER in a *T. reesei* transformant was carried out by fluorescence using confocal microscopy and the optical properties of the Venus fluorescence were investigated in detail. The accuracy of ER-labelling by the fusion protein approach was confirmed by EM studies. The expression of the *venus-bip1* fusion construct as a function of time was further examined by Northern blotting and Western analysis. Our success in targeting a relatively stable fluorescent protein to the ER in the filamentous fungus *T. reesei* provides a powerful tool for studies into the function of the ER in protein secretion in living fungal hyphae.

Cloning and expression of frutalin, an alpha-D-galactose-binding plant lectin, in *Pichia pastoris*

C. Oliveira[1], W. Felix[2], R. A. Moreira[2,3], J. A. Teixeira[1] and L. Domingues[1]

[1] IBB Institute for Biotechnology and Bioengineering, Universidade do Minho, Portugal
[2] Departamento de Bioquímica e Biologia Molecular, Universidade Federal do Ceará, Brasil
[3] Centro de Ciências da Saúde, Universidade de Fortaleza, Brasil

Frutalin is the alpha-D-galactose-binding Jacalin-related lectin from *Artocarpus incisa* seeds. This plant lectin was successfully used on the recognition of cancer metastasis, specifically those of breast and thyroid gland, due to its ability to interact with galactose complexes and thus potentially combine with cancer cells surfaces containing these sugars. It can be potentially used as a histochemical probe in the diagnostic of several cancers. However, Frutalin extraction from seeds is a long process with low yields and a mixture of many isolectins can be obtained. Frutalin is thought to be synthesized as a pre-pro-lectin, consisting of a signal sequence, a pro-peptide, a 20-amino-acid beta-chain, a 4-amino-acid linker peptide and a 133-amino-acid alpha-chain. In mature Frutalin, the signal sequence and the pro-peptide may be removed through post and/or co-translational processing and the linker excised to generate two chains, alpha and beta. Active Frutalin consists of a tetrameric protein and each one of its subunits is made of one alpha chain and one beta chain non-covalently linked.

The aim of this work is the production of recombinant biologically active Frutalin in the methylotrofic *Pichia pastoris* KM71H yeast strain. Frutalin synthetic gene, containing codons in preference in *P. pastoris*, was obtained using base synthesis and PCR approaches. Optimized codifying Frutalin sequence was cloned into the pPICZalphaA expression vector that contains the *Saccharomyces* alpha-factor preprosequence to direct recombinant protein into the secretory pathway. Soluble recombinant Frutalin was detected in the culture supernatants after optimized batch culture conditions. Frutalin was expressed as a single chain as the 4-amino-acid linker peptide (T-S-S-N), that connects α and β chains, was not cleaved. Furthermore, incomplete processing of the signal sequence resulted in recombinant Frutalin with one Glu-Ala N-terminal repeat derived from the alpha-factor prosequence, and part of recombinant Frutalin was highly N-glycosylated. Nevertheless, recombinant Frutalin was recognised by native Frutalin antibody and its ability to bind galactose was maintained. The recombinant lectin was purified on an *Adenanthera pavonina* cross-linked galactomannan column taking advantage of the galactose-binding property. Immunohistochemical studies for cancer diagnostic are now being conducted with purified recombinant Frutalin.

Heterologous expression of isotypically labelled *Trichoderma reesei* tyrosinase 2 in *Pichia pastoris*

A. Westerholm-Parvinen, M.-L. Mattinen, E. Selinheimo and M. Saloheimo

VTT Technical Research Centre of Finland, Finland

Tyrosinase (EC 1.14.18.1) is a copper-containing oxidase that is widely distributed in mammals, invertebrates, plants and microorganisms. In mammals the enzyme is essential for the formation of melanin pigments, whereas tyrosinases in fruit and vegetables are related to the browning reaction that occurs upon bruising and long term storage. Tyrosinase is of great interest for many applications in the field of medicine, biotechnology and food engineering. It is a promising target enzyme for prodrug activations in melanomas and in biotechnological applications including crosslinking of protein matrices. It is of great importance to find ligands and inhibitors for tyrosinase. Structural studies and screening for ligands and inhibitors can be carried out using NMR spectroscopy with isotopically labeled tyrosinase. Therefore, we cloned a novel tyrosinase from *Trichoderma reesei* and expressed it heterologously in the methylotrophic yeast *Pichia pastoris*.

A novel tyrosinase, tyrosinase 2 (TYR2), was cloned from *Trichoderma reesei*. The cDNA sequence was expressed under the control of the AOX1 promoter in the *Pichia pastoris* X-33 strain. The Saccharomyces cerevisiae alpha-MF prepro sequence was used for secretion and an N-terminal His6-tag was fused to the tyrosinase to facilitate the detection and purification of the recombinant protein. Heterologous expression was carried out in shake flask cultivations and the enzymatic activity was measured directly on the culture medium, using L-Dopa as a substrate. Extensive optimisation of the expression in shake flasks was carried out as the stable isotope labels are costly. Different temperatures, different CuSO4 and NH4SO4 concentrations and different shake flasks were tested. The expression level of recombinant TYR2 was increased tenfold as a result of the optimisation. Metabolic 15N–labeling of TYR2 was carried out with 15NH4SO4 in minimal medium to assess its suitability for investigations by NMR spectroscopy. Initial 3D heteronuclear 1H-15N HSQC NMR spectrum of TYR2 showed signals with chemical shifts typical of folded proteins.

The *Trichoderma reesei* tyrosinase 2 was successfully expressed and uniformly 15N-labeled in the yeast *Pichia pastoris*. This methylotrophic yeast is a suitable expression system for the production of recombinant proteins for NMR studies as it is cost-effective and possesses the ability to perform many of the posttranslational modifications of higher eukaryotes.

Production of the human carbohydrate binding module from laforin protein: A comparative study between bacterial and yeast expression systems

S. Moreira[1,2], L. Domingues[1], M..Gama[1] and M. Casal[2]

[1] IBB Instituto De Biotecnologia E Bioengenharia, Universidade Do Minho, Portugal
[2] Centro De Biologia, Braga, Portugal

The goal of this work is the production of a recombinant Carbohydrate Binding Module (CBM) from human origin aiming to improve the functional properties of biomaterials based on polysaccharides. When fused with the bioactive peptides, CBMs may dramatically improve the therapeutic efficiency of biomaterials such as biocompatibility, biomimetism and/or biodegradability. Laforin is a human protein associated with glycogen metabolism, composed of two distinct structural and functionally independent domains: a phosphatase and a substrate binding module. The Laforin-CBM sequence was originally cloned by PCR from a human muscle cDNA library. Commercially heterologous expression systems of *Escherichia coli* (pET 29a, pET 25b and pGEXT4-1) and of *Pichia pastoris* (pGAPZ alpha C and pPICZ alpha C) were used in order to obtain high levels of soluble protein that can be purified by affinity chromatography using 6xHis-tag or GST-tag.

With pGEXT4-1 expression system, CBM was fused with the GST protein, which in theory increases solubility, but the amount of recombinant protein obtained was very low. In pET29a the CBM was obtained in inclusion bodies, which after solubilization and refolding processes was soluble but easily aggregated. Using pET25b, in the presence of arginine and CHAPS in the lyses buffer, the amount of soluble protein was higher but again formed aggregates. Two *P. pastoris* expression systems, both with secretion signal alpha- factor, were utilized: pGAPZ alpha C (containing a constitutive promoter) and pPICZ alpha C (containing an inductive promoter). The integration of the CBM coding sequence, in yeast genome, was confirmed by slot-blot. Expression was analysed by northern-blot and confirmed by western-blot. Fermentations carried out at 18ºC were critical to achieve production in both systems. The utilisation of *P. pastoris* expression systems led to the production of soluble and stable CBM in extra cellular medium; however, this CBM was obtained at low expression level. Post-translation modifications, such as glycosylation and phosphorylation of the protein may explain the increased stability, at the expense of reduced functionality. Studies are underway to confirm this hypothesis.

This study was supported by the portuguese grant POCTI/BIO/45356/2002; Susana Moreira (nº SFRH/BD/18418/2004) received a FCT fellowship.

Production of endo-1,4-beta-mannanase of *Aspergillus fumigatus* in *Aspergillus sojae* and *Pichia pastoris*

G. Duruksu[1], B. Ozturk[2] and Z. B. Ogel[1,2]

[1] Biotechnology Department, The Middle East Technical University, Turkey
[2] Food Engineering Department, The Middle East Technical University, Turkey

Extracellular endo-1,4-beta-mannanase (EC 3.2.1.78) gene of *Aspergillus fumigatus* IMI 385708 (formerly known as *Thermomyces lanuginosus* IMI 158749) was cloned and transformed in *Aspergillus sojae* (ATCC 11906) and *Pichia pastoris* GS115. High level of expression was achieved in both expression systems. Comparison of the expression levels of heterologous mannanase reveals that *A. sojae* is a better expression system than *P. pastoris* with respect to extracellular mannanase activity. Highest expression of mannanase was in *A. sojae* after 6 days of incubation (351.85 U/ml). In *P. pastoris*, highest expression was observed after 8 hrs of induction with methanol (51 U/ml). Expressed enzymes were purified and analyzed. Both enzymes have specific activity *c.* 349 U/mg protein with a pH optimum of *c.* 5.0 and temperature optimum *c.* 50 °C. Mannanase production of recombinant *A. sojae* cultivated on glucose and sucrose was compared. Slightly higher production level was observed on glucose. During prolonged incubation of the mannanase with locust bean gum, transglycosylation activity was detected. Utilization of hydrolysis and transglycosylation activities of mannanase in the production of functional oligosaccharide is discussed.

Production of alpha-galactosidase from *Aspergillus fumigatus* in *Aspergillus sojae* under the control of the *gpd*A promoter and the influence of osmotic stress

S: Gurkok[1], B. Soyler[2] and Z. B. Ogel[1,2]

[1] Biotechnology Department, The Middle East Technical University, Turkey
[2] Food Engineering Department, The Middle East Technical University, Turkey

The enzyme α-galactosidase catalyzes the hydrolysis of α-1,6-linked galactose residues from oligosaccharides such as melibiose, raffinose, and stachyose and from polymeric galactomannans. The most important industrial application of α-galactosidases is presently in the sugar-making industry. Some α-galactosidases are also known to catalyze transgalactosylation, especially at a high concentration of substrate. Alpha-galactosidases have been isolated from a variety of eukaryotes and bacteria. The strain *Aspergillus fumigatus* IMI 385708, a thermotolerant fungus is a good producer of extracellular α -galactosidase. However, being a human pathogen, *Aspergillus fumigatus* can not be used in industrial applications. In this study, the gene encoding α-galactosidase (*agl*B) was isolated from *Aspergillus fumigatus* and cloned to the expression vector pAN52-4 (Acc. No: Z32699). The vector contains glyceraldeyhde 3-phosphate dehydrogenase (*gpd*A) promoter of *A. nidulans* and signal and propeptide region of the *A. niger* glucoamylase gene. Following transformation of *Aspergillus sojae* ATCC11906 with recombinant expression vector, heterologous expression was achieved in this GRAS in the presence of simple carbon compounds such as glucose. Further, osmotic stress was applied to enhance enzyme production. Recombinant *Aspergillus sojae* strain was gradually adapted to increasing salt concentrations. In the presence of 2M NaCl or Na_2SO_4 enzyme production was about 4-fold higher (U/g biomass) with respect to *A.fumigatus* grown on locust bean gum. Adaptation to osmotic stress yielded better results than non-adapted shocked cells.

Improvement of lipase production at different concentations of inductor and aeration rate

A. Cozea[1], S. Jurcoane[1] and E. Sasarman[2]

[1] Faculty of Biotehnology, University of Agriculture and Veterinary Medicine, Bucharest, Romania
[2] Faculty of Biology, University of Bucharest, Romania

Lipases are secreted by many bacteria and fungi. The biotechnological potential of these enzymes is steadily increasing with various industrial applications. Recently attention has been turned to nonconventional yeasts (NCY). The aim of the study was to investigate the values of inductor concentration and specific rate aeration for lipase production with *Yarrowia lipolytica* yeast. The culture medium was containing glucose for carbon source and the most suitable nitrogen source was peptone and yeast extract for the production of the extracellular lipase. We also demonstrate that in the presence of olive oil in media like inductor, in diferent concentration, the lipase activity variate. Higher activity levels of lipase production 11.972 U/ml were obtained with an 0.05% concentration of olive oil comparative with 5.975 U/ml activity with 2% olive oil concentration. In case of aeration rate the best lipase activity was 2.99 U/ml with 1:10 aeration rate comparative with 2.39 U/ml lipase activity with 1:4 aeration rate. This work provides a better understanding of the extracellular mechanism lipase production of the yeast *Yarrowia lipolytica*.

Improving recombinant cyprosin B protease production in batch and fed-batch culture of a strain *Saccharomyces cerevisiae*

P.N. Sampaio[1,2]; L.P. Fonseca[2] and M.S. Pais[1]

[1] Unit of Molecular Biology and Biotechnology of Plant, Institute of Applied Science and Technology, Portugal
[2] Biologycal and Chemistry Engineering Center, Biochemical and Biotechnological Institute, Instituto Superior Técnico, Portugal

The batch and fed-batch strategies carried out were aimed at providing fundamental knowledge on the physiological aspects involved in the optimization of recombinant cyprosin B production by *Saccharomyces cerevisiae* BJ1991 strains in bioreactor. Cyprosin B is an aspartic protease present in *Cynara cardunculus* flowers which are widely used in Portugal, Spain and other Mediterranean countries for cheese making. The productivity of fermentations employing recombinant micro-organisms depends on multiple factors, like microbial physiology, copy number and stability of the plasmid, gene expression and cell viability. The growth of the transformed yeast strain was tested in 2 liters on bioreactor with 50% of oxygen saturation and pH 5.5 at 30ºC. In the batch strategy, the fermentation was made using YPGal/Glu medium with yeast extract (10 $g.l^{-1}$), bactopeptone (20 $g.l^{-1}$), glucose (20 $g.l^{-1}$) and galactose (20 $g.l^{-1}$) for 24 hours. The biomass, protein concentration and enzymatic activity obtained were 10.6 g $dcw.l^{-1}$, 25 $mg.l^{-1}$ and 176 $U.ml^{-1}$, respectively. The increase expression of recombinant protein was obtained after the total consumption of glucose and ethanol which allows the consumption of galactose. It was observed that the consumption of galactose is coincident with increase of the expression and secretion of heterologous protein because the enzyme gene transcription is controlled by this promoter.

The fed-batch fermentation was carried out in two phases: the initial phase was performed along 24 hours in YPGlu medium with glucose (20$g.l^{-1}$). The biomass produced was 9.1$dcw.l^{-1}$. The protein concentration and enzymatic activity were very low. In the second feed phase, after total consumption of glucose and ethanol, analyzed by HPLC, 500 ml of YPGal medium containing galactose (40 $g.l^{-1}$) were added for 6 hours. During this phase biomass reached 34.6 $dcw.l^{-1}$. The total protein concentration and enzymatic activity obtained were 408 $mg.l^{-1}$ and 201 $U.ml^{-1}$, respectively. The fed-batch strategy allowed 3.2 times increase the biomass, in 16 times protein concentration and in 1.2 times enzymatic activity. These results suggested that for improving the cyprosin B production in bioreactor is important to develop the strategy in fed-batch in two phases: the first phase, with growth only in glucose which enables the increase of biomass; the second phase, in medium containing the inducer enables the increasing of expression and secretion of the recombinant cyprosin B.

Improve enzyme expression in *Saccharomyces cerevisiae*

J. Lin, B. Cherry, T. Heu, P. Harris, E. Bashkirova, and D. Yaver

Novozymes, Inc., Davis, USA

Saccharomyces cerevisiae is an excellent organism for creating libraries and high throughput screening. However, low protein expression sometimes can cause problems for high throughput screening systems in *S. cerevisiae.* We therefore tested several methods to increase expression in *S. cerevisiae* in 96 well plate formats. We first examined swapping signal sequences, and found *Humicola insolens* cutinase signal sequence can increase enzyme expression for certain reporter genes. We also determined media components and growing conditions for optimal *Thermomyces lanuginosus* lipase variant expression. *Thermomyces lanuginosus* lipase variant expression from different promoters was determined. Finally, we tested the use of the *CUP* promoter in the *S. cerevisiae* expression system in 96 well plates. We found that lipase variant from *Thermomyces lanuginosus*, cutinase from *Humicola insolens*, plectasin from *Pseudoplectania nigrella*, and laccase from *Myceliophthora thermophila* showed much higher expression from the *CUP* promoter when compared to expression from the *TPI* or *GAL* promoter. Alpha-amylase from *A. oryzae* and glucoamylase from *A. niger* expressed from the *CUP* promoter did not show higher expression when compared to expression from the *TPI* promoter. Combinations of these expression systems can be beneficial for high throughput screening in *S. cerevisiae.*

Heterologous protein production in *Coprinopsis cinerea*

P. C. Rittershaus[1,2], P. Hoegger[3], M. Ruehl[3], L. Fischer[2], U. Kues[3], U. Dechert[1] and J. Eck[1]

[1] BRAIN Aktiengesellschaft, Zwingenberg, Germany
[2] Department of Biotechnology, Institute of Food Technology, University of Hohenheim, Germany
[3] Molecular Wood Biotechnology, Institute of Forest Botany, Georg-August-University of Gottingen, Germany

Coprinopsis cinerea was choosen as a model organism for the production of homologous laccases in basidiomycetes (Kilaru et al. 2006). Due to the high production and secretion potential it is also a promising candidate for the production of heterologous proteins. With the objective to show the stability of heterologous transcripts, we were able to use green fluorescent protein (eGFP) as reporter gene. Since *C. cinerea* secretes various endogenous ones, it seems in particular to be adapted for the secretory expression of heterologous eukaryotic oxidoreductases. Because the expression of heterologous genes is also thought to be limited by proteolytic degradation, we present in this poster the attempt to develop a *C. cinerea* strain with reduced extracellular proteolytic activity as a host for the secretory expression of eukaryotic oxidoreductases.

Development of tailor made *Chrysosporium lucknowense* strains for the saccharification of lignocellulosic biomass

Jan C. Verdoes[1], Arkady P. Synitsyn[2] and Glenn E. Nedwin[3]

[1] Dyadic Nederland BV, The Netherlands
[2] Department of Chemistry, Moscow State University, Moscow, Russia
[3] Dyadic International Inc. Jupiter, Florida, USA

Large-scale production of bioethanol from biomass, an alternative to fossil fuels, is primarily done by recombinant *Saccharomyces cerevisia* strains. The major polysaccharides comprising different lignocellulosic residues are cellulose and hemicellulose. Both are hydrolyzed into the monomers like glucose, xylose and other hexoses and pentoses by the combined action of cellulases, hemicellulases and an additional set of accessory enzymes. Key factors in this process are the composition and activities of (hemi)cellulases that convert the plant lignocellulosic fibers. Finally the monomeric sugars are fermented into ethanol by the yeast.

Scientists of Dyadic Nederland, in collaboration with scientists in other research centers have developed and patented strains of the fungal microorganism *Chrysosporium lucknowense* – or C1 – to rapidly discover and express both eukaryotic and prokaryotic genes; and to manufacture the novel products of those genes, using C1 as the host organism from beginning to end (Burlingame *et al.* 2003, PharmaGenomics 25–29). The C1 genome has been sequenced and the annotation that is now in progress has identified a large set of potentially interesting genes encoding biopolymer degrading enzymes.

The extracellular proteins content of *C. lucknowense* is a mix of cellulases and hemicellulases. Specific enzymes are being purified, characterized and compared with those obtained from other established (hemi)cellulase producers like *Trichoderma* sp.. This resulted in the identification of an extremely active cellobiohydrolase from *C. lucknowense*. The use of artificial multi-enzyme compositions to guide construction of tailor-made (hemi)cellulases production strains of *C. lucknowense* will be presented.

Characterization of novel thermostable fungal cellobiohydrolases

T. Puranen[1], S. Voutilainen[2], M. Siika-aho[2], J. Kallio[1], S. Hooman[2], L. Viikari[2], A. Koivula[2] and J. Vehmaanperä[1]

[1] Roal Oy, Rajamäki, Finland
[2] VTT Technical Research Centre of Finland, Finland

Cellulose degradation requires sequential or simultaneous synergic action of three types of hydrolytic enzymes: cellobiohydrolases, endoglucanases and beta-glucosidases. Various filamentous fungi produce these enzymes in order to hydrolyze insoluble cellulose into glucose. Cellulases are currently extensively studied in enzyme industry for cellulosic biomass conversion to ethanol. Here, molecular cloning of three different cellobiohydrolase genes from thermophilic ascomycetes is presented together with their heterologous expression in *Trichoderma reesei*. The recombinant cellobiohydrolases that belong to the glycosyl hydrolase (GH) family 7 were purified and characterized in terms of pH optimum, thermal stability and kinetic parameters. Thermostable cellulases such as described here have been proposed to improve the overall process economy of the biomass conversion with favourable impact on enzyme need, hydrolysis performance and flexibility of the process.

Bottlenecks in bacterial transglutaminase production in *Trichoderma reesei*

M. Paloheimo[1], S. Mäkinen[1], J. Kallio[1], K. Langfelder[2] and J. Vehmaanperä[1]

[1] Roal Oy, Rajamäki, Finland
[2] AB Enzymes GmbH, Darmstadt, Germany

Transglutaminases catalyse cross-linking in proteins via an acyl transfer reaction between peptide-bound glutamine and lysine. TGases are widespread in mammals and they have also been found in microorganisms, plants, invertebrates, fish and birds. The cross-linking activity of TGases is exploited in several food applications, e.g. in improving the texture of processed meat and fish products. There is a growing interest to use TGases in technical applications, such as use in the textile industry. TGases could be used in modification of silk and wool fibres. For technical applications a cheap source of TGase must be available. For this reason a preliminary study was performed to determine whether *Trichoderma reesei* is a suitable host for production of a bacterial transglutaminase. The *S. mobaraensis* tg gene was used as a test gene. The TGase is activated after secretion by two-step cleavage of the pro-peptide from the precursor. Constructs encoding the full-length and mature and the corresponding inactive TGase forms were expressed in *T. reesei*. The TGase encoding sequences were expressed in *T. reesei* as 3´-fusions to a sequence encoding a carrier polypeptide. The results obtained will be shown and discussed.

This work was part of the STReP project HIPERMAX/NMP-3-CT-2003-505790.

Problems with proteases in a recombinant filamentous fungus, *Aspergillus niger* B1-D

Q. Li, L. M Harvey and B. Mcneil

Strathclyde Fermentation Center, Strathclyde Institute of Pharmacy and Biomedical Sciences, University of strathclyde, UK

Filamentous fungi have attracted great research interest recently and several genetically modified organisms have been successfully constructed to express recombinant proteins, including industrial enzymes and therapeutic proteins. The advantages of using filamentous fungi as hosts include, (1) the ability to secret large amount of proteins, (2) post-translational modifications which are essential to proper protein function, but may be neglected in prokaryotic hosts, (3) fast growing and inexpensive cultures compared to insect, plant and mammalian cells. In the formation of recombinant proteins in filamentous fungi, one of the bottlenecks may be the extracellular proteases, which can potentially digest recombinant proteins at a high rate.

In the present work, we cultivated a recombinant filamentous fungus, *Aspergillus niger* B1-D, genetically modified to secret hen egg white lysozyme (HEWL), in a 15L fermentor, and investigated the properties of its extracellular proteases. Our results indicate that the extracellular proteases are exclusively acid proteases, with an optimal temperature at 45^{o}C. The use of specific protease inhibitors indicated the presence of four different kinds of acid proteases in the medium, ie. cysteine, aspartic, serine and metallo-proteases. In addition, we found the secretion of proteases is largely regulated by the carbon source using *Aspergillus* Complete Medium. Also protease secretion was independent of dissolved oxygen tension (DOT) and cultivation temperatures, though shifting of DOT or temperature leads to a small amount of secretion.

Our results clarify the effects of proteases on recombinant protein production in *Aspergillus*, and add to our understanding to the factors influencing protease appearance in these cultures. This should aid the bioprocessing of recombinant proteins using filamentous fungi, and help challenge the current recombinant protein production methods which are dominated by mammalian cell expression systems.

An improved and markerfree™ expression system for *Aspergillus niger* enzyme production

I. de Lange, J. G. Bakhuis, L. F. de Lange, R. Verwaal, N. N.M.E. van Peij

DSM Food Specialties, The Netherlands

For many decades, *Aspergillus niger* has been safely used in the commercial production of various food enzymes, such as glucose oxidase, pectinase, alpha-amylase and glucoamylase. Genetically modified industrial strains of *A. niger* have been used as a host to over-express food and feed enzymes such as phytase and xylanase. Traditionally, the genes encoding these enzymes are integrated in a random fashion in the genome of the host organism. Technological developments have enabled us to construct a new generation of *A. niger* CBS513-88 lineage strains according to a "design and build" concept, in which the genes of interest are targeted integrated in the host genome. A recombinant strain was derived from a glucoamylase production strain, in which the glucoamylase (*gla*A) genes were deleted, creating so-called delta *gla*A loci. Each of these loci was designed in such a way that it can be detected individually by gel electrophoresis. Therefore, targeted integration of the gene of interest in all seven loci can be monitored, allowing selection of strains with multiple integrated expression units of the gene of interest. Homologous integration frequency was improved by disruption of the *A. niger hdf*A and/or *hdf*B genes, homologues of the human KU70 and KU80, that are essential for non-homologous end joining of DNA in double strand break repair. Deletion of *hdf* genes greatly reduced the frequency of non-homologous integration of transforming DNA fragments leading to dramatically improved gene targeting. The *hdf* knockout has contributed to a highly controlled strain construction process, with both increased targeting frequency and increased co-transformation percentage.

Development of *Trichoderma reesei* transformation technology with the aid of an episomal vector system

N. Aro, A. Westerholm-Parvinen, M. Penttilä and M. Saloheimo

VTT Technical Research Centre of Finland, Finland

In conventional transformation procedures of filamentous fungi the transformation frequencies have been modest and only integrative vectors have been available. In modern fungal molecular biology, however, screening approaches with the help episomal vectors have advanced the progress of the field greatly. For example, in enzyme discovery or protein engineering it is beneficial to be able to screen for new enzymes or improved mutant enzymes in the ultimate production host of the enzyme. Episomal vector systems have been developed for a few fungal species including the *Aspergilli*, but *Trichoderma reesei* has suffered from a lack of such a system.

We have developed *T. reesei* transformation technology for genetic and enzyme screening purposes. The transformation procedure was optimised to improve the transformation frequencies of any vectors. Telomeric episomal vectors were used, and with these we obtained transformation frequencies that are far above those obtained with integrative vectors and are feasible for genetic and enzyme screening purposes. Procedures for plasmid rescue, colony picking and microtiter plate cultivation were also developed. The use of the novel transformation system in enzyme and genetic screening experiments will be discussed.

Role of the unfolded protein response in *Candida albicans*

T. T. Wimalasena[1], T. Guillemette[1,3], A. Plumridge[1], B. Enjalbert[2], A. J. P. Brown[2] and D. B. Archer[1]

[1] School of Biology, University of Nottingham, UK
[2] Aberdeen Fungal Group, University of Aberdeen, Institute of Medical Sciences, UK
[3] Present address : UMR 77 Pathologie Vegetale, Faculte des Sciences, Université d'Angers, France

The Unfolded Protein Response (UPR) is a stress response that regulates the folding of proteins in the endoplasmic reticulum and the delivery of secretory proteins to the cell surface and exterior. *Candida albicans* is an opportunistic human fungal pathogen: its morphology and the secretion of enzymes and cell surface components are important factors in its virulence. We therefore aimed to describe the UPR in *C. albicans* and to determine its importance in nitrogen-dependent processes in *C. albicans*, such as the yeast-mycelial transition. In *S. cerevisiae*, the UPR induces the dimerization of a ER-resident type I transmembrane protein, Ire1p which activates splicing of *HAC1*^{u} to *HAC1*^{i} mRNA by excising a 252-bp non-conventional intron towards the 3'end of the mRNA, which produces the functional Hac1p. This activates transcription of UPR-sensitive genes via direct binding to the *cis*-acting UPR element (UPRE) to activate its target genes. In *Aspergillus niger*, a similar mechanism is observed, but the intron is limited to 20-bp. In addition to this unconventional splicing in *A. niger*, a 5'-UTR truncation occurs which affects the size of the transcripts. Here we report the characterization of the gene encoding Hac1p in *C. albicans.* In this study, the UPR was induced by treating *C. albicans* yeast cells with dithiothreitol (DTT) and tunicamycin. The analysis of cDNA made from *HAC1* mRNA shows that there is a difference between the *HAC1*^{i} and *HAC1*^{u} towards the 3' end of the mRNA: *HAC1*^{i} mRNA lacks a 19-base intron that is present in *HAC1*^{u}. Furthermore, we deleted both copies of the *HAC1* gene in *C. albicans* to create a null mutant strain. We confirm the role of the UPR in the delivery of cell surface proteins and show also that the UPR has a role in determining the morphology of *C. albicans*.

Engineering of multiple bottlenecks in *Rhizopus oryzae* lipase production in *Pichia pastoris*

K. Baumann[1], M. Mauer[2], D. Resina[1], H. Marx[2], F. Valero[1], D. Mattanovich[2] and P. Ferrer[1]

[1] Departament d'Enginyeria Química, Universitat Autònoma de Barcelona, Spain
[2] Institute of Applied Microbiology, Department of Biotechnology, University of Natural Resources and Applied Life Sciences, Austria

Methanol-free high cell density fed-batch cultivation strategies for the *P. pastoris* expression system have been recently developed by expressing a *Rhizopus oryzae* lipase (ROL) under the transcriptional control of the PFLD1 [1]. Intracellular active product accumulation and a decrease in the specific product secretion rate were observed along the induction phase of the fed-batch fermentation processes, i.e. suggesting the presence of bottlenecks throughout the ROL synthesis and secretion process. In this context, the passage of secreted proteins through the yeast cell wall is a potential bottleneck in protein secretion. Gas1p, a glycoprotein anchored to the outer leaflet of the plasma membrane through a glycosylphosphatidylinositol, plays a key role in yeast cell wall assembly. We have recently shown that inactivation of *P. pastoris' GAS1* gene leads to a supersecretory phenotype yielding a considerable increase in secreted ROL protein production [2]. In addition, recent studies [3] strongly suggest that heterologous overexpression of ROL in *P. pastoris* provokes an important accumulation of misfolded protein in the endoplasmatic reticulum causing the activation of the unfolded protein response (UPR) [4]. Interestingly, the constitutive overexpression of the *S. cerevisiae* unfolded protein response transcription factor Hac1p in *P. pastoris* may enable an important increase of the secretion of some heterologous proteins in this host [5]. In this study, we have constructed a double mutant *HAC1/deltaGAS1 P. pastoris* strain expressing ROL under the PFLD1 promoter. Furthermore, this engineered strain has been compared in terms of secretion capacity to a set of different engineered ROL-producing *P. pastoris* strains expressing ROL, namely i) a control *P. pastoris* X-33 strain, ii) a *P. pastoris* strain GS115 co-expressing the induced form of the *S. cerevisiae*'s *HAC1* gene under the control of the constitutive PGAP promoter, the iii) a *P. pastoris* strain X33 with its *GAS1* gene knocked out, and iv) the double mutant *HAC1*/delta*GAS1* GS115 strain.

[1] Resina, D., Cos, O., Ferrer, P., Valero, F. (2005) *Biotechnol. Bioeng*. 91: 760–767.
[2] Marx, H., Sauer, M., Resina, D., Vai, M., Porro, D., Valero, F., Ferrer, P., Mattanovich, D. (2006). FEMS Microbiol Lett. 264:40–47.
[3] Resina, D. PhD Thesis, Universitat Autònoma de Barcelona, Catalonia, Spain
[4] Hohenblum, H., Gasser, B., Maurer, M., Borth, N., Mattanovich, D. (2004) *Biotechnol. Bioeng.* 85: 367–375.
[5] Gasser, B., Maurer, M., Gach, J., Kunert, R., Mattanovich, D. (2006) Biotechnol Bioeng. 94:353–61.

M. Mauer and D. Resina equally contributed to this work.
This work was supported by a grant from the Spanish Program on Chemical Processes Technologies (CTQ2004-00300).

ABSTRACTS OF POSTER SESSION VI

Metabolite production

Posters P96 – P120

Strain robustness in engineered yeasts

P. Branduardi[1], T. Fossati[1], R. Pagani[1], M. Papini[2] and D. Porro[1]

[1] Dipartimento di Biotecnologie e Bioscienze, Università degli Studi di Milano-Bicocca, Italy
[2] Technical University of Denmark, Center for Microbial Biotechnology, Denmark

The advent of rDNA technology together with the broader utilization of microorganisms as cell factories are making more and more real the implementation or even substitution of the chemical synthesis with biotransformations. Metabolic engineering, thanks to the targeted optimisation of cellular activities and functions, seems to be the more suitable approach to face this challenge. Notably, for the development of a successful process not only the microorganism has to be engineered to acquire the desired capabilities, but it has to be robust enough to counteract the severe chemical and physical constraints that very often the process itself implies.

Our laboratory has been involved since years in this field of research, exploiting yeasts as host systems. Recently, we reported the construction of a recombinant *S. cerevisiae* strain able to convert D-glucose into L-ascorbic acid, naturally not produced by yeasts. This result was obtained by implementing endogenous enzymatic activities with heterologous ones, by the (over)expression of the known plant genes of the pathway. Thanks to the improved endogenous antioxidant levels, the engineered strain becomes remarkably more tolerant to oxidative stress driven by H_2O_2, organic acids or low pH, conditions which severely inhibit the growth of the control strains. Remarkably, this features is not strain dependent, but can be reproduced in different backgrounds. Moreover, by microscopic and flow cytometric analyses performed on cells grown under oxidative stress condition, we could correlate the higher intracellular antioxidant levels of the engineered strains with a lower accumulation of intracellular ROS (Reactive Oxygen Species) and with an higher cell viability.

We strongly believe that this aspect of “strain robustness” is of crucial importance for host optimization and industrial strain improvement.

A novel strategy for glycerol overproduction in *Saccharomyces cerevisiae*

H. T. T. Nguyen and E. Nevoigt

Department of Microbiology and Genetics, Institute of Biotechnology, University of Technology Berlin, Germany

Glycerol is formed in yeast as a by-product during the fermentation of sugars to ethanol in a NADH-dependent manner. Glycerol overproduction by redirecting the glycolytic flux towards glycerol biosynthesis is limited to a maximum theoretical yield of 1 mol·mol^{-1} glucose (50 % of glucose carbon) as the NADH needed in the enzymatic conversion of glycolytic dihydroxyacetone phosphate to L-glycerol 3-phosphate is generated in the pyruvate/ethanol formation branch of glycolysis. With the aim to exceed this maximum yield, we embarked on a novel approach to engineer yeast's carbon catabolism: i) redirecting the carbon flux through the pentose phosphate pathway (PPP) by strongly reducing the activity of the phosphoglucose isomerase (PGI), thereby generating an excess of NADPH and ii) introducing a NADPH-dependent glycerol 3-phosphate dehydrogenase (GDH). Using this strategy, glycerol yield could reach, in theory, 83% of glucose consumed. Here we show that the *gpsA* gene from *Bacillus subtilis* encoding a NADPH-dependent GDH was successfully expressed in a *S. cerevisiae* mutant possessing 1% residual PGI activity. The presence of the *B. subtilis* GDH improved the growth of the *pgi1* deletion mutant from 22% to 48% of the wild-type level. The glycerol yield of the *pgi1* deletion mutant expressing *gpsA* in oxygen limited small-scale batch fermentation was about 35% higher than in the corresponding *pgi1* deletion mutant with the empty vector and 20% higher than the wild-type level. The future challenge is to improve the specific activity of the NADPH-dependent GDH and to force the metabolic flux towards glycerol production by additional engineering approaches.

Production of xylitol and other five-carbon sugars and sugar alcohols from D-glucose with *Saccharomyces cerevisiae*

M. Toivari[1], A. N. Miasnikov[2], P. Richard[1], L. Ruohonen[1] and M. Penttilä[1]

[1] VTT Technical Research Centre of Finland, Finland
[2] Danisco Global Innovation, Kantvik, Finland

We constructed recombinant Sa*ccharomyces cerevisiae* strains deficient in either transketolase or phosphoglucose isomerase activities for production of xylitol, ribitol or ribose from D-glucose via the pentose phosphate pathway (PPP) intermediates D-xylulose 5-phosphate, D-ribulose 5-phosphate or D-ribose 5-phosphate.

The transketolase-deficient strains accumulated PPP sugar-phosphates and excreted D-ribulose/D-ribose to the culture medium. Introduction of xylitol dehydrogenase encoding gene *XYL2* from *Pichia stipitis* increased ribitol and xylitol production and concomitantly decreased the amount of D-ribulose/D-ribose. A sugar phosphate phosphatase Dog1p increased ribitol production 1.6-fold, but did not enhance xylitol excretion. Deletion of the xylulokinase encoding gene *XKS1* increased the amount of xylitol produced to 50% of the total sugar alcohols (ribitol plus xylitol) produced. The highest yield of xylitol and ribitol obtained with the various transketolase deficient strains studied was 3.6% (w/w) of consumed D-glucose.

S. cerevisiae strains deficient in phosphoglucose isomerase activity are unable to grow on D-glucose unless a NADPH-consuming reaction is introduced into the cell (1, 2). We introduced two NADPH-consuming reactions, a NADPH-utilizing glyceraldehyde 3-phosphate dehydrogenase of *Bacillus subtilis* (GapB) and a NAD-dependent glutamate dehydrogenase (GDH2) of *S. cerevisiae* into the *pgi1*-mutant strain in order to produce 5-carbon sugars and sugar alcohols from D-glucose via the PPP. Both of these enzymes enabled growth on D-glucose of the *pgi1*-mutant strains, as shown for the *GDH2* overexpression previously (1). The Dog1p phosphatase was, however, needed for production of 5-carbon sugars and sugar alcohols. On higher D-glucose concentrations the expression of *DOG1* together with either *GapB* or *GDH2* reduced growth. The Dog1p may create a futile cycle of phosphorylation and dephosphorylation of glucose 6-phosphate resulting in ATP depletion.

1. Boles, E., W. Lehnert, and F. K. Zimmermann. 1993. The role of the NAD-dependent glutamate dehydrogenase in restoring growth on glucose of a *Saccharomyces cerevisiae* phosphoglucose isomerase mutant. Eur. J. Biochem. 217:469–477.
2. Fiaux, J., Z. P. Cakar, M. Sonderegger, K. Wuthrich, T. Szyperski, and U. Sauer. 2003. Metabolic-flux profiling of the yeasts *Saccharomyces cerevisiae* and *Pichia stipitis*. Eukaryot. Cell. 2:170–180.

Proteome and transcription analysis of recombinant xylose-utilising *Saccharomyces cerevisiae*

L. Salusjärvi[1], M. Kankainen[2], H. Koivistoinen[1], M. Penttilä[1] and L. Ruohonen[1]

[1] VTT Technical Research Centre of Finland, Finland
[2] Institute of Biotechnology, University of Helsinki, Finland

Economically feasible production of fuel ethanol from lignocellulosic material relies on quantitative conversion of the carbon present in the biomass that may contain 30–40% hemicellulose. Xylose is the most abundant pentose sugar in the hemicellulose and it is the second only to glucose in natural abundance. Hexose sugars are readily utilised by most industrial micro-organisms but efficient utilization of pentoses present in the hemicellulose fraction is still a challenge.

Xylose fermentation by *Saccharomyces cerevisiae* has been enabled by introducing the genes encoding xylose reductase (*XYL1*, XR) and xylitol dehydrogenase (*XYL2*, XDH) from the yeast *Pichia stipitis* naturally utilising xylose. Over-expression of the endogenous xylulokinase-encoding gene (*XKS1*) of *S. cerevisiae* further enhances xylose consumption (1). Introduction of the xylose-utilisation pathway into *S. cerevisiae* not naturally fermenting pentose sugars has a major impact on the overall cellular metabolism as the carbon introduced will now flow through the pentose phosphate pathway. In addition, the introduction of redox enzymes into *S. cerevisiae* affects the redox balance of the cell as xylose reductase has a preference for NADPH, while xylitol dehydrogenase is specific for NAD^+. This has been attributed to be one of the major reasons for inefficient incorporation of xylose-derived carbon into the central carbon pathways leading to ethanol by the oxido-reductive pathway.

Genome wide approaches offer an attractive and global strategy to study the overall cellular metabolism under different physiological conditions. We have studied both on proteomic and genomic level and under different culture conditions the recombinant *S. cerevisiae* to reveal novel changes in the metabolism of xylose fermenting yeast (2, 3, 4).

1. Toivari, M.H., Aristidou, A., Ruohonen, L., and Penttilä, M. (2001). Metab. Eng. 3: 236.
2. Salusjärvi, L., Poutanen, M., Pitkänen, J.-P., Koivistoinen, H., Aristidou, A., Kalkkinen, N., Ruohonen, L, and Penttilä, M. (2003). Yeast, 20: 295.
3. Salusjärvi, L., Pitkänen, J.-P., Aristidou, A., Ruohonen, L, and Penttilä, M. (2006). Appl. Biochem. Biotechnol., 128: 237.
4. Salusjärvi, L., Kankainen, M., Koivistoinen, H., Soliymani, R., Baumann, M., Penttilä, M., and Ruohonen, L. The transcriptome and proteome of recombinant *Saccharomyces cerevisiae*: Derepression and induction in the regulation of xylose metabolism (2007). Submitted.

Deleting the alkaline phosphatase, *PHO13,* improves growth of recombinant *Saccharomyces cerevisiae* CEN.PK on D-xylose

J. Headman Van Vleet[1,2], T. W. Jeffries[2,3] and L. Olsson[1]

[1] Center for Microbial Biotechnology, Technical University of Denmark, Denmark
[2] Department of Bacteriology, University of Wisconsin-Madison, USA
[3] Forest Products Laboratory, Forest Service, USDA, Madison, Wisconsin, USA

Saccharomyces cerevisiae transformed with the xylose reductase and xylitol dehydrogenase (*XYL1* and *XYL2*) genes from the native xylose fermenting yeast *Pichia stipitis* grows slowly on D-xylose. Native *S. cerevisiae* contains a gene for D-xylulokinase (*XKS1*), but its level of expression is too low to allow strong growth and fermentation on this sugar. Several researchers have over-expressed endogenous *XKS1* in an attempt to improve growth, and have reported some capacity of the resulting transformants to produce ethanol from xylose. Other researchers have reported variable results from this approach – including growth inhibition on xylose. Previous studies (1) showed that strong over expression of the *Pichia stipitis* gene (*PsXYL3*) inhibited growth on xylose while moderate expression enabled optimal growth. More recent studies (2) showed that spontaneous or induced mutations in *S. cerevisiae* could overcome the inhibition caused by strong overexpression of *ScXKS1* or *PsXYL3*. Two prominent mutational events were up-regulation of *TAL1* and inactivation of *PHO13*. Deletion of the *PHO13* gene alleviated this inhibition in *S. cerevisiae* strain L2612; however this auxotrophic strain shows very poor growth on defined minimal medium. More recently the *pho13* deletion was constructed in a *S. cerevisiae* CEN.PK background. During aerobic batch cultivations, the xylose uptake rate increased dramatically. Quantitative PCR studies performed by Ni et al. showed that transcript levels for *TAL1* were elevated in a *pho13* mutant. This combined with the overall rates of glucose and sugar utilization during growth in bioreactors suggests that the pentose phosphate pathway is activated in these cells. Physiological characterization of the effects of this deletion on glucose, xylose, and a sugar mixture has been carried out and indicate that this gene may be useful in the construction of better xylose-fermenting *S. cerevisiae* strains.

1. Jin, Y. S., H. Ni, J. M. Laplaza, and T. W. Jeffries. 2003. Optimal growth and ethanol production from xylose by recombinant *Saccharomyces cerevisiae* require moderate D-xylulokinase activity. Appl Environ Microbiol 69: 495–503.
2. Ni, H., J. M. Laplaza, and T. W. Jeffries. 2007. Transposon Mutagenesis To Improve the Growth of Recombinant Saccharomyces cerevisiae on D-Xylose. Appl Environ Microbiol 73: 2061–6.

Efficient production of L-lactic acid from xylose by metabolically engineered yeast *Pichia stipitis*

M. Ilmén[1], K. Koivuranta[1], L. Ruohonen[1], P. Suominen[2] and M. Penttilä[1]

[1] VTT Technical Research Centre of Finland, Finland
[2] NatureWorks LLC, Minnetonka, USA

Pichia stipitis, a yeast which ferments naturally xylose, was genetically engineered for L-(+)-lactate production. A *P. stipitis* strain expressing the L-lactate dehydrogenase (LDH) from *Lactobacillus helveticus* under the control of the *P. stipitis* fermentative *ADH1* promoter was constructed. Either xylose or glucose was used as the carbon source for lactate production under oxygen restricted conditions. Remarkably, the constructed *P. stipitis* strain produced a higher lactate concentration and yield on xylose than on glucose. Lactate accumulated as the main product on xylose-containing medium: 58 g/l lactate was produced from 100 g/l xylose. Relatively efficient lactate production was also observed on glucose medium, 41 g/l lactate was produced at a yield of 0.44 g/g glucose. Lactate was produced at the expense of ethanol production which was decreased to approximately 20% of the wild type levels on xylose-containing medium and to 75% on glucose-containing medium. Thus, LDH competed efficiently with the ethanol pathway for pyruvate, even though the pathway from pyruvate to ethanol was intact.

Process optimization and strategies to minimize the effects of lactic acid on ethanol production by *Saccharomyces cerevisiae*

K. F. Oliveira[1], C. S. Souza[1], M. V. Capela[2], and C. Laluce[2]

[1] Programa de pós-graduação Interunidades em Biotecnologia; Brazil
[2] Depto de Bioquímica e Tecnologia Química, Instituto de Química da Unesp, Brazil

The optimization of ethanol production in agitated batch cultures at 34°C was obtained using a synthetic medium (1) and response surface methodology (RSM) with a 2^3 full central composite design. The initial pH of the medium containing 0.0 to 1.0 g/L of added lactic acid varied from 2.3 to 2.7 and these pH values were not raised to 4.5 after inoculation. A thermotolerant strain of *Saccharomyces cerevisiae* was used to study the effects of the independent variables on viability retention and ethanol formation. The independent variables were: added lactic acid varying form 0.0 g/L to 1.0 g/L; sucrose from 100 g/L to 200 g/L; inoculum from 30 to 40 g/L in dry weight. Aiming at retaining maximal values of the viability and ethanol produced at the end of a fermentation cycle, the MINITAB Statistical Software (Release 14.0) gave the following optimized values for the independent variables: 0.6 g/L added lactic acid, 172 g/L initial sucrose concentration, and inoculum concentration of 35 g/L. Under such a condition the viability was 88.6% and the ethanol production was 80.7 g/L (ethanol productivity of 20.2 $g.L^{-1}.h^{-1}$).

Minimization of the negative effects of lactic acid was studied at 34°C in pulse fed-batch cultures. In the medium having high inoculum (40 g/L), a total of amount of 200 g/L sucrose was added to the synthetic medium in which the concentration of lactic acid varied from 0.0 g/L to 4.0 g/L while the medium pH was restored to 4.5 by adding NaOH after the addition of the first sucrose pulse as required. A sequence of pulses having decreasing volumes was used. Under such conditions, the yeast cells were able to tolerate high concentrations of added lactic acid as shown by the retention of 93.7% viability and the production of 95.6 g/L ethanol at the end of the fermentation cycle carried out the presence of 3.0 g/L added lactic acid. This work shows that the negative effects of lactic acid bacteria can be minimized by keeping the pH at values $\geq$ 4.5, high cell densities within the reactors, and high concentrations of the carbon source. Sponsored grants nos.2005/01489-6 and 2005/03681-2 from FAPESP, SP, Brazil.

(1): Thomas *et al.*, J. Ind. Microbiol. Biotechnol. 21: 247, 1998.

Optimization of ethanol production by *Saccharomyces cerevisiae* and minimization of ethanol effects on cell survival at increasing temperatures

C. S. Souza[1], K. F. Oliveira[1], M. V. Capela[2], and C. Laluce[2]

[1] Programa de pós-graduação Interunidades em Biotecnologia USP/ I.Butantan/ IPT, Brazil
[2] Instituto de Química da Unesp, Brazil

The effects of the independent variables on viability retention and ethanol formation were analyzed in the synthetic medium (1) *via* response surface methodology (RSM), using a 2^3 full central composite design. The independent variables were: temperatures varying from 30°C to 40°C; sucrose from 100 g/L to 200 g/L; and inoculum size from 30–40 g/L in dry weight. A thermotolerant strain of *Saccharomyces cerevisiae* was used. Sucrose was completely exhausted from the medium after a four-hour fermentation period in all cases. Aiming at retaining both a maximal value of the viability and a maximal level of ethanol produced at the end of the fermentation cycle, the MINITAB Statistical Software (Release 14.0) gave the following optimized values for the independent variables: 30°C, initial sucrose concentration of 200 g/L and inoculum concentration of 40g/L. Under such a condition, the maximum value of viability was 93.5% and the ethanol produced was 84.9 g/L (21.2 $g.L^{-1}.h^{-1}$).

The minimization of the effects of increasing temperatures on viability retention and ethanol production was studied in pulse fed-batch cultures carried out in mini-reactors. Pulses of decreasing volumes were added to the synthetic medium over the first two hours for a total fermentation period varying from 3h to 5 h. When required, the initial pH was restored to 4.5 by adding NaOH after the addition of the first sucrose pulse. The lethal effect of ethanol produced on cell survival was overcome by reducing the amount of sucrose added to the fermentation system when temperatures were above 34°C. At 37°C, a high retention of viability (90–100%) was obtained at the end of a fermentation cycle by adding to the mini-reactors a total sucrose equivalent to 150 g/L. At 40°C, the concentration of added sucrose had to be reduced to 100 g/L in order to maintain a high viability. In the Brazilian alcohol factories, a long term succession of batch cultures with cell reuse is usually carried out above 30°C. Sponsored by grants from Fapesp (proc. 2005/01498-6) and Capes.

(1): Thomas et al., J. Ind. Microbiol. Biotechnol. 21: 247, 1998.

Improved ethanol tolerance and ethanol production of a self-flocculating yeast strain SPSC01 by uniform design of multiple media compositions

X. Zhao, C. Xue and F. Bai

Department of Bioscience and Bioengineering, Dalian University of Technology, China

High ethanol tolerance of industrial yeast is important for good performance of ethanol fermentation, especially for Very High Gravity (VHG) ethanol fermentation process. However, despite extensive reports on media optimization of ethanol production, little studies have been reported on the effects of media compositions on ethanol tolerance. In this study, the impacts of six metal ions, including potassium, magnesium, calcium, zinc, ferrous and cobalt on both ethanol tolerance and ethanol yield of a self-flocculating yeast strain SPSC01 were investigated using a minimal defined medium by One-Way ANOVA analysis. Firstly, the concentration of K_2HPO_4, $(NH_4)_2SO_4$, and vitamin mixture of the minimal medium was optimized for high ethanol production and ethanol tolerance; secondly, the effects of the six metal ions were investigated in the optimized minimal medium. It was found that magnesium, calcium, and zinc had significant effects on both ethanol tolerance and ethanol yield, and ferrous and cobalt influenced ethanol yield although they showed no effect on ethanol tolerance, while manganese did not exhibit significant effect on neither ethanol production nor ethanol tolerance. Furthermore, applying uniform experiment design, a predictive mathematical model was established by linear step-wise regression analysis and the improved ethanol tolerance (90.2%±0.89 cell viability after 1 h of exposure to 18% (v/v) ethanol at 30℃) and ethanol production level of 47.15g/l (±0.85) from 100 g/l glucose were obtained using the optimized nutrient combination. These results identified zinc as an important mineral element for both ethanol tolerance and ethanol production in SPSC01, and also demonstrated the coordinated effects of various nutrient factors in the ethanol tolerance of SPSC01. The present study also substantiated the usefulness of uniform design as an effective method for the optimization of multiple media compositions for both yeast ethanol tolerance and ethanol production.

Co-factor balancing in engineered *Saccharomyces cerevisiae* for bioethanol production from lignocellulosic feedstock

J. R. M. Almeida, B. Hahn-Hägerdal, and M. F. Gorwa-Grauslund

Applied Microbiology, Lund University, Sweden

Baker's yeast *Saccharomyces cerevisiae* is a model organism for the study of eukaryotic organisms but it is also a potential cell factory for the production of various biotechnological compounds, such as bulk chemicals and pharmaceuticals. *S. cerevisiae* is notably considered as the organism of choice for the production of bioethanol from lignocellulosic feedstocks. Efficient ethanol production by *S. cerevisiae* requires generating yeast strains that (i) can efficiently ferment all sugars that are present in the lignocellulosic hydrolysate, i.e. both hexose and pentose sugars and (ii) can tolerate the inhibitors (furans, acids and phenolics) that are released during the hydrolysis steps.

We have previously generated *S. cerevisiae* strains that are capable of fermenting xylose to ethanol, by introducing the fungal xylose pathway consisting of NAD(P)H-dependent xylose reductase (XR) and NAD^+-dependent xylitol dehydrogenase (XDH). While the level of the by-product xylitol that originates from the difference in co-factor usage between XR and XDH, is high in defined mineral medium, it is low when using undetoxified lignocellulosic hydrolysate (Karhumaa *et al*., 2007, Microbial Cell Fact. 6:5; Öhgren *et al.*, 2006, J. Biotechnol. 126: 488–498). It is suspected that the concomitant reduction of the furans 5-hydroxymethylfurfural (HMF) and furfural by endogenous yeast reductases changes the intracellular cofactor balance in favor of ethanol formation.

ADH6p has previously been identified as a NADPH-dependent enzyme responsible for HMF conversion in *S. cerevisiae* (Petersson *et al.*, 2006, Yeast, 23: 455–464). In the present work, we have constructed xylose-fermenting *S. cerevisiae* strains in which the *ADH6* gene is overexpressed. The strains were physiologically characterized under anaerobic conditions to analyze HMF uptake, xylose consumption and product distribution. By-product formation is discussed in relation to intracellular cofactor levels.

P106

Differential malic acid degradation by indigenous and commercial *Saccharomyces cerevisiae* wine strains

L. Pereira, D. Schuller and M. Casal

Centro de Biologia, Departamento de Biologia, Universidade do Minho, Portugal

One thousand six hundred and twenty yeast isolates were obtained from spontaneous fermentations performed with grapes collected in three vineyards of the Vinho Verde Wine Region in northwest Portugal during three subsequent harvest seasons. All isolates were analyzed by mitochondrial DNA restriction fragment length polymorphism (mtDNA RFLP) and a pattern profile was verified for each isolate, resulting in a total of 294 different profiles, all revealed to belong to the species *Saccharomyces cerevisiae*. A remarkable heterogeneity of phenotypical traits was found when this collection of indigenous *S. cerevisiae* strains was screened regarding ethanol tolerance, H_2S production, capacity to utilize acetic and malic acid.

Malic acid, together with tartaric acid, are the most abundant organic acids in wine contributing to its acidic taste. Excess malic acid removal is of enological interest in this wine region. From the screening of 294 strains, only 3 (318, 319 and 320) showed enhanced malic acid consumption in combination with other desirable phenotypic traits. Their fermentative profiles in a synthetic must medium containing glucose (20%, w/v) and D,L-malic acid (0.6%, w/v) were very similar to the ones observed for the commercial strains QA23 and 71B, but considerable differences were found regarding the activity of key enzymes involved in the metabolism of malic acid (malic enzyme, malate dehydrogenase, fumarase). The best malic acid degrading strain was 71B (40% at the end of fermentation), but from an applied and enological point of view it is still desirable to improve this trait.

In order to enhance malic acid consumption, cells were transformed with genetic constructs containing both *Kluyveromyves lactis* dicarboxylate permease KlJEN2 and the MAE2 malic enzyme from *Schizosaccharomyces pombe* under constitutive expression. Data will be presented showing how the physiology of malic acid utilization is associated with the distinct yeast genetic backgrounds.

Financially supported by the programs POCI 2010 (FEDER/FCT, POCI/AGR/56102 /2004), and AGRO (ENOSAFE, Nº 762).

New molecular genetic tools for the non-conventional yeasts *Candida sonorensis* and *Candida methanosorbosa*

M. Ilmén[1], K. Koivuranta[1], P. Suominen[2], V. Rajgarhia[2,3], L. Ruohonen[1] and M. Penttilä[1]

[1] VTT Technical Research Centre of Finland, Finland
[2] NatureWorks LLC, Minnetonka, USA
[3] Mascoma, Lebanon, USA

New molecular genetic tools were developed for *Candida sonorensis* and *Candida methanosorbosa* enabling their genetic engineering for the first time. Transformation was based on antibiotic markers and, alternatively, a dominant non-antibiotic marker, the *MEL5* gene from *Saccharomyces cerevisiae*, enabling growth on melibiose as sole carbon source. Endogenous promoters were cloned from *C. sonorensis* and used to control the expression of the heterologous genes in both hosts. Introduction of the lactate dehydrogenase gene (*ldhL*) of *Lactobacillus helveticus* into *C. sonorensis* and *C. methanosorbosa* resulted in moderate (~10 g/l, ~20% yield) production of lactic acid with co-production of ethanol. Targeted gene deletions were accomplished in *C. sonorensis*. Two genes encoding pyruvate decarboxylase (PDC) were isolated. Strains deleted of *PDC1*, *PDC2*, or both *PDC* genes were constructed generating *C. sonorensis* strains producing reduced amounts or no ethanol. The *PDC2* encoded enzyme was identified as the major activity contributing to ethanol production. Integration of the *PDC1* and *PDC2* replacement constructs into both homologous and non-homologous sites occurred in *C. sonorensis*. This work clearly demonstrates the feasibility of genetically engineering novel, non-conventional yeast species, and illustrates the usefulness of the molecular tools for analysing gene function.

Adaptive evolution of a lactose-consuming *Saccharomyces cerevisiae* recombinant

P. M. R. Guimarães[1], J. François[2], J. L. Parrou[2], J. A. Teixeira[1] and L. Domingues[1]

[1] IBB – Institute for Biotechnology and Bioengineering, Centre for Biological Engineering, Universidade do Minho, Portugal
[2] Laboratoire d'Ingenierie des Sistèmes Biologiques et Procédés, France

The construction of *Saccharomyces cerevisiae* strains with the ability to efficiently ferment lactose has biotechnological interest, particularly for the alcoholic fermentation of cheese whey (a high pollutant by-product of dairy industries). A flocculent lactose-consuming *S. cerevisiae* recombinant expressing the *LAC12* (lactose permease) and *LAC4* (beta-galactosidase) genes of *Kluyveromyces lactis* was previously constructed, but presented poor efficiency in the fermentation of lactose. Thus, it was subjected to a long-term adaptation experiment (serial transfer/dilution in lactose media), which yielded an evolved recombinant strain with strongly improved lactose fermentation phenotype. The lactose (25 g/L) fermentation parameters of the evolved strain were similar to *K. lactis* wild-type strain CBS2359. The evolved recombinant showed increased beta-galactosidase activity (>20-fold) and also improved lactose uptake rates, compared to the original recombinant. We found a 1593 bp deletion in the intergenic region between *LAC4* and *LAC12* (which works as common promoter region for these genes) in the plasmid isolated from the evolved recombinant. The results strongly suggest that the intact promoter does not mediate activation of transcription in the original recombinant, whereas the deletion triggered transcriptional activation of both the *LAC* genes in the evolved strain. We also found evidence that plasmid copy number is lower (about 10-fold difference) in the evolved strain compared to the original recombinant. We suggest that tuning of the heterologous *LAC* genes expression in the evolved recombinant was accomplished by interplay between decreased copy number of both genes, as consequence of decreased plasmid copy number, and different levels of transcriptional induction for *LAC4* and *LAC12*, resulting from the changed promoter structure. This study illustrates the usefulness of simple evolutionary engineering approaches in the improvement of genetically engineered strains that display poor efficiency. The evolved strain obtained displays a stable lactose fermentation phenotype and constitutes an attractive alternative for the fermentation of lactose-based media. This strain was able to completely ferment 140 g/L lactose (mineral medium, batch) yielding 63 g/L of ethanol. It also fermented completely cheese whey powder solution containing 140 g/L of lactose, producing 55 g/L of ethanol.

Roles of the aminoacid transaminases in volatile sulfur compounds production in the yeast *Yarrowia lipolytica*

S. Landaud, A. Hebert, D. Cernat-Bondar, N. Merault, P. Bonnarme.
and J.M. Beckerich

Agroparistech, UMR INRA Génie et Microbiologie des Procédés Alimentaires, UMR INRA-CNRS Microbiologie et Génétique Moléculaire, France

Cheese flavour is the result of complex biochemical transformations attributed to bacteria and yeasts grown in cheese curd. Volatile sulphur compounds (VSCs) are responsible for the characteristic aromatic notes of several cheeses, e;g;, smear-ripened cheeses. The enzymatic degradation of L-methionine and subsequent formation of volatile sulfur compounds (VSCs) is essential for the development of this typical flavor. *Yarrowia lipolytica* is one of the yeasts most frequently isolated from the surface of ripened cheeses. A survey of the annotated genome of this yeast allowed building of the metabolic pathways for sulphur aminoacids. The first step of the methionine catabolism is a transamination. There is no specific methionine transaminase. The roles of aminotransferases for aromatic aminoacids and for branched chain aminoacids in the biogenesis of VSCs were investigated. There are two branched chain aminoacid aminotransferases BAT1 and BAT2, two aromatic aminoacids aminotransferases, ARO8 and ARO9 with different intracellular locations. The contribution of each was estimated by overproducing these enzymes under the control of a constitutive strong promoter. These construction were integrated in a strain displaying a weak production of VSCs. Under these conditions, we have shown that BAT1 overproduction increased the yield of these compounds but not ARO8 overproduction. These results will be discussed.

Yeasts of environmental origin as biocatalysts for the asymmetric reduction of electron-poor alkenes

M. Goretti[1], C. Gasparetti[1], C. Ponzoni[2], M. R. Cramarossa[2], P. Buzzini[1], B. Turchetti[1] and L. Forti[2]

[1] Dipartimento di Biologia Vegetale e Biotecnologie Agroambientali, University of Perugia, Italy
[2] Dipartimento di Chimica, University of Modena and Reggio Emilia, Italy

Biotransformation processes using GRAS microbial cells as biocatalysts for the production of fine chemicals gained in recent years an increasing interest as emerging environmentally-soft technologies allowing the production of regio- and stereo-selective compounds. One of the most interesting area for exploration in biocatalysis is the asymmetric reduction of electron-poor alkenes using whole-cell containing enoate reductase.

In agreement with a recent overview underlining the biotechnological potential of yeast biodiversity (1), this study refers of a large-scale survey over the aptitude of yeasts of environmental origin to express enoate reductase activity. Furthermore, although the bioconversion of monoterpenes has been widely studied by using prokaryotic and eukaryotic cells, only a little attention has been so far devoted on the ability of yeasts to convert cyclic ketonic monoterpenes (2). Accordingly, a large-scale screening on the enoate reductase activity of 146 strains of the genera *Candida, Cryptococcus, Debaryomyces, Hanseniospora, Kluyveromyces, Pichia, Saccharomyces* and *Zygosaccharomyces* (deposited on the Industrial Yeast Collection DBVPG, Italy) has been performed by using (*4S*)-(+)-carvone as a model substrate. Yeasts were used under different physiological conditions: growing cells, resting cells, and lyophilized cells. The results revealed that enoate reductase activity is a broad distributed character among such yeasts. A few strains exhibiting good conversions and chemoselectivity either at the C=C- or the C=O-bond level of the model substrate has been observed.

[1] Buzzini, P., Vaughan-Martini, A. (2006) Yeast biodiversity and biotechnology. In: *Biodiversity and Ecophysiology of Yeasts*. (Rosa C & Peter G eds.), Springer, Berlin, pp. 533–559.
[2] de Carvalho, C.C.C.R, da Fonseca, M.M.R (2006) Biotransformation of terpenes. *Biotechnol. Adv.* 24: 132–142.

Carotenoid pigment production at *Rhodotorula* yeasts

P. Patáková, T. Krulikovská and M. Lukčo

Institute of Chemical Technology Prague, Czech Republic

Two species of *Rhodotorula* yeasts: *R. glutinis* and *R. mucilaginosa* were selected as model microorganisms for comparison of image analysis results with parameters determined by usual analyses. The yeasts were cultivated in shaken flasks in cultivation media containing different carbon and nitrogen sources. At the work, a method for carotenoid pigments isolation and determination was optimalized. Carotenoid pigments were extracted from DMSO treated cells by petrolether and the amount of pigments was determined spectrophotometrically and by HPLC analysis. At the same time samples of cultivation media after cultivation end were poured on Petri dishes and photographed at constant conditions in dark room. The colour characteristics of the photographs (wavelength and colour deviation defined by L, a, b parameters) were evaluated by LUCIA image analysis software (Laboratory Imaging Ltd., Czech Republic). Results obtained by classic determinations were compared to image analyses and the comparison was discussed.

Glutamate dehydrogenase in *Penicillium chrysogenum* is involved in regulation of beta-lactam production

J. Thykaer[1], K. Rueksomtawin[1] H. Noorman[2] and J. Nielsen[1]

[1] Center for Microbial Biotechnology, Technical University of Denmark, Denmark
[2] DSM- Anti-Infectives, Industrial Pharmaceutical Products Division, The Netherlands

Metabolic engineering has proven to be a rational alternative for improving betalactam producing strains. Several research groups have been working specifically on optimisation of the betalactam production of *Penicillium chrysogenum* e.g. through gene dosage studies. It is however, of equal importance to gain insight into other aspects of the metabolism to establish a general overview in order to apply metabolic engineering for further improvement of the production strains. The redox metabolism is essential when characterising the metabolism of biological cells. It functions as a tightly controlled connection between the different parts of the central metabolism and provides an important link between the primary and the secondary metabolism. In this work, the interactions between the redox metabolism, the central metabolism and the secondary metabolism were investigated. Especially the role of NADPH-dependent glutamate, in this perspective, was examined. This was pursued by disruption of the *gdhA*-gene, encoding the NADPH-dependent glutamate dehydrogenase, in two different industrial strains of *P. chrysogenum.*

Detailed physiological characterisation was performed focussing on cellular performance i.e. growth, yield and productivity. The disruption of *gdhA* resulted in significant decreases in the maximum specific growth rates of around 30% for both the strains. Furthermore, disruption of *gdhA* had a severe effect on the production as no production of betalactam was measured in any of the cultures carried out with the constructed strains. Consequently, deletion of the NADPH-dependent glutamate dehydrogenase resulted in significantly lower maximum specific growth rates and complete elimination of beta-lactam production. However, the specific growth rate of the references strains could be restored in the transformant strains by addition of glutamate, but despite the glutamate addition, the mutant strains remained non-productive. From the results of this work it is argued that besides being the key enzyme in ammonium assimilation, the NADPH-dependent glutamate dehydrogenase appears to be involved in regulation of betalactam production in industrial strains of *P. chrysogenum*.

In vivo transport of the intermediates of the penicillin biosynthetic pathway in tailored strains of *Penicillium chrysogenum*

C. García-Estrada[1], I. Vaca[1], M. Lamas-Maceiras[2] and J. F. Martín[1,2]

[1] Instituto de Biotecnología (INBIOTEC), León, Spain
[2] Área de Microbiología, Departamento de Biología Molecular, Facultad de CC. Biológicas y Ambientales, Universidad de León, Spain

Penicillium chrysogenum npe10 (*deltapen*; lacking the 56.8-kbp amplified region containing the penicillin gene cluster), complemented with one, two or the three penicillin biosynthetic genes, was used for *in vivo* studies on transport of benzylpenicillin intermediates. 6-APA (6-aminopenicillanic acid) was taken up efficiently by *P. chrysogenum* npe10 unlike exogenous ACV (delta[L-alpha-aminoadipyl]-L-cysteinyl-D-valine) or IPN (isopenicillin N), which were not taken up or very poorly. Internalisation of exogenous IPN and 6-APA inside peroxisomes was tested by quantifying their peroximal conversion into benzylpenicillin in strains containing only the *penDE* gene. Exogenous 6-APA was transformed efficiently into benzylpenicillin, whereas IPN was converted very poorly into benzylpenicillin due to its weak uptake. IPN was secreted to the culture medium. IPN secretion decreased when increasing levels of phenylacetic acid were added to the culture medium. The *P. chrysogenum* membrane permeability to exogenous benzylpenicillin was tested in the npe10 strain. Penicillin is absorbed by the cells by an unknown mechanism, but its intracellular concentration is kept low.

The unprocessed preprotein form IAT^{c103s} of the isopenicillin n acyltransferase is transported inside peroxisomes and regulates the self-processing

C. García-Estrada[1], I. Vaca[1], F. Fierro[2], K. Sjollema[3], M. Veenhui[3] and J. F. Martín[1,2]

[1] Instituto de Biotecnología (INBIOTEC), León, Spain
[2] Área de Microbiología, Departamento de Biología Molecular, Facultad de CC. Biológicas y Ambientales, Universidad de León, Spain
[3] Laboratory of Eukaryotic Microbiology, Groningen Biomolecular Sciences and Biotechnology Institute, University of Groningen, The Netherlands

The isopenicillin N acyltransferase (IAT) of *Penicillium chrysogenum*, that catalyzes the last step of penicillin biosynthesis, is formed as an inactive 40-kDa proIAT that is activated by self-processing forming the alpha-beta heterodimer (11 + 29 kDa). Previous comparative studies in *P. chrysogenum* and *Aspergillus nidulans* suggested that the efficiency of self-processing is an important differential factor in these two fungi, being very efficient and fast in *P. chrysogenum* but slow in *A. nidulans*. A variant IAT unable to be self-processed (IAT^{C103S}) was expressed from the strong *gdh* promoter in *P. chrysogenum*. The unprocessed 40-kDa IAT^{C103S}, like the wild-type processed protein, was detected by immunoelectron microscopy inside peroxisomes (microbodies), indicating that transport of the proIAT inside these organelles is not dependent on the processing state of the protein. The IAT^{C103S} lacked the isopenicillin N acyltransferase (benzylpenicillin-forming) activity. *P. chrysogenum* transformants containing both the endogenous (processed) wild-type IAT and the unprocessed IAT^{C103S} (Wis54-*DE*^{C103S} strain) produced lower benzylpenicillin levels than the Wis54-1255 control strain, but i*n vitro* assays indicated that this decrease was not due to a reduction of the IAT activity. Changes in the wild-type IAT processing profile (beta subunit formation) were observed in the Wis54-*DE*^{C103S} strain when compared with the Wis54-1255 parental strain, suggesting a putative regulatory role of the unprocessed IAT^{C103S} on the processing of the wild-type IAT. This delay in the processing of the IAT was confirmed in *E. coli*, where these two proteins were efficiently co-expressed. In summary, our results indicate that IAT is post-translationally regulated by its preprotein, which interferes with the self-processing.

Introduction of the penicillium biosynthesis pathway in the methylotrophic yeast *Hansenula polymorpha*

L. Gidijala, J. A. K. W. Kiel, I. J. van der Klei and M. Veenhuis

Eukaryotic Microbiology, Groningen Biomolecular Sciences and Biotechnology Institute (GBB), University of Groningen, The Netherlands

The need for novel semi-synthetic β-lactam antibiotics is increasing due to the growing resistance of micro-organisms towards known antibiotics. Because of the high cost of chemical production, industry currently focuses its research to produce these new compounds using white biotechnology. The penicillin biosynthetic pathway in the filamentous fungus *Penicillium chrysogenum* is well characterized both genetically and biochemically. In *P. chrysogenum*, this pathway is compartmentalized and the last steps in penicillin biosynthesis take place in microbodies.

We aim to introduce the penicillin biosynthesis pathway in the methylotrophic yeast *Hansenula polymorpha*. Yeast species have the advantage of being versatile and easy to handle and cultivate. *H. polymorpha* has the additional advantage that the number, protein content and volume fraction of microbodies are readily manipulated, allowing better control over the microbody located enzymes and their function. Previously, we have constructed strains each producing one of the penicillin biosynthetic pathway enzymes δ-(L-α-aminoadipyl)-L-cysteinyl-D-valine synthase (ACVS), isopenicillin N synthase (IPNS) and isopenicillin N:acyl CoA acyltransferase (IAT).To allow simultaneous expression of the multiple genes of the penicillin biosynthetic pathway, additional markers were required. To this end, we have constructed a novel host/vector system based on methionine auxotrophy and the *H. polymorpha MET6* gene that encodes a putative cystathionine β-lyase. Using this new host/vector system, the *P. chrysogenum pcl* gene, encoding peroxisomal phenylacetyl CoA ligase (PCL), was expressed in *H. polymorpha*. PCL has a potential C-terminal peroxisomal targeting signal type 1 (PTS1, SKI-COOH). Our data demonstrate that a GFP.PCL fusion protein has a dual location in the heterologous host in the cytosol and in peroxisomes. Mutation of the PTS1 of PCL to SKL-COOH restored sorting of the fusion protein to solely peroxisomes. Additionally, we demonstrate that peroxisomal PCL.SKL produced in *H. polymorpha* displays normal enzyme activities.

This project is financially supported by the Netherlands Ministry of Economic Affairs and the B-Basic partner organizations (www.b-basic.nl) through B-Basic, a public-private NWO-ACTS programme (ACTS = Advanced Chemical Technologies for Sustainability).

1. R. P. Elander, *Appl. Microbiol. Biotechnol*. 61, 385–392. 2003.
2. Lutz, M.V., Bovenberg, R.A.L., van der Klei, I.J., Veenhuis. M., *FEMS Yeast Res*. 5, 1063–1067. 2005.

Antibacterial activity of K9 yeast killer toxin

I. Ochigava[1] and G. M. Walker[2]

[1] LtD Biopharm-L. Tbilisi, Georgia
[2] University of Abertay Dundee, Scotland, UK

The increasing incidence of antibiotic-resistant bacterial infections has made identification and characterization of new antibacterial therapies major goals of the pharmaceutical industrial sector. Our attention was drawn to the killer toxin of *Williopsis saturnus var. mrakii* K 9 (*W. mrakii*) because of its high theromstability (100^0C, for 10 min) and activity retention at wide pH values (pH 2~11). This work describes the antibacterial spectrum of crude K9 killer toxin (KT) preparation against a range of Gram positive as Gram negative bacteria.

The killer yeast, *Williopsis saturnus var. mrakii* - NCYC 500, was tested against the following bacterial cultures: *E.coli* – NCIMB 10000; *Staphylococcus epidermidis* – NCIMB 12721; *Streptomyces griseus*, *Streptococcus pyogenes* – NCIMB 8884; *Bacillus subtilis* – NCIMB 1043, NCIMB 6633. Agar diffusion assays were employed for killer toxin antibacterial activity using *W. mrakii* live cells. For more detailed assessment of antibacterial activity, we used a flow cytometric assay of crude K9 toxin preparation using a fluorescent viability probe, DiSBAC2(3). Both approaches confirmed *W. mrakii* and its K9 toxin exhibited antibacterial activity against Gram positive bacteria. Although the flow cytometric assay revealed high numbers of dead cells after the treatment with killer toxin, this activity was sensitive-strain dependent, particularly in the case of *Bacillus* spp.

These results provide one of very few reports of effective killer yeast action against bacteria and form the basis of further development of stable yeast toxins as novel agents in the fight against drug resistant infections.

Studies on the *Aspergillus flavus* aflatoxins production and the predictive model of the aflatoxinogenesis

R.C. Toma[1], M. Cirlan[1], M. Avram[2], A. Gageanu[1] and F. Radoi-Matei[1]

[1] USAMV Bucharest, Faculty of Biotechnology, Romania
[2] Institute of Food Bioresources Bucharest, Romania

Three *Aspergillus flavus* strains isolated from food and feed products have been studied in order to obtain a predictive model of the aflatoxins production depending on environmental factors. The primary model is based on the rate of aflatoxins production on a synthetic or natural medium. Initially, it has been studied the rate of appearance of aflatoxins on plates, on synthetic media (PDA + cyclodextrine) by halo formation. The data obtained through "on plate" method are not mathematically reproductible employing usual equation, such the Rosso one. New equations should be designed.

After cultivation of the moistures on natural media (wheat) and by using semi-quantitative "ELISA" immunological tests for aflatoxins detection, the obtained data allowed to obtain a primary model of the secondary toxinogen metabolite production depending on the temperature (from 4^oC to 36^oC, passing through 10 different levels of the parameter). Experimental results have been processed both with the help of statistical functions and the "SlideWrite" software application.

A correlation between the moulds contamination level and the mycotoxin content in stocked corn harvest

I. Vatuiu[1], A. Stanciu[2], M. Avram[3] and F. Radoi-Matei[2]

[1] CCAI Bucharest, Laboratory of Microbiology, Romania
[2] USAMV Bucharest, Faculty of Biotechnology, Romania
[3] Institute of Food Bioresources Bucharest, Romania

The grains in general, corn especially, are one of the most important culture in Romania. Last decade main part of the harvest has been lost during the storage because of the moulds contamination. Moreover, depending on the storage conditions mycotoxins production occurred. Three years harvests, from 2004 to 2006, have been supervised in Iasi region during 5 months keeping in warehouses.

Non-contaminated to 100 % contaminated samples with different spoiling fungi and bacteria have been analyzed from a microbiological point of view. By semi-quantitative immunological tests, the total content of mycotoxins has been measured. A correlation between the degree of the contamination and the content of the mycotoxins has been established.

The highest level of the total mycotoxins after 5 months of warehouse keeping was 0.83 ppb, measured on a 90% moulds contamination sample. This value it is not significant for the animal or human health, as long as the legal accepted level for the corn is 10 ppb (µg/kg).

The incidence of toxinogenic fungi in Romanian food products of intermediary humidity

F. Radoi-Matei[1], I. Vatuiu[2], A. Cozea[3], S. Cristea[1], A. Gageanu[1] and S. Jurcoane[3]

[1] USAMV Bucharest, Faculty of Biotechnology, Romania
[2] CCAI Bucharest, Laboratory of Microbiology, Romania
[3] Center of Microbial Biotechnology, Bucharest, Romania

Belonging to a wider national project regarding the prevention of the mycotoxin contamination in feed and food, the aim of this study it was to identify the main species involved in the alteration of food products of intermediary activity. A high number of intermediary humidity feed and food samples (flours, dehydrated vegetables, flakes, spices, premixes, peanuts, food ameliorators, etc) have been analyzed for their microbial content. A special attention was paid to the phylamentous fungi with toxinogenic activity.

Finally, 54 moulds strains have been insolated, belonging to the following genders: *Aspergillus (A. flavus, A. niger, A. candidus), Alternaria (A. solani.), Fusarium (F. graminearum), Penicillium, Rhizopus, Cladosporium, Macrosporium.* The strains have been screened for their toxinogenic activity (aflatoxins, ochratoxin and deoxynivalenol production) by on-plate method and the toxins were quantified by Elisa Immunologic tests. 38 of those moulds showed toxinogenic activity under laboratory tests.

Further, new studies on the growth and their toxinogensis are envisaged, identifying the genes involved into the secondary metabolite formation, and the expression of these genes will be described by molecular techniques (Q-RT-PCR).

P120

Structure analysis of computer generated homologous models of pm3 protein in *Triticum aestivum* (Wheat)

D. B. M. Virupakshaiah, S. H. Ameresh and B. M. Sphoorthi

Depart of Biotechnology, Basaveshwar Enineering College, Karanataka, India

Most of the plant protein structures have not been structurally characterized.(either by NMR or X-ray crystallography). In our research we made an effort to generate computer built model for powdery mildew resistant protein(f allele) found in triticum aestivum (bread wheat) cultivar, michigan amber and also find homologous models to this protein. This protein is also called as pm3f protein and belongs to a group of pathogenesis related (pr) proteins. Structural analysis of this protein and its homologous models can help in studying their interaction with the target macro molecules in the attacking fungi with advent of artificial gene synthesis technique, the information retrieved from such a structural analysis can help in cloning and gene transfer experiments in those wheat cultivars which lack the resistance to powdery mildew.

Participants

ABRASHEV Radoslav
Bulgarian Academy of Science Institute of Microbiology, Bulgarian
rabrashev@microbio.bas.bg

ACAR Seyda — P68
Middle East Technical University, Turkey
seydaacar@gmail.com

AHTINEN Jouni — P58
Medicel Oy, Finland
jouni.ahtinen@medicel.com

AHUJA Manmeet — P40
Indian Institute of Technology, India
manmeeta@gmail.com

ALAMÄE Tiina — P53
Institute of Molecular and Cell Biology, Estonia
talamae@ebc.ee

ALAPURANEN Marika
Roal Oy, Finland
marika.alapuranen@roal.fi

AL-FALIH Abdullah — P43
Teachers College, Saudi Arabia
alfalih@hotmail.com

ALKIM Ceren — T38, P27
Istanbul Technical University, Turkey
alkim@itu.edu.tr

ALMERING Marinka
Technical University Delft, The Netherlands
m.j.h.almering@TUDelft.nl

ANDERSEN Mikael Rørdam — T4, P2
Center for Microbial Biotechnology – DTU, Denmark
mr@biocentrum.dtu.dk

ARCHER David — P12, P94
University of Nottingham, United Kingdom
david.archer@nottingham.ac.uk

ARO Nina — P93
VTT Technical Research Centre of Finland
nina.aro@vtt.fi

ARVAS Mikko — T3
VTT, Technical Research Centre of Finland
mikko.arvas@vtt.fi

BAKKER Barbara T18, P56
Vrije Universiteit, Dept. Molecular Cell Physiology,
The Netherlands
barbara.bakker@falw.vu.nl

BALOGH Gabor
TEVA Pharmaceutical Works Ltd, Hungary
Gabor.Balogh@teva.hu

BASTOS LOURENÇO Artur
Institute for Biotechnology and Bioengineering, Portugal
arturbl@ist.utl.pt

BAUMANN Kristin P95
Universitat Autònoma de Barcelona,
Departament d'Enginyeria Química (ETSE), Spain
kristin.baumann@uab.cat

BECKERICH Jean-Marie T32, P109
INRA, France
jean-marie.beckerich@grignon.inra.fr

BILLS Gerald P39
Merck Sharp & Dohme de Espaňa, Spain
gerald_bills@merck.com

BJØRN Sara
Fluxome Sciences, Denmark
sb@fluxome.com

BOLES Eckhard T17
Institute of Molecular Biosciences, Goethe-University,
Germany
e.boles@bio.uni-frankfurt.de

BONINI Beatriz M. T14, P51
VIB Department of Molecular Microbiology,
K.U. Leuven, Belgium
beatriz.bonini@bio.kuleuven.be

BOONE Charlie T2
University of Toronto, Canada
charlie.boone@utoronto.ca

BRAAKSMA Machtelt P59
TNO Quality of Life, The Netherlands
machtelt.braaksma@tno.nl

BRAMBILLA Luca P17, P24
Università di Milano-Bicocca, Italy
luca.brambilla@unimib.it

BROCK Matthias P70, P72
Leibniz Institute for Natural Product Research
and Infection Biology (HKI), Germany
matthias.brock@hki-jena.de

BURGSTALLER Wolfgang T26, P50, P75
Institute of Microbiology, Austria
wolfgang.burgstaller@uibk.ac.at

BUYUKKILECI Ali Oguz P48
Middle East Technical University, Turkey
buyukkileci@gmail.com

BUZZINI Pietro P110
University of Perugia, Italy
pbuzzini@unipg.it

CADIERE Axelle P57
INRA, France
cadiere@supagro.inra.fr

CAKAR Zeynep Petek T38, P27
Istanbul Technical University, Turkey
cakarp@itu.edu.tr

CANELAS André P52, P61, P66
T.U. Delft, The Netherlands
A.Canelas@tudelft.nl

CASAL Margarida T21, P81, P106
Universidade do Minho, Dep. Biologia, Portugal
mcasal@bio.uminho.pt

CHOOLUCK Sunthorn P28
School of Biological Sciences, The University
of Liverpool, United Kingdom
s.chooluck@liverpool.ac.uk

COZEA Andreea P84, P119
Microbial Biotehnological Centre, Bucharest, Romania
andreea.Cozea@yahoo.com

DANKMEYER Lydia
Genencor, a Danisco Division, The Netherlands
lydia.dankmeyer@danisco.com

DARAN Jean Marc
Technical University Delft, The Netherlands
J.G.Daran@TUDelft.nl

DARAN-LAPUJADE Pascale T24, P61
Technical University Delft, The Netherlands
P.A.S.Lapujade@TUDelft.nl

DATO Laura T11, P25
Università degli Studi di Milano, Italy
laura.dato@unimib.it

DAUM Guenther T22
Institute of Biochemistry, Graz University of Technology,
Austria
guenther.daum@tugraz.at

DE BOER Paulo P8
Add2X Biosciences BV, The Netherlands
deboer@add2x.com

DE HULSTER Erik T24
Technical University Delft, The Netherlands
a.f.dehulster@TUDelft.nl

DE VRIES Ronald T15
Microbiology, Utrecht University, The Netherlands
r.p.devries@bio.uu.nl

DE WINDE Han P61
Kluyver Centre Delft, The Netherlands
j.h.dewinde@tudelft.nl

DEGRÉ Richard
Lallemand Inc, Canada
rdegre@lallemand.com

DEKKER Peter T30
DSM Food-Specialties, The Netherlands
peter.dekker@dsm.com

DIAS Paulo P26
IBB-Institute for Biotechnology and Bioengineering,
Portugal
pjdias@hotmail.com

DIDERICH Jasper
Heineken Supply Chain, The Netherlands
jasper.diderich@heineken.com

DIGUTA Camelia
University of Agronomical Sciences and Veterinary Medicine,
Romania
cameliadiguta@yahoo.com

DOMINGUES Lucília P79, P81, P108
Institute of Biotechnology and Bioengineering,
Portugal
luciliad@deb.uminho.pt

DURUKSU Gokhan P82
The Middle East Technical University, Turkey
e109870@metu.edu.tr

EICHHORN Heiko
SANDOZ GmbH, Germany
heiko.eichhorn@sandoz.com

FACCIO Greta
VTT Technical Research Centre of Finland
greta.faccio@vtt.fi

FERRER Pau P95
Universitat Autònoma de Barcelona, Spain
pau.ferrer@uab.cat

FLORES Carmen-Lisset T19
Instituto de Investigaciones Biomédicas, CSIC-UAM, Spain
clflores@iib.uam.es

FRANÇOIS Jean Marie T5, T25, T38,
Laboratoire d'Ingenierie des Systèmes Biologiques P11, P54, P108
et Procédés UMR –CNRS, France
fran_jm@insa-toulouse.fr

FREIMOSER Florian T16, P46
ETH Zurich, Switzerland
ffreimoser@ethz.ch

GANCEDO Carlos T19
Instituto de Investigaciones Biomédicas CSIC-UAM, Spain
cgancedo@iib.uam.es

GANCEDO Juana M. P47
Instituto de Investigaciones Biomédicas CSIC-UAM, Spain
jmgancedo@iib.uam.es

GASSER Brigitte T31
IAM BOKU, Institute for Applied Microbiology,
University of Natural Resources and Applied Life Sciences, Austria
brigitte.gasser@boku.ac.at

GEERTMAN Jan-Maarten
Iogen Corporation, Canada
jan-maartenG@iogen.ca

GHILLEBERT Ruben T20
Katholike Universiteit Leuven, Belgium
Ruben.Ghillebert@student.kuleuven.be

GIDIJALA Loknath P115
University of Groningen, The Netherlands
l.gidijala@Rug.nl

GJERMANSEN Claes
Carlsber Research Laboratory, Denmark
clgj@crc.dk

GOMBERT Andreas
Technical University Delft, The Netherlands
a.gombert@tudelft.nl

GORETTI Marta P110
University of Perugia, Italy
martago22@hotmail.com

GORWA-GRAUSLUND Marie F. P105
Applied Microbiology, Lund University, Sweden
marie-francoise.gorwa@tmb.lth.se

GUAIS Olivier T5
LISBP, INSA, France
olivier.guais@insa-toulouse.fr

GUDYNAITE-SAVITCH Loreta
Iogen Corporation, USA
loretag@iogen.ca

GUIMARAES Pedro
University of Minho - Centre of Biological Engineering, Portugal
prguimaraes@deb.uminho.pt P108

GURKOK Sumeyra
The Middle East Technical University, Turkey
sumeyra@metu.edu.tr P83

HAESENDONCKX Steven
K.U.Leuven, Belgium
steven.haesendonckx@bio.kuleuven.be T14

HANSEN Kim
Novozymes A/S, Denmark
kimh@novozymes.com P5

HATICE OZLEM Ercin
Graduate School of Natural and Applied Sciences,
Middle East Technical University, Turkey
ozbuyucu@yahoo.com

HE Susu
LBB/INSA-FRANCE, France
susu.he@insa-toulouse.fr P54

HEADMAN VAN FLEET Jennifer
Center for Microbial Biotechnology, Biocentrum-DTU, Denmark
jvv@biocentrum.dtu.dk P100

HELLMUTH Karsten
Chr.Hansen GmbH, Germany
karsten.hellmuth@de.Chr-Hansen.com

HIGUCHI Yujiro
Department of Biotechnology, University of Tokyo, Japan
aa56122@mail.ecc.u-tokyo.ac.jp T12

HILDITCH Satu
VTT Technical Research Centre of Finland
satu.hilditch@vtt.fi P69

HUUSKONEN Anne
VTT Technical Research Centre of Finland
anne.huuskonen@vtt.fi P49, P60, P62, P63

ILMÉN Marja
Technical Research Centre of Finland
marja.ilmen@vtt.fi T37, P101, P107

JOUHTEN Paula
VTT Technical Research Centre of Finland
paula.jouhten@vtt.fi P60, P62, P63

JOYET Philippe T32
Lab. de Microbiologie et Génétique Moléculaire
CBAI, France
philippe.joyet@grignon.inra.fr

JUNTUNEN Kari
Roal Oy, Finland
kari.juntunen@roal.fi

JURCOANE, Stefana P16, P84, P119
Microbial Biotechnological Center, Romania
jstefana@yahoo.com

KALLIO Jarno P89, P90
Roal Oy, Finland
jarno.kallio@roal.fi

KARA Burcu
Graduate School of Natural and Applied Sciences,
Ege University , Turkey
burcukara@hotmail.com

KEMPPAINEN Minna P9
Universidad Nacional de Quilmes, Argentina
mkemppainen@unq.edu.ar

KILIAN Stephanus
University of the Free State, South Africa P41
kiliansg.sci@mail.uovs.ac.za

KITAMOTO Katsuhiko T12
Department of Biotechnology, The University of Tokyo, Japan
akitamo@mail.ecc.u-tokyo.ac.jp

KITANOVIC Ana T13, T19, P29
Institute for Pharmacy and Molecular Biotechnology,
Ruperto-Carola University of Heidelberg, Germany
ana.kitanovic@urz.uni-heidelberg.de

KLAASSEN Paul
DSM Gist, The Netherlands
paul.klaassen@dsm.com

KOIVURANTA Kari T37, P101, P107
VTT Technical Research Centre of Finland
kari.koivuranta@vtt.fi

KREPS Joel
Gene Expression Department, Diversa Corp, USA
jkreps@Diversa.com

KRUCKEBERG Arthur
Amyris Biotechnologies, USA
kruckeberg@amyrisbiotech.com

KRULIKOVSKA Tereza P32, P44, P111
Institute of Chemical Technology Prague, Czech Republic
tereza.krulikovska@vscht.cz

LALUCE Cecilia
Instituto de Química da UNESP, Brasil
claluce@iq.unesp.br P102, P103

LAPLAZA Jose
Cargill, USA
jose_laplaza@cargill.com

LEE Heather
University of Nottingham, United Kingdom
heather.lee@nottingham.ac.uk P12

LEGISA Matic
National Institute of Chemistry, Slovenia
matic.legisa@ki.si P67, P71

LI Qiang
University of Strathclyde, United Kingdom
qiang.li@strath.ac.uk T27, P91

LIN Janine
Novozymes, Inc., USA
jlin@novozymes.com P86

LIRAS Paloma
Universidad de León - Área de Microbiología, Spain
paloma.liras@unileon.es

LONDESBOROUGH John
VTT Technical Research Centre of Finland
john.londesborough@vtt.fi P49

LOSIO Dario
Università di Milano-Bicocca, Italy
dario.losio@unimib.it P17

LUTTIK Marijke
Technical University Delft, The Netherlands
m.a.h.luttik@tudelft.nl

MAAHEIMO Hannu
VTT Technical Research Centre of Finland
hannu.maaheimo@vtt.fi P60, P62, P63

MACHIDA Masayuki
National Institute of Advanced Industrial Science
and Technology (AIST), Japan
m.machida@aist.go.jp T1

MARTIN Juan F.
Universidad de León - Área de Microbiología, Spain
jf.martin@unileon.es P34, P113, P114

MATTANOVICH Diethard
IAM BOKU, Austria
diethard.mattanovich@boku.ac.at T31, P95

MEULENBERG Rogier
DSM Food Specialties/R&D/FPD, The Netherlands
rogier.meulenberg@dsm.com

MIASNIKOV Andrei — P98
Genencor, a Danisco Division, USA
andrei.miasnikov@danisco.com

MIRA Nuno P. — T10, T14, P1, P15
Institute for Biotechnology and Bioengineering,
Instituto Superior Técnico, Portugal
nuno.mira@ist.utl.pt

MUNRO Carol
University of Aberdeen, United Kingdom
c.a.munro@abdn.ac.uk

MUTSCHLECHNER Wolfgang — T26, P75
Institute of Microbiology, Austria
wolfgang.mutschlechner@uibk.ac.at

MÄKINEN Susanna — P90
Roal Oy, Finland
susanna.makinen@roal.fi

NEEFE Paulien
Genencor, A danisco Division, The Netherlands
paulien.neefe@danisco.com

NEVALAINEN Helena — P78
Macquarie University, Sydney, Australia
henvalai@els.mq.edu.au

NEVOIGT Elke — P65, P97
Technische Universität Berlin, Germany
E.Nevoigt@LB.TU-Berlin.de

NGUYEN Huyen Thi Thanh — P97
Berlin University of Technology, Germany
h.nguyen@lb.tu-berlin.de

NGUYEN Quoc Khanh
AB ENZYMES GmbH, Germany
nguyen.khanh@abenzymes.com

NISAMEDTINOV Ildar — P18
Competence Centre of Food and
Fermentation Technologies, Estonia
inisamedtinov@lallemand.com

ÖGEL Zümrüt Begum — P23, P82, P83
The Middle East Technical University, Turkey
zogel@metu.edu.tr

OLIVEIRA Carla — P79
Universidade do Minho, Portugal
carlaoliveira@deb.uminho.pt

OLIVEIRA Karen Fernanda — P102, P103
Instituto de Ciências Biológicas, Brasil
ka_fernanda2005@yahoo.com.br

OLSSON Lisbeth — T23, P100
Center for Microbial Biotechnology, Biocentrum-DTU, Denmark
lo@biocentrum.dtu.dk

OZTURK Bengu — P82
Graduate School of Natural and Applied Sciences,
Middle East Technical University, Turkey
e145220@metu.edu.tr, benguozturk@gmail.com

PAALME Toomas — P18
Tallinn University of Technology, Estonia
tpaalme@staff.ttu.ee

PADDON Chris — T36
Amyris Biotechnologies, Inc., USA
paddon@amyrisbiotech.com

PAKULA Tiina — T29
VTT Technical Research Centre of Finland
tiina.pakula@vtt.fi

PALKOVA Zdena — P31, P45
Charles University in Prague,
Charles University, Czech Republic
zdenap@natur.cuni.cz

PALOHEIMO Marja — P90
Roal Oy, Finland
marja.paloheimo@roal.fi

PARDO Alejandro — P9
Universidad Nacional de Quilmes, Argentina
apardo@unq.edu.ar

PARROU Jean-Luc — T5, P11, P54, P108
LISBP / UMR INSA, France
Jean-Luc.Parrou@insa-toulouse.fr

PATÁKOVÁ Petra — P32, P111
Institute of Chemical Technology Prague, Czech Republic
petra.patakova@vscht.cz

PENALVA Miguel A. — T8
Centro de Investigaciones Biologicas CSIC, Spain
penalva@cib.csic.es

PENTTILÄ Merja — T3, T37, P60, P62, P63, P76, P69, P93, P98, P99, P101, P107
VTT Technical Research Centre of Finland
merja.penttila@vtt.fi

PETRANOVIC Dina
BioCentrum-CMB, DTU, Denmark
dp@biocentrum.dtu.dk

PIASECKA-JOZWIAK Katarzyna P42
Institute of Agricultural and Food Biotechnology, Poland
piasecka@ibprs.pl

PITKÄNEN Juha-Pekka P58
VTT Technical Research Centre of Finland
juha-pekka.pitkanen@iki.fi

PÓCSI István P22, P30
University of Debrecen, Hungary
istvanpocsi@yahoo.com

PORRO Danilo T11, P17, P24,
University of Milano Bicocca, Italy P25, P96
danilo.porro@unimib.it

PRONK Jack T24, T34, P61
Kluyver Centre for Genomics and Industrial Fermentation
The Netherlands
j.t.pronk@tudelft.nl

PUNT Peter J. T33, P59, P77
TNO Quality of Life, The Netherlands
peter.punt@tno.nl

PURANEN Terhi P89
Roal Oy, Finland
terhi.puranen@roal.fi

RADOI-MATEI Florentina P16, P117,
USAMV Bucharest, Faculty of Biotechnology, Romania P118, P119
flore_radoi@hotmail.com

RAUDASKOSKI Marjatta P33
Plant Physiology and Molecular Biology,
University of Turku, Finland
marjatta.raudaskoski@utu.fi

RIBEIRO Orquidea
Universidade do Minho,
Institute of Biotechnology and Bioengineering, Portugal
ext-orquidea.ribeiro@vtt.fi

RICHARD Peter P69, P98
VTT Technical Research Centre of Finland
peter.richard@vtt.fi

RINTALA Eija P60, P62, P63
VTT Technical Research Centre of Finland
eija.rintala@vtt.fi

RISCHATSCH Riccarda P13
Biozentrum University of Basel, Switzerland
Riccarda.Rischatsch@unibas.ch

RITTERSHAUS Philipp C. P87
BRAIN AG, Germany
pr@brain-biotech.de

RUOHONEN Laura
VTT Technical Research Centre of Finland
laura.ruohonen@vtt.fi
T37, P60, P62, P63, P98, P99, P101, P107

RYDING Jamie
Diversa Corporation, USA
jryding@diversa.com

SALOHEIMO Markku
VTT Technical Research Centre of Finland
markku.saloheimo@vtt.fi
T3, P4, P76, P80, P93

SALUSJÄRVI Laura
VTT Technical Research Centre of Finland
laura.salusjarvi@vtt.fi
P60, P62, P99

SAMPAIO Pedro
ICAT – IST, Portugal
psampaio@icat.fc.ul.pt
P85

SAVILAHTI Harri
Department of Biology, University of Turku, Finland
harri.savilahti@utu.fi
T7, P10

SCHÖBEL Felicitas
Leibniz Institute for Natural Product Research
and Infection Biology (HKI), Germany
felicitas.schoebel@hki-jena.de
P70

SHEMATOROVA Elena
Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry, Russia
elenashe@ibch.ru
P38, P39

SHPAKOVSKI George V.
Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry,
Russian Academy of Sciences, Russia
gvs@ibch.ru
P38, P39

SMITS Gertien
Swammerdam Insitute for Life Science, University of Amsterdam,
The Netherlands
gertien@science.uva.nl
T6, P3

SNOEK Ishtar
Technical University Delft, The Netherlands
i.s.i.snoek@tudelft.nl

SOARES-SILVA Isabel
Universidade do Minho, Dep. Biologia, Portugal
ijoao@bio.uminho.pt
T21

SOLAR Tina
National Institute of Chemistry, Slovenia
tina.solar@ki.si
P67, P71

STAM Hein
DSM Food Specialties, The Netherlands
Hein.Stam@hccnet.nl

STECKA Krystyna — P42
Institute of Agricultural and Food Biotechnology, Poland
stecka@ibprs.pl

TEIXEIRA DE MATTOS, Joost — P64
University of Amsterdam, The Netherlands
teixeira@science.uva.nl

THEVELEIN Johan — T14, P6, P51
K.U. Leuven & VIB, Belgium
johan.thevelein@bio.kuleuven.be

THYKAER Jette — P112
Center for Microbial Biotechnology,
Biocentrum-DTU, Denmark
jt@biocentrum.dtu.dk

TITORENKO Vladimir — T9, P21, P73, P74
Concordia University, Canada
vtitor@alcor.concordia.ca

TOIVARI Mervi — P60, P62, P63, P98
VTT Technical Research Centre of Finland
mervi.toivari@vtt.fi

TOMA Radu Cristian — P117
University of Agronomical Sciences and
Veterinary Medicine Bucharest, Romania
radu.toma@gmail.com

TURAKAINEN Hilkka — P10
Institute of Biotechnology, University of Helsinki, Finland
hilkka.turakainen@helsinki.fi

TURCHETTI Benedetta — P110
University of Perugia, Italy
bturchetti@unipg.it

TUZUN Isil — P64
SILS-UVA, Amsterdam, The Netherlands
tuzun@science.uva.nl

VACHOVA Libuse — P31, P45
Institute of Microbiology AS CR, v.v.i., Czech Republic
vachova@biomed.cas.cz

VALKONEN Mari — P76
VTT Technical Research Centre of Finland
mari.valkonen@vtt.fi

VAN DE VONDERVOORT Peter — P7
DSM, The Netherlands
Peter.Vondervoort-van-de@DSM.com

VAN DEN BRINK Hans
Chr Hansen A/S, Denmark
hansvanden.brink@dk.chr-hansen.com

VAN EUNEN Karen T18, P56
Vrije Universiteit Amsterdam, The Netherlands
karen.van.eunen@falw.vu.nl

VAN GULIK Walter T35, P61, P66
Delft University of Technology, The Netherlands
w.m.vangulik@tudelft.nl

VAN MULDERS Sebastiaan P36, P37
Katholieke Universiteit Leuven, Belgium
sebastiaan.vanmulders@biw.kuleuven.be

VANMIDDELEM Jan P6
K.U. Leuven, Belgium
jan.vanmiddelem@biw.kuleuven.be

VATUIU Ioana P118, P119
CCAI (Compania de Cercetari Aplicative si Investitii SA)
Romania
flore_radoi@hotmail.com

VEHKOMÄKI Maija-Leena
VTT Technical Research Centre of Finland
maija-leena.vehkomaki@vtt.fi

VEHMAANPERÄ Jari P89, P90
Roal Oy, Finland
jari.vehmaanpera@abenzymes.com

VERDOES Jan P88
Dyadic Nederland BV, The Netherlands
jverdoes@dyadic-group.com

VERWAAL Rene P92
DSM Food Specialties, The Netherlands
rene.verwaal@dsm.com

VITIKAINEN Marika
VTT Technical Research Centre of Finland
marika.vitikainen@vtt.fi

VON WEYMARN Niklas
VTT Technical Research Centre of Finland
niklas.weymarn@vtt.fi

VONGSANGNAK Wanwipa P5
Center for Microbial Biotechnology, Biocentrum-DTU, Denmark
wav@biocentrum.dtu.dk

VRABL Pamela T26, P50
Institute of Microbiology, Austria
pamela.vrabl@student.uibk.ac.at

WALSH Mike
Heineken Supply Chain, The Netherlands
mike.walsh@heineken.com

WALTHER Thomas
INSA Toulouse, France
thomas.walther@insa-toulouse.fr T25

WARD Michael
Genencor - Danisco, USA
mick.ward@danisco.com

WIEBE Marilyn
VTT Technical Research Centre of Finland
marilyn.wiebe@vtt.fi P60, P62, P63

WILDT Stefan
Merck & Co., Inc., US
stefan_wildt@merck.com T28

WINKLER Ron
BIRD Engineering BV, The Netherlands
a.a.winkler@tudelft.nl

WÖLFL Stefan
Institute for Pharmacy and Molecular Biotechnology,
Ruperto-Carola University of Heidelberg, Germany
wolfl@uni-hd.de T13, T19, P29

WU Liang
DSM, The Netherlands
liang.wu@dsm.com T35

YÜZÜGÜLLÜ Yonca
Middle East Technical University M.E.T.U., Turkey
yyonca@metu.edu.tr P23

Series title, number and
report code of publication

VTT Symposium 245
VTT- SYMP -245

Author(s)
Kuokka-Ihalainen, Annemari, Saloheimo, Markku & Pakula, Tiina (eds.)

Title

3rd European Federation of Biotechnology Conference Physiology of Yeasts and Filamentous Fungi PYFF3

Abstract

The 3rd Conference on Physiology of Yeasts and Filamentous Fungi (PYFF3) belongs to a meeting series organised by the Microbial Physiology Section of the European Federation of Biotechnology (EFB). More than 200 participants from 32 countries around the world participate in the conference.

The aim of the meeting is to bring together scientists from academic research institutes and industrial laboratories to discuss the latest achievements in research on physiology of eukaryotic microbes. Yeasts and filamentous fungi are important in a number of biotechnical processes including food processing, brewing, and production of protein products such as industrial enzymes and therapeutic proteins, and production of metabolites such as antibiotics, polymer precursors and biofuels. The current trend of using lignocellulose biomass as raw material for new products in biorefineries has made yeasts and filamentous fungi more important than before. The PYFF3 conference covers comparative analysis of the expanding genomic sequence information in yeasts and filamentous fungi, as well as the experimental systems biology approaches taken to understand the global cellular regulation cues. Bioprocess-level analysis of production processes based on yeasts and filamentous fungi is also discussed, including its correlation with the physiological knowledge obtained with systems-wide analysis.

The main financial supporters of the meeting are: EFB Microbial Physiology Section, Federation of European Microbiological Societies (FEMS) and companies using yeasts and filamentous fungi in industrial processes.

ISBN
978-951-38-6313-5 (soft back ed.)
978-951-38-6314-2 (URL: http://www.vtt.fi/publications/index.jsp)

Series title and ISSN	Project number
VTT Symposium 0357-9387 (soft back edition) 1455-0873 (URL: http://www.vtt.fi/publications/index.jsp)	15643

Date	Language	Pages
May 2007	English	193 p.

Name of project	Commissioned by

Keywords	Publisher
yeast, filamentous fungi, microbial physiology, systems biology, genomics, proteomics, metabolomics, modelling, stress responses, metabolic engineering, protein production	VTT Technical Research of Finland P.O.Box 1000, FI-02044 VTT, Finland Phone internat. +358 20 722 4404 Fax +358 20 722 4374

We are very grateful to the following organisations and companies for their support:

EFB Microbial Physiology Section of the European Federation of Biotechnology
FEMS The Federation of European Microbiological Societies
University of Helsinki
VTT Technical Research Centre of Finland
AB Enzymes
Diversa-Celunol
DSM
Dyadic
Genencor
NatureWorks LLC
Oy Sinebrychoff Ab
Roal Oy

Julkaisu on saatavana

VTT
PL 1000
02044 VTT
Puh. 020 722 4404
Faksi 020 722 4374

Publikationen distribueras av

VTT
PB 1000
02044 VTT
Tel. 020 722 4404
Fax 020 722 4374

This publication is available from

VTT
P.O. Box 1000
FI-02044 VTT, Finland
Phone internat. + 358 20 722 4404
Fax + 358 20 722 4374

ISBN 978-951-38-6313-5 (soft back ed.)
ISSN 0357-9387 (soft back ed.)

ISBN 978-951-38-6314-2 (URL: http://www.vtt.fi/publications/index.jsp)
ISSN 1455-0873 (URL: http://www.vtt.fi/publications/index.jsp)