

'Microbial physiology is gaining power through integrated studies': physiology of yeasts and filamentous fungi III (PYFF-III), Helsinki, Finland, 13–16 June, 2007

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First published online October 2007.

DOI:10.1111/j.1567-1364.2007.00318.x

Over a period of three days, about 200 scientists from 32 different countries met at the Marina Congress Center in Helsinki to discuss and exchange their views and experiences on a classical but still very dynamic research field, 'Physiology of yeasts and filamentous fungi'. There were 39 oral presentations from which 27 were selected from 140 abstracts by an international scientific committee. Overall, the balance between talks on yeasts and filamentous fungi shifted from *c.* 3/1 during the last PYFF-2 conference (Olsson, 2004, Anglet, France, 2004) to 2/1 in this meeting, and 30% of the abstracts were dealing with filamentous fungi. This indicates a clear inclination towards increasing research interests in these latter microorganisms. A likely reason for this may be that the genomes of several filamentous fungi genomes have been sequenced, which has highlighted the great biotechnological potential of these microorganisms. Themes set out at this 3rd conference, nicely organized by Drs Merja Pentilla and Markku Solaheimo from VTT, covered the present driving force in this field, from global genomic and functional analysis to biotechnological applications focused on proteins and metabolites production, with a detour on basic, though challenging topics on signaling, nutrient transport, stress responses and cellular energetics.

In the session 'Genomics and Functional analyses', comparative genomics was shown to be a powerful tool to explore the complexity of biological systems and to provide testable hypothesis. For instance, comparative genome analysis of *Aspergillus oryzae* and other fungal species highlighted the occurrence of nonsynthetic blocks (NSBs) that turned out to be expressed specifically under conditions of solid-state cultivation (Machida *et al.*, Japan). Also, a comparative analysis of protein-coding genes between the fungal phyla *Pezizomycotina* and *Saccharomycotina* predicted the former to be more suitable to produce secondary metabolites and to secrete enzymes (Arvas *et al.*, Finland). Meta-analysis using T-profiler algorithm to GO-ontology of thousands of published transcriptomic and genome-wide

mutant analyses in yeast revealed a novel nontranscriptionally regulated general stress response in yeast (Smits *et al.*, The Netherlands). Charles Boone (Canada) reviewed the power of synthetic genetic arrays to map the genetic interactions in yeast and informed the scientific community about gene deletions in the Σ 1278 b strain that will be used for deciphering the genetic complexity underlying invasive growth and biofilm formation that are typical for this strain.

Changes in the environmental pH are likely one of the most frequently encountered situations during the life of fungi. Adaptation to environmental pH was a general focus of the second topic 'Fundamental Cell Functions and Stress'. As reviewed by M. Penálva (CSIC, Madrid), ambient pH alkalization is signaled in yeast and filamentous fungi by the conserved *pal/RIM* transduction pathway. Recent progress on our understanding of this complex regulatory system showed the function of β -arrestins as endocytic adaptors that mediate down-regulation of the pH signaling by endocytosis of the plasma membrane sensor modules PalH/Rim21 and PalF/Rim8. Lowering the pH using weak acids such as acetic or propionic acids involved transcriptional regulation by Haa1 and Rim101, respectively, which leads to resistance to these weak acids (I. Sá-Correia and collaborators, Portugal). On the other hand, D. Porro and coworkers (Italy) proposed that overproduction of the H^+ -ATPase could improve growth initiation under acidic conditions. Direct evidence, however, that this improvement was related to ATPase activity remained to be demonstrated. A final keynote in this session was given by A. Kitanovic from S.Wölf's lab (Germany) who proposed the implication of the gluconeogenic enzyme FBPase1 in DNA damage defense, aging and apoptosis, which was found to be independent to the catalytic activity of this enzyme.

Besides harsh environmental conditions that often penalize the physiological performances of yeasts and filamentous fungi, the growth efficiency of fungi relies on their capacity to cope with essential nutrients. Whether the same signaling pathway is sensing the availability of different

nutrients, or whether as many sensing systems as nutrients exist, remains a recurrent question which was addressed by J. Thevelein (Belgium) at the beginning of the third session on 'Nutrient signaling and transport'. In the yeast *Saccharomyces cerevisiae*, the presence of a fermentable carbon source is sensed by the PKA signaling cascade through stimulation of cAMP synthesis. Evidence was presented that this pathway is also activated by amino acids and Pi, which does not require increase of cAMP, but does require a specific amino acid or Pi-transporter that functions as a receptor. This hypothesis was substantiated by the existence of agonist molecules that cancelled the PKA activation by competitively inhibiting the amino acid transport. Binding however is not sufficient for activation, and the signaling system from the transporter to PKA needs further clarification. On the other side, the fate of intracellular Pi is intriguing in many aspects of yeast physiology. It is mainly stored as inorganic polyphosphate (Poly P) in the vacuole, which could represent 20% of the cell dry mass. A systematic screen of the yeast haploid deletants collection identified 255 genes implicated in maintenance of normal level of Poly P, most of them encoding vacuole proteins and proteins functioning in intracellular transport and cell homeostasis. F. Freimoser (Switzerland) reported on the role of *PHO* pathway components in the metabolism and homeostasis of Poly P in yeast, showing that the high affinity Pho84 permease can solely accumulate poly P from Pi. The low affinity Pho90 and Pho91 may function as Pi exporter at the vacuole membrane, thereby controlling intracellular Pi. Sugar uptake was also addressed in this session on a more applied perspective by E. Boles (Germany). Using a *S. cerevisiae hxt* null mutant, which lacked 20 genes encoding sugar transporters, he cloned *AraT* gene by screening a DNA library from a *Pichia stipitis* library for the ability of the *hxts* mutant to growth on L-arabinose. Quite amazingly, this transport, which was highly specific to L-arabinose, was not functional when expressed in wild type *S. cerevisiae*. The mechanism of this selective repression has not yet been characterized. In contrast to yeast, filamentous fungi can thrive on a large variety of complex plant polysaccharides. However, as reviewed by R.P. de Vries (The Netherlands), this requires complex regulatory systems, which are necessarily dependent or triggered by the sugar moieties of the polysaccharide in order to secrete hydrolytic enzymes in the growth medium. This was illustrated for regulatory systems under *XlnR* for xylose and *AraR* for arabinose utilization.

The characterization of metabolic pathways and the control of cellular energetics has been studied for a long time in microbial physiology. This fourth session illustrated clearly our limited understanding of these processes. A good illustration was given by G. Daum (Austria) on the metabolic pathways for neutral lipids (triacylglycerol and steryl esters), which are stored in subcellular fractions called lipid

particles. Synthesis and mobilization of these droplets are catalyzed by a large number of enzymes with apparent overlapping activities. The function of these lipid droplets in yeast physiology is even more challenging as no clear-cut phenotype is observed in mutants totally defective in their production. According to L. Olson (Denmark), new metabolic pathways can be instigated by a global approach using Metabolomics that aimed at quantifying extracellular and intracellular metabolites (within the limit of the technical tool) under a given condition and analyzing the data using machine learning tools. This approach led these authors to propose the existence of a phosphoketolase pathway in *Aspergillus niger*, although the gene remains to be characterized. With respect to cellular energetics, the paradoxical drop of ATP and loss of total nucleotides shortly after addition of glucose to respiring or starving yeast cells remains a long-standing paradox. Although the exact physiological meaning of this drop is still unclear, the pronounced drop of AXP is now explained by the activation of the purine salvage pathway that divert transiently the nucleotide through inosine, which is then reassimilated into AMP back to ATP. This purine salvage pathway was shown to be a regulated process rather than a passive response of the metabolism (Th. Walther and coworkers, France). Transition from aerobiosis to anaerobiosis is also a physiological situation inducing important energetic and metabolic modifications. The group of J.T. Pronk (P. Lapujade and collaborators, The Netherlands) showed that this shift led to dramatic proteomic changes that were largely independent from the transcriptomic response. Major posttranslational changes concerned proteins implicated in glycolysis, purine-nucleotide synthesis and amino acid biosynthesis. This finding raises new challenging questions with respect to the mechanism that control protein production and stability in yeast.

Advances in postgenomic tools enable a more rational redesign of metabolic pathways for 'Protein and metabolites production'. Several case-studies in the last two sessions of this conference pointed to a renewed interest in using microorganisms as 'cell factories' for the production of e.g. therapeutic proteins and pharmaceutical products. Re-engineering the glycosylation machinery of *Pichia pastoris* to create human-like glycosylation is now operational (S. Wildt). Also, redesigning the mevalonate pathway in *S. cerevisiae* by integrating two specific genes from the original plant producer to perform the last steps of oxidation of the artemisinic precursor to produce artemisinic acid, an antimalarial drug, has been successful. This opens the way for further replacement of chemical production methods by microbial ones (C. Paddon *et al.*). Evolutionary engineering is another attractive method for improving or tailoring cellular properties for a desired phenotype. Examples were given for improving glycerol production of a

previously engineered yeast strain for glycerol (J.T. Pronk, The Netherlands) and for the efficient riboflavin-producing yeast strain based on increased resistance to cobalt (Z.P. Cakar, Turkey). Besides these main achievements, important efforts are still devoted to the recalcitrant problem of protein secretion, as this is a major bottleneck in the production of enzymes and heterologous proteins at the industrial scale. Boosting heterologous proteins production originating from distantly related organisms provokes the activation of the unfolded response pathway (UPR), which in turn causes a feedback mechanism to reduce the protein load in the secretory pathway. It was shown that the UPR response was not the same depending on whether the expressed protein was from human or fungi (T. Pakkula, Finland). Another limitation for protein secretion has been attributed to a low folding rate, and, hence, a rational solution is to overproduce proteins supporting folding, such as protein disulfide isomerase Pdi1 and unfolded protein response transcription factor Hac1. These effects obviously will displace the limitation to another step of the process. To identify other 'helper' factors that would support secretion, B. Gasser (Austria) compared transcriptome profiling of a *Pichia pastoris* overexpressing human trypsinogen vs. a nonexpressing strain. This gave rise to a set of candidate genes that support secretion. One of them, corresponding to the *S. cerevisiae* homolog *SSE1* was tested and shown to moderately increase the secretion of human Fab antibody when overexpressed together with *PDII* and *HAC1* in *P. pastoris*. An alternative route to circumvent the secretory pathway is to engineer 'ex nihilo' a novel protein secretion pathway. This approach is currently tested with *Aspergillus niger* by DSM, but so far it has been limited to intracellular enzymes that were forced to be secreted by this system. Protein export typically bears N-terminal signal peptides that target them for secretion. However, recent proteomic studies have reported a large set of proteins at the cell surface that transgress this canonical mechanism for secretion. A nice illustration was reported by the group of J.M. Beckerich (France) for the yeast enolase, a typical glycolytic enzyme. There are in *S. cerevisiae* five genes encoding enolase isoforms, but only enolase 2 encoded by *ENO2* is exported. This specificity seems to be due to the N-terminal region of

this isoform that is structurally different from the other enolases. In addition, this export required an automodification of a lysine residue (K345) with 2-P glycerate (2PGA), which forms a peptide bond between the ϵ -NH₂ of the lysine and the carboxyl group of 2PGA, and is likely mediated by lipid rafts.

This conference was closed by an open discussion between project leaders from biotechnological industries and the audience on the future of 'Biorefinery'. To decrease our dependency on fossil fuels reserves and to boost rural development, biorefineries are seen as a promising route to meet our aims for sustained prosperity and preserving the environment. However, a consensus remains to be found on which critical efforts are needed, e.g. related to the increase, modification or development of new raw materials, the engineering of more efficient enzymes for biomass pretreatment, and reaching a commitment between biochemical and thermochemical conversion of biomass, in order to make this enterprise economically viable.

To conclude, this PYFF-3 conference has shown tremendous progress in our understanding of the physiology of yeasts and filamentous fungi. The increasing number of fully sequenced microbial genomes, the access to 'omics' tools and the development of user-friendly data mining and treatment tools are likely the basic reason for this progress. As the common target of researchers attending this meeting is an in-depth understanding of the microorganisms living in natural and industrial environments in order to maximize their biotechnological value, it can be foreseen that this kind of conference will move smoothly towards even more integrated research in microbial physiology, e.g. Systems Biology. I am already eager to attend the next PYFF-4 that will be organized in 3 years by the group of Jack Pronk in The Netherlands, to see how our understanding of microbial systems will progress.

References

- Olsson L (2004) Physiology of yeasts and filamentous fungi II (PYFF-2), Anglet, France, March 24–28. *FEMS Yeast Res* 4: 891–892.