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Conference report

The 22nd International Specialized Symposium on Yeasts (ISSY 2002) 'Yeast Fermentations and Other Yeast Bioprocesses'

1. Introduction

The 22nd edition of the International Specialized Symposium on Yeasts, ISSY 2002 was held from 25 to 28 March at the beautiful and well-equipped Kwa Maritane (Land of the Rock) conference resort at Pilanesberg National Park, some 150 km northwest of Johannesburg, South Africa. In line with thriving and blooming fundamental and applied yeast research all over the world [1], and despite the relatively 'out of bounds' conference venue, ISSY 2002 attracted over 160 international delegates. Indeed, the organizing committee had done a marvelous job in attracting a relatively large number of acknowledged leading researchers from all over the world and had succeeded in putting together interesting and stimulating scientific sessions on various topics. As already foreseen by James du Preez, chair of the organizing committee, in his welcoming address, "... being 'captive' within a game lodge with wild animals roaming free ..." indeed fostered stimulating and productive interactions between the delegates.

This report does not pretend to give a full coverage of the whole Symposium, but tries to highlight important issues and examples of the impact of modern research on yeast fermentation science and technology. Primarily, the report should breathe the lively and informal, but nonetheless fruitful atmosphere that characterized this meeting.

2. Message through the grapevine ...

Various species and types of yeast have traditionally played – and are still playing – important roles in biotechnology. The intrinsic robustness, culturability and specific physiological characteristics of many yeasts have been adapted and utilized for production of food and beverages and of various non-food products. With its well-accessible genetics and rapid adaptation to varying growth conditions, *Saccharomyces cerevisiae* as baker's, wine or brewer's yeast has become a hallmark of both fundamental and applied research on yeasts. This obviously and notably bears the inherent danger of having other important and interesting yeasts fully overlooked by the majority of the yeast research community. Which historically has happened more than once ...

Two opening lectures, by *Isak Pretorius* and *Morten Kielland-Brandt*, clearly set the stage for this symposium. Wine yeast and brewer's yeast have a long and important history in the preparation of alcoholic beverages. In spontaneous wine fermentations an initially rich growth of various indigenous yeasts is subsequently replaced by dominant growth of alcohol-tolerant wine yeast strains of *S. cerevisiae*. Beer fermentations usually are initiated by the addition of specialized brewer's yeast strains. With the rise of modern and more sophisticated wine and beer markets, the demand for specialized yeast strains with optimized and novel properties has launched considerable efforts in strain improvement and development. Both classical and recombinant strain improvement strategies require increasingly detailed insight into cellular and molecular control mechanisms regulating yeast growth and fermentation characteristics and development of taste, flavor and wholesomeness of wine and beer. Therefore, current research efforts are focusing more and more on mechanisms of environmental signal transduction and intracellular regulatory networks.

Amongst other matters of interest, control of cellular morphology, flocculation, sugar utilization, nutritional starvation responses and flavor formation have become major research topics. Flavor, off-flavor and shelf life of alcoholic beverages are for a large part determined by the relative levels of higher alcohols, being side products of amino acid metabolism. In addition, amino acid metabolism is central to nitrogen-dependent control of yeast cell growth and morphology. These notions thus have set a stage for detailed molecular analysis of the underlying regulatory mechanisms, in order to find genetic and physiological targets for dedicated strain improvement by holistic, integrated 'engineering' approaches.

3. Molecular systematics

Yeast taxonomy historically relies on the discrimination of specific morphological and physiological properties of the different species. Non-traditional growth tests like assimilation of imidazole, methanol, or even *n*-alkanes forc-

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ing yeast species to rely on specific and relatively extreme metabolic characteristics are of high value in 'classical' phylogenetic determination (*Wouter Middelhoven*). However, in recent years, molecular approaches have presented major contributions to reliable identification of species and the reconstruction and extension of previously sometimes erroneous phylogenetic relationships. As presented at the conference, sequence analysis, RAPD fingerprinting and PCR-RFLP of ribosomal DNA fragments have played important roles in accurately typing relatively related yeast strains (*Elena Naumova*, and [2]). Additionally, sequence analysis and comparison of a number of variable genes greatly enhances the accuracy of phylogenetic determination and allows for fine mapping of genera and groups (*Cletus Kurtzman*). Thus, over 80 species of the *Saccharomyces* clade were phylogenetically arranged using sequence information of six genes. Further resolution of the lineage trees will be obtained using an increased number of gene comparisons. With world-wide ongoing efforts on microbial genome sequencing (e.g. [3,4]), molecular foundation of taxonomic lineages is rapidly becoming routine business. Given the various examples presented in a separate lecture session and in the poster sessions, molecular typing has become equally important for determination of indigenous fermentations (e.g. African sorghum beer, Mexican tequila) and starter cultures.

4. Basics of food and beverage yeast

The implementation of molecular cell biology in yeast research has revolutionized our understanding of yeast physiology and growth control in brewing, wine making and bakery applications. Several interesting and beautiful examples were given at this conference.

Senescence and mortality of brewer's yeast are important determinants for yeast strain management and fermentation performance in the brewing industry (*Katherine Smart*, and [5]). Yeast senescence is pre-determined genetically and is directly related to the number of cell cycle divisions an individual yeast cell has gone through. In addition to genetic determination, environmental impact and stress factors may grossly influence the replicative lifespan. Several biomarkers of yeast 'old age' have been identified and are being used to further investigate this phenomenon: severe increase in cell size, accumulation and 'stretching' of bud scars and even the formation of wrinkles on the surface (who said yeast doesn't look like man?). Since cell cycle control appears to play an important part in yeast senescence, the putative involvement of the G_1 cyclin Cln3 was investigated. Over-expression of *CLN3* led to an average cell volume reduction of 50% but, however, did not have any effect on lifespan.

In both the wine and brewery industries, cell–cell adhesion, flocculation and pseudohyphal morphology are important determinants of fermentation, productivity and quality. All three phenotypical characteristics are controlled through the same network of signal transduction pathways, responding to the (non-)availability of nutrients (*Florian Bauer*, and several posters). At the end of the signaling cascade several lectin-like cell wall proteins play their specific roles in one or the other process. Specificity is obtained through subtle variations and cross-regulations in the signaling network.

Divalent metal ions function as important biominerals in many organisms and also for yeast, molecular details of transport and homeostasis of Ca^{2+}, Mg^{2+}, Cu^{2+}, Zn^{2+} and Fe^{2+} are rapidly becoming available. Mg^{2+} has been found to play an essential role in glycolysis and fermentation (i.e. conversion of sugars into ethanol) and in maintaining viability under stressful conditions like elevated growth temperatures and high alcohol concentrations (*Graeme Walker*, and [6]). Moreover, supplementation of brewer's or wine yeast with Mg^{2+} significantly enhanced stress resistance as well as fermentative capacity and ethanol production. Although not all details are understood, this provides a fine example where knowledge-based physiological engineering and pre-conditioning of the yeast lead to immediate process improvement.

In frozen dough applications, the resistance of baker's yeast strains to freezing is lost to a large part in the prefermentation period that is unavoidable during preparation of the dough (*Patrick Van Dijck*). A dedicated hunt for mutants that specifically retain their stress resistance during the onset of fermentation identified the adenylate cyclase gene *CYR1* as a key player in the reciprocal control of stress resistance and growth initiation. Additional mutants were obtained in a polyploid strain of baker's yeast through repeated freeze–thaw treatment. The most promising mutant obtained combined an enhanced freeze stress resistance with maintenance of fermentative capacity and good growth yield. However, the mutant appeared to be a diploid derivative of the parental strain and consequently had a smaller cell size that may cause problems in centrifugation and downstream processing on a large scale. Nevertheless, by genome-wide expression analysis it was found that this freeze-resistant mutant displayed enhanced expression of the aquaporin gene *AQY2*. Subsequently it was shown that vectorial over-expression of both *AQY1* and *AQY2* indeed improves freeze resistance in various baker's yeast strains.

5. Non-food yeast applications

Several yeast species have long been used for the production of both food and non-food products, like (homologous and heterologous) proteins, amino acids and organic acids. Judging from the plenary contributions and the number of posters related to the subject, the use of yeasts for non-food products and processes is rapidly increasing. Various yeast species are able to tolerate and accumulate relatively high levels of trace elements like Cr(III), incorporating those into organic compounds which may be useful for pharmaceutical purposes (*Peter Raspor*). Several yeasts and yeast-like fungi can be applied as efficient biocatalysts for the bioconversion of diverse agricultural residues and wastes, thus opening an abundant potential of novel feed stocks for fermentation industry (*Timothy Leathers*). In line with this, essentials and mechanisms of xylose and xylitol fermentation are being studied in various yeasts, including *Hansenula polymorpha*, *Pichia stipitis* and *S. cerevisiae* (*Andrei Sibirny*, *Gunnar Lidén*, *Bärbel Hahn-Hägerdal*). The latter yeast requires extensive recombinant technology input and, therefore, has largely contributed to our current understanding of fermentation control of alternative carbon sources. Further novel applications are the use of *Saccharomyces* spp. for enantioselective redox conversions (*Marie Gorwa-Grauslund*) and redox cofactor regeneration (*Peter Richard*), and for the heterologous production of a novel class of antibodies originating from camel (*Yvonne Thomassen*). As rightfully hinted upon by one of the contributors, the Yeast kingdom is only beginning to reveal a wealth of versatility and efficacy in bioconversion potential that will undoubtedly bring important progress in the development of bio-refineries of the future.

6. Metabolic engineering and control of yeast bioprocesses

Today, molecular biology and genetics have brought us the ability to engineer at want single or several steps in metabolic pathways in various yeasts. With *S. cerevisiae* as the well-known and hence most-engineered model yeast running up front, other yeasts are rapidly catching up because of larger versatility or flexibility. At the same time, 'classical' application of baker's, wine or brewer's yeast is still subject to physiological and process improvement. Novel methods like DNA micro-array analysis (transcriptome analysis), 2D protein analysis coupled with mass spectrometric detection (proteome analysis), sophisticated high-throughput measurement of intracellular metabolites (metabolome analysis) are increasingly being used in routine analysis of yeast fermentations. Jointly referred to as Functional Genomics technologies, these analyses can provide a more or less 'genome-wide' overview of physiological and metabolic regulatory networks which are operative in various yeast strains under various growth conditions. For industrial R&D, a Functional Genomics approach promises to enhance knowledge development on cellular physiology, and to accelerate strain and process improvement.

Several examples of the implementation of genome-wide expression analysis were presented, the majority aiming at the control of sugar utilization and fermentative capacity in baker's and wine yeast. In addition, several studies focused more specifically on the control of one or a few interrelated metabolic pathways. In many cases there was a clear tendency to zoom in on regulatory mechanisms operating on groups of genes and gene products rather than on individual genes. Accordingly, many efforts on metabolic pathway engineering are tackling regulatory components and mechanisms of enzyme pathways rather than individual components within the pathways. For example, engineering of xylose metabolism in non-xylose-consuming *S. cerevisiae* concomitantly involved modification of ammonium assimilation and rearrangement of redox balance (*Jens Nielsen*, *Merja Penttilä*).

7. Yeast in modern biotechnology

The general take-home message of this 22nd International Specialized Symposium on Yeasts was adequately put together in *Jens Nielsen*'s closing lecture. As indicated above, future challenges in yeast fermentations and bioprocesses are in further exploiting and refining current knowledge and applications, at the same time inquisitively looking for new species and/or novel characteristics that may lead to innovative applications and new processes. The combined implementation of metabolic engineering and functional genomics promises to yield important steps in strain and process improvement [7]. A true systems approach in yeast cell biology will be the key to the construction, identification and implementation of strains with new or improved properties and to the unraveling of novel functionalities. With this, yeast bioprocesses will have an unbridled future, as will the ISSY symposia ...

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