YEAST AS A PLATFORM CELL FACTORY IN FUTURE BIOREFINERIES

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Introduction. Microbial fermentations are the core of biorefineries as this process ensures value addition when the raw material is converted to desired fuels and chemicals. The development of efficient cell factories that can be used as biocatalysts in microbial fermentations is often the most time consuming and R&D intensive part in the development of a biorefinery. Most current biorefineries involves ethanol production, but there is much interest to upgrade the production of ethanol to other more valuable fuels like butanol and diesel. This can be done through simple replacement of the biocatalyst, which in this case is the yeast *Saccharomyces cerevisiae*, and replacement of the biocatalyst can often be done in a plug-and-play fashion with little requirement for retrofitting of the production facility.

Besides its classical application in the production of bread, beer, wine and bioethanol, the yeast *Saccharomyces cerevisiae* is a widely used cell factory for production of fine chemicals such as resveratrol, and for production of pharmaceutical proteins such as human insulin and vaccines. In connection with the development of biorefineries for sustainable production of fuels and chemicals, there is much interest to use yeast as a cell factory due to its general acceptance in the industry, its robustness towards contaminations, its high alcohol tolerance, and its low pH tolerance. Further advantages of using yeast are that a large number of molecular biology techniques are available for this organism, there is a large experimental database available and there is an excellent research infrastructure.

In this lecture tools for advancing the design and engineering of novel, efficient yeast biocatalysts will be presented. This will involve both a number of novel **synthetic biology** tools that allows for rapid reconstruction of heterologous pathways, for tuning expression using promoter libraries and different expression vectors. Also different tools from **metabolic engineering** will be presented, e.g. methods for flux quantification and mapping of flux control. Finally methods from **systems biology** will be presented, as these may offer completely computerized design of cell factories in the future using so-called genome-scale metabolic models. Examples for how the metabolism of yeast can be engineered for production of novel fuels and chemicals will be presented, and it will be demonstrated how yeast can be engineered to produce a range of different products and hence can serve as a platform cell factory in modern biorefineries.

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References.

1. I.-K. Kim; A. Roldao; V. Siewers; J. Nielsen (2012) A system-level approach for metabolic engineering of yeast cell factories. *FEMS Yeast Res.* **12**:228-248
2. K.-K. Hong; W. Vongsangnak; G.N. Vemuri; J. Nielsen (2011) Unravelling evolutionary strategies of yeast for improving galactose utilization through integrated systems level analysis. *Proc. Nat. Acad. Sci. USA* **108**:12179-12184
3. K.-K. Hong; J. Nielsen (2012) Recovery of phenotypes obtained by adaptive evolution through inverse metabolic engineering. *Appl. Environ. Microbiol.* **78**:7579-7586
4. R. Agren; L. Liu; W. Vongsangnak; I. Nookaew; J. Nielsen (2013) Use of the RAVEN toolbox for generating the genome-scale metabolic network for *Penicillium chrysogenum*. *PLoS Computational Biol.*, in press