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Primary Metabolism and Metabolic Engineering

Expansions and reductions in fungal primary metabolism studied across 100 fungal species. Julian Brandl, Jane Nybo, Tammi C. Vesth, *Mikael Rørdam Andersen* Department of Biotechnology and Biomedicine, Technical University of Denmark, Kgs Lyngby, DK.

The primary metabolism of fungi is the power house that drives nearly all cellular functions. Primary metabolism is involved in converting the surroundings of the fungus to a food source as well as delivering metabolite precursors for everything from cellular growth and maintenance over biological responses to external stimuli to producing secreted secondary metabolites and protein effectors. Furthermore, it is known that fungal metabolism is highly versatile. Saphrophytic fungi can grow on a very wide range of carbon and nitrogen sources and utilize this for production of even more diverse range of secondary metabolites and secreted proteins.

Within this project, we have been interested in two main things: 1) What is the "roadmap" of fungal metabolism within a single species? What is the total sum of genes involved in primary metabolism? For studying this, we have been focusing on the industrial workhorse and model fungus Aspergillus niger. 2) What is the diversity of metabolism across fungi? Which gene functions are expanded, reduced, added and lost across species? For this we have used the metabolic "roadmap" of *Aspergillus niger*, and used it to query the genomes of approximately 100 different species, primarily of the genus Aspergillus.

For the initial roadmap of fungal metabolism, we have reconstructed the metabolism of *A. niger* ATCC 1015 at genome-scale. The metabolic reconstruction covers 1801 metabolic conversions, 997 genes, and 1411 metabolites across six compartments. Phenotype arrays have been applied to evaluate the ability to germinate on 180 carbon sources and 92 nitrogen sources. Examining the metabolism shows a high degree of isoenzymes across both central and outer metabolism.

Employing this metabolic network to our database of fungal genomes allows us to examine the diversity of metabolic strategies through the identification of orthologs across species and map this to the species. Our analysis shows that some specialized fungi have a more reduced genome and survives with 1-2 isoenzymes for most of central metabolism, while others apply a diversification strategy and often have 2-5 isoenzymes, even for the highly conserved functions in central metabolism.

Engineering the smut fungus *Ustilago maydis* for the degradation of pectin. P. Stoffels[1,3], E. Geiser[2,3], L.M. Blank[2,3], M. Feldbrügge[1,3], N. Wierckx[2,3], *K. Schipper*[1,3] 1) Institute for Microbiology, Heinrich Heine University Düsseldorf, Düsseldorf, Germany ; 2) iAMB – Institute of Applied Microbiology, ABBt – Aachen Biology and Biotechnology, RWTH Aachen University, Worringerweg 1, 52074 Aachen, Germany ; 3) Bioeconomy Science Center (BioSC), c/o Forschungszentrum Jülich, 52425 Jülich, Germany.

The microbial conversion of plant biomass components to valuable products in a consolidated bioprocess would greatly increase the ecologic and economic impact of a biorefinery. Plant-pathogenic fungi are promising candidates for biomass valorization, because they contain a vast repertoire of hydrolytic enzymes to sustain their lifestyle. However, expression of the corresponding genes is usually tightly regulated and mostly restricted to the pathogenic phase. We use the biotrophic smut fungus *Ustilago maydis* for the degradation of plant cell wall components by activating its intrinsic enzyme potential during axenic growth. This fungal model organism is equipped with a potent set of hydrolytic enzymes, and moreover, it naturally produces value-added substances such as organic acids and biosurfactants. To achieve the deregulated expression of hydrolytic enzymes during the industrially relevant yeast-like growth in axenic culture, the native promoters of the respective genes were replaced by constitutively active synthetic promoters. This led to an enhanced conversion of biomass components like xylan, cellobiose, and polygalacturonic acid to fermentable sugars. Currently, the intrinsic fungal repertoire is activated and in parallel supplemented with potent heterologous enzymes for the degradation of pectin. Importantly, this can be achieved using both conventional secretion for enzymes of eukaryotic and unconventional secretion for enzymes of bacterial origin. In summary, our results demonstrate the potential applicability of activating the expression of native hydrolytic enzymes from phytopathogens in a biocatalytic process.

A trehalose-regulatory subunit moonlights to regulate cell wall homeostasis through modulation of chitin synthase activity in *Aspergillus fumigatus*. *Arsa Thammahong*, Alayna Caffrey, Sourabh Dhingra, Josh Obar, Robert Cramer Microbiology and Immunology, Dartmouth College, Hanover, NH.

Purpose: Trehalose biosynthesis is a metabolic pathway found in fungi but not humans. Proteins involved in trehalose biosynthesis are essential for human and plant fungal pathogen virulence. Loss of canonical trehalose biosynthesis genes in the human pathogen *Aspergillus fumigatus* alters virulence and cell wall integrity through undefined mechanisms. Here we characterize additional genes, herein called *tslA* and *tslB,* which encode proteins that contain similar protein domains as OrlA (a trehalose-6-phosphate phosphatase), but lack critical catalytic residues for phosphatase activity.
Methods: We utilized a genetics approach to generate null mutants of *tslA* and *tslB*. To observe the phenotypes of these mutants, we used trehalose assays and cell wall perturbing agents. Furthermore, LC-MS/MS and co-immunoprecipitation were performed to define protein-protein interactions of TslA . To further characterize the phenotype of *tslA* null mutant, chitin synthase activity assay and spinning-disk confocal microscope were used to study the chitin content and the localization of CsmA. A chemotherapeutic murine model was utilized to study the host-pathogen interaction.
Results: Loss of *tslA* reduced trehalose content in both conidia and mycelia, impaired cell wall integrity, and significantly altered cell wall structure. Unexpectedly, immunoprecipitation assays coupled with LC-MS/MS revealed a protein interaction between TslA and CsmA, a type V chitin synthase enzyme. TslA regulates not only chitin synthase activity but also CsmA localization. Loss of TslA directly affected the host immune response to *A. fumigatus* characterized by an increase in murine mortality likely due to enhanced immune cell recruitment.
Conclusion: Our data provide a mechanistic model whereby proteins in the trehalose pathway play critical roles in fungal cell wall