Tools for Exploiting Fungi as Production and Discovery Platforms

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Fungi have served as efficient cell factories of a wide range of products including primary- and secondary metabolites as well as therapeutic peptides and industrial enzymes. For example, many fungi are natural producers of bioactive secondary metabolites, and several of presently used block-buster drugs have a fungal origin. Genome sequencing has demonstrated that fungi have genetic potential to produce a repertoire of secondary metabolites and enzymes that by far exceeds the number of metabolites and enzymes that are produced by the same fungi at standard laboratory conditions. Considering that hundreds of fungal genomes are now fully sequenced and that more are constantly added to this collection; it is fair to assume that many bioactive fungal small molecules and enzymes await discovery and that some of them may possess important pharmaceutically or industrially relevant properties. At the same time it is important to stress that bioactive fungal metabolites may also act as mycotoxins, which may cause human disease or negatively impact human food resources. A fuller understanding of fungal secondary metabolisms is therefore highly desirable. Discovery of new fungal compounds and elucidation of their biosynthetic pathways have been hampered by a general lack of genetic tools. The same lack of genetic tools is similarly a barrier for optimizing production of an interesting product in a natural producer by metabolic engineering. One solution to this problem has been to reconstruct secondary metabolite production in well characterized fungal model systems like the yeast *Saccharomyces cerevisiae* or a filamentous fungus like *Aspergillus nidulans* where elaborate genetic toolboxes are in place. In our laboratory, one point of focus is to develop genetic tools and methods to engineer model and non-model fungi for gene discovery and for metabolic engineering. To this end, the recent introduction of CRISPR/Cas9 technology promises to be a game changer that will revolutionize our genetic engineering strategies for understanding basic fungal biology, for discovering new fungal products like secondary metabolites and enzymes, and for improving fungal cell factories. The nuclease Cas9 introduces DNA double strand breaks at defined loci. These breaks may be mis-repaired by the non-homologous end-joining DNA repair pathway to introduce mutations in a gene of interest, or serve as substrate for homologous recombination setting the stage for efficient gene targeting. Cas9 is a riboprotein, and a specific section of Cas9 associated RNA, the protospacer, directs Cas9 to the target DNA locus, hence, accounting for Cas9 specificity. Since the sequence of the protospacer can be easily modified, virtually any sequence can be targeted for CRISPR based gene editing. Cas9 mediated genetic engineering is very efficient and even multiplexing is possible if several guide RNA species are produced simultaneously during a CRISPR experiment. Together this will speed up gene discovery by reverse genetics tremendously. Another set of CRISPR based activities exploit that catalytically dead Cas9, dCas9, can be used as a basis for making synthetic transcription factors; and such transcription factors can be used to rewire e.g. the metabolic network of a secondary metabolite producing organism or cell factory. Examples of how different fungal CRISPR tools can be used for gene discovery, pathway elucidation, and metabolic engineering will be presented.