Unraveling the Secondary Metabolism of the Biotechnological Important Filamentous Fungus Trichoderma reesei (Teleomorph Hypocrea jecorina)

The filamentous fungus Trichoderma reesei (teleomorph Hypocrea jecorina) is one of the most important industrial production organisms, owing to its highly efficient (hemi-)cellulase synthesis and secretion machineries. These enzymes, which in nature allow the fungus to utilize energy bound in cellulosic biomass, have wide application in the pulp and paper, textile and biofuel industries. The genomic sequence for T. reesei was published in 2008 and provides the basis for introducing targeted genetic alterations that could for example increase cellulase production yields. Like all other filamentous fungi, also T. reesei synthesizes a range of small bioactive metabolites which are not required for growth, but can broaden the ecological niche of the fungus. Since these bioactive metabolites are not involved in the primary metabolism, they are often referred to as secondary metabolites. These metabolites include some of the most potent toxins of natural source and can therefore have a vast implication on human health and also on the economy if crops are contaminated or livestock affected. Luckily, the high bioactivity for these metabolites also provides a range of beneficial activities and they therefore constitute one of the most important sources for pharmaceuticals; including drugs for treatment of infections, for lowering of the cholesterol level in the bloodstream and for minimizing undesirable immune responses. The primary objective of this study was to gain more knowledge about the genetic mechanisms leading to biosynthesis of two of the most prominent types of secondary metabolites produced by T. reesei, polyketides and non-ribosomal peptides. Since the molecular tools for T. reesei are not as well-developed as for many other species, the study was initiated with creation of new genetic tools that would enable pursuance of the primary objective. The developed molecular tools were assembled into an expression system for high-throughput construction of defined integrated T. reesei mutants and combined inactivation of the non-homologous end joining pathway that facilitates ectopic integration of exposed DNA fragments, and a color maker so that the mutants, in which the substrate had been integrated correct, could be identified by their phenotype. A new bidirectional selective marker system was developed based on the pyr2 gene, involved in the pyrimidine biosynthesis pathway, and was included in the expression system. The developed expression system was subsequently utilized to overexpress several transcription factor genes located in the proximity of polyketide synthase- or non-ribosomal synthetase genes in T. reesei. Overexpression of one of these transcription factor genes led to a significantly increased production of 78 different metabolites, some of which had previously been described and belong to the polyketide derived sorbicillinoids. Genetic deletions performed subsequently, demonstrated that two polyketide synthase genes, located near the overexpressed transcription factor gene, were both essential for biosynthesis of the sorbicillinoids. Hence, genes involved in biosynthesis of this group of polyketides were identified for the first time. Comparative genomics was subsequently used to identify a highly similar polyketide synthase gene cluster in another well-known sorbicillinoid producer, Penicillium chrysogenum. Twenty three T. reesei polyketide synthase- and non-ribosomal peptide synthetase genes were selected for heterologous expression in Aspergillus nidulans, using a previously developed expression system. Several of the resulting mutants exhibited altered phenotypes and could be divided into two groups; one with mutants producing a pigment and another where the mutants had changed colony morphology. Unfortunately, no new metabolite products could be identified with the applied extraction- and analysis methods. Nevertheless, the isolated mutants could constitute an interesting source of knowledge about the T. reesei secondary metabolism after optimization of the applied methods.

General information
Publication status: Published
Organisations: Department of Systems Biology, Eucaryotic Molecular Cell Biology
Contributors: Jørgensen, M. S.
Number of pages: 164
Publication date: 2013

Publication information
Place of publication: Kgs. Lyngby
Publisher: Technical University of Denmark (DTU)
Original language: English
Electronic versions:
Ph.d._afhandling_Mikael_Skaanning_Jrgensen..PDF