An LC-HRMS Based Metabolomics Workflow to Investigate the Sorbicillinoid Biosynthesis in *Trichoderma reesei*

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The role of selected genes which were assumed to be involved in the biosynthetic pathway of polyketides from the group of sorbicillinoids was investigated by means of a metabolomics experiment with LC-HRMS. For this purpose, genetically modified strains of *Trichoderma reesei* were used, in each of which one of the genes of interest was deleted. The knockout strains and a wild type were cultivated on synthetic minimal liquid medium and the culture medium supernatants as well as the mycelia were extracted and measured by LC-HRMS. Since no authentic standards are available for sorbicillinoids, the annotation of the target metabolites was based on accurate mass and the comparison of theoretical and measured natural isotope distribution as well as MS/MS spectra. True fungal metabolites were distinguished from matrix fractions by a global labeling approach, where cultivation was performed on U-^{13}C labeled glucose as the sole source of carbon in the medium and in parallel on native glucose. For a more reliable annotation of the sorbicillinoids sought, MS/MS spectra of all metabolites found were then compared and grouped by similarity to the only MS/MS spectrum of a sorbicillin available in a public spectra database (sorbicillin itself), according to the assumption that related substances or those of a specific biosynthetic pathway should have very similar MS/MS fragment spectra. The sorbicillinoids annotated in this way were quantified in the knockout strains and the wild type and analyzed by univariate statistics in order to be able to determine significant differences in the concentrations, with which it may be possible to deduce the role of the corresponding genes in the synthetic pathway.