Project Ulrich Kück

Engineering of the filamentous fungus Acremonium chrysogenum to convert cephalosporin C into 7-aminocephalosporanic acid by a single enzymatic step

**Background and preliminary work:** The world’s major anti-infective agents are β-lactam antibiotics, with an estimated world market of about 22 billion US$. With a market share of 50%, semisynthetic derivatives of cephalosporin C play an important role in the pharmaceutical industry. Like most β-lactam antibiotics, cephalosporin C is produced by fermentation and the filamentous fungus *Acremonium chrysogenum* is exclusively used for industrial production. The complex cephalosporin C biosynthesis has been well studied. It requires seven enzymes and takes place in different cellular compartments. These enzymes are encoded in two different gene clusters on different chromosomes. Cephalosporin C itself has an antibacterial activity against Gram-positive and Gram-negative bacteria and contains a 7-aminocephalosporanic acid (7-ACA) nucleus, which is of no clinical value. However, 7-ACA is the substrate for the generation of semi-synthetic cephalosporins (e.g. cefazolin). These are produced by modifying the side chains linked to the 7-ACA nucleus. In industrial productions, the 7-ACA nucleus is obtained from cephalosporin C chemically or enzymatically using D-amino acid oxidase and glutaryl-7-aminocephalosporanic acid acylase. Both methods have major disadvantages, for example the production of toxic waste products (chemical process) or the expensive production costs (enzymatic process).

**Work planned:** In order to circumvent these problems, this project is aimed at engineering *A. chrysogenum* strains that are able to convert cephalosporin C to 7-ACA enzymatically in a single step. The optimal expression of bacterial genes in eukaryotic
microorganism for metabolic engineering is a highly challenging approach. In order to achieve this aim, a gene for a modified glutaryl-7-aminocephalosporanic acid acylase (cephalosporin C acylase), which binds specifically and deacylates cephalosporin C more preferentially over glutaryl-7-aminocephalosporanic acid will be used. The gene sequence for the corresponding modified bacterial enzyme was commercially patented and is thus publicly available. The prerequisites for metabolic engineering of A. chrysogenum by genome editing have been established. As a prerequisite for genome editing, functional genomic approaches based on non-homologous end joining were used in several projects with diverse β-lactam producers. Further, tools were developed for targeted gene disruption/deletion for functional analysis and marker recycling, using the FLP/FRT recombination system. The experimental objective of the project will be to obtain a cephalosporin C acylase that is functional in A. chrysogenum. To this end a codon-optimized variant of the modified bacterial cephalosporin C acylase will be synthesized and recombinant A. chrysogenum strains constructed that express the mutated gene. Growth conditions will be optimized for cephalosporin acylase and 7-ACA biosynthesis. Finally 7-ACA will be isolated and purified from the fungal host for further enzymatic analysis.

**Selected references:**


Terfehr D, Dahlmann T, Kück U (2017) Transcriptome analysis of the two unrelated fungal β-lactam producers *Acremonium chrysogenum* and *Penicillium chrysogenum*: Velvet-regulated genes are major targets during conventional strain improvement programs. BMC Genomics. DOI: 10.1186/s12864-017-3663-0

Terfehr D, Kück U (2017) Deactivation of the autotrophic sulfate assimilation pathway reduces substantially high level β-lactam antibiotic biosynthesis and arthrospore formation in a production strain from *Acremonium chrysogenum*. Microbiology 163:817-828