EPOS

Enzyme production in optimized streptomyces

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The demand for high level production of industrial enzymes and for the development of suitable production platforms is growing continuously. Streptomycetes are a rich source of industrial-relevant enzymes and are potentially very attractive hosts for the production of those enzymes.

The hosts for enzyme production are Streptomyces lividans 1326 and strains derived from this wild type by genome engineering. Reporters for enzyme expression levels are secreted enzymes, such as xylanase, cellulase and tyrosinase.

One of the aims of the consortium is to develop a new generation of expression vectors. A prototype has been constructed that is based on a toxin-anti-toxin system and therefore does not require antibiotics to maintain the vectors in the host.

For high level expression, several promoters have been isolated that show a higher transcription activity than the promoter sequences available at the moment in Streptomyces. Furthermore, translation efficiency is enhanced by modification of the untranslated region of the mRNA and by selection of high performance ribosome-binding sites. The last phase in the development of a stable expression vector is to combine all these features into a single expression vector, and this process is currently underway. Following their expression, enzymes should be protected as much as possible against degradation by cytoplasmic and/or extracellular proteases. Since the extracellular proteolytic activity of the selected host, (Streptomyces lividans) is relatively low, we focussed on intracellular degradation.

A new tagging system in Streptomyces – Pupylation, which directs tagged proteins for degradation to the 20S proteasome – was studied in detail.

The on-going study aims at identifying the role of this system in protein degradation during the over-expression of enzymes and how the integrity of the expressed enzyme can be secured. In the work package on strain development, we reduced the clump size of the mycelium in liquid cultures.

The targets for engineering are the SALP family (SsgA Like Proteins) and enzymes involved in tip extension. The aim is to improve on the two-fold increase in enzyme production that has already been obtained. The final production strains will be analyzed by RNA-seq to determine if the production strains have acquired limitations at the genome expression level and need further strain development.

The final goal of EPOS – the delivery of a new production platform based on the combination of a S. lividans-derived host and expression vectors capable of industrial-level protein production and secretion – will be demonstrated in the last phase of the project, using industrial-relevant enzymes.

The EPOS consortium aims at developing Streptomyces lividans as a production platform, which will include new and stable expression vectors. To reach these objectives, the research groups led by the following experts in the field have joined forces in EPOS: Dr. Ramon Santamaria (Spain, expression vectors), Dr. Jean-Luc Pernodet and Dr. Philippe Mazodier (France, genetics and proteases), Professor Jozef Anné (Belgium, secretion and "omics"), Professor Gilles van Wezel and Dr. Erik Vijgenboom (The Netherlands, coordinators, morphology and expression). The Proteonic B.V. company (The Netherlands) is responsible for translation.

This international consortium brings together the latest know-how needed to tackle the sub-optimal elements in existing enzyme production, related in particular to expression (transcription & translation), secretion (pathways and signals), and the stability of the expressed protein (protease activity), as well as to the organism itself (morphological engineering). The research within EPOS is still on-going and will continue until October 2013 for the coordinators, January 2013 for the French partners and September 2012 for the other members.

S. lividans expression system