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Controlling growth and morphogenesis of the industrial enzyme producer Streptomyces lividans

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Chapter 1

Introduction

Streptomyces are Gram-positive, soil dwelling bacteria that raised interest in the last 50 years for their high potential in antibiotic (Hopwood 2007) and protein production (Vrancken and Anné 2009; Anné et al. 2012). The work presented in this thesis is part of a project funded by the European Research Area for Industrial Biotechnology (ERA-IB) under the acronym EPOS (Enzyme Production in Optimized *Streptomyces*). According to the ERA-IB, the definition of Industrial Biotechnology is "the application of biotechnology for the environmentally-friendly production and processing of chemicals, pharmaceuticals, materials and bio-energy", thus using biological catalysts (enzymes) instead of the classical chemical processes. It therefore aims at sustainable production of goods, with less dependency on non-renewable fossil resources.

The number and amount of enzymes needed for industrial applications has been increasing steadily in the last years. The preferred production platforms are fungi and bacteria, with a predominant role of *Escherichia coli* and *Bacillus* spp. in the second category. The aim of this project is to extend the currently available range of enzyme production platforms by optimizing *Streptomyces* and, in particular, the best protein producer *Streptomyces lividans*.

Thanks to their saprophytic nature, streptomycetes secrete a massive amount of industrial enzymes. They have a relatively low level of endogenous extracellular proteolytic activity when compared to other expression hosts (e.g. *Bacillus*), they are generally more suited to produce proteins encoded by high G+C actinomycete genes in their native form, coupled to efficient secretion so as to avoid that the proteins end up in inclusion bodies (often a problem when using e.g. *E. coli*) and making downstream processes easier. Despite their attractive potential, *Streptomyces* present several constraints which so far limit their application in industry. The first constraint is morphology: by growing as a network of hyphae, they produce dense pellets in liquid cultures that hold *Streptomyces* back from being one of the first choice cell factories in large scale fermentations. In addition, the limited availability of efficient expression systems for high-level transcription/translation and subsequent secretion is a further bottleneck. A review on recent developments in strain improvement from

the perspective of a host-vector optimization is presented in **Chapter 2**.

A rational morphological engineering by addressing proteins involved in growth and morphology had proven successful in the past. The overexpression of SsgA, an activator of cell division, led to a fragmenting phenotype with higher growth rate and protein secretion when compared to the wild type (van Wezel et al. 2006). Following this idea, genes involved in growth and development where identified and the related mutant strains analyzed. In particular, a cellulose-like protein CslA and a copper oxidase GlxA were shown to be functional partners in the process of synthesis and accumulation of an extracellular, chitin-like glycan at hyphal tips, whose absence leads to disruption of pellet aggregation in liquid cultures. These findings, presented in **Chapter 3**, allowed identifying new potentially interesting strains for protein production and expanding further our understanding of the processes and contributors in bacterial morphogenesis.

To further analyze the morphology and physiology of these strains, we took advantage of the next generation sequencing technologies applied to the transcriptome, such as RNA-Seq. **Chapter 4** presents an overview of the expression state at mRNA level of the *glxA* and *cslA* mutants compared to wild type, providing potential novel hints for future engineering.

In addition to strain improvement, the design of an efficient expression vector remains to be solved. We addressed this issue by identifying new strong promoters to drive high levels of transcription in our system. In **Chapter 5** a new pipeline is described, starting from the identification of strong constitutive promoters from RNA-Seq and microarray data, screening via a newly optimized Lux system and validation with the production of a small laccase (SLAC) from *S. coelicolor*. This allowed us to expand the list of available strong promoters and optimizing/developing new reporter systems.

The success of SsgA for morphological engineering and strain design also prompted analysis of the SsgA-like proteins (SALPs) in *S. lividans*, as a primer towards future rational strain engineering approaches. Genes for SALPs other than SsgA were deleted and the effect on *S. lividans* tested in terms of phenotype and

enzyme production. The results are presented in **Chapter 6**.

A general discussion is presented in **Chapter 7**, with reflections on how the experiments presented in this thesis – combined with the current state of the art – can provide insight on how to obtain better *Streptomyces* production hosts.