# Multi-omics studies of the control of growth and antibiotic production of streptomyces

Du, C.

# Chapter 1

## Introduction

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Since the discovery of penicillin by Alexander Fleming in 1928 (Fleming, 1929), numerous lives have been saved from bacterial infections by the use of antibiotics in the clinic. However, as Fleming already predicted, antibiotic resistance emerged with the increased usage of antibiotics. At this moment in time, bacterial infections are once again a huge threat to human health (WHO, 2014). Bioactive natural products (NPs) are essential weapons in the battle between human and drug-resistant bacteria, and new drugs are constantly needed. The vast majority of the NPs are produced by microbes and have a wide variety of chemical skeletons, including polyketides synthesized by polyketide synthases (PKS), complex peptides produced by non-ribosomal peptide synthases (NRPS) or ribosomally produced and post-translationally modified peptides (RiPPs), terpenes or aminoglycosides. After the initial boom in antibiotic discovery in the so-called golden age (roughly 1950-1970), there has been a sharp decline in the number of new antibiotics discovered (Cooper & Shlaes, 2011, Baltz, 2008). This is largely due to replication in the search of novel NPs, in other words many of the compounds that are found have already been seen before. It has led to a substantial reduction in the return of investment of high-throughput screening, while at the same time synthetic antibiotics have failed to meet the needs (Kolter & van Wezel, 2016). Hence, there is an urgent need for new strategies to discover novel antimicrobial compounds from nature. To this end, a fundamental, systems-based understanding of the biosynthesis and regulation of antibiotic production is required.

Most antibiotics are secondary metabolites produced by microbes, and they are often produced in low amounts. Actinobacteria are the producers of approximately two-thirds of all known antibiotics and many other medical relevant NPs (Barka *et al.*, 2016, Hopwood, 2007). Actinobacteria have a complex mycelial life cycle that is unique as compared to other bacteria. It starts with the germination of a spore that grows out to form vegetative hyphae via tip extension and branching (Barka *et al.*, 2016, Chater & Losick, 1997). When the environmental situation requires reproduction, for example due to nutrient starvation, actinomycetes start developing areal hyphae, which eventually differentiate into chains of unigenomic spores (Claessen *et al.*, 2014, Flärdh & Buttner, 2009). The production of antibiotics correlates temporally to the developmental growth phase (Bibb, 2005, van der Heul *et al.*, 2018).

The production of antibiotics is often facilitated by a group of proteins encoded by several genes clustered in large gene cluster, which we call a biosynthetic gene cluster (BGC). The BGCs of Actinobacteria can be 100 kb or even larger in size, encoding both core enzymes and those carrying out the decorating steps, as well as resistance and transport genes (Cimermancic *et al.*, 2014). Each type of NP thereby has its own unique features that can be used to find and predict new BGCs specifying similar molecules. Bioinformaticians have made and are still improving

*in-silico* tools that are able to predict the possible BGCs from genome sequences, utilising the information extracted from known BGCs (Blin *et al.*, 2017, Skinnider *et al.*, 2016, Rottig *et al.*, 2011, Agrawal *et al.*, 2017). Harnessing the power of the artificial intelligence boom across every aspect of human life, these tools provide more information about predicted gene clusters and their likely product(s), whereby prediction accuracy is continuously being improved. The development of these *in-silico* tools combined with the rapidly increasing number of available genomes have shown that the ability of Actinobacteria to produce bioactive NPs have been grossly underestimated (Hopwood, 2007, Fedorova *et al.*, 2012).

One way to explore the hidden treasures from Actinobacteria is to try to understand the biosynthetic cell factory as a whole system and tackle the cellular machineries which are blocking the biosynthesis of interesting compounds. It is highly likely that a large part of the NP repository is expressed under specific environmental conditions, responding to interactions with other microbes and higher organisms, as well as to biotic and abiotic stresses (Seipke *et al.*, 2012, van der Meij *et al.*, 2017). At the same time, there are non-traditional NPs like peptidic NPs, which were not a focus in traditional screening methods but may still prove very valuable. Exploring their potential requires a systems-level investigation of the regulation network of the target Actinobacteria, requiring different 'omics' technologies. Proteomics technologies, which directly study the global protein levels of metabolic enzymes and other proteins, are becoming increasingly important in these studies. A systematic review of utilizing systems biology, especially proteomics related methods and results, in microbial NP research is provided in **Chapter 2**. In this review, it is discussed how recent advances in genomics, metabolomics and bioinformatics have helped NP discovery, with a particular focus on the application of advanced proteomics, including both biased and unbiased proteomics pipelines.

In **Chapter 3**, natural product proteomining, an important NP research pipelines developed in our laboratory (Gubbens *et al.*, 2014), was put into practice. This chapter includes two studies that both achieved fluctuations in the metabolome of a specific target strain. A workflow combining metabolomics and bioinformatics allowed us to connect a compound to its cognate BGC, namely a prenylated isatin antibiotic in *Streptomyces* sp. MBT28 and C-glycosyl-pyranonaphthoquinones in *Streptomyces roseifaciens* (MBT76). Their corresponding BGCs were confirmed by proteomics and mutagenesis studies, respectively.

In **Chapter 4**, elicitors were added to enforce fluctuation of the secondary metabolome of the gifted NP producer *S. roseifaciens*, and the concomitant changes in the protein levels analysed using high coverage full proteomics studies. We aimed to provide an overview of whole proteomics response patterns of different types of small molecules in *S. roseifaciens*. The elicitors used in this study

should mimic the environmental factors present in the natural habitat of *Streptomyces*. These molecules we used included plant hormones, oligosaccharides and pathogen-related compounds. The results show that the response to these molecules can be categorized into three main clusters, which not necessarily corresponded to the elicited antimicrobial activity. We then focused specifically on the effect of the addition of the plant hormone jasmonic acid via a growth phase-dependent proteomics study. A jasmonic acid response (*jar*) gene cluster was found that related to the antimicrobial activity of *S. roseifaciens*.

A different method in activating the expression of cryptic BGCs is heterologous gene expression. This method has the advantage of allowing highly modulated expression and simplified identification. However, to achieve this goal, an optimised production host suitable for the efficient production of NPs is required. In **Chapter 5**, the background-reduced host *Streptomyces coelicolor* M1152 (Gomez-Escribano & Bibb, 2011) was investigated using quantitative proteomics. The effects caused by the optimization in this strain on growth and production of *S. coelicolor*, including the removal of major native BGCs and introduction of an additional *rpoB*[C1298T] mutation, were analysed by comparing *S. coelicolor* M1152 to its parent *S. coelicolor* M145. This revealed interesting differences that might provide guidance in future strain optimization of *S. coelicolor* and other *Streptomyces* hosts, including a delayed response of members of the PhoP regulon and enhanced production of ectoine biosynthetic enzymes, which relate to stress responses.

Antibiotic production correlates temporally to the onset of development (Bibb, 2005, van der Heul *et al.*, 2018). In order to systematically investigate the regulation of NP production in Actinobacteria, more knowledge on the global control of development is important. In **Chapter 6**, we describe the discovery of a new family of DNA binding proteins, represented by SCO1839. SCO1839 is a nucleoid associated protein (NAP) that belongs to a family of Actinobacteria-specific regulators. The protein is expressed in the later stages of development and is highly abundant in spores. We found that the protein binds specifically to a small DNA motif centred around the palindromic sequence GATC. ChIP-Seq experiments revealed that SCO1839 binds to 1000s of sites on the chromosome and may affect the expression of some 10% of all genes in *S. coelicolor*. A positive feedback loop between SCO1839 and the global antibiotic activator AtrA was also proposed in this study.

A summary of the most important discoveries of this thesis is provided in **Chapter 7**, as well as a brief discussion and future perspectives.