CHAPTER I

GENERAL INTRODUCTION

Streptomyces are fascinating bacteria with a complex multicellular life cycle, which display beautifully diverse morphologies. Though predominantly considered to be soil dwellers, they are found in most environmental niches including aquatic habitats and in endophytic interactions (van der Meij *et al.*, 2017). Their life cycle begins with the germination of a single spore which expands into a scavenging network of vegetative hyphae. Stress conditions, such as nutrient depletion, initiate development; this morphological differentiation leads to the creation of aerial hyphae which ultimately form chains of spores that can then be dispersed to new habitats. The onset of development is closely linked to and coincides with the production of antibiotics and other bioactive molecules (Bibb, 2005; van Wezel & McDowall, 2011). Indeed, streptomycetes are avid producers of enzymes and secondary metabolites, including over 50% of all clinical antibiotics, which makes them highly interesting for medical, biotechnological and industrial purposes (Bérdy, 2005; Hopwood, 2007). However, a large portion of the biosynthetic gene clusters responsible for the production of antibiotics are poorly expressed under standard laboratory conditions.

A complicated and intertwining network of sensory and regulatory proteins is involved in the control of primary metabolism, development and antibiotic production in response to a multitude of stimuli and stressors occurring in the heterologous environments that streptomycetes live in. Identifying the triggers that activate secondary metabolism is of utmost importance for screening purposes. Thus we need to unravel the regulatory mechanisms that link biosynthesis of antibiotics, development and growth to the responses to biotic and abiotic changes in the environment. In this thesis, I have explored how the model organism *Streptomyces coelicolor* senses environmental signals and cues, and translates these into appropriate responses.

SENSING THE SURROUNDINGS

The aminosugar *N*-acetylglucosamine (GlcNAc) differentially regulates the growth, development and antibiotic production of *S. coelicolor* under different environmental conditions. The presence of this pleiotropic signal in a nutritionally poor environment, in contrast to a rich one, activates antibiotic production and development (Rigali *et al.*, 2008). Presumably, the presence of GlcNAc in a rich environment is interpreted as nutritional abundance, in the form of GlcNAc-polymer chitin, and in a nutrient-depleted environment it is perceived as a signal of development. Monomeric GlcNAc, from the cell wall's peptidoglycan, is released into the environment at the onset of development due to the autolytic degradation of part of the vegetative hyphae which provides the resources for the building of the aerial mycelium. GlcNAc is transported via the phosphoenolpyruvate-dependent phosphotransferase system (PTS) that simultaneously phosphorylates it to *N*-acetylglucosamine 6-phosphate (GlcNAc-6P), which is then deacetylated by NagA to form glucosamine-6P (GlcN-6P) (Nothaft *et al.*, 2010; Świątek *et al.*, 2012a). These phosphosugars are important in the GlcNAc activation of antibiotic production; both metabolic intermediates inhibit the ability of global pleiotropic regulator DasR from repressing antibiotic production (Tenconi *et al.*, 2015b; Rigali *et al.*, 2008; Rigali *et al.*, 2006).

The control of antibiotic production, development and other essential process is not limited to the regulatory power exerted by DasR. In addition to DasR, at least a handful of other regulatory proteins have been implicated in the control aminosugar utilisation, including AtrA and Rok7B7 (Nothaft *et al.*, 2010; Swiatek *et al.*, 2013; van Wezel & McDowall, 2011; Urem *et al.*, 2016a). In fact, the *Streptomyces coelicolor* genome alone encodes around 700 proteins with a predicted regulatory function (Bentley *et al.*, 2002), many of which regulate development and secondary metabolism in response to environmental conditions. Chapter

II reviews the global regulators that control antibiotic production and development, with emphasis on (amino)sugar-related nutrient sensory pathways, and explores the complexity of these regulatory networks given their antagonistic and/or cooperative behaviour and the extensive cross-talk amongst them.

Responding to stress

Streptomycetes require quick sensing machinery and adaptive responses to survive the fluctuating landscape of their environments. Streptomycetes may suffer from oxygen depletion as a result of environmental hypoxia or due to poor oxygen transfer within densely grown mycelia (van Dissel *et al.*, 2014). To survive in low oxygen conditions, the aerobic *Streptomyces* must active its stress responses and anaerobic respiration pathways to survive (Fischer *et al.*, 2010; Fischer *et al.*, 2014; van Keulen *et al.*, 2007). Two-component systems (TCS) are important bacterial mechanisms for sensing and responding to environmental changes and stressors; a signal is detected by the sensory kinase (SK) which then phosphorylates its cognate response regulator (RR) to activate the appropriate response.

Chapter III describes the function of the novel TCS pair OsdRK (SCO0203-0204) that senses and responds to oxygen stress, and links this to the control of development. The depletion of oxygen is likely sensed by OsdK, which shares high similarity with the signal recognition domains of the sensory kinases DosT/DevS, responsible for the sensing of oxygen depletion in *Mycobacterium tuberculosis* (Honaker et al., 2009; Sousa et al., 2007). The response regulator OsdR recognises and binds targets of the *M. tuberculosis* dormancy regulator DevR, which recognises a binding site very similar to that of OsdR (Chauhan *et al.*, 2011). OsdR is phosphorylated by OsdK and in response positively regulates genes involved in stress response, anaerobic respiration and development. The regulon of OsdR is described in detail in this Chapter.

New proteins in aminosugar metabolism

With improved understanding of the regulatory networks controlling antibiotic production and development, it becomes possible to genetically manipulate strains. To exploit the inhibitory effect of GlcNAc metabolic intermediates on the DasR repression of antibiotic production, metabolic engineering efforts involved the creation of metabolic mutants that accumulate GlcN-6P and/or GlcNAc-6P. The *S. coelicolor nagA* deletion mutant, that accumulates GlcNAc-6P, had increased antibiotic production when grown on GlcNAc (Świątek *et al.*, 2012a; Świątek *et al.*, 2012b). The *nagB* deletion mutant accumulates GlcN-6P when grown in the presence of either GlcNAc or its deacetylated form glucosamine (GlcN), and as a consequence fails to grow. Selecting for suppressor mutants of the *nagB* deletion mutant, that relieve the aminosugar sensitivity, previously identified novel enzymes and regulators of aminosugar metabolism (Swiatek, 2012).

Phospho(amino)sugar isomerase SCO4393

The suppressor mutant analysis lead to the discovery of SCO4393, a phosphosugar isomerase not previously associated with aminosugar metabolism. Mutation of SCO4393 in *S. coelicolor* relieved the toxicity of both GlcN and GlcNAc to *nagB* mutants. This strongly suggests that SCO4393 plays a role in synthesizing the toxic intermediate that accumulates in *nagB* mutants when grown on GlcN or GlcNAc. Functional and structural studies of SCO4393 are described in Chapter IV. The crystal structure of SCO4393 was resolved and strongly suggests that the substrate is a phosphoaminosugar. Binding studies

identified GlcNAc-6P as a substrate, in contrast to related sugars GlcNAc, GlcN-6P, Fru-6P or GlcNAc-1P.

UNDERSTANDING GLCN METABOLISM

A suppressor mutant screen, specifically aimed at identifying novel components of GlcN metabolism, is described in Chapter V. The surprising role of GlcNAc metabolic genes in GlcN metabolism is explored further and also shown is how the toxicity of only GlcN but not GlcNAc to *nagB* mutants is alleviated by disruption of SCO1447, which encodes ROK-family regulator RokL6. The chapter also highlights how the selection and analysis of suppressor mutants that relieve the aminosugar sensitivity proved an indispensable tool for identifying novel proteins involved in aminosugar metabolism.

RokL6 is likely involved in the regulation of the response to GlcN and its transport and/or metabolism. In Chapter VI, proteomic analysis of the *rokL6* mutant of *S. coelicolor* suggested that GlcN sensing is independent of RokL6 function and that RokL6 regulates development and antibiotic production. Resistance of the *rokL6-nagB* mutant to the anticancer drug 2-deoxyglucose may provide new insights into the connection between glucose and aminosugar metabolism.

Finally, all data and new insights are discussed and summarized in Chapter VII**.**