Chapter 1

General introduction

Although invisible to our eyes, bacteria constitute the largest and most biodiverse domain of life on earth (*1*, *2*). Similar to animals and plants, bacteria also display a broad diversity of morphologies, ranging from unicellular species with different shapes to multicellular groups taking distinct forms and manners of organization (*3*, *4*). The physiology and morphology of bacteria reflect how they adapt to their living environment, offering a gigantic repository for microbiologists to study. Within the diversity of bacteria, *Streptomyces* are particularly noteworthy because of several exceptional features. The filamentous *Streptomyces* are a large genus within the phylum Actinobacteria. *Streptomyces* predominantly live in soil but can also be found in aquatic sediments (*5*, *6*). Different from many other bacteria, *Streptomyces* produce spores that germinate to produce branching vegetative mycelia. Hyphae extend into the soil, growing from their tips, and secrete a large repertoire of proteases, cellulases and chitinases that allow these bacteria to break down insoluble organic materials arising from the decay of fungi, plants and animals. When these resources are exhausted, mycelia undergo a developmental shift from vegetative growth to aerial growth, followed by sporulation leading to another cycle of life (*7–9*) (Fig. 1). This shift is accompanied by the production of an enormous diversity of secreted secondary metabolites (*10*, *11*), including many antimicrobial compounds; indeed, *Streptomyces* antibiotics and antifungals include a majority of the antimicrobials used in clinical practice (*12*, *13*). For example, streptomycin, produced by *Streptomyces griseus*, was one of the first discovered antibiotics in this genus (*14*). Daptomycin which is used as one of the last resort antibiotics is also produced by *Streptomyces roseosporus* (*15*). Although these antimicrobials have important value in human medicine, they are also ecologically important for the bacteria that make them. They provide advantages for ecologically invasion and defense (*16*). But as this thesis will show, antibiotics are metabolically expensive to produce which has led to novel evolutionary strategies to mitigate these costs.

Both because of their multicellular lifestyle as well as their prolific production of secondary metabolites, streptomycetes are of unique fundamental and applied importance. However, an important challenge to continue the commercial exploitation of these organisms is the fact that streptomycetes display enormous genomic instability, an attribute that can lead to the alteration of functions that are relevant to their economic value, especially the production of antibiotics (*17–20*).

Phenotypic heterogeneity due to genomic instability has been recognized for more than half a century (*21*). This was initially identified through observation of frequent loss of certain phenotypes, including formation of aerial hyphae and sporulation, pigmentation, antibiotic resistance and amino acid synthesis. Instability occurs spontaneously at a frequency of higher than 0.1% per spore in numerous species (*22*), while DNA damaging agents can further increase it several-fold (*23*, *24*). This latter aspect suggested that phenotypic changes were caused by underlying genetic changes. Later, the development

of more modern molecular methods (e.g., pulsed-field gel electrophoresis and DNA hybridization) allowed scientists to directly associate phenotypic changes with genomic rearrangements, often revealed to be massive amplifications and deletions to the genome (*25–32*). Studies since the 1980s highlighted hotspots where amplifications tend to arise, named as the amplifiable unit of DNA (AUD) (*33*). These AUD structures exist in many different species and can be amplified to a few to thousands of copies designated as amplified DNA sequences (ADS), which regularly coincide with extensive deletions at the edges of genome (*25–30*). At the beginning of the 21st century, the advances of whole genome sequencing improved our understanding of the linear nature of *Streptomyces* chromosomes (*34*, *35*). The ~9Mb linear chromosome in *Streptomyces* contains a centrally located origin of replication and core functions and two arms on the two sides that contain more dispensable functions. At the end of the two arms, terminal inverted repeats (TIRs) are found that bear covalently bound terminal proteins (TPs). These arms are typically where the gross genomic rearrangements occur (*36*). Other than the general description of the fact that genomic instability arises from extensive genomic rearrangements, the precise mechanisms that either trigger or suppress these diverse phenomena remain unknown, thereby placing limits on our ability to control these processes for commercial gain. Moreover, until the work in this thesis, there has been very little effort to understand these phenomena in evolutionary or ecological terms.

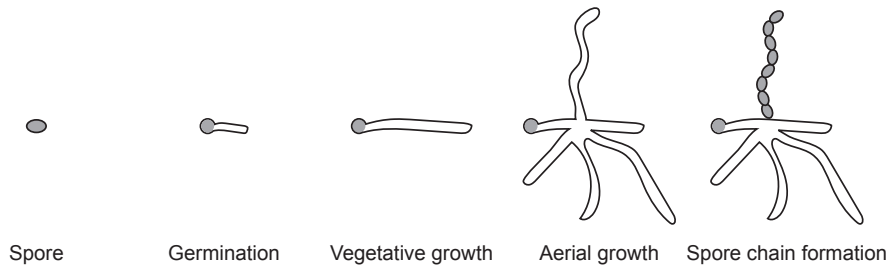

Fig. 1. A schematic representation of the *Streptomyces* life cycle on solid surfaces. A spore germinates to form vegetative mycelia which extend into the substrate to absorb nutrients. Afterwards, aerial mycelia grow out from the vegetative mycelia. During this transition, secondary metabolites including antibiotics are produced for their ecological benefits. Aerial hyphae will eventually form spore chains that stay dormant until the next life cycle.

Beyond earlier studies focusing on commercially important streptomycetes, we have found that bacteria freshly isolated from the soil (*37*) also display genomic instability (Fig. 2). This implies that the genomic instability seen in an industrial setting is not an artifact of this condition and leads to the question of the natural role of the exceptionally high rates of phenotypic heterogeneity and genomic rearrangements in streptomycetes.

More specifically, what are the evolutionary and ecological consequences of genomic instability in these bacteria? Both theory and experiments have shown that bacterial mutation rates can evolve (*38–40*). On the one hand, because most new mutations are likely to be deleterious, natural selection will tend to reduce the mutation rate (*41*, *42*). This occurs by increasing the accuracy and efficiency of mechanisms of DNA replication and repair (*43*). On the other hand, mutations are the ultimate source of adaptive change, thus an increased mutation rate can sometimes be adaptive because this will facilitate the fixation of beneficial mutations (*44*, *45*). This is best exemplified in a medical setting where many bacterial pathogens evolve mutator genotypes, leading to more rapid evolution of antibiotic resistance (*46*). However, the rates of mutation, even in these mutators, are several orders of magnitude lower than genomic instability appears to be in wild-type streptomycetes (*47*, *48*). Why are these bacteria so prone to variation? Does genomic instability offer benefits and in which ecological contexts? And what are the costs of this instability?

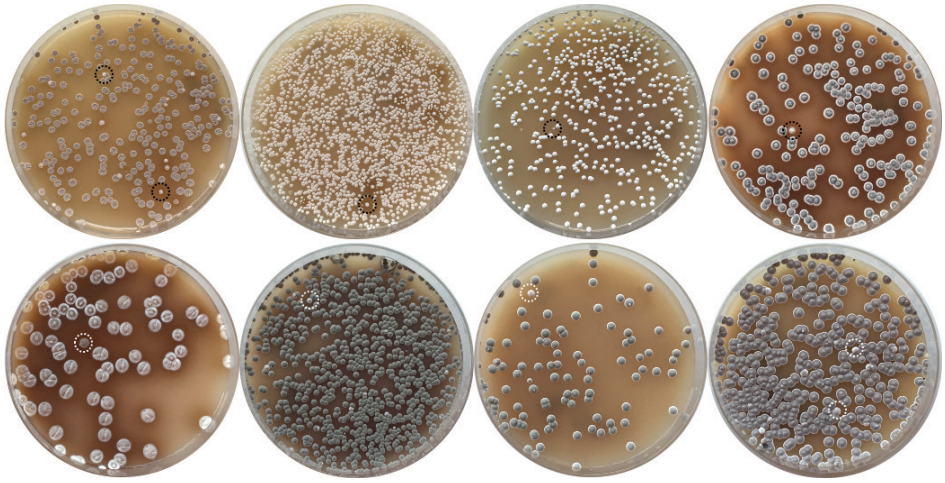

Fig. 2. Genomic instability is common in freshly isolated actinomycetes from soil. Plates show colonies of eight individual actinomycete strains after 3-4 days of growth. While the majority of colonies shows a wild-type morphology, aberrant phenotypes with altered growth and / or pigmentation are present at a high frequency (indicated by white and black cycles).

Understanding genomic instability in streptomycetes therefore sits at the intersection of many important applied and fundamental questions in microbiology. The aim of this thesis is to elucidate the evolutionary functions, mechanisms and consequences of genomic instability in *Streptomyces*, by focusing on the model species *Streptomyces coelicolor*.

Division of labor is used in multicellular organisms to coordinate mutually incompatible functions and to increase group efficiency. It is defined as the situation where individual cells within a body or sub-populations within societies perform complementary tasks to increase fitness of the organism or colony (*49–52*). This definition ideally requires identifying the extent and causes of preexisting phenotypic variation, cooperation and/or altruism and a quantitative estimate of adaptative benefits (*50*). Microbes offer unique opportunities to assess these features, and the last several years have seen a significant increase in cases of microbial division of labor. To understand this concept better, and to frame our later discussion of division of labor in *Streptomyces* colonies, in **Chapter 2**, we review recent articles and discuss causes and implications of division of labor in microbes. First by focusing on multicellularity as a representative example of germ:soma division of labor in *Myxococcus* and *Dictyostelium*, we debate how the idea of caste ratios in social insects can be compared to spore production in microbes and how kin selection can work as a mechanism in maintaining cooperation against cheating. Later, we consider division of labor in patterned multicellular bacteria, using examples from cyanobacteria and streptomycetes. This is followed by a more general discussion of additional possibilities for division of labor in *Streptomyces* and how these types of studies have been performed in other bacteria.

Hyperpigmentation is frequently observed in colony variants of *S. coelicolor* generated due to genomic instability. The few known antibiotics produced by *S. coelicolor* are pigmented. We therefore investigated the hypothesis that instability coordinated a division of labor related to antibiotic production in *S. coelicolor*. Through utilizing diverse techniques from microbial evolution and different omics approaches, **Chapter 3** provides a new insight into how antibiotic production is coordinated in *S. coelicolor* through terminal differentiation of their genome. We show that the emergence of mutants, at a rate of approximately 1%, creates variants that have reduced fitness but elevated amounts and diversity of antibiotics. These changes scale with the size of genome deletions, resulting in a clear trade-off between antibiotic production and fitness. By performing competition experiments in a mixture of mutants and wild-type cells, we confirmed that a division of labor occurs between mutants and wild-type that increases colony-wide fitness.

Since mutants are less fit than their parental wild-type, they are rapidly eliminated in the colony by competition. In **Chapter 4**, in order to learn the genetic fate of mutants after their emergence, we extended our study to a series of transfers to simulate spore-to-spore reproduction happening across colonies. Results show that mutants with initial genome deletions continue to suffer from further deletions. This reveals the same trade-off, as above, namely that continued deletions to both left and right chromosome arms reduce the fitness of strains, by decreasing spore production markedly. Mutants also tend to become mutators which accelerates the base-substitution mutation rate, and therefore the rate of deleterious mutations. Taken together, these results suggest that mutants in

S. coelicolor are similar to sterile castes in social insects. Due to diverse and continuous genomic damage, they are readily eliminated during colony growth and therefore need to be reestablished anew in every developing colony. These data add a new dimension to the idea of mutational meltdown, since gross genomic deletions work together with the emergence of increased mutation rates and competitive declines to guarantee that mutant lineages rapidly go extinct.

In **Chapter 5** we used mass spectrometry-based proteomics and metabolomics to study the effects of genomic rearrangements in *S. coelicolor*. We confirmed that the increased antibacterial activity is caused by overproduction of their antibiotics. More specifically, we observed upregulation of many proteins in three known antibiotic biosynthetic gene clusters. Additionally, several key developmental proteins are downregulated in mutants, leading to their reduced fitness. This chapter provides detailed molecular information of how the trade-off between antibiotics and fitness is mediated by genomic differentiation, which helps us to better understand the division of labor in *S. coelicolor* colonies

Results obtained in the other chapters are discussed in **Chapter 6** as are future perspectives for studying genomic instability in *Streptomyces* and other multicellular bacteria.