Scope of Thesis

The discovery of antibiotics and antibiotic era revolutionized the treatment of infectious diseases worldwide. Majority of antibiotics are isolated from soil bacteria and fungi. In particular, 80% of antibiotics are sourced from the genus *Streptomyces* and rare Actinomycetes, and only 20% are produced by fungal species. Antibiotics are believed to be synthesized in late log phase and stationary phase of the producers. Traditionally, it is considered that secondary metabolites – antibiotics often having very complex structure and are secreted at tail end of the growth under nutrient starvation condition may not contribute to the active growth or survival of the producer. This belief presents a conundrum why would an organism harbor genes for complex molecules and begin excreting in large amounts in nutrient starvation condition. Thus the true biological role of antibiotics for the producer remains inscrutable and mysterious as ever. Reports on second roles of antibiotics apart from 'biological warfare' started emerging during last couple of decades, clearly warranting re-examination of secondary status of these molecules. Several of these studies infer that the world of sub-inhibitory concentration of antibiotics is indeed different from the concentration required for killing. This applies to both - organisms being affected and the organism that produces it. However, the latter effect is not obvious and thus the direct benefit to the producer organism is not fully appreciated.

The results of the present work indicate that chromomycin synthesized by *Streptomyces flavisclerotic*us benefits the physiology, metabolism and survival of its producer at concentration permissive for growth. The study presumably clarifies the 'secondary' status of the secondary metabolite and unambiguously demonstrates the profound importance of chromomycin for survival of the producer in its antioxidant defense arsenal. The results are also consistent with an intimate link between oxidative stress and the life span of a prokaryote.

The thesis is structured into seven chapters.

Chapter 1 – General Introduction: provides insights on the aspects of life cycle of *Streptomyces*, their physiology and production of secondary metabolites. This is then followed by a description of production of chromomycin by *S. flavisclerotic*us.

Chapter 2 summarizes detailed materials and methods used to carry out experiments.

Chapter 3 describes the construction and characterization of genetically verified chromomycin deletion mutant, JP1, of *S. flaviscleroticus* which fails to produce chromomycin. The chromomycin non-producer mutant exhibits the expected phenotype of loss of chromomycin production along with many unexpected and overt phenotypes such as reduced growth rate, complete absence of sporulation, early loss of viability and lack of growth on minimal medium with glucose as the sole carbon source. The complementation of the disrupted biosynthetic genes for chromomycin by plasmid DNA containing the corresponding genes in JP1, rescues all the phenotypes of the mutant.

Chapter 4 addresses the rationale behind early loss of viability in the JP1 mutant. It is reasoned that the early loss of viability of the JP1 in stationary phase may be the result of the generation of reactive oxygen species causing oxidative stress. By multiple ways it is shown that JP1 experiences constitutive oxidative stress.

Chapter 5 provides evidence that JP1 undergoes metabolic rewiring in response to oxidative stress. This chapter also describes inherent preference of *Streptomyces flaviscleroticus* for amino acids over glucose i.e. preference for oxidative metabolism over glycolysis. The chromomycin null mutant is unable to grow on minima media with glucose as sole carbon source because of the additive effects of inherent preference of the wild type for amino acid along with constitutive oxidative stress. Moreover, in response to oxidative stress in JP1 carbon flux of glycolysis is shunted to pentose phosphate pathway to generate more NADPH. Such metabolic rewiring allows JP1 to mitigate the oxidative stress.

Chapter 6 shows *in vitro* antioxidant property of chromomycin indicating its *in vivo* relevance. The supplementation of sub-inhibitory concentration of chromomycin in the growth medium rescues viability defect of JP1 and many of the metabolic alterations in JP1 such as enhancement of the expression and activity of the ATP-PFK, Kreb cycle enzymes and catalase in the stationary phase, resetting the levels of the reducing agent NADPH, and decreasing the intracellular levels of H_2O_2 and ROS.

Chapter 7 describes isolation and partial characterization of chromomycin overproducer mutant of *Streptomyces flaviscleroticus* which overproduces chromomycin by ~176 fold. The overproducer mutant exhibits high level resistance to the aminoglycoside antibiotic apramycin unselectively. The chapter describes likely

mechanisms for the mutation causing single step chromomycin overproduction and apramycin resistance.

Present study provides ample evidence that chromomycin plays important role in the physiology, metabolism and survival of its producer. However, it is limited to study of selected representative enzymes assays. Since oxidative stress and adaptation require the coordinated expression and function of multifarious proteins and small molecules, transcriptomic/proteomic and metabolomic approaches are required to better understand global changes occurring during oxidative stress and after chromomycin treatment in JP1 in comparison to wild type.