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Three approaches were taken in order to study the regulation of actinorhodin:
(1) investigation of the influence of protein tyrosine phosphatase on actinorhodin biosynthesis
(2) modification of regulation on actinorhodin cluster by randomizing the native act II orf4 promoter and
(3) modification of the redox levels inside the cell

SCO3700 is a tyrosine phosphatase

The genome of Streptomyces coelicolor contains 40 kinases and a few annotated phosphatases. Little is understood about their role in regulatory cascades and relation to secondary metabolite production. We have investigated the role of phosphotyrosine-protein phosphatases (PTPs). SCO3700 might be taking over the function of PtpA in SCO ΔPtpA mutant. In SCOM145 exp PtpA mutant onset of Antibiotic synthesis is early compared with wild type while in SCOM145 ΔPtpA mutant onset of UDP synthesis is delayed, while the Actinorhodin synthesis starts at around same time as that of SCO M145

In vitro Tyrosine Phosphatase activity using PNPP as substrate

In vitro Tyrosine Phosphatase activity using PtkA

Antibiotic production is known to be induced when cell experiences stress. Hence, NADH oxidase (Streptomyces pneumoniae), NADH kinase (Saccharomyces cerevisiae) were expressed in Streptomyces coelicolor to study the effect of redox stress on antibiotic synthesis. The constructed strains carrying these enzymes have been assessed for physiological behavior.

Synthetic Promoter Library

In the current study the native promoter of actII orf4 was modified by randomizing the spacer sequence between the -35 box and -10 box and 5 nucleotide before and 5 nucleotides after -35 box and -10 box, respectively (see below). Furthermore, to ensure the stability of RNA the leader sequence was replaces with that of the glycolytic gene pgii2.

Visual screening of ca. 10,000 colonies

200 selected colonies further characterized

Detailed characterization of SPL20

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