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Regulation of secondary metabolism in *Streptomyces coelicolor* A 3(2)

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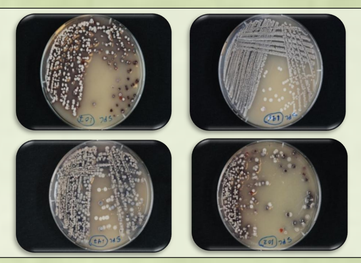
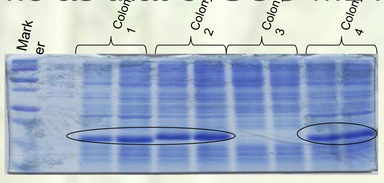
Intro

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

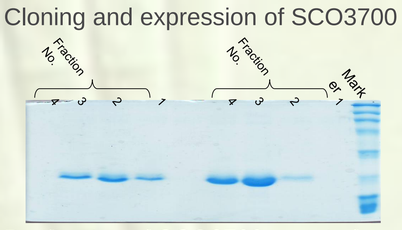


SCO3700 is a tyrosine phosphatase

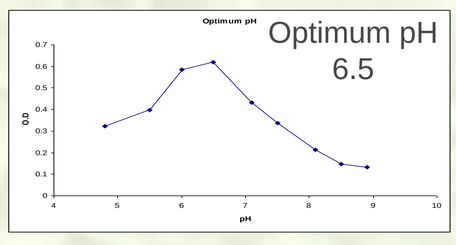
The genome of *Streptomyces coelicolor* contains 40 kinases and a few annotated phosphatases. Little is understood about their role in regulatory cascades and relation to secondary metabolite production. We have investigated the role of phosphotyrosine-protein phosphatases (PTPs). SCO3700 might be taking over the function of PtpA in SCO ΔPtpA mutant. In SCOM145 oxp 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

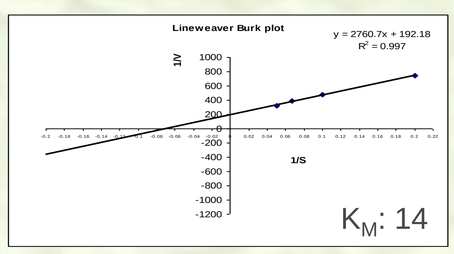


Cloning and expression of SCO3700 E. coli

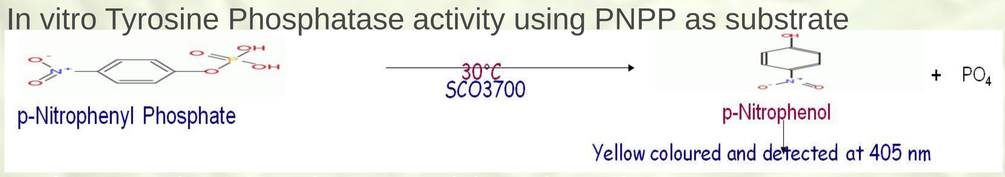


Purification of SCO3700 protein from E.coli

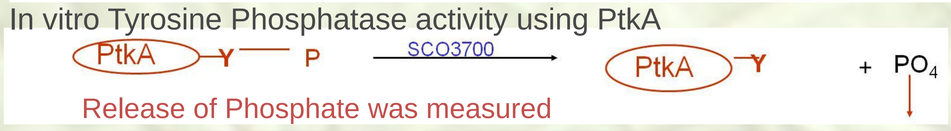




In vitro Tyrosine Phosphatase activity using PNPP as substrate

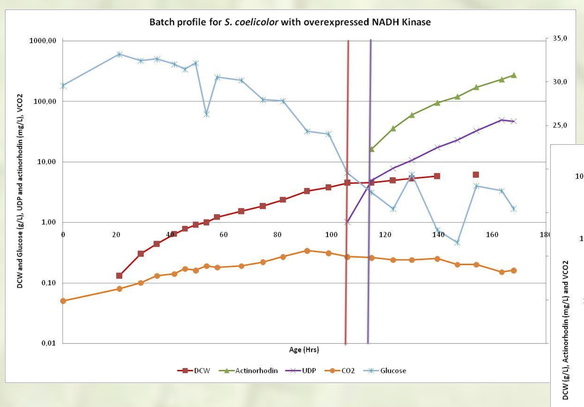


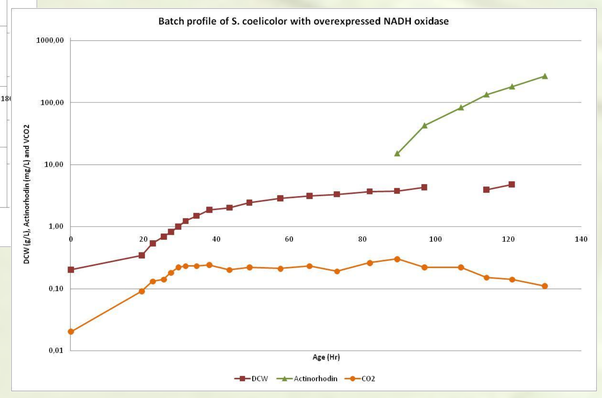
In vitro Tyrosine Phosphatase activity using PtkA



Modification of redox levels

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 pgi2.

-35 -10

TTGTGACGGCAAGCACATTGAAATCTGTTGAGTAGGCCTGTTATT~~GTCGCCC~~
~~CCAGGAGACGGAGAATCTCGACGGGGGCGCAG~~ATGAGATTCAACTTATTGGG
ACGTG

SD

Native *actII ORF4;* The bases that are striked through correspond to the leader sequence that was replaced when generating the syntetic promoters, in blue in figure 3B.

RE site -35 -10

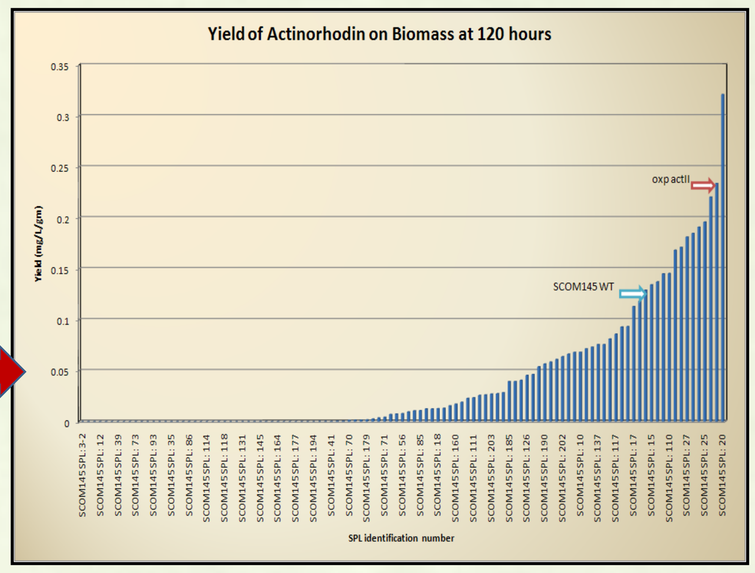
GTCATATGCTTGANNNNNTTGAAANNNNNNNNNNNNNNNNNNTTATTN
NNNNAGCTGTACAGGGGACAGCTGGGACACCCAAGGAAGAAGGCTGACGTC
CGACATGAGATTCAACTTATTGGGACGTG

SD

Synthetic *actII ORF4*

Visual screening of ca 10.000 colonies

200 selected colonies further characterized



Detailed characterization of SPL20

Strain	μexp (h-1)	μUDP (h-1)	μACT (h-1)	r P UDP (mg/L/hr)	r P ACT (mg/L/hr)	-rS (g/g)	Y P/X ACT (g/g)
Sco M145	0.1±0.01	0.06	-	1.5	15.1±1.4	1.8±0.01	0.13
Sco M145 oxp act II orf4	0.12	-	0.12	-	38.1	2.2±0.01	0.23
Sco SPL20	0.13	0.04	0.05	1.6±0.1	**70.9±1.5**	**2.5±0.01**	**0.32**

ACKNOWLEDGEMENTS

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