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To cite this version:

A Millan-Oropeza, C Henry, C Lejeune, M David, M.-J Virolle. A system biology approach revealed the nature and the cause of the different metabolic features of weak and strong antibiotic producers of the Streptomyces genus. FOSBE2019 8th IFAC Conference on Foundations of Systems Biology in Engineering, Oct 2019, Valencia, Spain. 10.1371/journal.pone.0126221 . hal-02383962

HAL Id: hal-02383962

https://hal.science/hal-02383962v1

Submitted on 25 Nov 2020

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A system biology approach revealed the nature and the cause of the different metabolic features of weak and strong antibiotic producers of the *Streptomyces* genus.

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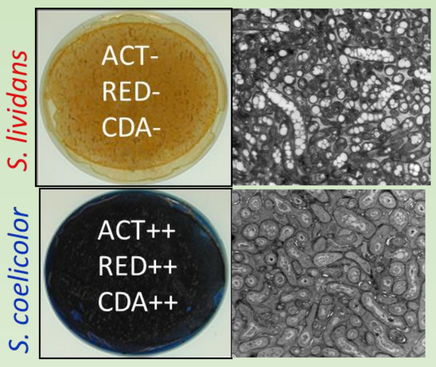
Introduction

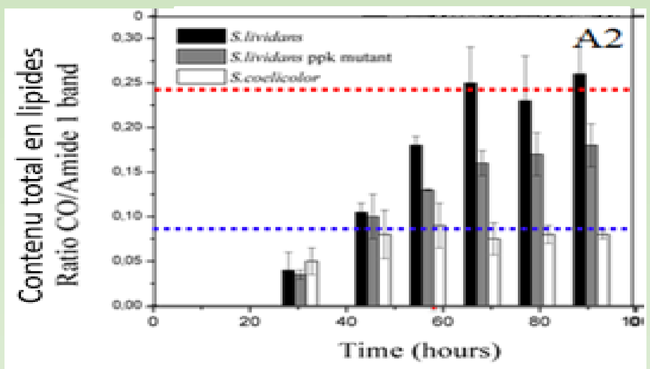
Streptomycetes are efficient producers of chemically diverse bio-active molecules useful to human health including life-saving antibiotics. The biosynthesis of these so called "secondary metabolites" usually occurs in the period of slow or no growth. It is thought to be triggered by nutritional limitations, especially in phosphate, that are correlating with energy shortage. Despites numerous important scientific contributions over the past 40 years, a systemic understanding of the regulation of the biosynthesis and of the function of these bio-active metabolites for the producing bacteria remain incomplete.

The model strains, *Streptomyces lividans* (*SL*) and *Streptomyces coelicolor (SC)*, are extensively studied by the scientific community to address these questions. These closely related species possess identical biosynthetic pathways directing the synthesis of three well characterized secondary metabolites (CDA, RED and ACT) but only *S. coelicolor* expresses them at a high level. A comparative proteomic analysis of these strains, grown on phosphate limited R2YE medium, with glucose as main carbon source, was carried out in order to establish the **specific metabolic features linked to weak (*SL*) and strong (*SC*) antibiotic production and a possible cause of such differences.** Furthermore, a novel view of the **function of the antibiotics for the producing bacteria** in the specific context of their production is proposed.

Results

The ability to produce antibiotics and that to accumulate storage lipids of the TriAcylGlycerol family are anti-correlated in *SL* and *SC*





The two component system PhoR/PhoP is weakly expressed in *S. coelicolor*

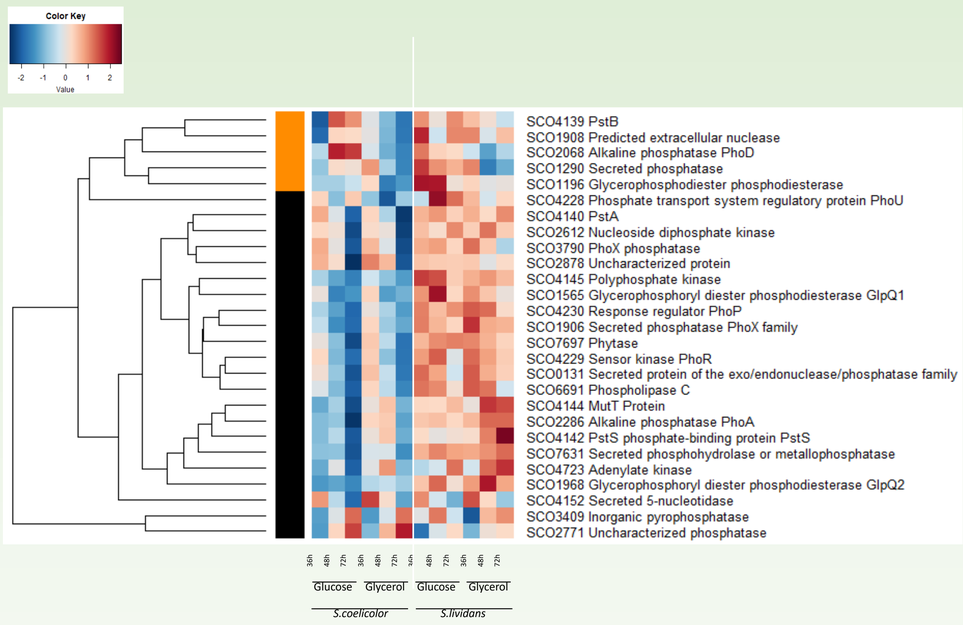


Figure 1: Heatmap representation of proteins of the Pho regulon belonging to phosphate metabolism with significant abundance change (ANOVA, adjusted p value < 0.01) between *S. coelicolor* and *S. lividans* grown on R2YE medium limited in phosphate (1mM) with either glucose or glycerol as main carbon source, for 36, 48 and 72 h at 28°C. The quantification methods are displayed in the vertical bar indicating those proteins quantified by SC (orange) or XIC (black).

The two component system PhoR/PhoP is know to regulate positively phosphate (Pi) scavenging and uptake and negatively nitrogen (N) assimilation (1). Consequently in *SC*, the toning down of PhoR/PhoP regulatory role was correlated with low abundance of proteins involved in Pi scavenging and uptake leading to s**evere Pi limitation** as well as with **high N availability**. High N availability is likely to be responsible for the inhibition of TAG biosynthesis and thus for the low TAG content of this strain (2, 3). Inhibition of TAG biosynthesis is predicted to lead to high acetylCoA availability, that together with high N availability, would fuel the Krebs cycle to support the oxidative metabolism characteristic of this strain (2). The up-regulation of enzymes generating reduced co-factors belonging to the Krebs cycle or to amino acid degradative pathways in *SC* is consistent with the previously reported high ATP content of this strain (2).

In *SC*, the activation of the oxidative metabolism constitutes an attempt for the strain to **restore its energy balance**. To do so most of the acetylCoA, generated by glycolysis or amino acids degradation, will be used to feed the Krebs cycle rather than being stored as TAG.

However, an active oxidative metabolism requires sufficient Pi to generate ATP and even if *SC* was shown to actively consume its polyphosphate stores (2), at some point, it will face a situation of Pi starvation linked to the weak expression of proteins of the Pho regulon involved in Pi supply. Oxidative phosphorylation cannot proceed in this context and should be stopped.
We propose that the benzochromane quinone ACT might fulfill such a function.

ACT biosynthesis occurs in a context of active oxidative metabolism and is correlated with an abrupt drop in the ATP content of *S. coelicolor*

ACT, like other molecules possessing quinone groups (melanine, humic acid …) is proposedto act as electron acceptor reducing electron flow through the respiratory chain and thus respiration efficiency.

The function of ACT would thus reduce ATP synthesis in order to adjust it to low Pi availability.

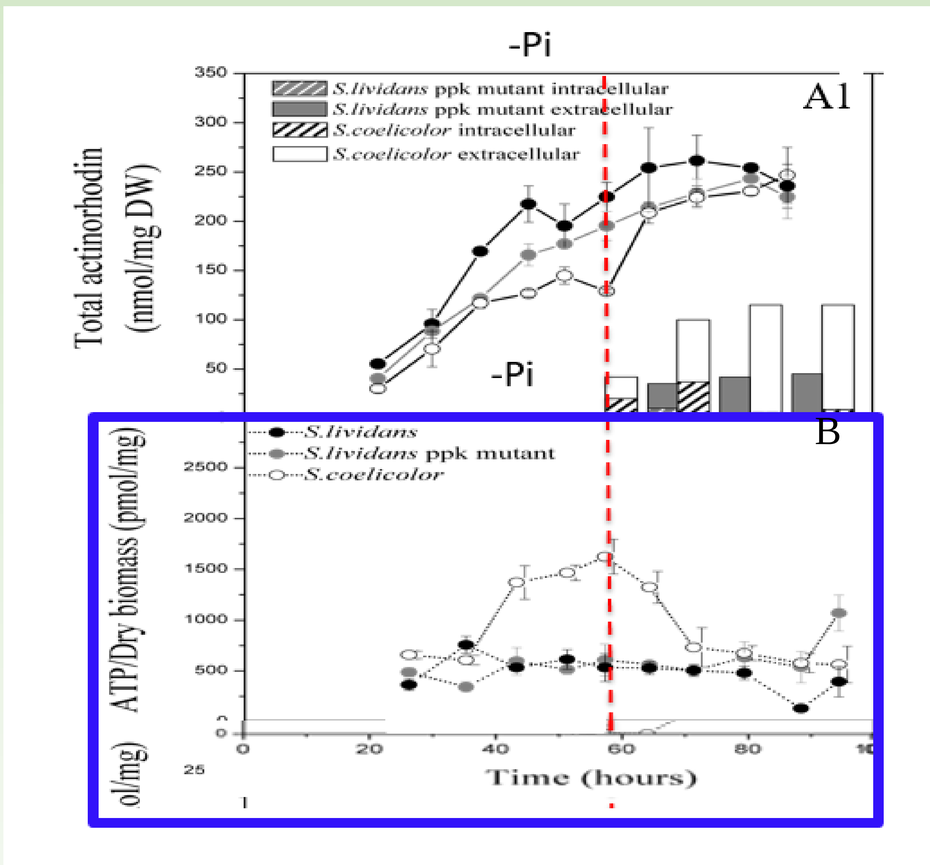


Figure 2: Cultures of *S. lividans*, its *ppk* mutant (3, 5, 6) and *S. coelicolor* grown on R2YE glucose limited in phosphate (1mM). Estimation of growth (continuous lines, mg of dry biomass per plate) of *S. lividans* (black circles), its *ppk* mutant (grey circles) and *S. coelicolor* (white circles), and evolution of the levels of ACT produced extracellularly and intracellularly by the *ppk* mutant of *S. lividans* (grey and grey hatched histograms, respectively) and *S. coelicolor* (plain white and hatched histograms, respectively) (**A**). Quantification of intracellular ATP levels in *S. lividans* (black circles), its *ppk* mutant (grey circles) and *S. coelicolor* (open circles). The error bars represent the standard deviation of at least three independent experiments. (2)

Conclusions

We demonstrated that the ability of *SL* and *SC* to produce antibiotics was anti-correlated with their ability to accumulate TAG (2, 3). The low expression of the two component system in *SC* lead to a severe Pi limitation and thus energy shortage that the strain tries to compensates by activating its oxidative metabolism. The activation of the oxydative metabolism requires the feeding of the Krebs cycle by acetylCoA resulting into a very low TAG content of this strain. TAG and antibiotics biosynthesis compete for common precursors, acetylCoA or its derivatives. This is true for the biosynthesis of polyketide antibiotics (ACT) that are directly using acetylCoA or malonylCoA as precursors but that is also true for that of peptidic antibiotics (CDAand RED). Indeed these are synthesised from amino acids, often derived from intermediates of the Krebs cycle, and acetylCoA is feeding the Krebs cycle for the synthesis of the latters.
Furthermore the produced antibiotics are proposed to play important roles in the regulation of the energetic metabolism of the bacteria in condition of phosphate scarcity. For instance, CDA and RED were proposed to promote cell death and lysis of a fraction of the population, *via* membrane damages, to feed and especially provide phosphate to the surviving one (7). The provided Phosphate would allow the temporary activation of the oxidative metabolism of the strain to re-establish its energy balance. However when phosphate is exhausted, oxydative phosphorylation has to be stopped and ACT was proposed to fulfill such function.

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