IDENTIFICATION OF ROS RELATED BOTTLENECKS IN PIMARICIN BIOSYNTHESIS

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Introduction. *Streptomyces* are Gram-positive bacteria known by their ability to produce a great variety of secondary metabolites with a wide range of biological activities. Although oxygen is indispensable to streptomycetes metabolism, its usage is not costless, due to the formation of the potentially hazardous reactive oxygen species (ROS). In a previous study we have shown a crosstalk between intracellular ROS homeostasis and pimaricin production in *Streptomyces natalensis* ATCC 27448 [1]. The strains defective for the KatA1 and the AhpCD proteins revealed a pimaricin overproducing phenotype, while the strain defective for the SodF protein was a pimaricin underproducer.
This work aims to unveil, at the molecular level, the mechanisms that lie behind this crosstalk. With this purpose, the transcriptomes of the mutant strains CAM.02 (Δ*sodF*) and CAM.04 (Δ*ahpCD*) were compared with the wild-type through an interspecies microarray.

Methods. For the interspecies microarray it was used customized microarray chips (Agilent) containing probes for the genome of the closely related strain *S. avermitilis* MA-4680 and for the *S. natalensis* ATCC 27448 genes whose sequence is publically available. Specific enzymatic activities of glyceraldehyde 3-phosphate dehydrogenase (GAPDH), glucose 6-phosphate dehydrogenase (G6PDH) and valine dehydrogenase (VDH) were determined as previously described [2-4]

Results. The results showed that both mutant strains had the carbon flux preferentially rerouted to the Pentose Phosphate Pathway (PPP), increasing the production of NADPH as a compensatory mechanism against oxidative stress. Moreover, CAM.02 (Δ*sodF*) presented an impaired TCA cycle which may lead to a decrease of the NADH intracellular levels. Consequently, the transcription of the *nuo* operon, encoding the NADH dehydrogenase, was downregulated. Both strains also presented the transcription of phosphate related genes upregulated, although at different growth phases.
Opposite behaviors were observed regarding the branched-chain amino acid (BCAA) metabolism and pimaricin biosynthetic gene cluster transcription: both pathways were downregulated in CAM.02 (Δ*sodF*) strain and upregulated in CAM.04 (Δ*ahpCD*) strain.

Conclusions. Modulation of intracellular ROS levels showed that in addition to the transcription of pimaricin biosynthetic gene cluster, NADPH, ATP levels and BCAA degradation products, are important bottlenecks of pimaricin biosynthesis in *S. natalensis* ATCC 27448.

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