ENGINEERING TACROLIMUS PRODUCTION BY MODULATING THE OXIDATIVE STRESS RESPONSE

Sílvia D.S. Pires [1,2], Pedro Moradas-Ferreira[1,2],Marta V. Mendes[1]
1-IBMC – Instituto de Biologia Molecular e Celular, Universidade do Porto, 4150 Porto; 2-ICBAS – Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto, 4050 Porto;
silvia.pires@ibmc.up.pt

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Introduction. Tacrolimus (FK-506) is a macrolide widely used to prevent graft rejection in organ transplanted patients. The biosynthesis of tacrolimus in *Streptomyces* submerged cultures is controlled by networks regulated by different factors such as dissolved oxygen (DO). Exposure of microorganisms to high levels of DO increases the formation of reactive oxygen species (ROS) that can damage cellular components. To counteract these effects, microorganisms have response mechanisms at different levels, from modulation of gene expression to changes in enzymatic and non-enzymatic activities in order to sense, detoxify and repair the damage caused by ROS. Previously we have shown a crosstalk between intracellular ROS homeostasis and pimaricin production in *Streptomyces natalensis* ATCC 27448 (1).
The objective of this work was to assess the effect of intracellular ROS homeostasis in tacrolimus biosynthesis.

Methods. To induce an alteration in ROS intracellular levels in *S. tsukubaensis* NRRL 18488, mutant strains on the main enzymatic anti-oxidant defences were constructed. Knock-out mutants were obtained using the PCR-targeting system REDIRECT© (2) and over-expression was achieved using the integrative vector, pIB139 (3) that contains the heterologous constitutive promoter *ermE**p. Catalase activity, intracellular H_2O_2 levels and tacrolimus production were determined as previously described (1,4).

Results. The characterization in submerged cultures showed that *S. tsukubaensis* displayed high levels of total catalase specific activity throughout the growth curve. Moreover, a temporal overlap is observed between the growth-phase dependent increase in catalase activity, the decrease in H_2O_2 intracellular levels and the onset of tacrolimus production.
Regarding tacrolimus production, the *S. tsukubaensis* Δ*sodA* and *S. tsukubaensis* pIBkatA1 (overexpressing the monofunctional catalase) strains displayed an overproducing phenotype (a 30% increase on average).
In the Δ*sodA* strain we detected an increased transcription of the tacrolimus pathway specific regulators, of the catalase encoding gene (*katA1*) and the PhoRP regulon. Conversely, pIB*katA1* strain showed a decrease transcription of the genes coding for the TCS PhoRP.

Conclusions. Deletion or over-expression of key genes in the enzymatic anti-oxidant defence system of *S. tsukubaensis* showed that tacrolimus biosynthesis can be modulated by intracellular ROS levels. Together with our previous results regarding pimaricin biosynthesis in *S. natalensis* (1), we provide further evidence for the role of ROS in the regulation of secondary metabolism in *Streptomyces* strains.

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