TRANSCRIPTOMIC APPROACH TO CARBON CATABOLITE REPRESSION OF TACROLIMUS IN *STREPTOMYCES TSUKUBAENSIS*

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Introduction. Tacrolimus (FK506) is a macrolide polyketide with immunosuppressant activity used in the prevention of graft-rejection (1) and, more recently, in the treatment of dermatitis (2). This secondary metabolite acts as a calcineurin inhibitor and is produced by *Streptomyces tsukubaensis.* Production of secondary metabolites is frequently repressed at the transcriptional level by preferred carbon sources, a phenomenon known as carbon catabolite repression (CCR). This mechanism is not completely understood at the molecular level in the genus *Streptomyces* (3) despite its great importance.
The objectives of this work were: 1) to determine the carbon source/s producing CCR on tacrolimus production in *S. tsukubaensis,* and 2) to analyze the effect of this/these carbon source/s at the transcriptional level, including the tacrolimus biosynthetic genes.

Methods. *S. tsukubaensis* was grown in phosphate limited MG-3.2 medium (4), in 500 ml flasks, at 28ºC and 220 rpm. Several sugars were added at the mid-exponential growth phase, at final concentration of 3%. Samples for dry weight, RNA extraction and tacrolimus detection were taken. Tacrolimus was extracted from the samples with methanol, detected by bioassay using *Saccharomyces cerevisiae* TB23 and quantified by HPLC. Custom Agilent microarrays were designed for transcriptomic analyses using the annotated genome sequence (5).

Results. Tacrolimus production was detectable at 112 hours of growth in all cases, except when glucose or glycerol was added. In the last cases no tacrolimus production was detected along the fermentation. Several samples from the glycerol- and glucose-addition fermentations were selected for microarray analysis. These samples covered different culture stages such as the exponential growth phase (in which the sugar is added), the metabolic switch from primary to secondary metabolism and the stationary phase.

Conclusions. Among the sugars tested, only glucose and glycerol are able to arrest tacrolimus production in *S. tsukubaensis*.
From dry weight curves we were able to conclude that such an effect was not due to a growth arrest. Both glucose and glycerol are common effectors of CCR in the genus *Streptomyces* (3). Transcriptomics data are being currently analyzed.

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