SCIENCESPRINGDAY

FACULDADE DE CIÊNCIAS E TECNOLOGIA
UNIVERSIDADE NOVA DE LISBOA

Departamento de Ciências da Vida

Regulation of *ara* gene's expression and sugar-P toxicity in *Bacillus subtilis*

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Objectives

The Gram positive model organism *Bacillus subtilis* comprises at least 13 genes that respond to the sugar signal arabinose. We aim to characterize two genes, *araL* and *araM* belonging to a large *B. subtilis* cluster of genes involved in the catabolism of the pentose arabinose and unveil their biological function.

Elucidation of the regulatory mechanisms involved in *araL* and *araM* gene expression and protein production is an additional goal.

The study of the mechanisms that underlie arabinose-induced cell toxicity caused by sugar-phosphate stress will also be targeted.

Methodology

Amplification of DNA sequences by Polymerase Chain Reaction (PCR) to clone the genes of interest, measurement of gene expression by transcription/translational fusions (Fig. 1) and by RNA analysis (Fig.2), such as real-time PCR (Fig. 3).

Quantification of intracellular protein levels using a specific antibody (Western Blot analysis) and determination of protein function (AraL) by enzymatic activity assays.

Measurements of phosphorylated intracellular metabolites by Nuclear Magnetic Ressonance (NMR) (in collaboration).

Expected Results

Identification of the specific role of AraL (a sugar phosphatase) and AraM (a glycerol 1-phosphate dehydrogenase) in bacterial carbohydrate metabolism.

Establish a correlation between accumulation of sugar-phosphate intermediates and cell toxicity.

Contribute to studies of *B. subtilis* metabolism, which are of special interest due to the extensive utilization of this organism for the production of industrial enzymes.

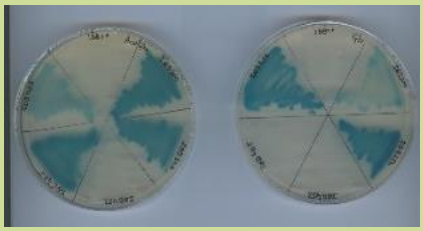

Fig. 1 – Gene expression analysis on solid media – Bacterial strains containing translational fusions of the gene of interest to a reporter gene.

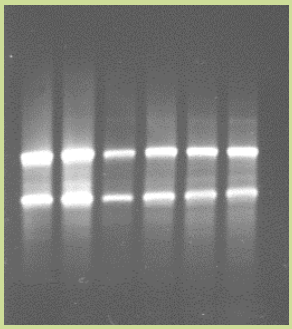

Fig. 2 – RNA analysis – evaluation of total RNA extracted from *B. subtilis* strains on an agarose gel.

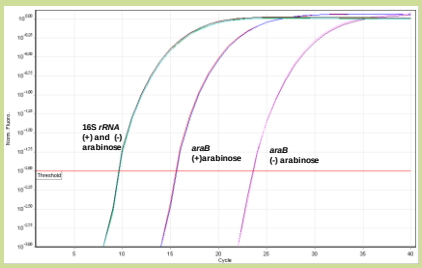

Fig. 3 – Normalized amplification profile of 16S *rRNA* and *araB* cDNA in the presence and absence of the sugar arabinose.

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