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FCT FACULDADE DE CIÊNCIAS E TECNOLOGIA UNIVERSIDADE NOVA DE LISBOA

Departamento de Ciências da Vida – Life Sciences Department

Characterization of *Bacillus subtilis* hemicellulases and sugar transport systems

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Collaboration:

requimte rede de química e tecnologia

Objectives

Bacillus subtilis possesses an enzymatic consortium involved in the degradation of arabinose-containing polysaccharides (Figure 1), playing an important role in plant biomass degradation. Albeit being important, the regulation mechanisms behind the expression of genes encoding endo-arabinanases isn't fully understood. Thus, the mechanisms that modulate gene expression and enzyme production of will be identified.

The uptake of arabinooligosaccharides is accomplished via the ABC-type transporter AraNPQ-MsmX (Figure 2). In this work, the specificity of this transporter to its substrates will be determined. Furthermore, the unusual ability of the ATPase MsmX to interact with multiple transporters will be assessed. In addition, the characterization of new sugar ABC-type transporters in *B. subtilis* will also be targeted.

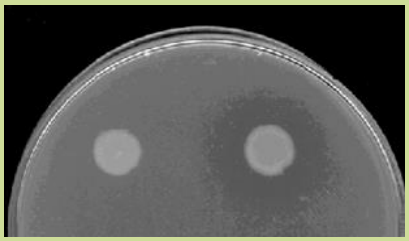

Figure 1 – Arabinan degradation by endo-arabinanases from *B. subtilis*. Growth of a bacterial strain on solid medium containing arabinan. The clear halo is the result of the hydrolytic activity of an endo-arabinanase.

Methodology

To determine the regulation mechanisms of endo-arabinanases, genes encoding putative regulator proteins will be inactivated. To analyze expression levels of endo-arabinanse genes in different metabolic conditions, RT-qPCR technique will be used.

The analysis of the specificity between AraN (the substrate-binding protein of AraNPQ-MsmX) and arabinooligosaccharides will be performed by ITC (Isothermal Titration Calorimetry) and X-ray Crystallography (in collaboration).

Site-directed mutagenesis will be used to determine protein-protein interactions and identify catalytic amino acid residues.

Gene inactivation of putative ABC-type transporters and phenotype analysis will be used to identify and characterize new sugar importers.

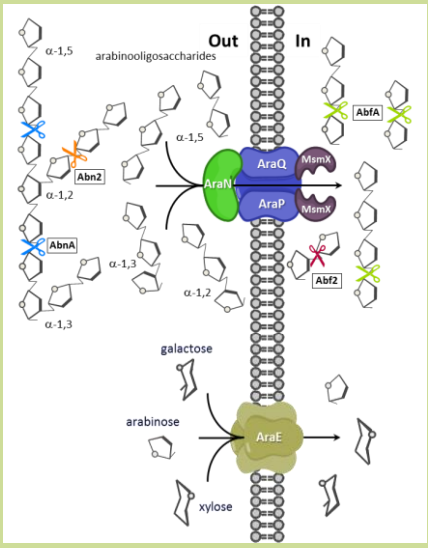

Figure 2 – Transport of arabinan in *B. subtilis*. Arabinan is extracellularly degraded by two endo-arabinanases, AbnA and Abn2. The arabinose and arabinooligosaccharides resulting from arabinan are transported through two different systems. The AraE permease is responsible for the uptake of arabinose (and also xylose and galactose) and the ABC-type importer AraNPQ-MsmX is involved in the uptake of arabinooligosaccharides.

Expected Results

- Identification of signal(s) and protein(s) that modulate the expression of genes encoding endo-arabinanases, and if a regulator protein is involved, determination of the DNA sequences to which it binds in order to repress/activate gene expression.
- Determination of the specificity of the interaction between AraN and the arabinooligosaccharides transported by AraNPQ-MsmX.
- Identification of the amino acid residues important for AraNPQ-MsmX assembly.
- Identification and characterization of new sugar ABC-type importers.

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