M.Sc. Project

Title:

A reverse genomics approach to identify the genes involved in the synthesis of rare sugars produced by bacteria isolated from Antarctica

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Host Laboratory: Cell Physiology & NMR, ITQB-UNL, Oeiras

Duration: 1 year

Introduction

Neotrehalose and its non-reducing glycosylated derivative, gluconeotrehalose, are scarce in nature. The occurrence of neotrehalose has been reported in honey [1], while gluconeotrehalose was recently found in *Carnobacterium* strain 17-4, a bacterium able to grow at cold temperatures [2]. Interestingly, the organisms that produce neotrehalose and consequently the respective biosynthetic pathways remain a mystery.

These metabolites are chemically related with trehalose, a disaccharide that is widespread in Nature and is currently used in a broad range of applications, namely in food, pharmaceutical and cosmetic industries. Therefore, it is important to explore the biotechnological applications of neotrehalose and gluconeotrehalose. Up to date, commercial neotrehalose is obtained by chemical synthesis and gluconeotrehalose has been purified from its natural producer, *Carnobacterium* strain 17-4; however, these strategies result in low yields and high production costs. In this context, the development of efficient producers is mandatory.

The **goals** of the present work plan comprise the: (i) identification of the genes and enzymes involved in the synthesis of gluconeotrehalose and neotrehalose; and (ii) genetic engineering of *Escherichia coli* to produce both sugars at competitive costs.

Using reverse genomics approaches and *Carnobacterium* strain 17-4 biomass, we will discover the genes encoding the enzymes involved in the synthesis of both sugars. Once this information is obtained, we will use the

model organism *Escherichia coli* as a host to produce the heterologous enzymes and hopefully the desired products. This organism is transformed with high efficiency and several commercial vectors for cloning and expression are available. The levels of neotrehalose and gluconeotrehalose produced by the engineered strains will be determined by Nuclear Magnetic Resonance. The efficacy of these compounds to protect model enzymes against heat denaturation, cold stress and dehydration (freeze-drying), will be assessed.

Methodologies

- Protein purification
- Screening of enzyme activities
- Gene cloning and mutant construction
- Nuclear Magnetic Resonance for quantification of the desired end-products

References

[1] Swallow KW, Low NH (1990) Analysis and quantification of the carbohydrates in honey using high-performance liquid chromatography. J Agric Food Chem 38:1828–1832

[2] Lamosa P, Mingote AI, Groudieva T, Klippel B, Egorova K, Jabbour D, Santos H and Antranikian G. (2011) Gluconeotrehalose is the principal organic solute in the psychrotolerant bacterium *Carnobacterium* strain 17-4. Extremophiles, 15:463-472.

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