M.Sc. Project

Title:

Development of microbial cell factories for the production of di-*myo*-inositol phosphate, a compatible solute typical of organisms adapted to hot environments

Supervisors: Dr. Carla Jorge/ Prof. Helena Santos

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

Duration: 1 year

Introduction

Di-*myo*-inositol phosphate (DIP) is an organic solute widespread in microorganisms adapted to hot environments, especially in hyperthermophiles. This compound has never been found in organisms with optimal growth temperatures bellow 60ºC [1]. The observation that the level of DIP generally increases in response to heat stress suggests that this compound has a thermoprotective role in the stabilization of proteins and other cell components. In fact, the superior ability of DIP to protect model proteins against thermal denaturation was amply illustrated by our group [2]. These findings highlight the potential application of DIP as a protein stabilizer in biotechnological processes. Currently, DIP is purified from its natural producer, *Pyrococcus furiosus*, at a high final cost. In this context, it is advantageous to construct efficient DIP producers.

Our group has identified the genes and enzymes involved in the synthesis of DIP [3]. The main goal of the present work is to develop microbial cell factories for the production of DIP at a competitive cost. For that, the genes involved in the synthesis of DIP will be cloned and expressed in model organisms. We selected *Thermus thermophilus* and *Halomonas elongata* as the hosts for the production of DIP. *T. thermophilus* is a thermophilic bacterium with a high efficiency of transformation and several cloning and expression vectors are commercially available. The main advantage of using this organism is that it displays an upper growth temperature (up to 82ºC) closest to the optimal conditions for the activities of the enzymes involved in DIP synthesis. *H.*

elongata is a moderate halophilic bacterium for which the genetic tools are also available. This organism has been used for the industrial production of compatible solutes, namely for ectoine, by a process called “bacterial milking” [4]. In this process, the organism responds to a hypo-osmotic shock by the rapid release of the accumulated solutes. Therefore, solutes are easily separated from the cell mass, lowering the costs of purification. We will assess the level of DIP in the engineered strains by Nuclear Magnetic Resonance.

Methodologies

- Gene cloning and mutant constructions
- Optimization of DIP production in the engineered strains
- Nuclear Magnetic Resonance for DIP quantification

References

[1] H. Santos, P. Lamosa, T. Q. Faria, N. Borges, and C. Neves. (2007) The physiological role, biosynthesis, and mode of action of compatible solutes from (hyper)thermophiles. p. 86-104. In C. Gerday and N. Glandorff (ed.), Physiology and biochemistry of extremophiles. ASM Press, Washington, DC.

[2] T. Q. Faria, A. Mingote, F. Siopa, R. Ventura, C. Maycock, H. Santos. (2008) Design of new enzyme stabilizers inspired by glycosides of hyperthermophilic microorganisms. *Carbohydr. Res.* 343:3025-3033.

[3] M. V. Rodrigues, N. Borges, M. Henriques, P. Lamosa, R. Ventura, C. Fernandes, N. Empadinhas, C. Maycock, M. S. da Costa, H. Santos. (2007) Bifunctional CTP:inositol-1-phosphate cytidylyltransferase/ CDP inositol:inositol-1-phosphate transferase, the key enzyme for di-*myo*-inositol-phosphate synthesis in several (hyper)thermophiles. *J. Bacterol.* 189:5405-5412.

[4] Sauer T, Galinski EA. (1998) Bacterial milking: A novel bioprocess for production of compatible solutes. *Biotechnol. Bioeng.* 57:306-313.

Contacts

Helena Santos (santos@itqb.unl.pt; 214469541)

Carla Jorge (cjorge@itqb.unl.pt; 214469545)