M.Sc. Project

Title: A biocatalytic approach towards the industrial production of mannosylglycerate, a compatible solute with great biotechnological interest

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Duration: 1 year

Mannosylglycerate is a compatible solute present in a variety of marine organisms, especially those inhabiting hot environments [1]. Since its first discovery, many studies demonstrated the capacity of mannosylglycerate to stabilize cellular components such as proteins. Our group demonstrated the ability of mannosylglycerate to protect diverse model proteins against heat denaturation and to prevent protein aggregation, both *in vivo* and *in vitro* [2,3, and our unpublished results]. The potential biotechnological interest of mannosylglycerate is constrained by the lack of an economically viable production process. Recently, Nidetzky and coll. [4,5] found a sucrose phosphorylase that is able to synthesize compounds structurally related to mannosylglycerate, i.e., glucosylglycerol and glucosylglycerate, from sucrose and glycerol or glycerate, respectively. However, the attempts to produce mannosylglycerate with the same enzyme were unsuccessful.

The bacterium *Agrobacterium tumefaciens* accumulates an analogue of sucrose, mannosucrose, which contains a mannosyl group instead of a glucosyl group [6]. The biosynthesis of mannosucrose has been clarified, but the catabolism of this compound remains unknown. In a genomic survey of *A. tumefaciens* we found a gene coding for a protein with high sequence similarity with sucrose phosphorylase that has not been characterized yet. We think that this enzyme is involved in the conversion of mannosucrose into mannose-P and fructose and it may also be able to use mannosucrose and glycerate to produce mannosylglycerate.

The **goals** of the present work plan comprise: (i) cloning, overproduction and purification of the putative sucrose phosphorylase of *A. tumefaciens*; (ii)

biochemical characterization of the enzyme; and (iii) evaluation of mannosylglycerate production and optimization of reaction conditions.

The recombinant enzyme will be produced in *Escherichia coli*. The enzyme will be characterized concerning substrate specificity and kinetic parameters, using Nuclear Magnetic Resonance (NMR).

In parallel, other promising candidates will be examined with respect to mannosylglycerate synthesis, namely a glucan phosphorylase of *Rhodothermus marinus*, a natural producer of mannosylglycerate. The student will be encouraged to search the genome data bases for other potentially useful biocatalysts.

Methodologies

- Molecular Biology techniques for gene cloning and protein production;
- Biochemical characterization of the enzyme;
- Nuclear Magnetic Resonance for quantification of substrates and/or end-products

References

[1] Santos H, da Costa MS (2002) Compatible solutes of organisms that live in hot saline environments. Environ Microbiol 4:501-9

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

[3] Jorge CD, Ventura R, Maycock C, Outeiro TF, Santos H, Costa J (2011) Assessment of the efficacy of solutes from extremophiles on protein aggregation in cell models of Huntington's and Parkinson's diseases. Neurochem Res 36:1005-11

[4] Goedl C, Sawangwan T, Mueller M, Schwarz A (2008) A high-yielding biocatalytic process for the production of 2-O-(α-D-glucopyranosyl)-sn-glycerol, a natural osmolyte and useful moisturizing ingredient. Angew Chem Int Ed 47:10086-9

[5] Sawangwan T, Goedl C, Nidetzky B (2009) Single-step enzymatic synthesis of (R)-2-O-α-D-glucopyranosyl glycerate, a compatible solute from micro-organisms that functions as a protein stabiliser. Org Biomol Chem 7:4267-70

[6] Smith LT, Smith GM, Madkour MA (1990) Osmoregulation in *Agrobacterium tumefaciens*: accumulation of a novel disaccharide is controlled by osmotic strength and glycine betaine. J Bacteriol 172:6849-55

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