technology opportunity

Trehalose phosphorylases useful for the production of trehalose-analogues

Ghent Bio-Energy Valley, a consortium of research laboratories of Ghent University, is seeking partners interested in the industrial and enzymatic production of trehalose-analogues using thermostable trehalose phosphorylases having broad acceptor specificity and high activity.

Introduction

Trehalose analogues have beneficial physicochemical and biological properties. Lactotrehalose, for example -when compared to trehalose-, does not contribute to the caloric content of food preparations as it is not hydrolysed in the intestine. In addition, it is able to lower the metabolic conversion of trehalose as it functions as a competitive inhibitor of trehalase. Unfortunately, trehalose-analogues are not yet produced at an industrial scale as chemical synthesis results in low yields and acceptable enzymatic methods are not yet available.

Technology

Researchers at Ghent University have identified a trehalose phosphorylase from the marine bacterium *Caldanaerobacter subterraneus* that is highly thermostable at 70°C and displays a high activity towards acceptors such as D-glucose, D-xylose, L-arabinose, L-fucose and/or D-galactose. The acceptor specificity of this enzyme and variants of this enzyme was further optimized by the introduction of mutations.

Applications

Enzymatic and/or recombinant methods to produce several trehalose-analogues at elevated temperatures.

Advantages

- Cost-effective and high-yield enzymatic production of several trehalose-analogues
- being able to produce trehalose-analogues at high temperatures avoids microbial contamination

Status of development

The trehalose phosphorylase of the bacterium *Caldanaerobacter subterraneus* has been expressed in E. coli and purified. The synthetic activity of the enzyme was assayed in a buffer at 60°C using glucose-1-phosphate as donor and different acceptors. The acceptor specificity of this enzyme and of a variant enzyme from *Thermoanaerobacter brockii* was optimized by mutagenesis within specific amino-acid coding regions. A production process for lactotrehalose has been developed with the wild-type from *C. subterraneus* obtaining a yield of 75% at 60°C.

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Partnership

UGent is seeking a partner for further validation and upscaling of the present methodology.

Intellectual property

A European application covering a trehalose phosphorylase from *C. subterraneus*, mutated forms of this enzyme and variant trehalose phosphorylases, and uses thereof, was filed on December 14, 2010. A PCT application claiming priority from this European application was filed on December 12, 2011.

Figure

Relative activity of TP from *C. pacificum* (at 60°C and pH 6)

Acceptor (500 mM)	Relative activity (%)
D-Glucose	100
D-Xylose	136
L-Arabinose	109
D-Galactose	115
L-Fucose	109
D-Mannose	< 5
D-Fructose	< 5
D-Glucosamine	< 5

The Inventors

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