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TECHNOLOGY OFFER

Ethanol production from pentoses by yeasts possessing a modified phosphofructokinase

*Institute Jozef Stefan, Slovenia, has developed a gene encoding a modified 6-phosphofructo-1-kinase, the key regulatory enzyme in glycolysis. The modified enzyme enables fermentative pentose sugar utilization and enhanced production of ethanol by the yeast *Saccharomyces cerevisiae*. The researchers are looking for partners in the field of bioethanol production interested in further development of the technology through research or technical cooperation agreements, and/or license agreements.*

Key words: Fermentation, Microbiology, Genetic Engineering, Protein Engineering, Gene Expression, Proteome Research

Glycolysis is one of the central metabolic pathways in all organisms, responsible for generating energy from sugars. In yeast cells, pentose sugars such as xylose and xylitol are first catabolised to products entering the so-called pentose phosphate pathway. After the transformation, fructose-6-phosphate (F6P) is formed and enters glycolysis. The enzyme phosphofructokinase (PFK) has an important regulatory function as it catalyses the transformation of F6P to the next intermediate, fructose-1,6-bisphosphate (F-1,6-BP), using adenosine triphosphate (ATP) in the reaction. PFK is stimulated by a further intermediate, fructose-2,6-bisphosphate (F-2,6-BP), whereas citrate and ATP act as strong inhibitors.

Experiments have shown that the native PFK has a low activity and low maximal velocity when pentose

sugars are used as substrates for the yeast. The technology solves this problem by introducing gene fragments encoding modified PFK enzymes, all of which show higher activity compared to the native enzyme. The activator, F-2,6-BP, causes a marked increase in the maximal velocity of the modified PFK whereas no such effect could be recorded with the native enzyme. This is particularly relevant in situations where levels of F6P are low, in which case the F6P cannot be metabolized with the native PFK, but it is successfully metabolized by the modified PFK. Consequently, the yeast cells were able to anaerobically grow on pentose sugars such as xylose and xylitol.

Enhanced flux through the glycolysis pathway leads to increased biosynthesis rates, which in turn leads to higher biomass yields, and increased production of homologous and heterologous proteins, primary metabolites (such as ethanol, acetate, lactate,

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organic acids, amino acids, polyols), and/or secondary metabolites (such as antibiotics, ergot alkaloids, statins, vitamins, immunomodulators, cytostatics, insecticides or herbicides). Adding the modified PFK gene to already engineered yeast strains with altered genes for various enzymes might further improve fermentative bioethanol production from pentose carbohydrates by *Saccharomyces cerevisiae*. In addition to *S. cerevisiae*, increased activity of the modified PFK was initially shown in the fungus *Aspergillus niger*, and later in human cancer cells, indicating a wide array of applications of the use of PFK gene fragments, from enhanced production of secondary metabolites to investigating pathways for cancer treatment.

Since the technology aims to reach its full potential in cleaning products, industrial partners, such as detergent producers, are sought. Technical cooperation is sought in order to facilitate continuous development rather than just routine production. License agreements and / or agreements for technical cooperation will enable the researchers to maintain their focus on the research behind the technology whereas up-scaling to industrial level will be carried out in the industrial partner's setting.

Advantages:

- **enhancing the rate of cell biomass synthesis**
- **enhancing the excretion of extracellular enzymes**
- **increasing the productivity of**

primary and secondary metabolites

Consequently, biotechnological processing time is reduced, as are the production costs.

The researchers are among the leading scientists in their respective departments, and regularly publish in high-impact scientific journals. They are experts in biochemical processes, particularly molecular recognition, signal transduction, and primary metabolism. They were the first to show that the PFK enzyme can be post translationally modified.

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STAGE OF DEVELOPMENT

Available for demonstration

INTELLECTUAL PROPERTY

Patent(s) applied for but not yet granted
Patents granted

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