Institut "Jožef Stefan" Ljubljana, Slovenija

TECHNOLOGY OFFER

ETHANOL PRODUCTION FROM PENTOSES BY YEASTS POSSESSING A MODIFIED PHOSPHOFRUCTOKINASE

Fields of use

Fermentation, Microbiology, Genetic Engineering, Protein Engineering, Gene Expression, Proteome Research

Current state of technology

Available for demonstration

Type of cooperation

Industry partners in the field of bioethanol production, interested in licensing in the technology, with the focus on, and capacity for, industrial-scale production.

Intellectual property

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

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Summary

A Slovenian research institute 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.

Description of the invention

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, fructose6-phosphate (F6P) is formed and enters glycolysis. The enzyme phosphofructokinase (PFK) has an important regulatory function as it catalyzes the transformation of F6P to the next intermediate, fructose-1,6-bisphosphate (F1,6-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, 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 form 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.

KONZORCIJ ZA PRENOS TEHNOLOGIJ IZ JRO V GOSPODARSTVO

EUROPEAN UNION
EUROPEAN REGIONAL DEVELOPMENT FUND

REPUBLIC OF SLOVENIA
MINISTRY OF EDUCATION, SCIENCE AND SPORT

The investment is co-financed by the Republic of Slovenia and the European Union under the European Regional Development Fund.

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Since the technology aims to reach its full potential in an industrial-scale bioethanol production, industrial partners are sought to license in the technology. The technology is in the field of microbial biotechnology, therefore technical cooperation is sought in order to facilitate continuous development as well as 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. Research cooperation agreements will, conversely, aid the researchers in further development in the laboratory setting.

Main 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

Partner Sought

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. The researchers are looking for industry partners in the field of bioethanol production, interested in licensing in the technology, with the focus on, and capacity for, industrial-scale production.

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