CLONING NOVEL SUGAR TRANSPORTERS FROM THE XYLOSE-FERMENTING YEAST SCHEFFEROMYCES STIPITIS

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Introduction:
Lignocellulosic biomass is an attractive raw material for bioethanol production. The major fermentable sugars found in this biomass are glucose and xylose, and abundant research has been devoted to improve xylose utilization by recombinant Saccharomyces cerevisiae strains, an industrial yeast that it is not able to ferment this sugar.

Objective:
Since limited uptake is one of the bottlenecks for xylose fermentation, we aimed to identify novel sugar transporters from the xylose-fermenting yeast Scheffersomyces stipitis that allow xylose uptake and fermentation by engineered S. cerevisiae.

Materials and Methods:
An hxt-null S. cerevisiae strain, lacking the major hexose transporters (hxt1Δ-hxt7Δ and gal2Δ) but having high xylose reductase, xylitol dehydrogenase and xylulokinase activities, was transformed with a genomic DNA library from S. stipitis. The genomic DNA from S. stipitis strain NBRC1687 was digested with BamHI, and 3-5 kb fragments were isolated by gel electrophoresis. These DNA fragments were cloned into the BamHI site of plasmid pPGK (URA3 PGK1 p-MCS-PGK1t) containing a strong and constitutive promoter. Strains transformed with the pPGK-derived plasmids were selected in synthetic complete medium lacking uracil with 2% xylose. Plasmids were extracted from the recombinant yeast strains, analyzed by restriction digestion and sequenced.

Results and Discussion:
Four plasmids allowing growth on xylose contained three genes encoding sugar transporters: the previously characterized XUT1 permease, and two new genes (HXT2.6 and QUP2) not previously identified as xylose transporters. High cell density fermentations with the recombinant strains showed that the XUT1 gene allowed ethanol production from xylose or xylose plus glucose as carbon sources, while the HXT2.6 permease produced both ethanol and xylitol, and the strain expressing the QUP2 gene produced mainly xylitol during xylose consumption.

Conclusion:
Cloning novel sugar transporters not previously identified from the S. stipitis genome using an hxt-null S. cerevisiae strain with a high xylose-utilizing pathway provides novel promising target genes for improved lignocellulosic ethanol production by yeasts.

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