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Generation of *Saccharomyces cerevisiae* Capable to Ferment Hexoses and Pentoses Derived from Lignocellulosic Biomass by Protein- and Metabolic-engineering

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Research aims

Generation of microorganism (s) with an increased ability to produce ethanol from lignocellulosic biomass is one of most important technological problems for industrial bioethanol production. Since lignocellulosic biomass contains a large amount of xylose, microorganisms capable of efficiently fermenting xylose have been sought. Although strains of *Saccharomyces cerevisiae* transformed with xylose-metabolic genes from other yeasts such as *Pichia stipitis* is the most potent recombinant strains capable of fermenting xylose, their ability to produce ethanol is not sufficient for industrial applications because their consumption rate of xylose is much slower than that of glucose. Therefore, in this study, I first sought *P. stipitis* genes involved in sugar transport and/or sensing by bioinformatic genome analysis, then characterized the putative transporter genes and finally introduced those genes into *S. cerevisiae* to improve the pentose fermentation.

Methods

Protein-BLAST search was performed against the *P. stipitis* genome sequence using the *S. cerevisiae* HXT1 protein as a query. Thirty-six genes with over 30% similarity were individually subcloned between the phophoglycerate kinase (PGK) promoter and terminator of the yeast multi-copy vector YEpPGK. *S. cerevisiae* KY73 strain (*hxt1-7 gal2 ura3*) was a kind gift from Prof. Toshiko Kasahara (Teikyo University, Japan). Metabolite analysis for fermentation experiments was carried out by using HPLC and gas chromatography.

Results

The *S. cerevisiae* genome has the highest numbers of sugar (hexose) transporter (*HXT*) genes in all sequenced genomes, and a null mutant of *S. cerevisiae* deleted for all of the *HXT* genes, incapable to grow on any hexose, is now available. Furthermore, since several *HXT* genes also play a (potential) role in pentose transport, the *hxt*-null mutant lacks the ability to transport not only hexose (s) but also pentose (s) such as xylose and L-arabinose. *P. stipitis* can metabolize (and ferment) pentoses much more efficiently than *S. cerevisiae*, indicating the existence of an efficient pentose transport system (s) in *P. stipitis*, which is missing in *S. cerevisiae.*

I first identified thirty-six putative sugar transporter genes in *P. stipitis* by bioinformatic analysis and then evaluated physiological function of each gene products by expressing the identified genes in the *S. cerevisiae hxt*-null mutant, KY73 and examining their phenotypes (Fig. 1). Although it was found that *P. stipitis* has a limited number of transporters capable of transporting hexose, I could identify

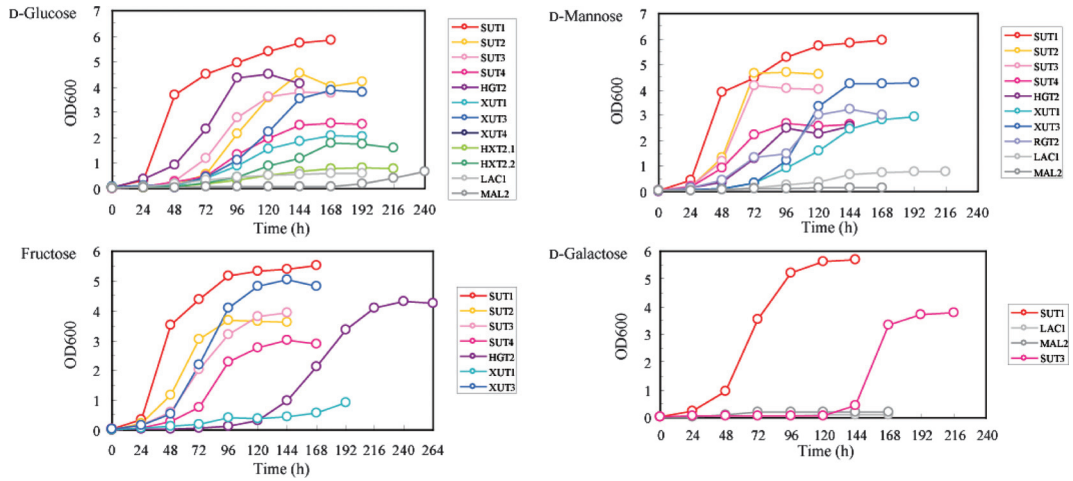

Fig. 1. Identification of hexose transporters from *P. stipitis.*

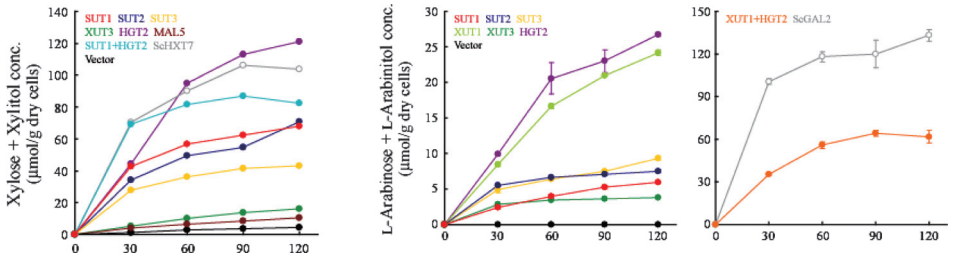

Fig. 2. Identification of pentose transporters from *P. stipitis.*

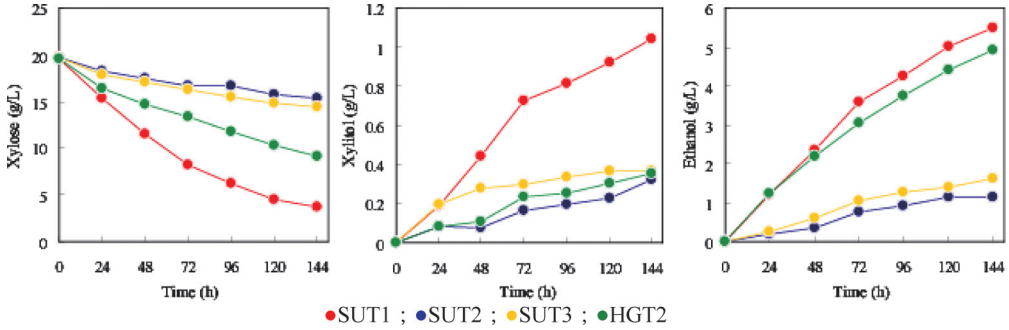

Fig. 3. Xylose fermentation by *P. stipitis* xylose transporters in *S. cerevisiae* KY73

several novel *P. stipitis* hexose transporters in addition to the known transporters, SUT1-3 [1]. In particular, HGT2 was shown to be the most abundantly expressed transporter gene in *P. stipitis* cells grown on all the hexoses (and also the pentoses) tested in this study. Although RGT2 and SNF3 had been annotated as "sugar sensor", this study revealed that RGT2 had the ability to at least transport mannose, indicating some difference (s) in sugar-sensing mechanism between *P. stipitis* and *S. cerevisiae.*

I next examined transformants expressing each of the putative *P. stipitis* transporter genes for their ability to transport pentose by measuring intercellular pentose concentration using HPLC (Fig. 2). In a previous study[1], it was shown that certain transporters of *P. stipitis* (SUT1, SUT2 and SUT3) have an ability to transport xylose and that SUT1, in particular, exhibits a relatively high ability for xylose transport. In addition, the previous observation that the *sut1*-disrupted strain exhibited a similar transport activity for xylose compared to the wild-type strain suggested that *P. stipitis* may possess additional major xylose transporter (s) other than SUT1. Transport activity for xylose observed with HGT2, a newly identified transporter from *P. stipitis*, was similar to that observed with SUT1, suggesting an important role of HGT2 for xylose metabolism in *P. stipitis*. Significant transport activity for L-arabinose was found only with HGT2 and XUT1, but not with SUT1, SUT2 and SUT3. Interestingly, when HGT2 and XUT1 were co-expressed, an additive effect was observed. On the other hand, their L-arabinose uptake ability was found to be less than that observed for the *S. cerevisiae* GAL2. Furthermore, HGT2 transported xylose ~5-fold faster than L-arabinose. Indeed, L-arabinose has previously been shown to be a poorer carbon source for *P. stipitis* than xylose. This is probably due to low uptake ability of the *P. stipitis* transporters for L-arabinose.

The function of the *P. stipitis* xylose transporters were evaluated in the *S. cerevisiae* KY73X strain, which was constructed by introducing the three xylose-metabolic genes (XR, XDH and XK) into the *hxt*-null mutant, KY73[2] (Fig. 3). Significant xylose fermentation was found only with transformants expressing SUT1 or HGT2. Although the xylose consumption rate observed for the SUT1-expressing strain was ~2-fold higher that observed for the HGT2-expressing strain, there was no difference in ethanol production between the two strains, indicating that a part of xylose incorporated by the SUT1-expressing strain was accumulated in cells as by-products such as xylitol and glycerol.

Conclusion

In this study, I carried out a systematic search for sugar transporters in *P. stipitis,* a pentose-metabolizing yeast, and identified several novel pentose transporters. Based on the results obtained in this study, I already had applied for national and international patents.

References

1. Weierstall, T., Hollenberg, C.P., and Boles, E. (1999) Mol. Microbiol. 31, 871–883.
2. Matsushika, A., Inoue, H., Watanabe, S., Kodaki, T., Makino, K., and Sawayama, S. (2009) Appl. Environ. Microbiol. 75, 3818–3822.