Enhanced xylose fermentation capacity related to an altered glucose sensing and repression network in a recombinant *Saccharomyces cerevisiae*

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The co-fermentation of glucose and xylose is one of the issues in decreasing the price of biofuel or chemicals produced from lignocellulosic materials. A glucose and xylose co-utilizing *Saccharomyces cerevisiae* was obtained through rational genetic manipulation. Non-rational evolution in xylose was performed, and the xylose utilization efficiency of the engineered strain was significantly enhanced. The results of transcriptome study suggested that Snf1/Mig1-mediated regulation, a part of glucose sensing and repression network, was altered in the evolved strain and might be related to the enhancement of xylose utilization.

With the worldwide interest in producing fuel ethanol from lignocellulosic feedstock, the utilization of xylose, the second most abundant component in lignocelluloses, has been widely studied. *Saccharomyces cerevisiae* is an ethanol-producing microorganism with high tolerance to stressful environment. This microorganism has excellent capacity to produce ethanol from hexose and can be endowed with xylose-utilizing capacity by introducing heterogeneous xylose metabolic pathways.1-3 Xylose is usually converted to xylulose by xylose reductase-xyitol dehydrogenase or xylose isomerase (XI). The produced xylulose is phosphorylated by endogenous xylulokinase (XK) and enters the endogenous pentose phosphate pathway (PPP) in recombinant *S. cerevisiae*. The produced xylose is normally converted to xylulose by xylulokinase activity, the effects of several functional genes, such as *PFK27*, *PDC6*, and *PHO13*, to xylose fermentation were further confirmed. The results suggested that these genes are not the only ones endowing the evolved strain with efficient xylose metabolism capacity.

So far, the repression of glucose to xylose was mainly described at the absorption level because the native hexose transporters in charge of the xylose uptake in *S. cerevisiae* but has much lower affinity to xylose than to glucose.1 Nonetheless, as a novel substrate for *S. cerevisiae*, xylose metabolism is suggested to be sub-optimal...
because the xylose-grown cells fail to activate appropriate genes. In *S. cerevisiae*, the enzymes required for the utilization of alternative carbon source were absent or kept in a low level when glucose was present. This phenomenon is known as carbon catabolite repression or glucose repression. Glucose repression mainly occurs at the transcriptional level, although it is also related to the alteration of protein synthesis and degradation. In the present addendum, the possible mechanisms underlying xylose metabolism in the glucose repression system through transcriptional analysis were further discussed.

The RNAs used for transcriptional analysis were extracted from the cells cultured in a mixture of 10 g·L⁻¹ glucose and 20 g·L⁻¹ xylose, and then collected during chemostat cultivation, we proposed that the altered Snf/Mig1 repression network might affect the xylose utilization in some aspects. Nonetheless, how this benefit to the xylose utilization of *S. cerevisiae* has remained to be elucidated.

In conclusion, the results of transcriptional analysis suggested that the alteration in glucose sensing and repression network occurred in our evolved strain. This global alteration might contribute to the enhancement of xylose utilization capacity.

Disclosure of Potential Conflicts of Interest

No potential conflict of interest was disclosed.
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Table 1. Transcriptional changes of the genes involved in the utilization of alternative carbon source

| Genes | Description | Fold-change (BSPX013 vs. BSPC095) |
|-------|-------------|---------------------------------|
| HXK1  | Hexokinase isoenzyme | 0.47 |
| HKT16 | Protein of unknown function with similarity to hexose transporter family members | 0.13 |
| GAL1  | Galactokinase | 0.42 |
| GAL2  | Galactose permease | 0.18 |
| MAL12 | Alpha-glucosidase | 0.45 |
| MAL31 | Maltose permease | 0.33 |
| MAL32 | Alpha-glucosidase | 0.36 |
| SUC2  | Invertase | 1.23 |
| FBP1  | Fructose-1,6-bisphosphatase | 1.16 |

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