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A novel pathway construction in *Candida tropicalis* for direct xylitol conversion from corncob xylan

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HIGHLIGHTS

- A new β-xylanase gene was discovered from *Aspergillus terreus*.
- The co-expression plasmid of xylanase and xylosidase was constructed.
- A novel xylan transforming pathway was integrated in *Candida tropicalis* BIT-Xol-1.
- Xylanase and xylosidase were secretively expressed in the engineered *C. tropicalis*.
- It simultaneously saccharify and transform xylan to xylitol efficiently.

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ABSTRACT

In this study, an integrated xylitol production pathway, directly using xylan as the substrate, was constructed in *Candida tropicalis* BIT-Xol-1 which could efficiently convert xylose into xylitol. In order to consolidate this bioprocessing, a β-1,4-xylanase gene (*atn*) and a β-xylosidase gene (*atl*) were cloned from *Aspergillus terreus*, and were constructed onto episomal plasmid pAUR123. Additionally, combination of the individual *atn* and *atl* expression cassette was also cloned onto pAUR123. After transforming, the positive *C. tropicalis* transformants co-expressing xylanase and xylosidase produced larger hydrolysis zones than those expressing xylanase alone, when incubated on xylan-congo red plates. The engineered *C. tropicalis*/pAUR-*atn*-*atl*-3 (*C. tropicalis* PNL3) secrete heterologous xylanase and xylosidase simultaneously, with the activities of 48.17 and 11.56 U/mL, respectively. The xylitol yields by *C. tropicalis* PNL3 utilizing xylan and corncob were 77.1% and 66.9%, respectively. The integrated pathway of xylitol production was feasible and efficient in utilization of xylan-rich renewable biomass via combining saccharification and transformation of xylan in engineered *C. tropicalis*.

1. Introduction

Xylitol, a natural occurring five carbon sugar alcohol, is one of the most expensive polyol sweeteners and has specific health claims in the world market. As an alternative sugar, owning the properties of low energy and inhibition against the metabolism of dental plaque formation, xylitol was widely used in oral hygiene and pharmaceutical products to reduce tooth decay and ear infection (Mäkinen, 2000). Additionally, xylitol also works as a sucrose substitute for diabetics since it does not require insulin for its metabolic regulation (Emodi, 1978).

The traditional production of xylitol involves direct chemical hydrogenation of D-xylose derived from hemicelluloses xylan hydrolysates of biomass materials over a Raney–Nickel catalyst, which includes high pressure and temperature as well as expensive separation and purification steps. Some research efforts have focused on xylitol production using *Escherichia coli* by xylose reduction during growth on glucose (Cirino et al., 2006; Khankal et al., 2008) or xylose (Cirino et al., 2006; Cirino and Akinterinwa, 2009). Additionally, the biotransformation of D-arabitol into xylitol was also investigated with focus on the conversion of D-xylulose into xylitol (Zhou et al., 2012). Alternately, xylitol production from D-xylose through bioconversion has been proposed as an alternative process utilizing microorganisms such as yeasts, bacteria and filamentous fungi (Chang and Knight, 1960; Antti et al., 2005; Converti and Dominguez, 2001). Among these, yeasts are generally considered to be more efficient producers of xylitol than bacteria or filamentous fungi. Many studies have investigated biological methods of xylitol production by three different types of yeasts (Jin et al., 2005). First, there are wild type xylose utilizing yeasts, such as *C. guilliermondii* (Meyrial et al., 1991), *C. boindii* (Vandeska et al., 1995), *C. tropicalis* (Kim et al., 1999), *C. parapsilosis* and *Debaryomyces hansenii* (Prakash et al., 2011). In addition, recombinant

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