Surface tethered xylosidase activity improves xylan conversion in engineered strainsof *Saccharomyces cerevisiae*

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Introduction: Second-generation biofuel production strategies require utilization of the largest possible fraction of available sugars in renewable biomass. Xylose can make up as much as 35% of plant DCW, primarily in the form of the hemicellulose polymer xylan. Xylo-oligosaccharides (XOS) are breakdown products of xylan released during pretreatment and hydrolysis that may inhibit the fermentation process. In an attempt to enable growth on xylan and removal of inhibitory XOS, xylanase and GH43 xylosidase activity were conferred to xylose-assimilating *Saccharomyces cerevisiae*strains. Xylosidase activity was engineered to be cell free or tethered.

Methodology: CRISPR-Cas9 was used to engineer the *xyn2* and *xln43* genes into *Saccharomyces cerevisiae* strains. After confirmation of integration, enzymatic assays were performed to measure the activity levels of the expressed enzymes. All of the strains then underwent growth trials on xylan and XOS and fermentation trials on xylan to determine the effects of xylanolytic enzyme activity on growthand ethanol production respectively.

Results: Gene-integration and expression of both xylanolytic enzyme-encoding genes was successful. Secretion yielded higher overall xylosidase activity when strains were cultivated on glucose, but cell- associated activity was higher when cultivated in the presence of xylose. Growth trials on both xylan and XOS showed that a combination of both enzymes, with cell-associated xylosidase production, resulted in the highest growth on both substrates. The increased growth on xylan also translated to substantial improvements in ethanol production from polymeric xylan as sole carbon source.

Discussion and Conclusion: Increased enzyme production, growth capabilities and hemicellulosic substrate conversion due to cell-tethered activity over secreted activity was shown for the first time. The use of a GH43 xylosidase that may help avoid problematic transglycosylation for this purpose is also novel. The development of *S. cerevisiae* strains capable of xylan utilization and fermentation brings the ideal of utilizing the full range of sugars in biomass feedstocks for large scale ethanolproduction closer.

References: La Grange *et al*. (2001), Mert *et al*. (2016), Liu *et al*. (2015)

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