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The prospect for developing a consolidated bioprocessing (CBP) strain using xylan as the substrate: the case study of *Yarrowia lipolytica*

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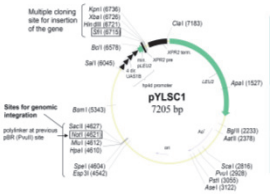
Abstract

Yarrowia lipolytica **was used to investigate its potential for being developed as a CBP strain for biofuel production by expressing cellulase and xylanase enzymes. *Y. lipolytica* is known to accumulate lipids intracellularly and is capable of metabolizing glucose and xylose to produce lipids; however, due to the lack of the biomass degrading enzymes, it cannot directly utilize lignocellulosic substrates as carbon sources. While research is continuing on the development of a *Y. lipolytica* strain able to degrade cellulose, in this study, we present successful expression of several xylanases in *Y. lipolytica*. To the best of our knowledge, this is the first study introducing heterologous hemicellulase genes into the genome of *Y. lipolytica*. SDS-PAGE showed that the endo-xylanase gene XynII and exo-xylosidase gene XlnD were successfully expressed and secreted, and the expressed xylanases were likely either not or sparsely glycosylated, which is advantageous for expression of heterologous proteins. Enzymatic activity tests further demonstrated active expression of XynII and XlnD in *Y. lipolytica*. Furthermore, synergistic action on converting xylan to xylose was observed when XlnD worked in concert with XynII. XlnD was able to work on the xylo-oligomers generated by XynII, enhancing the xylan conversion to monomeric xylose. The successful expression of these xylanases in Yarrowia further advances us towards our goal to develop a direct microbial conversion process using this organism.**

Heterologous expression of xylanases in yeasts

Genes and source species	MW (k Da)	Host	References
Xyn II, *Trichoderma reesei*	21 kDa	*Saccharomyces cerevisiae*	La Grange DC et al. (2001)
XlnD, *Aspergillus niger*	85 kDa	*Saccharomyces cerevisiae*	La Grange DC et al. (2001)
XynA, *Aureobasidium pullulans*	21 kDa	*Saccharomyces cerevisiae*	XL Li et al. (1996)
XynB, *Bacillus pumilus*	61 kDa	*Saccharomyces cerevisiae*	La Grange DC et al. (1997)
Xyn II, *Trichoderma reesei*	21 kDa	*Pichia pastoris*	J He et al. (2009)
XylB, *Aspergillus niger*	21 kDa	*Pichia pastoris*	V Ruanglek et al. (2007)
TxXYN, *Thermobacillus xylanilyticus*	21 kDa	*Yarrowia lipolytica*	S Duquesne et al. (2014)

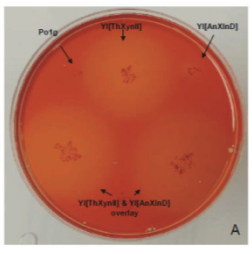
Expression of xylanase genes in Y. lipolytica

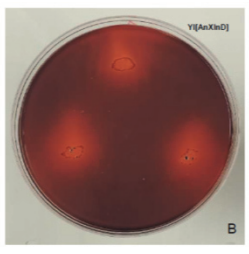


Proteins & source species	GenBank accession no.	MW of secreted mature protein	Transformant no., and recombinant strain designation
XynII; *T. harzianum*	ACF40831	21 kDa	131; Yl[ThXynII]
XlnD; *A. niger*	O00089	85 kDa	133; Yl[AnXlnD]

Host:** ***Y. lipolytica **Po1g (MatA, leu2-270, ura3-302:URA3, xpr2-332, axp-2)**

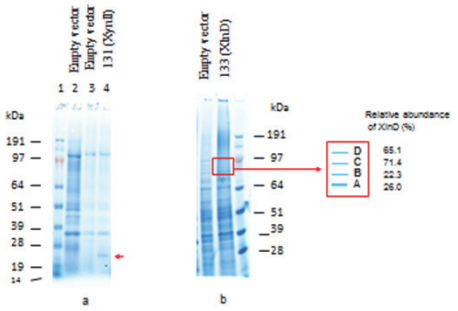
Transformants on xylan containing plates





- Clear halo zone around the colony indicates the release of the reducing sugars which therefore reflected the activity of the xylanase;
- Xylosidase XlnD are usually cell wall bound as demonstrated by the small clear zone in Fig. B

SDS-PAGE of secreted proteins



- Both xylanases were successfully expressed in *Y. lipolytica*; The target protein bands were further characterized by LC-MS;
- Xylanases expressed in *Y. lipolytica* were likely less glycosylated;
- Expressed XlnD were glycosylated to different level due to more glycosylation sites.

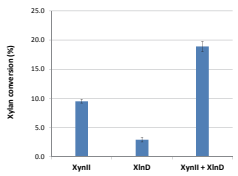
Xylanase activities in transformants

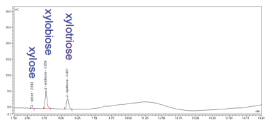
Transformant	Gene	Substrate	Activity
T 131	XynII, endo-1,4-beta-xylanase	Birchwood xylan	Supernatant, +++++, 1396 nkat/mL
T 133	XlnD, exo-1,4-beta-xylosidase	*pNPX*	culture supernatant: + cell culture, +++++, 14 nkat/mL

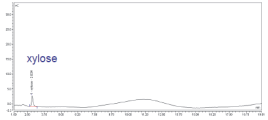
* 72h culture; One katal was the amount of enzyme needed to produce 1 mol of product from substrate per s.

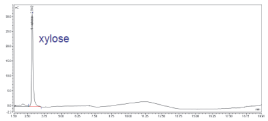
Synergistic effects between XynII and XlnD

Birchwood xylan digestion (5 min)

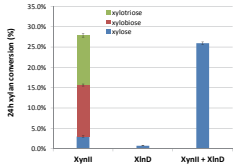








24h digestion of xylan in pretreated corn stover



HPLC chromatograms of birchwood xylan digestion

- XlnD was able to work on the xylo-oligomers generated by XynII, enhancing the xylan conversion to monomeric sugars.

Conclusions

- **Successfully demonstrated the heterologous expression of xylanases in *Y. lipolytica*;**
- **XynII and XlnD transformants showed positive results from the enzymatic activity assays suggesting XynII and XlnD were successfully expressed; Moreover, a synergistic action on converting xylan to xylose was observed when XlnD worked in concert with XynII;**
- **The successful expression of the xylanases in *Yarrowia* further advances us toward our goal to develop a direct microbial conversion process using this organism.**

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