Strain improvement of native *Saccharomyces cerevisiae* LN ITCC 8246 strain through protoplast fusion to enhance its xylose uptake

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Research Article

Keywords: Protoplast fusion, bioethanol, yeast, lignocellulose, co-fermentation

Posted Date: February 24th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-231041/v1>

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Version of Record: A version of this preprint was published at Applied Biochemistry and Biotechnology on March 25th, 2021. See the published version at <https://doi.org/10.1007/s12010-021-03539-3>.

Abstract

Co-utilization of xylose and glucose and subsequent fermentation using *Saccharomyces cerevisiae* could enhance ethanol productivity. Directed engineering approaches have met with limited success due to interconnectivity of xylose metabolism with other intrinsic, hidden pathways. Therefore, random approaches like protoplast fusion were used to reprogram unidentified mechanisms. *Saccharomyces cerevisiae* LN, the best hexose fermenter, was fused with xylose fermenting *Pichia stipitis* NCIM 3498. Protoplasts prepared using glucanex were fused under electric impulse and fusants were selected using 10% ethanol and cycloheximide (50 ppm) markers. Two fusants, 1a.23 and 1a.30 showing fast growth on xylose and tolerance to 10% ethanol, were selected. Higher extracellular protein expression observed in fusants as compared to parents was corroborated by higher number of bands resolved by twodimensional analysis. Overexpression of *XYL1*, *XYL2*, *XKS* and *XUT4* in fusants as compared to *S. cerevisiae* LN as observed by RT-PCR analysis was substantiated by higher specific activities of *XR*, *XDH* and *XKS* enzymes in fusants. During lignocellulosic hydrolysate fermentation, fusants could utilize glucose faster than the parent *P. stipitis* NCIM 3498 and xylose consumption in fusants was higher than *S. cerevisiae* LN.

Full Text

Due to technical limitations, full-text HTML conversion of this manuscript could not be completed. However, the latest manuscript can be downloaded and [accessed as a PDF](#).

Tables

Table 1. Enzyme activities of parents and fusants

Strains	Specific activity (U mg ⁻¹ protein)		
	XR	XDH	XKS
<i>P.stipitis</i> NCIM 3498	0.007±0.0002	0.003±0.00011	0.0013±0.0002
<i>S. cerevisiae</i> LN	0.003±0.00016	0.0012±0.00013	0.0002±0.000011
1a.23	0.006±0.00014	0.0011±0.00011	0.0097±0.0001
1a.30	0.006±0.00018	0.0014±0.0002	0.0022±0.0001

Table 2. Fermentation potential of fusants on 2% xylose at 72 h

Strains	Ethanol produced (g L ⁻¹)	xylose consumed (g L ⁻¹)	Fermentation efficiency (%)	Ethanol yield (g g ⁻¹)
1a.23	1.36±0.07	8.018±0.92	33.26	0.170
1a.30	1.99±0.08	8.358±0.68	46.69	0.238

Table 3. Comparison of ethanol yields of fusant strains and other genetically engineered strains (Source:{36})

Yeast Strain	Yeast improvement strategy	Sugar concentration	Ethanol yield (g g ⁻¹)	Reference
<i>S. cerevisiae</i> ScF2	Genome shuffling product	50 g L ⁻¹ glucose + 50 g L ⁻¹ xylose	0.40	[37]
<i>S. cerevisiae</i> Fusant 1	Protoplast fusant	30 g L ⁻¹ glucose + 20 g L ⁻¹ xylose	0.19	[38]
<i>S. cerevisiae</i> YD43-4	Protoplast Fusant	75 g L ⁻¹ glucose + 30 g L ⁻¹ xylose	0.35	[39]
<i>S. cerevisiae</i> and <i>C. intermedia</i>	Protoplast fusion and mutation	10 g L ⁻¹ glucose+ 10 g L ⁻¹ xylose	0.38 and 0.42	[14]
<i>S. cerevisiae</i> LN and <i>P. stipitis</i> NCIM 3498	Protoplast fusion	20 g L ⁻¹ xylose	0.24 and 0.17	This study

Figures

Xylose metabolic pathway

Figure 1

Schematic representation of xylose metabolic pathway in pentose fermenting yeasts

Figure 2

Growth of parents and fusants on 10% ethanol

Figure 3

Fold induction of genes in fusants with respect to *S. cerevisiae* LN. In the graph, each bar represent mean of three replicates and error bars represent standard deviation. Fold induction of genes in fusants with respect to *P. stipitis* NCIM 3498

Supplementary Files

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