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

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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                  |
| *P. stipitis* NCIM 3498 | 0.007±0.0002 | 0.003±0.00011 | 0.0013±0.0002 |
| *S. cerevisiae* 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 |
|--------------|-----------------------------|---------------------|------------------------|-----------|
| *S. cerevisiae* ScF2 | Genome shuffling product | 50 g L⁻¹ glucose + 50 g L⁻¹ xylose | 0.40 | [37] |
| *S. cerevisiae* Fusant 1 | Protoplast fusant | 30 g L⁻¹ glucose + 20 g L⁻¹ xylose | 0.19 | [38] |
| *S. cerevisiae* YD43-4 | Protoplast Fusant | 75 g L⁻¹ glucose + 30 g L⁻¹ xylose | 0.35 | [39] |
| *S. cerevisiae* and *C. intermedia* | Protoplast fusion and mutation | 10 g L⁻¹ glucose + 10 g L⁻¹ xylose | 0.38 and 0.42 | [14] |
| *S. cerevisiae* LN and *P. stipitis* NCIM 3498 | Protoplast fusion | 20 g L⁻¹ xylose | 0.24 and 0.17 | This study |

**Figures**
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 represents the 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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- SupplementaryData.docx