

Development of Thermotolerant Yeast for Simultaneous Saccharification and Co-Fermentation of Polysaccharides Present in Lignocelluloses for the Production of Bioethanol

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ABSTRACT

Second generation bioethanol has already been proved as a great alternative to the gasoline, and recommended to be blended with gasoline in transportation vehicles. In a particular, for the sustainable bioethanol production, xylose, the second highest monosaccharide in lignocellulosic residues, has to be converted in ethanol simultaneously with the glucose. Various xylose utilizing yeasts such as *Scheffersomyces stipitis*, *Candida shehatae*, *Candida intermedia* and *Kluyveromyces marxianus* have been reported for xylose-based ethanol production. However, various issues like slow xylose uptake, higher byproduct formation, low ethanol productivity, have been encountered during the process development. In this regard, targeted and non-targeted engineering have been approached by different researches to overcome the issues. The targeted engineering has been applied the different levels such as xylose metabolic and transporter engineering, co-factor regeneration, establishment of bypass pathway etc. The genetic engineering tools, applied for the improvement include gene insertion from one organism to potential xylose utilizing strain for xylose metabolism and uptake, site directed mutagenesis for glucose/xylose diauxic during sugar transport, to change the co-factor preference of xylose metabolic enzymes and inhibition of parallel pathway of other product formation. Apart from the above, intergeneric protoplast fusion and evolutionary engineering have also been applied as accessories to achieve the desirable. A thermotolerant yeast *Kluyveromyces marxianus* NIRE-FKS.A1 was developed through protoplast fusion of thermotolerant yeast *K. marxianus* NIRE-K3 and mesophilic yeast *Scheffersomyces stipitis* to ferment both glucose and xylose present in paddy straw. Fused thermotolerant yeast *K. marxianus* NIRE-FKS.A1 was then used to ferment sodium hydroxide pretreated (0.5 % w/v, 121°C, 15 min) paddy straw in simultaneous saccharification and fermentation (SSF) mode. *K.marxianus* NIRE-FKS.A1 showed the maximum ethanol concentration of 33.93 ± 1.69 g l⁻¹ after 36 h of incubation along with glycerol (2.94 ± 0.14 g l⁻¹), xylitol (3.39 ± 0.17 g l⁻¹), acetic acid (4.57 ± 0.22 g l⁻¹) and



xylose ($6.11 \pm 0.31 \text{ g l}^{-1}$) as by-products. The maximum ethanol yield of $237.54 \pm 12.31 \text{ g ethanol kg}^{-1}$ of raw paddy straw was obtained in SSF. It is concluded from the study that the novel developed thermotolerant yeast *K. marxianus* NIRE-FKS.A1 has the potential for bioethanol production utilizing pentoses in addition to hexoses of lignocelluloses such as paddy straw, sugarcane bagasse, etc. as feedstock.

Keywords: Sugarcane bagasse, Bioethanol, Thermotolerant, SSCF, SHF.

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