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Bioethanol production by thermotolerant yeast *Kluyveromyces marxianus* from sugarcane bagasse

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Second generation (2G) bioethanol is a clean and renewable source of energy, which could be produced by lignocellulosic biomass (LCB) such as agricultural, forestry, municipal, and industrial wastes. LCB contains cellulosic and hemicellulosic fractions, which is yielded into pentose and hexose sugars using pretreatment and enzymatic saccharification. Most of the ethanol producing microorganisms are either hexose sugars utilizing or utilize pentose sugars inefficiently along with hexose sugars. However, the utilization of both pentose and hexose sugars is required for economical fuel ethanol production. An isolated thermotolerant yeast Kluyveromyces marxianus NIRE-K3 is able to utilize both pentose and hexose sugars. However, the utilization of pentose sugar (xylose) is very slow as compared to hexose sugar (glucose). The present study was carried out to develop *K. marxianus* NIRE-K3.2 for enhanced xylose utilization through two-phase evolutionary adaptation, and analyzed the bioethanol production potential of adapted *K. marxianus* NIRE-K3.2 from sugarcane bagasse (SCB) in comparison to native yeast. The two-phase evolutionary adaptation was carried out: first in YEPX medium (20gl^{-1} xylose) for 60 generations followed by second in minimal salt medium containing 20gl-^{1} xylose for 55 generations. Liquid ammonia pretreated SCB was enzymatically saccharified using Novozyme Cellic Ctec2. The maximum concentrations of glucose and xylose in hydrolysate were found to be 35.45gl^{-1}, and 14.92gl-^{1}, respectively. The fermentation of enzymatic hydrolysate was carried out using native NIRE-K3 and adapted NIRE-K3.2, separately at 45oC and pH5.5. NIRE-K3 showed utilization of 43.23% xylose, whereas, NIRE-K3.2 utilized 75.06% xylose present in hydrolysate. The ethanol yields obtained by NIRE-K3.2 was equivalent to 92.15% of the theoretical yield, whereas, 60.78% in case of NIRE-K3. The adapted strain NIRE-K3.2 showed 34% improved ethanol yield by utilizing xylose efficiently along with glucose as compared to that of native strain. The aforesaid results show the importance of evolutionary adaptation to develop enhanced xylose utilizing thermotolerant yeast *K. marxianus* NIRE-K3.2 for bioethanol production by utilizing both pentose and hexose sugars in SCB.

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