

Assessing Non-Conventional Yeasts for Bioethanol Production: *Meyerozyma* and *Lodderomyces* Bio-Prospecting Potential

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Abstract

Bioethanol production from ligno cellulosic biomass by *Saccharomyces cerevisiae* is a matter of great concern due to its inability to ferment xylose which is present in abundance in biomass. *Saccharomyces cerevisiae* has undoubtedly been the organism of choice for ethanol production owing to its robustness, ethanol tolerance, inhibitor tolerance but its inability to utilize xylose is a limiting factor for the overall efficiency of the process. Henceforth, we look forward to searching non-conventional strains that are capable of utilizing both glucose and xylose. We isolated two yeast strains from rotten apple-*Meyerozyma* and *Lodderomyces*, as were identified by ITS sequencing. Fermentation of mixed sugar substrate (2:1 Glucose: Xylose) was carried out for 96 hours. Both the microorganisms depleted glucose and more than 33% xylose was consumed within 24 h. Ethanol produced was 0.344 g g⁻¹ and 0.327 g g⁻¹ by *Meyerozyma* and *Lodderomyces* respectively with an efficiency of about 65%. Strains were tolerant to inhibitors like 5-hydroxymethyl furfural and furfural at concentrations commonly found in pre-treated hydrolysates. This is the first report elucidating the potential of *Meyerozyma* and *Lodderomyces* for mixed sugar utilization, thereby producing ethanol.

Keywords: Non-conventional; Mixed sugar; Fermentation process

Introduction

The ever surging costs of fossil fuels and the resulting greenhouse effects have necessitated the need to search for alternative cheaper and eco-friendly biofuel resources as an approach to reduce global warming [1, 2]. One such method for the low-cost production of bioethanol is to make use of the lignocellulose biomass as they contain carbohydrates that must be first hydrolysed into simple sugars and then fermented into ethanol. The derivation of higher value added products like fine chemicals or bio-fuel from lignocellulose biomass generally requires a multi-step processing that includes (i) pre-treatment (chemical, biological or mechanical etc.) (ii) Enzymatic hydrolysis (iii) fermentation process.

Saccharomyces cerevisiae has undoubtedly been the paradigm for eukaryotic research. The yeast, being the workhorse of fermentation industry, has dominated alcoholic fermentations owing to its high tolerance to ethanol and also to organic acids, complemented with the exceptional ability to flourish even at low pH and limited oxygen availability [3-5]. Production of bioethanol using lignocellulosic biomass cannot be economically feasible if only the glucose present in the hydrolysate is converted to ethanol owing to the fact that lignocellulosic biomass consisting of ~30 to 45% glucan and ~20 to 35% xylan, which on subsequent pre-treatment and enzymatic hydrolysis, is converted to glucose and xylose, respectively. Despite the presence of gene homologs in the genome of *S. cerevisiae* encoding the necessary enzymes for xylose metabolism i.e. xylose reductase (XR), xylitol dehydrogenase (XDH) and xylulokinase (XKS), it cannot natively utilize xylose hydrolysed from plant biomass. Also, during pre-treatment and hydrolysis of lignocellulose biomass, different monomeric sugars along with a wide range of inhibitory substances are produced which limit microbial fermentation.

Two possible approaches to overcome this problem could be 1. To genetically engineer *S. cerevisiae*, 2. To use non-conventional microorganisms. Industrial strains of *S. cerevisiae* have been engineered worldwide in different ways. However, xylose fermentation in engineered *S. cerevisiae* brings with it the issue of co-factor imbalance followed by low metabolic flux. Thus, a highly promising alternative to engineering industrial friendly model hosts to efficiently utilize

mixed sugars is amending innate xylose-utilizing microorganisms. Non-conventional yeasts are a source of huge repository of barely exploited biodiversity, most of which have evolved independently from *S. cerevisiae* under extreme environmental conditions. They are highly suggestive of possessing novel mechanisms that are not present in the model yeast. Therefore, need to bio prospect native xylose utilizing/fermenting strains and assessing their potential cannot be undermined.

Conclusion

The yeast strains *M. caribbica* and *Lodderomyces sp.* used in this study showed promising mixed sugar fermentation (glucose + xylose) potential by producing 11.78 g L⁻¹ and 10.21 g L⁻¹ ethanol respectively within 24 hours of incubation. Their lower ethanol tolerance, as was observed in this study i.e., upto 6% concentration only could be overlooked since it is justifiable as ethanol titres obtained from biomass hydrolysates are generally not so high due to low sugar concentrations present. Also, these strains were tolerant to inhibitors-HMF and furfural at all studied levels, which definitely is advantageous over other strains, as they are a major hindrance to fermenting microorganism during ethanol production. Subsequent fermentation on pre-treated lignocellulose biomass will further validate the potentiality of this study in the scenario for bioethanol production on industrial scale. We still go with the ideology that improving a native pentose assimilating strain is far more logical than modifying a non-pentose assimilating strain, which otherwise fulfils all parameters as an ideal industrial strain.

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Conflict of Interest

Authors declare no conflict of interest.

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