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Leadership in Healthcare Organizations: Strategies for Effective Workforce Management

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Abstract

The production of bioethanol from lignocellulosic biomass remains a major challenge due to the high xylose content that conventional ethanol-producing yeasts, such as Saccharomyces cerevisiae, are unable to efficiently ferment. Although S. cerevisiae is widely favored for industrial ethanol production because of its robustness and tolerance to ethanol and fermentation inhibitors, its incapacity to utilize xylose significantly reduces overall process efficiency. This study focuses on exploring non-traditional yeast species capable of co-fermenting glucose and xylose to enhance bioethanol yield. Two yeast strains were isolated from decomposed apple samples and molecularly identified through ITS sequencing as Meyerozyma and Lodderomyces. Fermentation was carried out using a mixed sugar medium with a glucose-to-xylose ratio of 2:1 over a period of 96 hours. Both strains rapidly metabolized glucose, and more than one-third of the xylose was consumed within the first 24 hours. The resulting ethanol yields were 0.344 g/g for Meyerozyma and 0.327 g/g for Lodderomyces, corresponding to an approximate fermentation efficiency of 65%. In addition, these isolates demonstrated significant tolerance to common lignocellulosic inhibitors, including furfural and 5-hydroxymethylfurfural (HMF), at concentrations typically encountered in pretreated biomass hydrolysates. The findings highlight, for the first time, the ability of Meyerozyma and Lodderomyces to efficiently co-utilize mixed sugars for ethanol production, positioning them as promising candidates for industrial bioethanol processes. This study opens avenues for exploiting non-conventional yeasts to improve the conversion of lignocellulosic feedstocks into renewable fuels.

KEYWORDS: Non-conventional yeasts; Co-fermentation; Mixed sugar utilization; Bioethanol; Meyerozyma; Lodderomyces; Lignocellulosic biomass.

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INTRODUCTION

The continuous rise in fossil fuel costs and the environmental challenges arising from greenhouse gas emissions have intensified the global demand for alternative, sustainable, and cost-effective biofuels. Among various biofuel strategies, bioethanol production from lignocellulosic biomass has emerged as a promising solution due to the abundance of carbohydrates in plant cell walls. Lignocellulosic biomass is composed primarily of cellulose, hemicellulose, and lignin, which must undergo a series of processing steps—pre-treatment, enzymatic hydrolysis, and microbial fermentation—to yield fermentable sugars and, ultimately, ethanol. This multi-step approach not only enables bioethanol production but also allows for the generation of high-value products such as fine chemicals and biochemicals, thereby enhancing the economic feasibility of lignocellulosic biorefineries [1].

Saccharomyces cerevisiae has historically served as the industrial standard for ethanol fermentation due to its high ethanol tolerance, resilience under acidic conditions, and ability to grow

in low oxygen environments [2,3]. However, the yeast's inability to efficiently ferment xylose, a pentose sugar derived from hemicellulose, limits its efficacy for lignocellulosic bioethanol production. Although *S. cerevisiae* possesses endogenous genes encoding xylose-metabolizing enzymes—xylose reductase (XR), xylitol dehydrogenase (XDH), and xylulokinase (XKS)—it cannot naturally convert xylose into ethanol at industrially relevant rates. Additionally, inhibitory compounds generated during biomass pre-treatment, such as furfural and hydroxymethylfurfural (HMF), further reduce fermentation efficiency.

To overcome these limitations, two main strategies are typically considered: (i) genetic engineering of *S. cerevisiae* to enhance xylose metabolism, or (ii) exploration of non-conventional yeasts that naturally ferment xylose. Engineering approaches often face challenges related to co-factor imbalance and reduced metabolic flux. In contrast, non-conventional yeasts, which have evolved under diverse environmental conditions independent of *S. cerevisiae*, may possess unique metabolic pathways and stress tolerance mechanisms, making them excellent candidates for bioethanol production. Hence, bio-prospecting and assessing indigenous xylose-fermenting yeasts is a crucial step toward optimizing lignocellulosic ethanol processes [4].

METHODS

Isolation and Identification of Yeast Strains

Rotten apple samples were collected from local orchards as a potential source of xylose-fermenting yeasts. Samples were enriched in yeast extract-peptone-dextrose (YPD) broth and incubated at 30°C for 48 hours. Single colonies were isolated using streak plate techniques on YPD agar (Table 1). Morphological examination and ITS region sequencing were employed to identify the isolates at the species level [5].

Fermentation Substrate Preparation

Lignocellulosic hydrolysate was simulated using a sugar mixture containing glucose and xylose at a 2:1 ratio. The medium was supplemented with essential nutrients including ammonium sulfate, yeast extract, and phosphate buffer [6-9]. To assess inhibitor tolerance, furfural and 5-hydroxymethylfurfural (HMF) were added at concentrations typically observed in pretreated biomass.

Fermentation Conditions

Batch fermentation experiments were conducted in 250 mL Erlenmeyer flasks containing 100 mL of the sugar medium. Isolated yeast cultures were inoculated at an optical density (OD600) of 0.2 and incubated at 30°C with shaking at 150 rpm. Samples were withdrawn at 24-hour intervals up to 96 hours to monitor sugar consumption and ethanol production.

Analytical Methods

Glucose and xylose concentrations were determined using high-performance liquid chromatography (HPLC) equipped with a refractive index detector. Ethanol concentrations were quantified via gas chromatography (GC) using a flame ionization detector. Fermentation efficiency was calculated as the percentage of theoretical ethanol yield based on total sugar consumed (Tables 2 and 3) [10,11].

RESULTS

Isolation and Identification of Yeasts

Two yeast strains were successfully isolated from the rotten apple samples and identified as **Meyerozyma** sp. and **Lodderomyces** sp. ITS sequencing confirmed the taxonomic identity with >99% similarity to reference sequences.

Sugar Utilization and Ethanol Production

Both strains efficiently metabolized glucose, depleting it within 24 hours of fermentation. Xylose consumption reached over 33% of the initial content during the same period (Table 4). The

Table 1: Identification and Source of Non-Conventional Yeast Strains.

Yeast Strain	Source Sample	Morphological Features	Molecular Identification (ITS Sequencing)	Sequence Similarity (%)
Meyerozyma sp.	Rotten apple	Oval cells, single budding	ITS rDNA	>99
Lodderomyces sp.	Rotten apple	Ellipsoidal cells, multipolar budding	ITS rDNA	>99

Table 2: Sugar Consumption and Ethanol Production by Meyerozyma and Lodderomyces.

Yeast Strain	Glucose Consumed (%)	Xylose Consumed (%)	Ethanol Yield (g/g sugar)	Fermentation Efficiency (%)
Meyerozyma sp.	100	33	0.344	65
Lodderomyces sp.	100	33	0.327	65

Table 3: Tolerance of Yeast Strains to Common Lignocellulosic Inhibitors.

Yeast Strain	HMF Concentration Tolerated (g/L)	Furfural Concentration Tolerated (g/L)	Growth Impact	Ethanol Production Impact
Meyerozyma sp.	2	2	Minimal	Moderate
Lodderomyces sp.	2	2	Minimal	Moderate

Table 4: Ethanol Production Performance of Non-Conventional Yeasts.

Yeast Strain	Incubation Time (h)	Ethanol Concentration (g/L)	Maximum Ethanol Tolerance (%)	Notes
Meyerozyma caribbica	24	11.78	6	Efficient mixed sugar fermentation
Lodderomyces sp.	24	10.21	6	Effective inhibitor tolerance

ethanol yields were 0.344 g/g for **Meyerozyma** and 0.327 g/g for **Lodderomyces**, corresponding to approximately 65% of the theoretical maximum yield.

Tolerance to Inhibitors

Both yeast strains demonstrated remarkable tolerance to furfural and HMF at concentrations commonly found in pretreated biomass. Growth and fermentation performance were only moderately affected, indicating the robustness of these non-conventional strains under stress conditions typical of lignocellulosic hydrolysates [12-17].

DISCUSSION

The present study highlights the potential of non-conventional yeasts as viable alternatives to engineered *S. cerevisiae* for mixed sugar fermentation in bioethanol production. The rapid utilization of glucose and partial consumption of xylose by *Meyerozyma* and *Lodderomyces* demonstrates their inherent metabolic versatility. Unlike genetically modified *S. cerevisiae*, these yeasts naturally overcome co-factor imbalance issues, providing a practical route for industrial application [18].

Inhibitor tolerance is a critical factor in lignocellulosic ethanol production, as compounds generated during biomass pretreatment often inhibit microbial growth. The observed resilience of the isolated yeasts suggests that they could maintain fermentation efficiency without extensive detoxification steps, thereby reducing process costs. Furthermore, the ability of these yeasts to co-utilize glucose and xylose supports their potential for complete utilization of lignocellulosic hydrolysates [19]. This property could significantly enhance ethanol yields and contribute to the economic feasibility of second-generation bioethanol production. The study underscores the importance of exploring microbial biodiversity for discovering robust, non-conventional strains capable of addressing the limitations of model organisms in industrial biotechnology.

CONCLUSION

The non-conventional yeast strains *Meyerozyma caribbica* and *Lodderomyces* sp. demonstrated significant potential for mixed sugar fermentation, efficiently converting glucose and xylose into ethanol. Within 24 hours of incubation, ethanol concentrations of 11.78 g L^{-1} and 10.21 g L^{-1} were achieved by *M. caribbica* and *Lodderomyces* sp., respectively. Although the observed ethanol tolerance of these strains was limited to approximately 6%, this limitation is not critical for biomass-derived fermentations, as hydrolysates generally contain relatively low sugar concentrations, resulting in moderate ethanol titers. Importantly, both strains exhibited robust tolerance to fermentation inhibitors, including 5-hydroxymethylfurfural (HMF) and furfural, across the tested concentrations. This characteristic provides a significant advantage over conventional yeasts, as inhibitor accumulation often impedes industrial-scale ethanol production. Future studies involving the fermentation of pre-treated lignocellulosic biomass will be crucial to validate the industrial applicability of these strains for large-scale bioethanol production. Leveraging native pentose-assimilating yeasts appears to be a more pragmatic and effective strategy than attempting to genetically modify non-pentose-utilizing industrial strains. These findings reinforce the promise of *Meyerozyma* and *Lodderomyces* as viable candidates for sustainable and efficient bioethanol production.

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