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Study of novel *Saccharomyces cerevisiae* isolated from Finger millet for sustainable Bioethanol Production in Kenya

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ABSTRACT

Bioethanol is an important renewable energy source due to its cleaner combustion and reduced environmental impact compared to fossil fuels. It is biodegradable, less toxic, and contributes to lower emissions of harmful pollutants such as carbon monoxide and hydrocarbons. Despite these advantages, the economic feasibility of bioethanol production remains constrained by the relatively low ethanol yields obtained from commonly used commercial yeast strains, which increases production and processing costs. This study aimed to evaluate the fermentation performance of indigenous yeast strains isolated from pre-germinated finger millet (Eleusine coracana) and compare their ethanol production efficiency with that of the standard industrial strain, Saccharomyces cerevisiae. Two yeast strains (Y1 and Y2) were isolated, cultured, and purified from finger millet malt, while a commercial S. cerevisiae strain (Y3) served as the control. Fermentation experiments were conducted under static conditions for 48 hours across selected plant substrates. Ethanol production varied significantly among the tested strains (p = 0.0273). The mean ethanol concentrations obtained were 6.68% v/v for Y1, 8.15% v/v for Y2, and 6.96% v/v for Y3. Strain Y2 exhibited the highest ethanol yield, outperforming both the commercial strain and the other isolate, while Y1 showed performance comparable to the control. These findings demonstrate that indigenous yeast strain Y2 possesses superior fermentative capacity and holds strong potential for enhancing bioethanol production. The use of such locally sourced, high-performing strains could improve the efficiency, sustainability, and cost-effectiveness of biofuel production systems.

Keywords: *Biofuel, Yeasts, Hydrolysis, Finger millet, Fermentation.*

Introduction

Bioethanol (ethyl alcohol) is a renewable biofuel produced through the microbial fermentation of sugars derived from biomass sources such as sucrose, starch, and lignocellulosic materials [2]. As a sustainable alternative to fossil fuels, it has attracted significant attention due to its biodegradability, low toxicity, and reduced greenhouse gas emissions [8]. When blended with gasoline, bioethanol improves combustion efficiency and significantly lowers emissions of carbon monoxide (CO), unburned hydrocarbons (HC), and particulate matter [9]. Increasing ethanol content in fuel blends enhances engine performance while reducing environmental pollution [19]. In compression-ignition engines, bioethanol can also be applied in biodiesel–diesel dual-fuel systems, achieving high ethanol utilization under increased engine loads [11].

In addition to its role as a transport fuel, bioethanol has important domestic and industrial applications. Alcohol-based disinfectants containing more than 60% (v/v) ethanol are effective in inactivating pathogens such as SARS-CoV-2 [10]. Furthermore, bioethanol is increasingly used as a clean household cooking fuel, significantly reducing indoor air pollution compared to traditional fuels such as kerosene and charcoal, thereby lowering associated health risks [16].

Recent advances in biotechnology, including CRISPR–Cas systems and co-culture fermentation strategies, have enhanced the potential for improving bioethanol production [23,12]. However, the economic viability of bioethanol remains limited by the relatively low ethanol yields produced by conventional industrial yeast strains such as *Saccharomyces cerevisiae* [14]. Low ethanol concentrations increase downstream processing costs, particularly during distillation, making production less economical.

At the biochemical level, ethanol fermentation is a metabolic process in which sugars such as glucose are converted into ethanol and carbon dioxide by yeast.

Ethanol fermentation is a biochemical process in which sugars such as glucose are converted into ethanol and carbon dioxide by yeast under anaerobic conditions. The process begins with glycolysis, where glucose is metabolized to produce pyruvate, energy in the form of ATP, and reducing equivalents (NADH). This process can be represented as follows:

$C_6H_{12}O_6 + 2ADP + 2P_i + 2NAD^+ \rightarrow 2CH_3COCOO^- + 2ATP + 2NADH + 2H_2O + 2H^+$ [5]

In this step, one molecule of glucose is converted into two molecules of pyruvate (CH_3COCOO^-), producing a net gain of ATP and reducing NAD^+ to NADH [5]

The pyruvate formed is subsequently decarboxylated by the enzyme pyruvate decarboxylase to produce acetaldehyde and carbon dioxide:

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- $CH_3COCOO^- + H^+ \rightarrow CH_3CHO + CO_2$ [24]

Finally, acetaldehyde is reduced to ethanol by the enzyme alcohol dehydrogenase using NADH as a reducing agent:

$CH_3CHO + NADH + H^+ \rightarrow C_2H_5OH + NAD^+$ [24]

This final step regenerates NAD^+, which is essential for maintaining glycolysis under anaerobic conditions and ensuring continuous ethanol production [24].

The process begins with glycolysis, where glucose is broken down into pyruvate, producing ATP and NADH [5]. The pyruvate is subsequently decarboxylated to acetaldehyde and then reduced to ethanol, regenerating NAD^+ and allowing glycolysis to continue under anaerobic conditions [24].

Despite the widespread use of *Saccharomyces cerevisiae* in industrial fermentation, its performance is often limited in terms of ethanol yield and tolerance to stress conditions [14]. This has led to increasing interest in naturally occurring (wild) yeast strains, which may possess superior fermentation characteristics such as faster sugar utilization and improved stress tolerance [25].

Finger millet (*Eleusine coracana*), particularly during germination, provides a nutrient-rich environment that supports microbial growth and may harbor yeast strains with strong fermentative capabilities [3]. Exploring such local substrates offers an opportunity to identify efficient yeast strains that can enhance bioethanol production.

Therefore, this study evaluates the fermentation performance of wild yeast strains isolated from pre-germinated finger millet and compares their ethanol production efficiency with that of the industrial strain *Saccharomyces cerevisiae*.

Sample preparation

Cassava tubers, maize, sorghum, and Maerua shrub materials were collected and thoroughly washed with clean tap water to remove soil and surface contaminants. The cassava tubers were peeled and cut into small cubes. All samples were then subjected to sun drying for approximately 7 hours per day over two consecutive days to reduce moisture content. Thereafter, the partially dried samples were transferred to the laboratory and oven-dried at 80 °C for 72 hours to ensure complete drying. The dried maize, sorghum, and cassava samples were subsequently ground into fine powder using an electric grinder fitted with a 1 mm mesh sieve and stored in airtight containers until further use.

For the preparation of finger millet malt, 500 g of finger millet grains were soaked in 1000 mL of distilled water and left to stand for 24 hours. The water was then drained, and the soaked grains were transferred into perforated bags and incubated at 30 °C for 72 hours to allow germination. After germination, the grains were sun-dried for approximately 6 hours, ground into fine powder using a 1 mm sieve, and stored in sterile airtight containers.

Isolation and Purification of Yeast Strains

To activate the commercial yeast (Saccharomyces cerevisiae), 1 g of dry yeast was suspended in 50 mL of sterile 2% (w/v) dextrose solution and incubated at 30 °C for 24 hours. In parallel, ground finger millet malt was mixed with 50 mL of sterile distilled water and incubated under the same conditions. The two preparations were labelled as FM (finger millet) and SC (commercial yeast), respectively.

Potato Dextrose Agar (PDA) medium was prepared by dissolving 9.75 g of PDA in 250 mL distilled water and sterilized by autoclaving at 121 °C for 15 minutes.

After cooling, 3 mg of streptomycin was added to suppress bacterial growth. The incubated FM and SC samples were serially diluted up to 10^{-8}, and 0.1 mL aliquots were spread onto PDA plates in triplicate. The plates were incubated at 35 °C for 48 hours.

Distinct yeast colonies obtained from finger millet malt were identified based on morphology, with white colonies designated as Y1 and creamy colonies as Y2. These isolates, together with the commercial yeast (Y3), were purified by repeated streaking on fresh PDA plates under aseptic conditions. Pure cultures were then maintained on PDA slants and stored at 4 °C for preservation.

Preparation of Yeast Inoculum

A loopful of each purified yeast culture (Y1, Y2, and Y3) was inoculated into 500 mL of sterile Yeast Extract Peptone Dextrose (YEPD) broth (pH 6.0) contained in 1 L conical flasks. The cultures were incubated at 30 °C with shaking at 150 rpm for 48 hours to obtain active yeast biomass. The cells were then harvested by centrifugation at 5000 rpm and used as inoculum for fermentation.

Fermentation Process (Simultaneous Saccharification and Fermentation)

Fermentation was carried out using the simultaneous saccharification and fermentation (SSF) method in a batch system. Approximately 20 g of each plant substrate was weighed into 250 mL Erlenmeyer flasks, and 120 mL of distilled water was added. The mixtures were stirred thoroughly, and the liquid level was marked before sterilization by autoclaving at 121 °C for 15 minutes.

After cooling to approximately 40 °C, distilled water was added to restore the original volume. Each flask was then supplemented with 1 g of α-amylase enzyme to facilitate starch hydrolysis and 1 g of yeast inoculum (approximately 5% inoculum size). The flasks were covered with aluminium foil to prevent contamination and incubated at 35 °C for 48 hours under static conditions.

Ethanol Recovery and Quantification

After fermentation, the mixtures were filtered to remove solid residues. The filtrates were subjected to distillation using a thermostatically controlled distillation apparatus to recover ethanol.

Quantitative determination of ethanol concentration was performed using the acidified potassium dichromate method. Standard ethanol solutions (1–8% v/v) were prepared by diluting 96% analytical-grade ethanol with distilled water. A calibration curve was constructed by plotting absorbance against ethanol concentration.

For analysis, 0.5 mL of the distillate was diluted with 4.5 mL of distilled water. An equal volume of this diluted sample was reacted with 0.298 M acidified potassium dichromate solution, and the absorbance was measured using a spectrophotometer. Ethanol concentrations in the samples were determined by comparison with the calibration curve.

RESULTS AND DISCUSSION

Ethanol Concentration Produced by Different Yeast Strains

The ethanol concentrations produced by the three yeast strains are presented in Figure 1. Statistical analysis using one-way ANOVA showed that ethanol production differed significantly among the strains ($p = 0.0273$, $p < 0.05$), indicating that yeast type had a significant effect on fermentation performance.

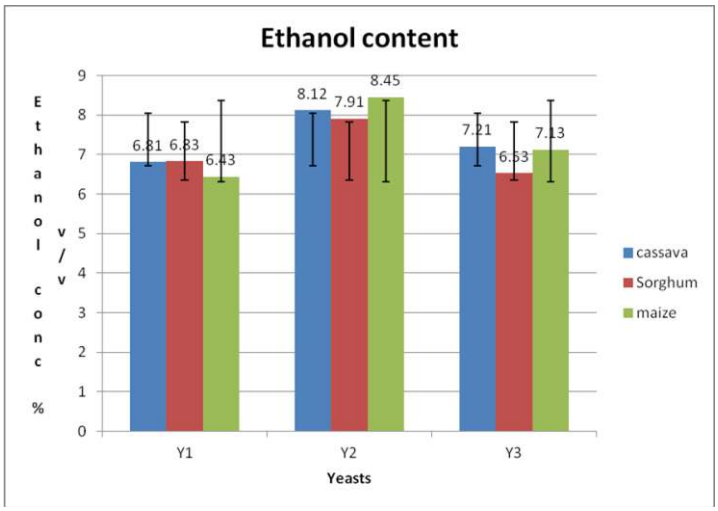

Figure 1: Bar graphs on ethanol concentration in (g/L) produced by yeasts from plants

The mean ethanol concentrations (% v/v) obtained were 6.68 for Y1, 8.15 for Y2, and 6.96 for Y3 (*Saccharomyces cerevisiae*). Among the strains, Y2 produced the highest ethanol concentration, significantly outperforming both Y1 and the commercial strain (Y3). In contrast, Y1 produced the lowest ethanol concentration, while the performance of Y3 was intermediate. However, the difference between Y1 and Y3 was not statistically significant, suggesting that Y1 exhibited fermentation efficiency comparable to the commercial yeast.

Across all plant substrates (cassava, maize, and sorghum), consistent differences in ethanol yield were observed among the yeast strains. The superior performance of Y2 indicates enhanced fermentative capacity, which may be attributed to improved sugar utilization efficiency, higher tolerance to ethanol accumulation, or greater enzymatic activity during fermentation [14, 25].

Analytical Reliability

The determination of ethanol concentration using the acidified potassium dichromate method demonstrated high analytical accuracy. The calibration curve constructed from standard ethanol solutions (1–8% v/v) exhibited strong linearity with a correlation coefficient ($R^2 \approx 0.998$), indicating that absorbance measurements provided a reliable estimate of ethanol concentration in the distillate samples [13].

Comparative Fermentation Performance

The results highlight the importance of yeast strain selection in bioethanol production. While all three strains were capable of fermenting the plant substrates, their efficiencies varied significantly. Statistical comparisons confirmed that Y2 produced significantly higher ethanol concentrations than both Y1 and Y3, whereas Y1 and Y3 showed comparable performance.

The enhanced performance of Y2 suggests that this wild isolate possesses favorable metabolic traits, such as rapid fermentation kinetics, efficient substrate utilization, and improved tolerance to fermentation stress conditions [25, 14]. In contrast, the relatively lower ethanol yield observed for the commercial strain may reflect limitations in its adaptability to the specific substrates used in this study.

Role of Indigenous Yeast Strains

The findings of this study support previous reports that wild yeast strains isolated from natural or traditionally fermented substrates can exhibit superior fermentation performance compared to commercial strains [4]. For example, studies on yeasts from traditional fermented beverages have identified strains with high ethanol-producing capabilities [2].

The present results similarly demonstrate that the wild yeast Y2, isolated from pre-germinated finger millet, outperformed the industrial standard. Finger millet malt provides a nutrient-rich and dynamic environment during germination, which may promote the development of yeast strains with enhanced stress tolerance and metabolic efficiency [3].

Effect of Fermentation Strategy (SSF)

The relatively high ethanol concentrations observed across all strains can also be attributed to the use of the Simultaneous Saccharification and Fermentation (SSF) process which improves sugar utilization and reduces feedback inhibition [15]. In this system, the addition of α-amylase enables the breakdown of starch into fermentable sugars, which are immediately utilized by yeast cells.

This simultaneous conversion reduces the accumulation of sugars in the medium, thereby minimizing feedback inhibition and maintaining a high rate of enzymatic activity throughout the fermentation period. As a result, SSF enhances overall ethanol yield compared to the separate hydrolysis and fermentation (SHF) approach [14].

Economic Implications

The significantly higher ethanol concentration produced by Y2 has important economic implications. In bioethanol production, downstream processing particularly distillation is energy-intensive. Higher ethanol concentrations in the fermentation broth reduce the volume of material that must be processed, thereby lowering energy requirements and overall production costs [14].

Therefore, the use of high-performing yeast strains such as Y2 can improve the efficiency and economic viability of bioethanol production systems [14].

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Finger millet malt provides a nutrient-rich and dynamic environment during germination, which may promote the development of yeast strains with enhanced stress tolerance and metabolic efficiency [3]. The creamy colony morphology of Y2 may also be indicative of physiological characteristics associated with improved ethanol production.

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Conclusion

This study demonstrated that ethanol production varied significantly among the three yeast strains evaluated. Two indigenous yeast strains (Y1 and Y2) were successfully isolated from pre-germinated finger millet, while Y3 represented the commercial Saccharomyces cerevisiae strain. Among the strains tested, Y2 exhibited the highest ethanol production (8.15% v/v), significantly outperforming both Y1 and the commercial strain. In contrast, Y1 showed ethanol production comparable to the industrial standard. These findings confirm that certain wild yeast strains possess superior fermentative capabilities and can serve as effective alternatives to conventional commercial yeasts [4,14].

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