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# CHARACTERIZATION OF INDIGENOUS *SACCHAROMYCES CEREVISIAE* STRAINS FOR THEIR POTENTIAL USE AS BAKER'S YEAST

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*Key words:* Baker's yeast, Indigenous, Dough leavening, Biomass yield, Dried yeast

## Abstract

This research isolated and characterized indigenous *Saccharomyces cerevisiae* strains. Additionally, this study optimized fermentation conditions for the potential yeast strains and compared their leavening efficacy and biomass production with locally available commercial dried yeast (DY1) in Bangladesh. A total of thirty-five yeast strains were isolated from twenty-five indigenous fruit samples such as Mango *(Mangifera indica* L.*),* Jackfruit *(Artocarpus heterophyllus* Lam.), Papaya *(Carica papaya* L.*)*, Litchi (*Litchi chinensis* Sonn.) and Banana (*Musa oranta* Roxb.). Based on bread leavening efficacy, four potential baker's yeast strains (Man5, Ban2, Man9, and Ban5) were selected for characterization as potential baker's yeast. Based on biochemical properties, API kit-based identification, and PCR-based molecular identification, all newly isolated yeast strains were identified as *S. cerevisiae*. None of these isolates produced $H_2S$. Man5 and Ban5 isolates flocculated at a level comparable to the DY1. All strains showed better temperature tolerance (up to 45°C) than DY1. Man5 and Man9 also showed maximum ethanol tolerance (up to 16%). Only DY1 and Man5 increased dough volume significantly compared with other strains ($p< 0.05$). Notably, in the optimized growth condition, the Man5 strain produced the highest biomass significantly compared with others ($p< 0.05$). From the present study, it is concluded that the indigenous strain Man5, Man9, and Ban5 have the potential to be used in the industry as a substitute for imported baker's yeast in Bangladesh and that will save a substantial amount of foreign currency.

## Introduction

*Saccharomyces cerevisiae* - a yeast species, has long been known for its multipurpose use in foods and alcoholic beverages. *S. cerevisiae* is used to produce fuels, chemicals, pharmaceuticals, food ingredients, and feed additives[1,2]. Food-grade yeasts offer a sustainable solution to the shortages of proteins and other valuable nutrients as protein supplementation in both human food and animal feed[3]. Commercial baker's yeasts (*Saccharomyces cerevisiae*) are mainly used for the fermentation of modern bread making, and it is used to leaven bread throughout the world[4,5]. The carbon dioxide produced by the yeast is responsible not only for the leavening of dough but also for improving the

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flavor and texture[6]. Besides, the yeast used in bread making contributes to nutritional benefits by providing amino acids and vitamins. Additionally, yeasts are also proved very promising for several biotechnological applications[7,8].

Baker's yeast and yeast products are used in the food industry to leaven bread and as nutritional supplements and food additives for human and animal health. Consequently, the demand for baker's yeast has tremendously increased over recent years. Today, in this first decade of the 21st century, baker's yeast is produced worldwide in 2.3 million tons per annum[9]. Modern industries require a considerable amount of selected baker's yeast to obtain high-quality, reproducible products. Every day, even the people in Bangladesh are rapidly leaning towards fast food, the primary component of which is the bread and bakery-related products. Therefore, the use of commercial baker's yeast for food production is increasing daily. Most of the baker's yeast used in Bangladesh is imported from abroad spending considerable foreign currency[10]. According to the foreign trade statistics of Bangladesh 2019-2020, Bangladesh imported different forms of active and inactive yeasts, which cost about BDT 917 Million each year (USD 0.9 Million)[11]. In 2021, the market size value for different forms of yeast was USD 4.02 Billion which is forecasted to reach USD 9.20 Billion by 2030[12]. If we could produce them locally, it would save a substantial amount of foreign currency and significantly reduce import dependency. It will also encourage local entrepreneurs to establish yeast industries in this country if they find it profitable.

So, it is crucial to find a good quality baker's yeast strain to fulfill this demand. Different fruits that are being used to isolate baker's yeast include grapes, apples, and oranges[10,13]. Other fruits that can be used to isolate baker's yeast include bananas, pears, and pineapples[14]. These fruits contain high levels of natural sugars, which can be used as a food source for yeast. In Bangladesh, we have an extensive collection of indigenous fruits[15]. These indigenous fruits could be explored to identify novel *S. cerevisiae* strains with excellent leaving activity and biomass-yielding capacity, which could replace commercial strains as potential baker's yeast.

Therefore, this study was aimed to isolate, identify, and characterize indigenous *S. cerevisiae* strains from regional fruit samples including optimization of the fermentation conditions and leavening capacity of the strains identified.

## Materials and Methods

*Sample collection and isolation of the indigenous yeast:* This study was done in Dhaka city in Bangladesh. A total of twenty-five samples, including Mango *(Mangifera indica* L.*)* (N = 9), Jackfruit *(Artocarpus heterophyllus* Lam.*)* (N= 4), Papaya *(Carica papaya* L.*)* (N= 4), Litchi (*Litchi chinensis* Sonn.) (N=3), and Banana (*Musa oranta* Roxb.) (N= 5) were randomly collected from local markets in Dhaka city for indigenous yeast isolation. Moreover, a commercially available dried yeast (DY1) and *S. cerevisiae* NRBC 2044 were also collected as reference strains. The yeast number was increased by enriching the sample in YPG

medium containing Chloramphenicol (10 g of sample in 100ml). Chloramphenicol was used to inhibit bacterial growth. The enriched culture broth was serially diluted appropriately and spread plated on Yeast Extract Peptone Glucose Chloramphenicol Agar (YPGC) or Rose Bengal Chloramphenicol Agar and incubated for 72 h. Creamy white-colored yeast colonies were selected based on their dough-leavening efficiency. Then distinct selected colonies were subsequently sub-cultured on YPGC to obtain pure isolates[16].

*Screening of potential baker's yeast:* A commercial baker's yeast (DY1) was used to compare with indigenous *S. cerevisiae* strains for screening as potential baker's yeast. Additionally, *S. cerevisiae* NRBC 2044 (obtained from Centre for Advanced Research in Sciences, CARS-DU) and was used as a positive control for all biochemical tests and screening.

*Microscopic observation:* A single yeast colony was mixed in a drop of 70% ethanol on a glass slide, and a smear was prepared. After that, using a pipette, one or two drops of diluted lactophenol cotton blue solution was added before the ethanol dries off. The stain was covered carefully with a clean, sterile coverslip without causing a bubble to the stain. The smear was observed at 100X magnification using a light microscope.

*Lactose utilization test:* Yeast cells were cultured in Yeast Fermentation Broth (YFB) with pre-sterilized lactose (6% w/v) for three days at 30°C[17]. Durham tubes were also introduced into the media to capture the carbon dioxide that was released. The transition of color indicated that lactose was being used by the yeast.

*Nitrate reduction test:* In nitrate broth, a well-isolated colony was inoculated and incubated for 48 hours at 30°C[18]. After incubation, the tube was filled with five drops of reactive 1 and reactive 2. After 5-10 min, the appearance of red color was not observed.

*H2S Production test*: In the KIA medium, the concentrations of lactose and sucrose are ten times higher than the glucose concentration (1.0 percent vs 0.1 percent). All test isolates were subjected to KIA tests and incubated for 48 hours at 30°C.

*Flocculation test:* The test isolates were inoculated into 10 ml of YPG broth and incubated for three days at 30°C. After incubation, agitation was performed to observe the flocculation[19].

*Carbohydrate utilization test:* The test for carbohydrate utilization was carried out in broth with inverted Durham tubes[19]. Glucose, dextrose, sucrose, galactose, raffinose, and maltose were the several sugars that were used. The yeast strains were inoculated into the media and incubated for 24 h. The color change showed the use of carbon sources for fermentation[17].

*Temperature tolerance test:* Candidate isolates were streaked on YPGC agar plate and incubated at 25, 30, 37, 45 and 55°C. After 24 hrs, their growth was observed and analyzed[20].

*Ethanol tolerance test:* Candidate isolates were inoculated in YPG broth containing ethanol (8, 10, 12, 14 and 16% v/v) and tested for ethanol tolerance[20].

*Invertase activity test:* The following ingredients (in grams per liter) are used to make the enzyme production media, i.e., sucrose 2%, yeast extract 1%, $(NH_4)_2SO_4$ 0.1%, $MgSO_4$ 0.075%, and $KH_2PO_4$ 0.35%, with a final pH of 5. Yeast isolates were cultured in media for 48 hours at 30°C at 120 rpm. The supernatant was collected after 48 hrs by centrifugation at 10000 rpm for 10 min. The supernatant was used as a crude invertase enzyme. Invertase activity was determined using a slightly modified technique of Sumner and Howells. In a 0.03 M acetate buffer (pH 5.0), 0.1 ml of enzyme solution was incubated with 0.9 mL of 10% sucrose for 5 minutes at 30°C. The reaction was halted by adding 1 ml of dinitrosalicylic acid reagent (DNS) and heating it for 15 min in a boiling water bath. After heating, the solution was diluted by adding 1ml of deionized water. Enzyme blank tubes are used as control. OD was measured against these enzyme blanks. Finally, using a Spectrophotometer, absorbance was measured at 540 nm.

*Dough leavening test:* 20 ml of sterilized water was taken in a 100 ml beaker for each isolate. Water was taken in five beakers. 0.5 g of yeast pellet was mixed with the water taken in a beaker. Then 25 g of flour, 2 g of sugar, and 0.5 g of salt were taken in the beaker. After that, ingredients, water and yeast cells were mixed well. The initial height of the dough was recorded. And finally, the increasing height of the dough was observed and recorded at one hour intervals.

*Identification of the potential yeast strains by* API confirmation: Four potential yeast strains were further confirmed biochemically using the 20 C AUX API (Analytical profile index) test. The 20C AUX API kit was purchased from Biomerieux, USA, and API detection was done for all presumptively identified yeast strains according to the manufacturer's instructions (https://www.biomerieux.com/). *S. cerevisiae* NRBC 2044 strain was used as a positive control for all identification tests.

*Genomic DNA extraction: S. cerevisiae* cultures were grown in 10 ml YPG media overnight. The cell was pelleted by centrifugation at 8000g for 5 minutes at room temperature. Cell pellets were re-suspended and transferred to a 1.5 ml micro-centrifuge tube. After that, the supernatant was discarded, and the pellet was taken to extract genomic DNA. Yeast DNA Extraction Kit (Thermo SCIENTIFIC, USA) was used for genomic DNA extraction according to the manufacturer's protocol.

*Polymerase chain reaction:* For *S. cerevisiae* species identification, a set of species-specific primers ScerF2 (5'–GCGCTTTACATTCAGATCCCGAG -3') and ScerR2 (5'-TAAGTTGGTTGTCAGCAAGATTG-3') were used[21]. DNA amplification was carried out in a final volume of 25 µl containing 0.2 mM of dNTPs, 0.5 µL of each primer, 1× PCR reaction buffer, 1.5 mM $MgCl_2$, and 1.25 U Taq DNA polymerase (BioRad, USA) and 2 µl of template DNA. PCR cycling conditions used were: initial denaturation at 94ºC for 4 min followed by 30 cycles of amplification, denaturation at 94ºC for 1 min, annealing at 55ºC for 1min, and extension at 72ºC for 1min; final extension at 72ºC for 2 min. Amplified DNA (150 bp) was separated on 1.5% agarose gel for one hour. The gels were stained ethidium bromide and visualized under UV light.

*Optimization of physicochemical parameters for yeast biomass production:* Optimization of temperature: Some studies were performed to determine the optimum temperature condition for each isolate. The inoculum with a similar concentration of each isolate was inoculated into 100 ml YPG broth and incubated in an orbital shaker for 24 hrs at 30°C with 120 rpm. The OD was tested the next day to check that each flask had the same number of yeast cells. After that, each sample was diluted $10^{-5}$ fold. Each sample was inoculated onto a YPG agar plate using the spread plate technique. For 24 hours, plates were incubated at 30, 37 and 40°C. After 24 hrs, colonies on each plate were counted for each temperature. The optimum temperature of each isolate was determined by counting the CFU on each plate incubated at different temperatures.

*Optimization of pH:* For pH optimization of yeast isolates, one single colony of each isolate was inoculated in 50 ml YPG broth and maintained in an orbital shaker at 30°C and 120 rpm for 24 hrs. The OD was checked after 24 hours to determine the cell concentration. After that, 1 ml of inoculum was inoculated in 100 ml of YPG broth having different pH levels (pH 4, 5, 6, and 7) and incubated in an orbital shaker at 30°C and 120 rpm. The following day, using a spectrophotometer, the OD was measured at 600 nm.

*Optimization of glucose concentration:* To optimize glucose concentration, fermentation was carried out in shake flasks containing the media with different glucose concentrations (0.5, 1, 2 and 3%). 1ml of inoculum for each strain was inoculated into each flask containing 100 ml YPG media (glucose concentration 0.5, 1, 2, 3%). These flasks were kept at 30°C and 120 rpm in a shaker incubator for 24 hrs. After 24 hrs, absorbance was measured using a spectrophotometer at 600 nm. Each sample was inoculated onto an YPG agar plate using the spread plate technique and incubated at 30°C. After 24 hrs, colonies on each plate were enumerated for each concentration.

*Measurement of biomass dry weight:* Each potential yeast strain was cultured at 100 ml YGP broth medium to estimate the dry biomass weight. The fermentation broth was centrifuged at 6000 rpm for 10 min and the pellet was collected by removing the supernatant. The wet weight of every isolate was measured carefully, and after that, the biomass was dried in a vacuum oven at 60°C for 6 hrs. After 6 hrs of drying, dry weight was measured carefully. By performing this experiment amount of biomass of each isolate was compared.

*Statistical analyses:* Data obtained from this study were taken in triplicate. ANOVA among the different baker's yeasts' properties was analyzed using SPSS version 24.0 for windows (IBM, Chicago, Il) and SAS software 9.1 (SAS Inst. Inc., NC, USA). All graphs were prepared using GraphPad Prism 8.0 (GraphPad Software, USA).

## Results and Discussion

*Isolation of the yeast:* Thirty five single colonies of yeast were isolated in Yeast extract Peptone Glucose (YPG) medium and Rose Bengal Chloramphenicol Agar media plate. Creamy to white colored colonies with fluffy and smooth margins were selected as a

tentative *Saccharomyces* species as described earlier[16,22,23]. Morphology of the each strain was observed and were found to be smooth and had a whitish cream color, convex and elevated elevation, undulated, round, and whole edges. After staining with lactophenol cotton blue, each yeast strain was examined under a 100X light microscope. Under microscopic view, they are unicellular, oval, and ellipsoid to the elongated shape. Among these 35 single colonies, only 12 strains were lactose and nitrate negative i.e., showed negative results in lactose utilization and nitrate reduction, therefore primarily identified as *S. cerevisiae*[19,24]. However, only four isolates showed promising dough leavening in preliminary test. Hence, those four isolates Man5 (isolated from Mango), Ban2 (isolated from Banana), Man9 (isolated from Mango), and Ban5 (isolated from Banana) were characterized further.

*Screening of potential baker's yeast:* Four presumptively identified strains of *S. cerevisiae* isolates were subjected to further screening tests to identify the best potential baker's yeast strain. *S. cerevisiae* NRBC 2044 was used as a positive reference in all identification/screening tests. Results of lactose utilization, nitrate reduction, $H_2S$ production, flocculation test, and carbohydrate utilization tests are presented in table 1. None of these yeast strains produced $H_2S$, an undesirable property of potential baker's yeast because $H_2S$ contributes a bad odor and flavor to the bread, lowering its quality[25,26]. Consequently, all of these tested strains were found to be as potential baker's yeast.

In this current study, all tested strains flocculated easily including Man5 and Ban5 isolates that were comparable with DY1. Flocculation is the tendency of yeast cells to aggregate together, forming a multicellular mass and sedimenting rapidly from the suspended medium or rising to the surface [27,28]. This particular property will make the downstream processing easier to separate the yeasts from the fermentation broth.

When the color changes to yellow in a carbohydrate utilization test, it means that the organisms are using sugar to make acidic compounds and gas. Because gas generation is one of the essential parameters for dough leavening in the baking industry[29], these strains showed promising results in gas production. To varying degrees, all four isolates were able to ferment glucose, dextrose, sucrose, galactose, raffinose, and maltose. Interestingly, Man5 and Ban5 produced gas from fermenting all the sugars that indicates their commercial potential comparable with DY1 strain.

In response to different temperature and alcohol tolerance tests, these four strains demonstrated variable responses (Table 2). All strains showed better growth at 30°C. However, newly isolated Man5, Ban2, Man9, and Ban5 showed similar growth at 37°C. These four strains were better thermo-tolerant than commercial yeast as they showed growth even at 45°C, where DY1 could not survive. However, none of these strains survived at 55°C. Baker's yeast might encounter very high temperatures during downstream processing of preparation of dried yeast[30]] and during baking and storage[31]. Additionally, temperature could get elevated during the fermentation of the dough, which would impair different metabolic processes causing deterioration of flavor

and taste quality[32]. Therefore, such thermotolerance properties of newly isolated yeasts made them promising candidates to be used as industrial baker's yeast.

**Table 1. Biochemical test results of potential yeast strains isolated from regional fruit samples.**

| Isolate ID | Lactose utiliza-tion test | Nitrate reduction test | $H_2S$ produc-tion test | Floccula-tion test | Carbohydrate utilization test | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Glucose | Dextrose | Sucrose | Galactose | Raffinose | Maltose |
| DY1 | - | - | - | + | + | + | + | + | + | + |
| PC | - | - | - | + | + | + | + | + | + | + |
| Man5 | - | - | - | + | + | + | + | + | + | + |
| Ban2 | - | - | - | + | + | + | + | + | + | + |
| Man9 | - | - | - | + | + | + | + | + | + | + |
| Ban5 | - | - | - | + | + | + | + | + | + | + |

DY1, commercial dried yeast; PC, positive control (*S. cerevisiae* NRBC 2044),

**Table 2. Growth and Inhibition of potential yeast strains at different temperature and ethanol concentration.**

| Isolate ID | Temperature tolerance | | | | | Ethanol tolerance | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 25°C | 30°C | 37°C | 45°C | 55°C | 8% | 10% | 12% | 14% | 16% |
| DY1 | + | +++ | ++ | - | - | + | + | + | - | - |
| Man5 | ++ | +++ | +++ | + | - | ++ | ++ | + | + | + |
| Ban2 | ++ | +++ | +++ | + | - | + | + | + | - | - |
| Man9 | + | +++ | +++ | + | - | ++ | + | + | + | + |
| Ban5 | + | +++ | +++ | + | - | + | + | + | - | - |

+++, intensive growth; ++, moderate growth; +, mild growth; -, no growth

Ethanol is produced as a secondary metabolites, which imparts bread flavor during leavening[33]. Therefore, the tolerance of potential yeast strains to ethanol was observed to be up to 16%. All tested strains grew well at 12% ethanol concentration (Table 2). Growth of DY1, Ban2, and Ban5 was inhibited at 14% ethanol level. The strains Man5 and Man9 showed the best tolerance to ethanol (up to 16%) than commercial yeast strain DY1. This trait indicated that those strains would achieve high cell density during biomass production in fermenters as they would survive such high ethanol concentrations in a large vessel.

Baking yeast, *Saccharomyces cerevisiae*, generally has an invertase activity and sucrose in the dough is quickly transformed to glucose and fructose. But invertase levels are usually low in strain that works best in high-sugar dough. From the assay it was found

that the invertase activity of all newly isolated strains were lower than that of the commercial strain DY1 (Fig. 1). The lowest invertase activity was found for Man5 strain. Other strains such as Man9 and Ban5 also had lower invertase activity therefore, these indigenous strains would be highly capable of overcoming osmotic pressure.

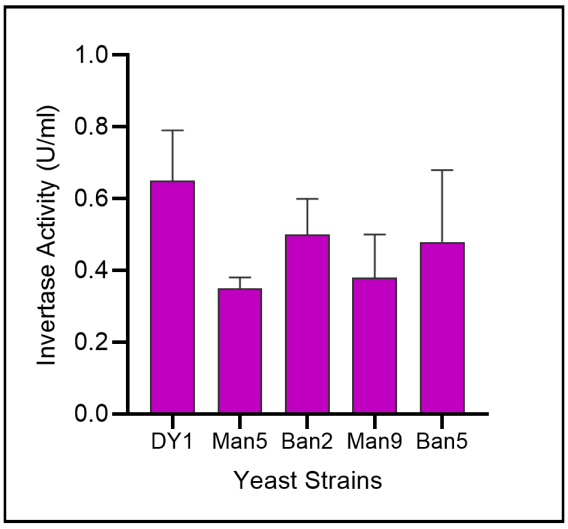


Fig. 1. Invertase activity of the potential yeast strains.

Dough leavening ability is the main characteristic of yeast to be used as a baker's yeast[34]. An increase in dough height for 2 hrs and differences in dough leavening efficiency of the tested yeast strains are presented in Fig. 2 and Table 3, respectively. Although all tested strains increased dough height to some extent for one hour, only DY1 and Man5 increased dough height significantly than other indigenous yeast strains after two hours of incubation. Only DY1 and Man5 increased dough volume significantly for dough leavening efficiency compared with other strains ($P < 0.05$). Maximum dough leavening activity was found for commercial dried yeast, DY1 (net increased volume 37.74 $cm^3$). Only Man5 had significant dough leavening activity (net increased volume 27.69 $cm^3$). On the contrary, Ban2, Man9, and Ban5 did not differ significantly on leavening action ($P > 0.05$). Developing yeast strains with better leavening capacity is essential for sweet and frozen sweet dough production; these indigenous yeast strains are proven to be promising candidates for use as industrial baker's yeast.

*Identification of the yeast:* After performing the screening tests of these potential baker's yeast strains, these tentative *S. cerevisiae* strains were confirmed by using the API 20C AUX kit. Newly isolated indigenous yeast strains were also confirmed as *S. cerevisiae* by molecular confirmation using a set of species-specific primers amplifying 150 bp amplicons[21]. *S. cerevisiae* NRBC 2044 was used as a positive control in API and PCR-based confirmation.

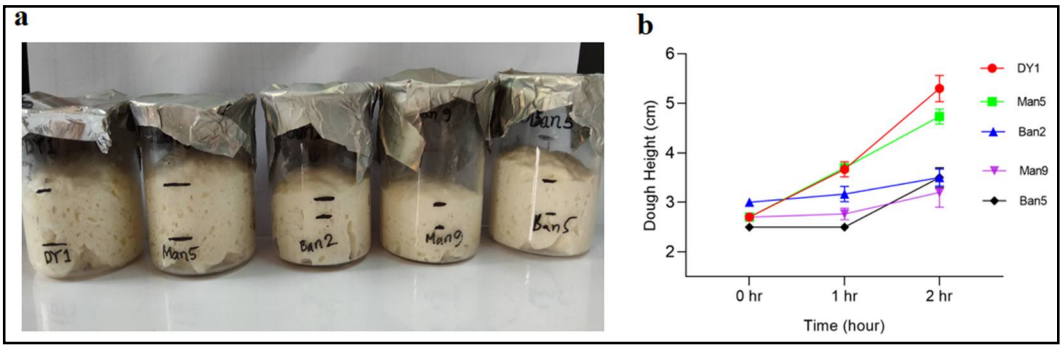


Fig. 2. Dough height increased by different potential yeast strains.

**Table 3. Dough leavening efficiency of the potential yeast strains.**

| Sample | π | Diameter of beaker d (cm) | Initial height Y (cm) | Final height X (cm) | Net increased volume ($cm^3$)* $V = \pi (d/2)^2(X-Y)$ $cm^3$ |
|---|---|---|---|---|---|
| DY1 | 3.14 | 4.3 | 2.7 | 5.3 | $37.74^a$ |
| Man5 | 3.14 | 4.2 | 2.7 | 4.7 | $27.69^b$ |
| Ban2 | 3.14 | 4.2 | 3 | 3.5 | $6.92^c$ |
| Man9 | 3.14 | 4.6 | 2.7 | 3.2 | $8.31^c$ |
| Ban5 | 3.14 | 4.2 | 2.5 | 3.5 | $13.85^c$ |

*According to Duncan's MRT test, Different letters as superscript indicates that different tested yeast strains had significantly different leavening actions (ANOVA, $P<0.05$).

*Optimization of physiological parameters for biomass production of Baker's yeast:* For industrial applications, these baker's yeast strains needed to be produced on a large scale for high biomass yield[35]. Therefore, to achieve maximum biomass production of the potential yeast strains, physiological parameters such as temperature, pH, and glucose concentration were optimized[36,37]. Different strains had different optimum temperatures for the highest biomass yield (Fig. 3a). The strain Man5, Ban5, and DY1 yielded the highest biomass at 37°C (7.7, 7.65, and 7 $Log_{10}$ CFU/ml, respectively), whereas Ban2 showed an optimum temperature of 30°C with the yield of 7.2 $Log_{10}$CFU/ml. Exceptionally, Man9 yielded the highest biomass at 40°C. For pH optimization, differences in biomass yield were also observed for these potential strains (Fig. 3b). DY1, Man5, and Ban5 strains yielded maximum biomass at pH 6.0, whereas the Ban2 showed maximum biomass yield at pH 4.0 and the optimum pH for Man9 was pH 5.0. Glucose concentration was optimized utilizing three different glucose concentrations (Fig. 3c). Commercial strain DY1 yielded maximum biomass (7.42 $Log_{10}$CFU/ml) at 1% glucose. However, indigenous Man5, Ban2, and Man9 yielded maximum biomass at 2% glucose (7.46, 7.70, 7.72 $Log_{10}$CFU/ml). Interestingly, Ban5 yielded maximum biomass (7.55 $Log_{10}$CFU/ml) at the lowest glucose concentration (0.5%).

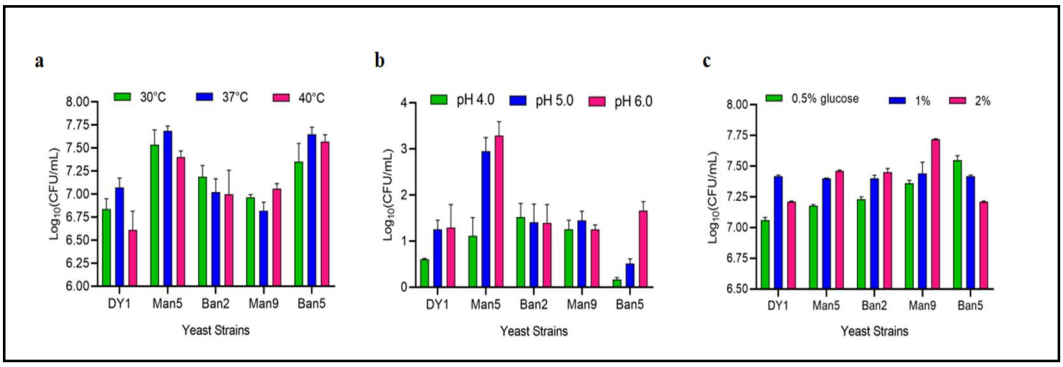


Fig. 3. Optimization of (a) temperature, (b) pH, and (c) glucose concentration for the production of yeast biomass.

*Biomass dry weight measurement:* To achieve the maximum biomass production, different culture media were prepared using these optimization results for physiological parameters for the tested yeast strains. Different indigenous yeasts yielded biomass comparable to the commercial DY1 (Fig. 4). Interestingly, Man5 produced the highest biomass as compared to others ($p< 0.05$). This newly isolated indigenous yeast strain yielded the maximum biomass (0.76 ± 0.06 g/100 ml) provided its best growth condition (Temp. 37°C, pH 6.0, and 2% glucose). Moreover, Man9 and Ban5 also yielded more biomass (0.57 ± 0.11 g/100ml and 0.60 ± 0.02 g/100 ml) than DY1 (0.43 ± 0.01 g/100ml). The lowest biomass production was observed for the Ban2 strain (0.42 ± 0.12 g/100ml). However, there were no notable differences among Ban2, Man9, and Ban5 with DY1's biomass production ability ($p > 0.05$).

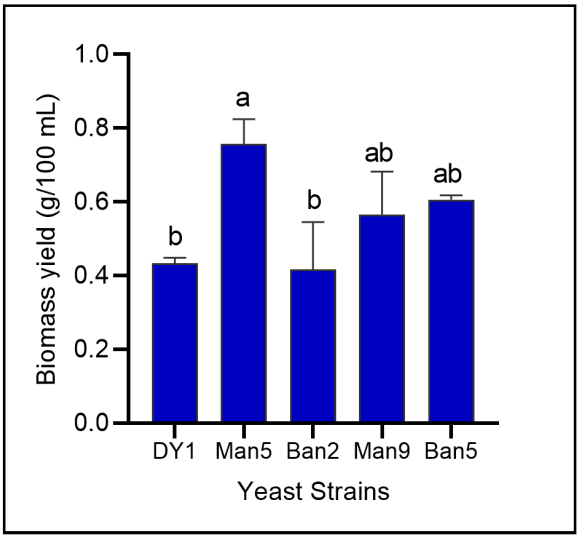


Fig. 4. Biomass yield of potential yeast strains. According to Duncan's MRT test, different letters in different column indicates significant differences (ANOVA, $p< 0.05$).

The biomass yield of baker's yeast is an important characteristic that determines the efficiency of yeast fermentation. A higher biomass yield means more yeast cells are produced for a given amount of sugar, resulting in a more efficient fermentation process. As Man5, Man9, and Ban5 yielded higher biomass than commercial baker's yeast, this property can be beneficial for industrial applications as these can increase yields and reduce production costs.

## Conclusion

In this study, four *S. cerevisiae* strains were collected from indigenous fruit sources (Man5 & Man9 from Mango, Ban2 & Ban5 from banana). Among these strains, Man5 and Ban5 proved to have comparable dough leavening efficiency, therefore, could be used as potential baker's yeast strains. Moreover, Man5 had the highest biomass-yielding capacity in small-scale fermentation, tolerance to a higher temperature, and ethanol concentration, demonstrating the strong potential to be commercial strains in the bakery industry. However, more detailed research is needed for the biomass optimization in large-scale fermentation and culture preservation for industrial application of these strains.

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