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Article

Isolation, Identification, Optimization of Baker's Yeast from Natural Sources, Scale-Up Production Using Molasses as a Cheap Carbohydrate Source, and Evaluation for Bread Production

A. N. M. Mamun-Or-Rashid [1,2,*], Tanzima Tarannum Lucy [2,*] and Md. Kamruzzaman Pramanik [3]

[1] Department of Biotechnology and Genetic Engineering, Islamic University, Kushtia 7003, Bangladesh
[2] Anti-Aging Medical Research Center and Glycative Stress Research Center, Graduate School of Life and Medical Sciences, Doshisha University, Kyoto 610-0394, Japan
[3] Microbiology and Industrial Irradiation Division, Institute of Food and Radiation Biology, Atomic Energy Research Establishment, Savar 1349, Bangladesh; kpramanik2003@yahoo.com
* Correspondence: amamun@mail.doshisha.ac.jp (A.N.M.M.-O.-R.); cygf2501@mail4.doshisha.ac.jp (T.T.L.)

Abstract: (1) Background: Bangladesh must has to spend a large amount of foreign currency to import commercial baker's yeast every year. We could save money by finding a potential *Saccharomyces cerevisiae* from natural sources compatible with commercial baker's yeast production. (2) Methods: Grapes, rice, pineapples were collected, processed, and inoculated on YMA plates and incubated at 30 °C for 48 h. Then 11 single morphologically well-formed colonies were isolated, purified, and identified, three as *S. cerevisiae*, three as *S. rouxii*, three as *S. bisporus*, and two as *S. exigus* based on standard cultural, morphological, and biochemical characteristics. Identified *S. cerevisiae* (designated as G2, P5 and R3) were then assessed for CO_2 production as a measure of their baking potential during bread production and compared with two commercial strains (designated as C1 and C2). (3) Results: Isolate-G2 produced the maximum of 1830 mm^3 of gas, whereas C1, C2, R3, and P5 produced 1520, 1680, 770, and 610 mm^3 gas, respectively. No strain produced H_2S which is associated with an off-flavor and unpleasant taste. These isolates showed maximum cell density at a pH range of 4–5.5 in 4–16% molasses broth at 30 °C after 4 days of incubation and maximum 4.75×10^9, 7.9×10^8, 1.472×10^{10}, 2.08×10^{10} and 5.24×10^9 CFU mL^{-1} were produced by C1, C2, G2, P5 and R3, respectively. Isolate-G2 was found to have the most potential, whereas isolate-R3 and P5 have satisfactory potential. (4) Conclusions: G2 could be a good candidate for commercial trials.

Keywords: baker's yeast; *Saccharomyces cerevisiae*; biochemical characteristics; baking parameters; bread production

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1. Introduction

Since the archaic days, long before mankind uncovered the existence of microbes; microorganisms are being applied unintentionally for food processing such as winemaking, beer manufacturing, bread making, food safeguarding and many more. About 4000 years ago, ancient Egyptians utilized yeast to make bread. Archaeologists investigating Egyptian heritage sites found early crushing rocks and baking chambers for producing yeasted daily bread as well as drawings of 4000-year-old bakeries and breweries [1–6].

Baker's yeast is utilized to leaven bread all over the world and it is the most familiar type of yeast [7–10]. Yeasts are unicellular eukaryotic microbes categorized in the kingdom of Fungi and are being utilized for distinct reasons, such as being reliant on their source of isolation [3,11–14]. They have been isolated from natural substrates like leaves, flowers, sweet fruits, peels of fruits and berries (such as grapes, apples, or peaches), grains, fleshy fungi, and exudates of trees, insects, dung, and soil [15–18].

Saccharomyces cerevisiae is mainly used as commercial baker's yeasts for fermentation during bread-making procedure [4,9,19–27]. These yeast products comprise almost 5% lactic bacteria to play an important role in the production of flavor quality of bread [13,22,28,29]. Today, in this first decade of the 21st century, baker's yeast is produced worldwide in quantities of 2.3 million tons per annum [21] revealing its huge market share.

Yeasts perform three main functions in a panary fermentation: (i) it produces carbon dioxide in sufficient quantities to inflate the dough and produce a light spongy texture that will result in a palatable bread when correctly baked; (ii) causing 'maturing' or 'ripening' of the bread; and (iii) produces a complex mixture of chemical compounds that contribute to the flavor of the bread [21]. In addition to producing carbon dioxide, the lactic acid-forming bacteria also produce acid. The acids contribute to the flavor of the finished bread and enhance the storage properties [21].

Molasses is commonly used as a feedstock for bioethanol production. Molasses, the non-crystallizable residue remaining after sucrose purification, has some advantages: it is a relatively inexpensive raw material, readily available, does not require starch hydrolysis, and is already being used for ethanol production. The molasses obtained after sugar beet processing contains about 60% sucrose and 40% other components. The non-sucrose substances include inorganic salts, raffinose, ketose, organic acids, and nitrogen-containing compounds [25,30–34]. Molasses is also used in baker's yeast production, in the fermentation technology for ethanol, citric, lactic, and gluconic acids production, as well as in glycerol, butanol, and acetone production, as a cheap and widely available ingredient of mixed feeds or in the production of amino acids [25,30–35]. The fermentative yeast *Saccharomyces cerevisiae* is largely used in ethanol and baker's yeast production using such renewable biomass as sugar cane or sugar beet molasses as the main carbon source [25,30–34,36–39].

Bangladesh is a developing country and a major part of the urban population is changing their food habits due to the fast-food culture of the western world and the very busy schedule of modern daily life. One of the basic ingredients of these fast foods is bread. It is being used in our breakfast, Tiffin as burger, sandwich, wrap. As a result, the use of commercial baker's yeast is increasing day by day. Baker's yeast is a very important ingredient in bread production and demand is met through import from abroad which reduces our foreign currency [21]. We can easily save the foreign currency by developing commercial plants for baker's yeast production. Nevertheless, this process is not established in our country. Recently some laboratories have started their research in this sector. Therefore, it is important to find out a good quality baker's yeast which would fulfill this demand. The objective of this study was to make a comparative evaluation among two commercial baker's yeasts and natural indigenous yeasts isolates from three different types of food samples using molasses as a sole carbon source for its cheapness, readily availability, and effectiveness to find out suitable indigenous yeast that would fulfill all desirable criteria of baker's yeast.

2. Materials and Methods

2.1. Collection of Samples

Samples of grape juice, pineapple juice, and rice were collected randomly from the local markets in different areas of Savar, Bangladesh in sterilized sample bottles and were transferred to Microbiology and Industrial Irradiation Division (MIID) of Institute of Food and Radiation Biology (IFRB), Atomic Energy Research Establishment, Ganakbari, Savar, Dhaka for further analysis. Two commercial yeast samples (Granular Yeast, DBL, China, and, Angel, Instant dry yeast, China) were used as positive control throughout this experiment.

2.2. Enrichment of the Samples

Enrichment was achieved by using an enrichment medium that encourages the growth of yeasts and inhibits the growth of undesired microorganisms. In this experiment, modified Yeast extract Malt extract broth (YMB) was used as an enrichment medium with the

following composition: glucose, 10 g/L; yeast extract, 3 g/L; peptone, 3 g/L; and malt extract, 3 g/L. pH was adjusted with 1N of HCl at 4.5 for gripping the growth of other microorganisms [25] and incubated at 30 °C for two days. Physiological saline (0.85% NaCl) was used to dilute the enriched medium up to 10^6 times.

2.3. Selection of Yeast Isolates

Spread plate technique was followed throughout this study. Diluted samples (0.1 mL) were transferred and spread on YMA plates of pH 4.5 and incubated at 30 °C for 48 h. Afterwards, single morphologically well-formed colonies that are like *S. cerevisiae* with respect to colony size, shape, color, opacity, edge, elevation were isolated [4,17].

2.4. Culture Purification

The selected isolates were then sub-cultured several times by streak plate technique on YMA plates and incubated at 30 °C for 48 h to check their purity.

Stock culture preparation: Stock cultures were prepared in the following ways:

a. The purified isolates were sub-cultured into two sets of YMA slants in test tubes and incubated at 30 °C for 48 h. Then one set was taken to apply sterile 60% glycerol above the cells grown in YMA slant to save the cells from dehydration. Then both sets were preserved at 4 °C as stock culture.
b. The purified isolates to be frozen were grown for about 24 h in 1 mL PDB (Potato Dextrose Broth) in a 5 mL vial before adding 400 µL of 60% solution of glycerol in water. Then these vials were shaken well to mix and then kept at −20 °C.

All these stocks were sub-cultured every month to check viability.

2.5. Identification of Yeast Isolates

Identification of yeasts up to the species level was carried out based on standard cultural, morphological, and physiological/biochemical tests proposed for each group (Figure A1) [2,36,40–48].

Cultural Characterization

Cultural characteristics of yeast isolates were performed by streak culturing of the isolates on YMA plates and incubated at 30 °C for 48 h and then the colonies were observed for the following characteristics:

Shape: Shapes of the isolated colonies were recorded as circular or irregular.

Color: Color was recorded as creamy, creamy white, or white.

Edge: The edge of the isolated colonies was recorded as entire, dentate, lobate, or undulate.

Opacity: The opacity of the isolated colonies was recorded as opaque, translucent, or transparent.

Elevation: Elevation was recorded as raised, convex or umbonate.

Surface: Surface was recorded as rough or smooth.

Consistency: Consistency was recorded as viscid.

2.6. Growth in Liquid Media

In liquid media, *Saccharomyces* accumulated as sediment or showed ring growth at the air-liquid-glass junction (Figure A1A) [49].

2.7. Morphological Characterization

To determine the morphology of yeast cells and reproduction type, the cultures were examined microscopically. Vegetative cells were observed after 3 days of incubation at 30 °C in a YMA medium.

2.8. Staining

Loeffler's methylene blue staining and Gram staining were accomplished by standard method to observe yeast cells and ascospores, respectively [49] (Figure A1B).

2.9. Microscopy

After Loeffler's methylene blue staining and Gram Staining, cells were observed under compound microscope (Nikon microscope, Japan) for morphological study of these yeast isolates [49] where ascospores appear as Gram-negative (pink) and the vegetative *Saccharomyces* cells appear as Gram-positive (violet) [49].

2.10. Biochemical Characterization

The biochemical characterization methods were based on the utilization of carbon and nitrogen sources (Figure A1C–G) [4,29,50–52]. The ability of strains to utilize glucose, fructose, sucrose, maltose, galactose, lactose, and ethanol as a sole carbon source and production of gas was determined in Durham's tubes on carbohydrate fermentation medium. These strains were then undergone urea, KNO_3 and $(NH_4)_2SO_4$ utilization tests as a sole nitrogen source. A positive reaction was detected by observation of color change from purple to yellow and gas formation in Durham tubes (for carbohydrate fermentation) and turbidity (for others) in the solution. Positive and negative controls were kept for all the tests to compare with the tested samples and to rule out any contamination.

2.11. Carbohydrate Fermentation

Bromocresol purple carbohydrate (2%) fermentation broth (tryptone 10 g, NaCl 5 g, yeast extract 5 g, Bromocresol purple 1% solution 2.5 mL per liter) was prepared by adding all the ingredients (except the indicator) to deionized water and dissolved by steaming. After cooling down, the pH was adjusted to 7.2 and the indicator was added. Later 5–10 mL of this media was distributed into screw cap tubes or test tubes (with inverted Durham's tube) and were sterilized by autoclaving. After cooling down at room temperature, these were inoculated with a 48-h fresh culture of the yeast isolates by sterile loop and incubated at 30 °C and examined every day for 5 days. Rapid change (within 2 days) of color from purple to yellow indicated a positive result, no change indicated a negative result, and slow change indicated as weak. Two un-inoculated tubes were kept as negative controls (Figure A1C–F) [49].

2.12. Ascospore Production

Ascospore production was induced by sub-culturing twice on nutrient agar containing 5% glucose and 0.5% tartaric acid, followed by subculturing onto an Agar medium containing 0.04% glucose and 0.04% anhydrous sodium acetate only [49].

2.13. Urease Test

This test was performed to determine the capability of the isolates to utilize urea as a nitrogen source. Three mL of urea broth (yeast extract 0.1 g, potassium dihydrogen phosphate 9.1 g, di-sodium hydrogen phosphate 9.5 g, urea 20 g, phenol red 0.01 g per liter and pH 6.8) was transferred into screw cap tubes. Subsequently, these tubes were kept under mild conditions [5 min in a current of steam] to sterilize. After cooling down, these sterilized tubes of urea broth were inoculated very lightly and incubated at 30 °C for 7 days. Negative controls were kept in compared with the inoculated tubes. A positive reaction was detected by observation of turbidity in the solution (Figure A1G).

2.14. Nitrate Reduction Test

The purpose of the nitrate reduction test was to determine the ability of yeast to reduce nitrate to nitrites or beyond the nitrate stage. As such, 10 mL nitrate broths (beef extract 3 g, peptone 5 g, NaCl 5 g, KNO_3 1 g, agar 1 g per liter, and pH 7.2) were taken into several test tubes and sterilized by autoclaving. Afterward, each of the isolates was inoculated into

test tubes and incubated at 30 °C. After 48 h, 5 drops of Sulfanilic acid solution (Sulfanilic acid 8 g per liter of 5 mmol L^{-1} acetic acid) and 5 drops of α-naphthyl amine solution (5 g α-naphthyl amine per liter of 5 mmol L^{-1} acetic acid) were added to all the inoculated test tubes. The tube, in which red color was developed, indicates nitrate reduction positive. On the other hand, zinc powder was added to those tubes which did not develop red color. After the addition of zinc, the tubes which developed red color indicated nitrate reduction negative and the tubes that did not develop red color indicated nitrate reduction positive (Figure A1H).

2.15. Utilization of Ethanol as a Sole Carbon Source

A sterilized tube of 3% ethanol broth (ammonium sulfate 1 g, potassium dihydrogen phosphate 1 g, hydrated magnesium sulfate 0.5 g, 1 g yeast extract per liter, and pH 4.5) and a tube of the medium without ethanol were inoculated very lightly and incubated at 30 °C for up to three weeks and examined at frequent intervals for growth. Since the yeast extract was used in the medium as a source of vitamins and growth factors, the growth of the examined yeasts was compared in the tubes with and without ethanol to ensure that false positives were not recorded (Figure A1I) [49].

2.16. Flocculation

All isolates were evaluated according to their ability to flocculate during cellular growth in Yeast extract Glucose Peptone broth. Cells were inoculated into tubes containing 4 mL YP medium and grown in a rotatory incubator at 140 rpm and 30 °C. Flocculent strains were selected by their capacity to sediment at the bottom of the tube at the end of fermentation [28,53,54].

2.17. Assessment of the Potency of Identified S. cerevisiae for Bread Production

The potency of S. *cerevisiae* was measured with respect to the level of CO_2 and H_2S gas production. More CO_2 production was considered as a more potent isolate with desired baking attribute. On the hand, more production of H_2S was considered as a less potent isolate as this property attributes the product with an off-flavor and unpleasant taste.

2.17.1. Production of H_2S

To examine the capacity to produce H_2S associated with an off-flavor and unpleasant taste [25], identified *S. cerevisiae* strains were streak cultured on Bismuth Sulfite Agar plates, and stab cultured on Kligler Iron Agar (KIA) slants and Sulfide Indole Motility (SIM) agar slants and incubated at 30 °C for 3 days. Colonies that exhibit brown or black color on Bismuth Sulfite Agar plates and any blackening of the KIA and SIM media along the line of inoculation or throughout the butt indicate hydrogen sulfide production [25]. Positive strains were discarded (Figure A1J) [45].

2.17.2. Measurement of Gas Production

Several vials were filled with 3 mL sucrose broth and capped with cotton. Then, these were sterilized by autoclaving and inoculated with a 200 µL solution containing the same number of tested isolates. The production of gas was measured by a lab-made special gas measuring apparatus made by attaching an inoculated vial to one end of a silicon adaptor and a 1 mL pipette containing 50 µL mercury at its tip on the other end. The vials were kept in a handmade rack where the pipettes were parallel to the ground to confirm the mercury is moving by the gas produced by the test isolates and that gravitation does not affect it. Gas production was measured by taking an initial reading of the mercury position and repeated after a regular interval (Figure A1K).

2.18. Optimization of Physicochemical Parameters for Maximum Growth of Isolates

All *S. cerevisiae* strains then underwent various tests to determine their optimum conditions for maximum growth and maximum biomass production. Inoculum cell numbers

of all the following tests were counted by a hemocytometer and then adjusted by the same number by diluting with autoclaved distilled water.

2.18.1. Optimum Carbon Sources

The best cheap carbon source for optimum growth and maximum biomass production was detected by measuring the optical density at 550 nm using a spectrophotometer of inoculated (3.86×10^5 cells/mL of culture broth) 2% sucrose and 4% molasses broth after 3 days of incubation at 30 °C.

2.18.2. Optimum pH

To examine the optimum pH, the yeast strains were cultured in liquid media of pH 3, 3.5, 4, 4.5, 5 and 5.5 containing 4% (*w*/*v*) molasses, 1% peptone, 0.5% yeast extract and 0.5% sodium chloride. The media were inoculated with the same number of actively grown yeast cells and incubated at 30 °C for 4 days. By measuring optical density at 550 nm using a spectrophotometer, optimum pH for growth and maximum biomass production was detected.

2.18.3. Optimum Molasses Concentration

Optimum molasses concentration was determined by culturing the yeast strains in liquid media of pH 4.5 (previously determined as optimum) containing 2%, 4%, 8%, 12%, 16% and 20% (*w*/*v*) molasses along with 1% peptone, 0.5% yeast extract and 0.5% sodium chloride. These were inoculated with the same number of actively grown yeast cells and incubated at 30 °C for 4 days. Optimum molasses concentration was determined by measuring optical density at 550 nm using a spectrophotometer.

2.18.4. Optimum Temperature

For determining the ability to grow at 25 °C, 30 °C, and 37 °C, and which temperature is optimum, the yeast strains were cultured in a liquid medium of pH 4.5 containing 4% (*w*/*v*) molasses (previously determined as optimum), 1% peptone, 0.5% yeast extract and 0.5% sodium chloride. The sterile media were inoculated with the same number of actively grown yeast cells and incubated at 25 °C, 30 °C, and 37 °C for 4 days. The optimum temperature for growth and maximum biomass production was determined by using a spectrophotometer at 550 nm.

2.19. Incubation Period

To determine the incubation time required for maximum biomass production with the highest number of viable cells by the yeast isolates, the yeast strains were cultured in liquid media of pH 4.5 containing 4% (*w*/*v*) molasses (previously determined as optimum), 1% peptone, 0.5% yeast extract and 0.5% sodium chloride. The sterile media were inoculated with the same number of actively grown yeast cells and incubated at 30 °C (previously determined as optimum) for 1, 2, 3, 4, 5 and 6 days. After incubation of each day, optical density was measured at 550 nm by using a spectrophotometer. The broth was vortexed, serially diluted up to 10^6-fold, and then spread plated to count viable cells (CFU mL^{-1}) on inoculated media. Five mL of inoculated media was then centrifuged at 5000 rpm for 10 min and the supernatant was decanted. The pellet was then weighted to determine cream cell mass and dried at 105 °C in a heat dryer until constant weight and then weighted to get dry cell mass. Later these data were plotted in Microsoft Office Excel Worksheet to obtain a yeast growth curve relating OD with CFU mL^{-1}, cream cell mass mL^{-1}, dry cell mass mL^{-1}, and incubation period. The minimum incubation period for maximum growth and maximum viable biomass production was determined from this curve.

2.20. Effect of Agitation

To determine the effect of shaking on biomass production by the yeast isolates, the same number of actively grown yeast cells were inoculated in a liquid medium of pH 4.5

containing 4% (*w*/*v*) molasses (previously determined as optimum), 1% peptone, 0.5% yeast extract and 0.5% sodium chloride. Then these were incubated at 140 rpm and 30 °C temperature (previously determined as optimum) in a shaking incubator. After 4 days, the culture broth was centrifuged at 5000 rpm and 4 °C for 10 min and the supernatant was decanted. The pellets were weighted to determine biomass production in shake culture. By comparing this with previous data, the effect of shaking was determined.

2.21. Preparation of Bread from Composite Flour

The dough was prepared by weighing 500 g wheat flour, 50 g sugar, 7.5 g salt, 10 g baking fat, and 5 g baker's yeast into a clean metallic mixing bowl. A mixer was used to mix the ingredients to homogenize the mixture. Then, 300 mL of water was added to the dough until the desired consistency was achieved. The dough was removed, weighed, and divided into equal portions. These were placed in separate baking pans of equal size and left for 30 min. They were transferred into an oven pre-heated to 180 °C and allowed to bake for 1.5 h. The baked products were left to cool [4,25,26,55–57]. Tasting of the baked breads were conducted by standard organoleptic test procedure. Evaluations were made as, pleasant < medium < good < better < best.

2.22. Measurement of Dry Cell Mass and CFU after Different Incubation Period

To measure the dry cell mass, inoculated molasses broth medium after different incubation periods were taken in test tubes and uniformly mixed with vortex. Then 5 mL of culture broth were centrifuged at 4000 rpm at 4.0 °C for 10 min and the supernatant were decanted. The pellets were dried at 105 °C until constant weight to obtain dry cell mass mL^{-1}. To determine CFU/mL, after different incubation periods, cell suspensions were uniformly mixed by vortex and then serially diluted upto 10^{-6}. Then spread plate method was used in YMA medium to determine the cell density as CFU/mL.

3. Results and Discussion

3.1. Selection of Yeast Strains with Desired Characteristics for Bread Production

The sources of *S. cerevisiae* are very common in Bangladesh. Three different samples such as grape, pineapple, and fermented rice were selected as good sources of *S. cerevisiae*. In this study, modified Yeast extract Malt extract broth (YMB) of pH 4.5 was used as an enrichment medium to grip the growth of other microorganisms and followed by culturing on YMA plates. After 48 h of incubation at 30 °C, eleven single morphologically well-formed colonies were selected [4,17,25,58]. To isolate and identify potential yeast isolate, we have used three different food samples and eleven morphologically well-formed single colonies that were like *S. cerevisiae* and they were designated as G1, G2, P1, P2, P3, P4, P5, R1, R2, R3, R4, respectively, and the controls were C1 (Granular Yeast, DBL, China) and C2 (Angel, Instant dry yeast, China).

Yeast isolates were selected based on their morphological differences in YMA plates. We selected only those that resemble the control yeast strains.

3.2. Characterization of Isolated Yeast Strains

Identification of microorganisms has changed dramatically over the past decade through direct examination of the tremendous variation present in DNA. Typing methods based on phenotypic characteristics are usually cheap and easy to perform but they have shown a lack in their reproducibility. On the contrary, genotypic methods are expensive and require sophisticated technology but they usually have good reproducibility [36,37,59–61].

In this study, the cultural, morphological, and biochemical properties of the isolated yeast strains were studied for characterization. Colony shape, color, elevation, consistency, edge were observed (Table 1). Morphologically (Table 1) they were unicellular spherical (C1, C2, G2, P5, R3), oval (G1, R1, R2), or elongated (P1, P2, P3, P4, R4) cells with terminal and lateral buds that are like Saccharomyces group [53,62–65]. Biochemical tests were conducted [51,52,66–68]. The biochemical tests include carbohydrate (Glucose, fructose,

sucrose, maltose, galactose, lactose) fermentation test, nitrate reduction test, $(NH_4)_2SO_4$ utilization test, urease test, utilization of ethanol as a sole carbon source, ascospore production test. After performing all the tests, three isolates (designated as G2, P5, and R3) were identified as *S. cerevisiae*. The other three isolates were identified as *S. rouxii*, three as *S. bisporus*, and two as *S. exigus* (Table 1). All the three *S. cerevisiae* strains along with two commercial baker's yeast strains were then continued for further studies. Using the methodology described, yeast isolates were isolated directly based on morphological characteristics from samples of diluted enrichment medium. The initial number of yeast isolates was reduced to 11 yeast isolates from several hundred. Among those, other strains did not follow the expected behavior typical for *S. cerevisiae*. Following standard methods for identification of the yeast genus Saccharomyces [49], three isolates were identified as *S. cerevisiae*, three as *S. rouxii*, three as *S. bisporus*, and two as *S. exigus* (Table 1). Then we chose only the *S. cerevisiae* isolates (approximately 27% of the total) along with the controls to perform further tests.

Table 1. Characteristics of isolated yeast isolates.

	Isolates	C1	C2	G1	G2	P1	P2	P3	P4	P5	R1	R2	R3	R4
Cultural characteristics	Shape	Circular	Circular	Circular	Circular	Circular	Circular	Circular	Circular	Circular	Circular	Circular	Circular	Circular
	Colony color	Creamy White	Creamy White	Creamy	Creamy White	Creamy	Creamy	Creamy	Creamy	Creamy White	White	White	White	Creamy
	Opacity	Opaque	Opaque	Opaque	Opaque	Opaque	Opaque	Opaque	Opaque	Opaque	Opaque	Opaque	Opaque	Opaque
	Elevation	Convex	Convex	Umbonate	Convex	Raised	Convex	Convex	Raised	Convex	Convex	Convex	Convex	Umbonate
	Surface	Smooth	Smooth	Smooth	Smooth	Rough	Smooth	Smooth	Rough	Smooth	Smooth	Smooth	Smooth	Smooth
	Edge	Entire	Entire	Entire	Entire	Entire	Entire	Entire	Entire	Entire	Dentate	Dentate	Dentate	Entire
	Consistency	Viscid	Viscid	Viscid	Viscid	Viscid	Viscid	Viscid	Viscid	Viscid	Viscid	Viscid	Viscid	Viscid
	Growth in liquid media	SG	SG	ALG	SG	ALG	ALG	ALG	ALG	SG	SG	SG	SG	ALG
Morphological	Cell shape	Spherical	Spherical	Oval	Spherical	Elongated	Elongated	Elongated	Elongated	Spherical	Oval	Oval	Spherical	Elongated
	Bud	Present	Present	Present	Present	Present	Present	Present	Present	Present	Present	Present	Present	Present
	Ascospore	Present	Present	Absent	Present	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Present
Biochemical characteristics	Glucose	+	+	+	+	w	w	w	w	+	+	+	+	+
	Fructose	w	w	w	+	−	−	−	−	+	+	w	w	w
	Sucrose	+	+	−	+	−	−	−	−	+	+	+	+	−
	Maltose	w	+	w	w	−	−	w	−	+	+	+	+	w
	Galactose	+	+	−	+	−	−	−	−	+	+	+	+	−
	Lactose	−	−	−	−	−	−	−	−	−	−	−	−	−
	KNO_3	−	−	−	−	−	−	−	−	−	−	−	−	−
	$(NH_4)_2SO_4$	+	+	+	+	+	+	+	+	+	+	+	+	+
	Urease	−	−	−	−	−	−	−	−	−	−	−	−	−
	Ascospore	+	+	−	+	−	−	−	−	−	−	−	−	+
	Ethanol as sole carbon source	w	w	+	w	+	+	+	+	+	+	+	+	+
	Identity	*S. cerevisiae*	*S. cerevisiae*	*S. rouxii*	*S. cerevisiae*	*S. bisporus*	*S. bisporus*	*S. rouxii*	*S. bisporus*	*S. cerevisiae*	*S. exigus*	*S. exigus*	*S. cerevisiae*	*S. rouxii*

ALG = Ring growth in Air-Liquid-Glass junction, SG = Sediment Growth, + = Positive, − = Negative, W = Weak.

3.3. Assessment on the Potency of Identified S. cerevisiae for Bread Production

In the highly competitive market of commercial bakers' yeast, fermentations are operated for maximum efficiency and minimum production cost. To maintain competitiveness, the fermentation must be highly consistent with minimum variation in yeast performance, the maximum yield on raw materials, and the minimum production of undesirable by-products [23,50,69,70]. So, the selection of optimum cultural and fermentation conditions with maximum viable biomass production and gas production at a moderate rate is mandatory for natural yeast isolates to be used as an effective commercial baker's yeast. Three identified *S. cerevisiae* strains and the two controls were then subjected to several tests to determine their potency for bread production.

3.4. Production of H_2S

H_2S is an undesirable compound associated with an off-flavor and unpleasant taste that must be absent in fermented foods [45]. Production of H_2S by test isolates was checked

thrice in three different media, Bismuth Sulfite Agar, Kligler Iron Agar (KIA), and Sulfide Indole Motility (SIM) Agar. No strain produces H_2S (Table 2).

Table 2. H_2S production test in three different media.

Isolates	H_2S Production		
	Bismuth Sulfite Agar	**Kligler Iron Agar (KIA)**	**Sulfide Indole Motility (SIM)**
C1	–	–	–
C2	–	–	–
G2	–	–	–
P5	–	–	–
R3	–	–	–

"–" means a negative result.

3.5. Measurement of Gas Production

A special apparatus (Figure A1K) was used to measure gas production by the test isolates in 2% sucrose broth. Among all isolates, G2 produced the highest (Figure 1) of 1830 ± 98.99 mm^3 of gas. Though initial gas production of G2, and R3 was highest 20 mm^3, their final production was 1830 ± 98.99, 770 ± 49.49 mm^3, respectively. The initial gas production by P5 was 10 mm^3 but it increased over time. Two positive controls, C1 and C2 showed moderately higher gas production of 1520 and 1680 mm^3, respectively.

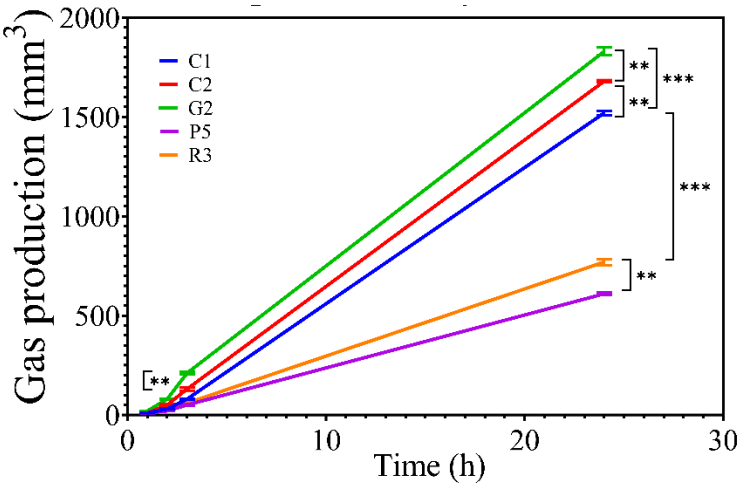

Figure 1. Gas production by test isolates. Data are shown as Mean ± SEM (Standard Error Mean). Isolate G2 produced highest amount of gas that significantly differ from both of the commercial strains. Tukey–Kramer test. $n = 3$, ** $p < 0.01$, *** $p < 0.001$.

3.6. Optimization

The successful production of baker's yeast on a commercial scale depends on the availability of a cheap suitable carbon source, one good strain of yeast, and an easily manageable fermentation process for maximum viable biomass production and the process development for preservation. The production efficiency of a strain depends on many factors such as nature, composition, the concentration of substrate, temperature, pH, inoculums size, incubation period, etc. [49]. The best cheap carbon source for optimum growth and maximum biomass production was detected by measuring optical density at 550 nm using a spectrophotometer of inoculated (3.86×10^5 cells mL^{-1} of culture broth) 2% sucrose and 4% molasses broth after 3 days of incubation at 30 °C. Highest OD for all the test isolates was found in molasses broth and it was relatively very low in sucrose broth (Figure 2A). G2 showed the highest optical density of 1.995 in molasses broth whereas it

was only 0.133 in sucrose broth which means molasses can be used as an effective cheap and widely available carbon source for baker's yeast production due to its wide availability as a byproduct from sugar industries.

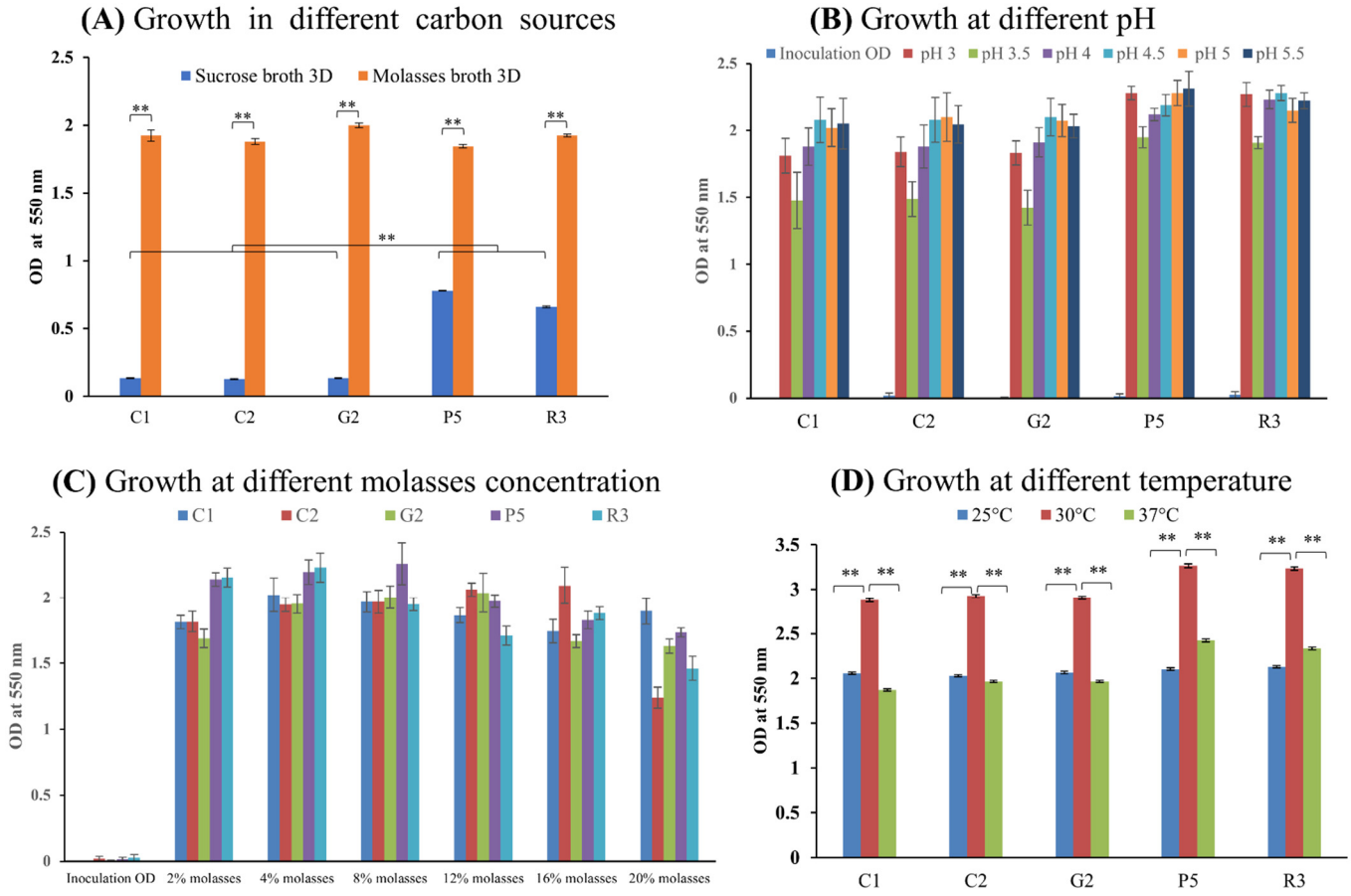

Figure 2. (**A**) Growth in molasses broth and in sucrose broth. (**B**) Growth at different pH. (**C**) Growth at different molasses concentration (**D**) Growth at different temperature. Tukey–Kramer test. $n = 3$, ** $p < 0.01$.

All the isolates showed moderate growth at a pH range from 4–5.5. Most of the isolates i.e., C1, G2, and R3 grew well at pH 4.5 (Figure 2B). C2 grows well at pH 5, and P5 at pH 5.5. This low pH helps grip the growth of undesirable microorganisms.

Optimum molasses concentration was different for different yeast isolates. A total of 4% molasses broth was determined as the optimum molasses concentration for two isolates, i.e., C1, R3. While on the contrary, P5, G2, and C2 showed maximum growth in 8%, 12%, and 16% molasses broth, respectively but it was very close to their growth in 4% molasses broth (Figure 2C).

All the test isolates showed better growth at 30 °C (Figure 2D) than at lower or higher temperatures.

3.7. Incubation Period

The OD of inoculated (inoculums size 3.86×10^5 cells mL^{-1}) 4% (w/v) molasses broth of pH 4.5 and incubated at 30 °C (previously determined as optimum) was highest for P5, R3 after 6 days; C1, G2 after 3 days; and C2 after 5 days of incubation (Figure 3). Cream cell mass mL^{-1} (Figure 3) and dry cell mass mL^{-1} (Figure 3) were maximum for C1, C2, and G2 after 2 days: P5, and R3 after 6 days of incubation. Nevertheless, all the test isolates produced maximum CFU mL^{-1} (Figure 3) after 4 days of incubation.

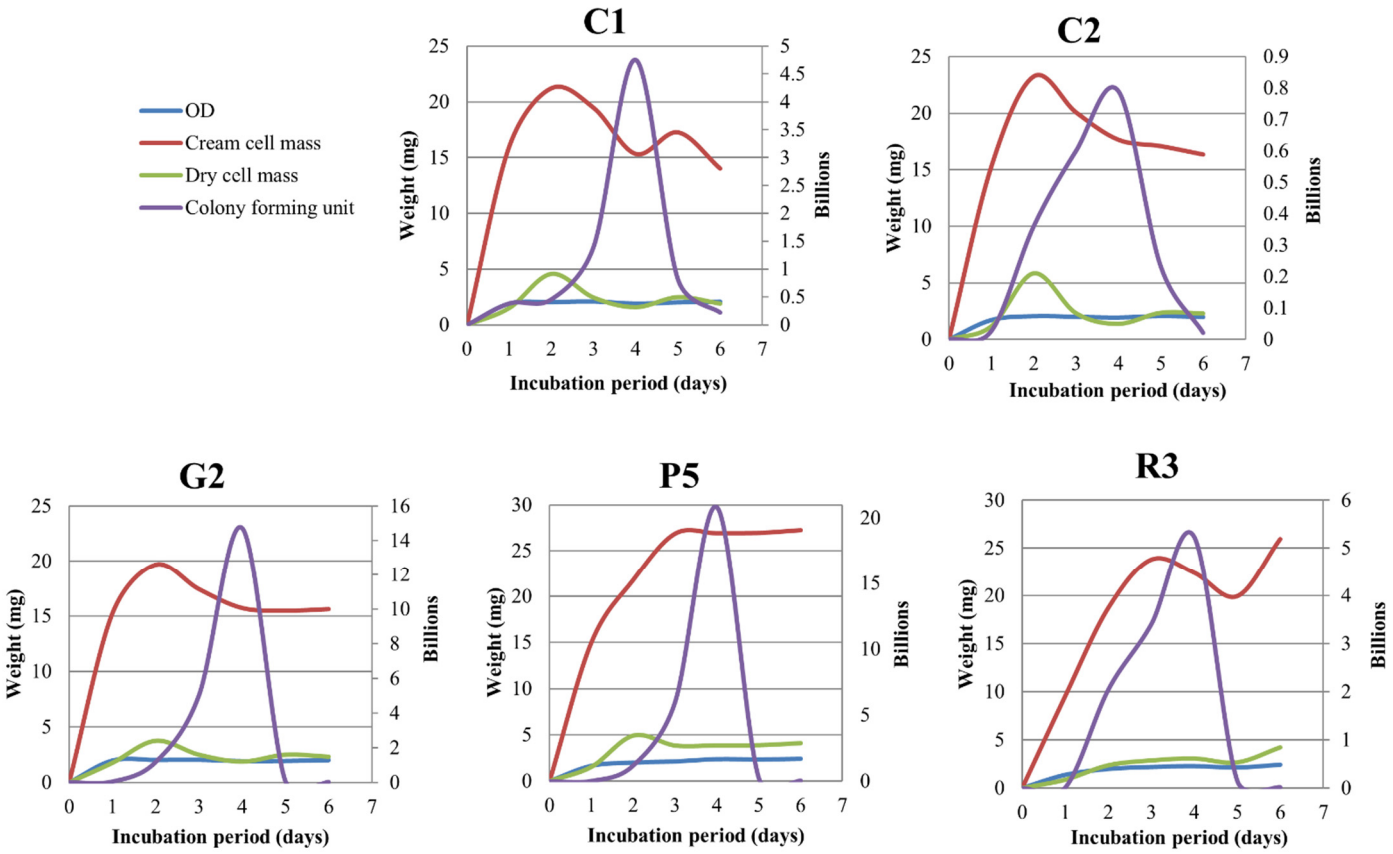

Figure 3. Biomass production curve of yeast isolates after different incubation periods.

3.8. Relationship between OD, Cream Cell Mass, Dry Cell Mass, CFU mL^{-1}, and Incubation Period

The incubation period required for maximum biomass production with the highest number of viable cells by the yeast isolates was determined by inoculating the yeast strains (3.86×10^5 cells mL^{-1} of culture broth) in 4% (w/v) molasses broth of pH 4.5 and incubated at 30 °C (previously determined as optimum). This culture broth was then tested for OD, cream cell mass mL^{-1}, dry cell mass mL^{-1}, and CFU mL^{-1} every day for 6 days. The data of OD, cream cell mass, dry cell mass and CFU mL^{-1} observed for each isolate after different incubation periods were plotted in Microsoft Office Excel Worksheet to obtain a yeast growth curve relating incubation period with OD, CFU mL^{-1}, cream cell mass mL^{-1}, and dry cell mass mL^{-1}. The minimum incubation period for maximum growth and maximum viable biomass production was determined from this curve (Figure 3). OD was highest for P5 and R3 after 6 days; C1 and G2 after 3 days; and C2 after 5 days of incubation at 30 °C (Figure 3). Cream cell mass mL^{-1} and dry cell mass mL^{-1} (Figure 3) were maximum for C1, C2, and G2 after 2 days: P5 and R3 after 6 days of incubation. However, all the test isolates produced maximum CFU mL^{-1} (Figure 3) after 4 days of incubation and fell drastically after 4 days. Then the cells died at a high rate due to accumulation of waste products, high cell concentration, and depletion of nutrients. All the yeast isolates have a maximum cell density above which the death phase begins (Figure 3). So fed-batch culture or continuous culture can be effectively used to produce baker's yeast instead of batch culture as it initiates ethanol production and may cause toxicity. As we need the maximum viable cell to make bread softer, 4 days of incubation is required (or the time required to reach the critical concentration in the case of different inoculum sizes) to have the highest number of viable cells in the lowest cell weight.

3.9. Effect of Shaking

Aeration and agitation significantly influenced the production of cell mass ($p < 0.01$). All the test isolates showed two to three times better biomass production in shake culture than in non-shake culture (Figure 4). This happens as shaking ensures the availability of nutrients and oxygen for all the cells and thus confirms maximum biomass production within a minimum period.

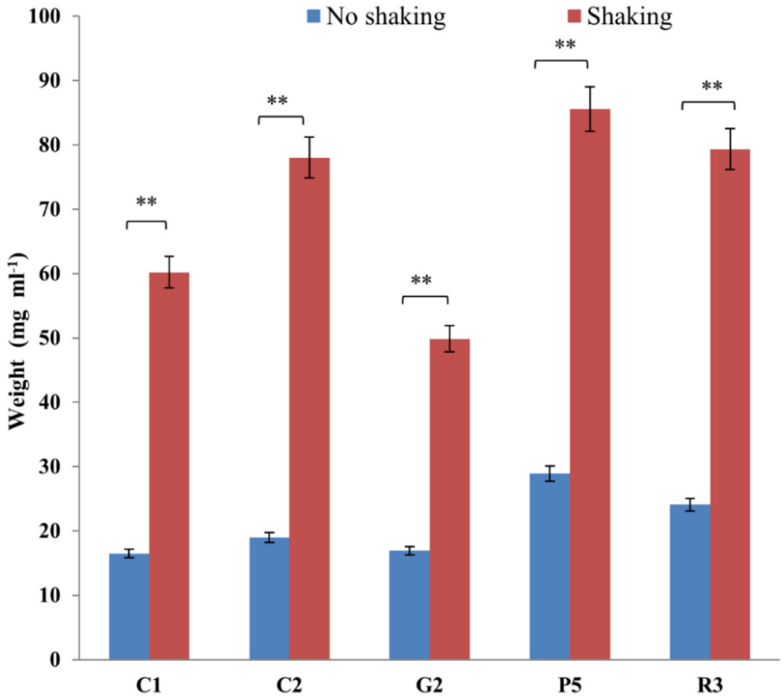

Figure 4. Effect of shaking on biomass production by test isolates. Tukey–Kramer test. $n = 3$, ** $p < 0.01$.

3.10. Preparation of Bread

The leavening ability of commercial baker's yeast on composite flour (wheat) was investigated. The leavening of dough during bread production is the result of carbon dioxide produced by the fermenting organism which is usually *Saccharomyces* Species [17,71–74]. The bread produced by G2 showed comparatively good results against those of Granular Yeast C1 and fresh culture of C1 (Table 3 and Figure 5). Yeast, specifically *Saccharomyces cerevisiae*, is used in baking as a leavening agent where it converts the fermentable sugars present in the dough into carbon dioxide. This causes the dough to expand or rise as the carbon dioxide forms pockets or bubbles. When the dough is baked it "sets" and the pockets remain, giving the baked product a soft and spongy texture. Dough mixtures are usually agitated by mixing to ensure that air pockets are created. It was found that the dough volume increased as agitation time increased but only to a limited extent as a longer period (40 min) caused a drastic drop in volume. Therefore, the period for mixing is important as it influences the size and quality of bread. The agitation enhances the ability of the dough to trap more air [4,9,26,29,34,56,57].

The bread produced by G2 showed a comparatively good result against those of Granular Yeast C1 and fresh culture of C1 (Table 3 and Figure 5). Granular C1 increased the dough volume to 114% whereas its fresh culture increased the dough volume to 108%, the same as the natural isolate G2 after 30 min of incubation at room temperature. After baking at 180 °C for 1.5 h, the bread volume of granular C1 was increased to 171% and the bread volume of fresh C1 and G2 was increased by 170% showing that G2 has similar potential to commercial yeast strains for bread production. In a previous report, yeast isolates from sugarcane juices increased the bread volume by 118–128% [25] which is lower than we observed in grape juice isolates. So, in all aspects, G2 can be produced commercially as an

effective baker's yeast and it has much potential to create a position by its characteristic flavor, texture, and taste in the competitive market of baker's yeast.

Table 3. Leavening profiles of dough using yeast isolates.

Samples		Granular Yeast, C1 (DBL, China)	Fresh C1	Fresh G2
Amount (gm)	Yeast	2.4	2.4	2.4
	Dough	356	356	356
Incubation	Temperature	Room	Room	Room
	Time	30 min	30 min	30 min
Increased dough volume (%)		114	108	108
Baking	Temperature	180 °C	180 °C	180 °C
	Period	1.5 h	1.5 h	1.5 h
Increased bread volume (%)		171	170	170
Color		Characteristics	Characteristics	Characteristics
Texture		Best	Good	Better
Taste		Best	Good	Better
Flavor		Pleasant	Pleasant	Better
Mouth feeling		Good	Medium	Best
Remarks		Longer incubation and baking period required	Longer incubation and baking period required	Longer incubation and baking period required

Figure 5. Samples after baking (left to right): Commercial granular yeast of C1, a fresh culture of C1, and G2.

4. Limitations

We could not identify the yeast isolates using molecular methods. Therefore, we have taken two commercial baker's yeast as control and compared all cultural, morphological, biochemical, potency assessment (as potential baker's yeast) data with these commercial strains to screen potential yeast isolates from different sources. This study provides evidence of having potential yeast isolates in grape and will lead researchers to investigate more on this source.

5. Conclusions

The biomass and viable cell production rate, and gas production by G2 were significantly higher than the two commercial yeast strains ($p < 0.01$) though the commercial granular yeast may contain some other chemical components that influence gas production. Although the production of viable biomass by P5 and R3 strains was higher ($p < 0.05$) than the commercial strains (C1 and C2), the levels of their gas production were significantly

lower than the controls ($p < 0.05$). So, in the above circumstances, G2 can be used as a potent yeast strain to be produced commercially as a baker's yeast. P5 and R3 have potential but they require some modification in their genetic level to produce more gases such as overproduction of the enzymes that break down sugar molecules into ethanol and carbon dioxide. Thus, the yeast isolates could be used as baker's yeast production as well as ethanol production to meet the demand of biofuel, and for industrial and laboratory usage.

As the isolate from grape juice showed promising results in bread production, more research is required focusing on different varieties of grape juice to isolate more promising yeast candidates for commercial baker's yeast production.

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Conflicts of Interest: The authors declare no conflict of interest.

Appendix A

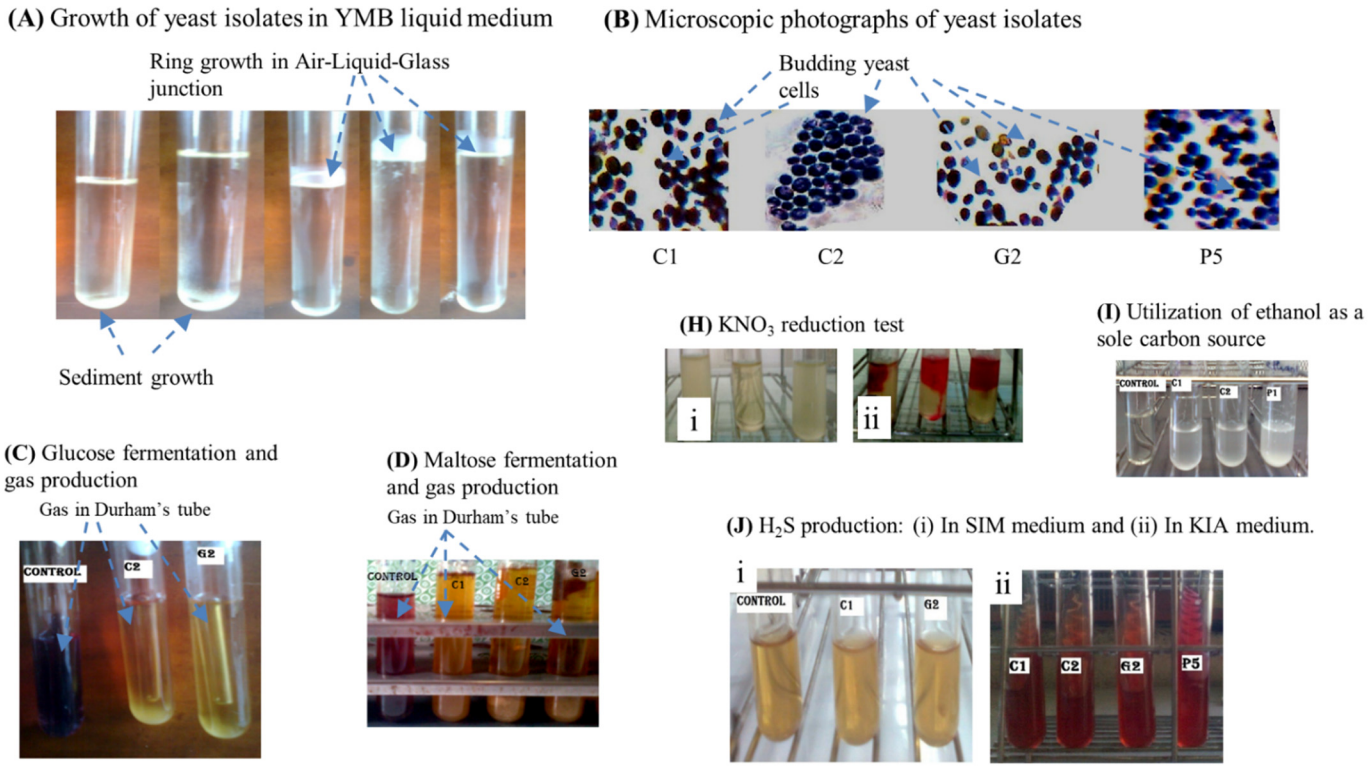

Figure A1. *Cont.*

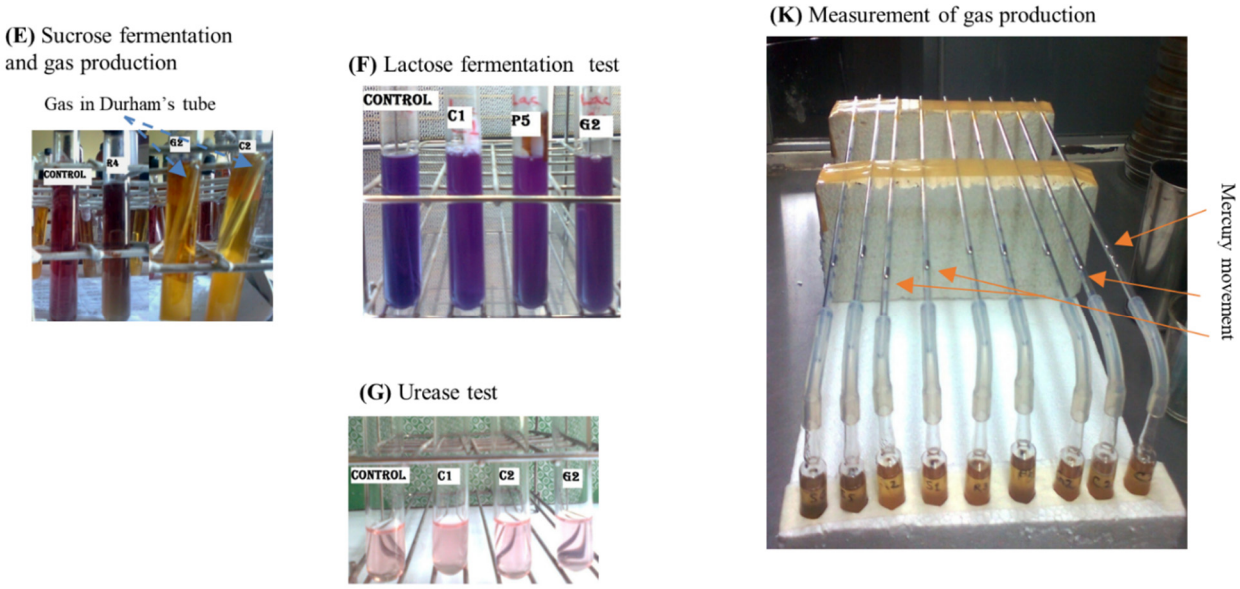

Figure A1. Different morphological and biochemical test results of fungal isolates. (**A**) Growth of yeast isolates in YMB liquid medium, (**B**) Microscopic photographs of yeast isolates showing budding yeast, (**C**–**E**) Glucose, maltose, sucrose fermentation test: Color changes from purple to yellow and Gas production in Durham's tube indicate a positive result, (**F**) Lactose fermentation test: no color change indicates a negative result, (**G**) Urease test: No color change indicates a negative result, (**H**) KNO_3 reduction test:(i)The test tubes did not develop red color after the addition of Sulfanilic acid solution and α-naphthyl amine solution, (ii)Soon after addition of zinc powder, these solutions develop red color, indicates negative results. (**I**) Utilization of ethanol as a sole carbon source, (**J**) H_2S production: (i) In SIM medium and (ii) In KIA medium. Any blackening of the KIA and SIM media along the line of inoculation or throughout the butt indicates hydrogen sulfide production, (**K**) Measurement of gas production by test isolates. The movement of mercury along the respirometer scale indicates the volume of gas produced by test isolates.

References

1. Zhou, N.; Semumu, T.; Gamero, A. Non-conventional yeasts as alternatives in modern baking for improved performance and aroma enhancement. *Fermentation* **2021**, *7*, 102. [CrossRef]
2. Gallone, B.; Steensels, J.; Prahl, T.; Soriaga, L.; Saels, V.; Herrera-Malaver, B.; Merlevede, A.; Roncoroni, M.; Voordeckers, K.; Miraglia, L.; et al. Domestication and Divergence of Saccharomyces cerevisiae Beer Yeasts. *Cell* **2016**, *166*, 1397–1410.e16. [CrossRef] [PubMed]
3. Landis, E.A.; Oliverio, A.M.; McKenney, E.A.; Nichols, L.M.; Kfoury, N.; Biango-Daniels, M.; Shell, L.K.; Madden, A.A.; Shapiro, L.; Sakunala, S.; et al. The diversity and function of sourdough starter microbiomes. *Elife* **2021**, *10*, e61644. [CrossRef] [PubMed]
4. Madden, A.A.; Lahue, C.; Gordy, C.L.; Little, J.L.; Nichols, L.M.; Calvert, M.D.; Dunn, R.R.; Smukowski Heil, C. Sugar-seeking insects as a source of diverse bread-making yeasts with enhanced attributes. *Yeast* **2021**, *39*, 108–127. [CrossRef]
5. Steensels, J.; Snoek, T.; Meersman, E.; Nicolino, M.P.; Voordeckers, K.; Verstrepen, K.J. Improving industrial yeast strains: Exploiting natural and artificial diversity. *FEMS Microbiol. Rev.* **2014**, *38*, 947–995. [CrossRef]
6. Xiao, M.; Wang, H.; Lu, J.; Chen, S.C.A.; Kong, F.; Ma, X.J.; Xu, Y.C. Three clustered cases of candidemia caused by Candida quercitrusa and mycological characteristics of this novel species. *J. Clin. Microbiol.* **2014**, *52*, 3044–3048. [CrossRef]
7. Bell, P.J.L.; Higgins, V.J.; Attfield, P.V. Comparison of fermentative capacities of industrial baking and wild-type yeasts of the species Saccharomyces cerevisiae in different sugar media. *Lett. Appl. Microbiol.* **2001**, *32*, 224–229. [CrossRef]
8. Hino, A.; Mihara, K.; Nakashima, K.; Takano, H. Trehalose levels and survival ratio of freeze-tolerant versus freeze-sensitive yeasts. *Appl. Environ. Microbiol.* **1990**, *56*, 1386–1391. [CrossRef]
9. Birch, A.N.; Petersen, M.A.; Arneborg, N.; Hansen, Å.S. Influence of commercial baker's yeasts on bread aroma profiles. *Food Res. Int.* **2013**, *52*, 160–166. [CrossRef]
10. Gelinas, P.; Gélinas, P. Inventions on baker's yeast storage and activation at the bakery plant. *Recent Pat. Food. Nutr. Agric.* **2010**, *2*, 1–11. [CrossRef]

11. Johansen, P.G.; Owusu-Kwarteng, J.; Parkouda, C.; Padonou, S.W.; Jespersen, L. Occurrence and Importance of Yeasts in Indigenous Fermented Food and Beverages Produced in Sub-Saharan Africa. *Front. Microbiol.* **2019**, *10*, 1789. [CrossRef] [PubMed]
12. Karim, A.; Gerliani, N.; Aïder, M. Kluyveromyces marxianus: An emerging yeast cell factory for applications in food and biotechnology. *Int. J. Food Microbiol.* **2020**, *333*, 108818. [CrossRef]
13. Bourdichon, F.; Casaregola, S.; Farrokh, C.; Frisvad, J.C.; Gerds, M.L.; Hammes, W.P.; Harnett, J.; Huys, G.; Laulund, S.; Ouwehand, A.; et al. Food fermentations: Microorganisms with technological beneficial use. *Int. J. Food Microbiol.* **2012**, *154*, 87–97. [CrossRef] [PubMed]
14. Shen, X.X.; Opulente, D.A.; Kominek, J.; Zhou, X.; Steenwyk, J.L.; Buh, K.V.; Haase, M.A.B.; Wisecaver, J.H.; Wang, M.; Doering, D.T.; et al. Tempo and Mode of Genome Evolution in the Budding Yeast Subphylum. *Cell* **2018**, *175*, 1533–1545.e20. [CrossRef] [PubMed]
15. Pozo, M.I.; Lachance, M.A.; Herrera, C.M. Nectar yeasts of two southern Spanish plants: The roles of immigration and physiological traits in community assembly. *FEMS Microbiol. Ecol.* **2012**, *80*, 281–293. [CrossRef]
16. Gutiérrez, A.; Boekhout, T.; Gojkovic, Z.; Katz, M. Evaluation of non-Saccharomyces yeasts in the fermentation of wine, beer and cider for the development of new beverages. *J. Inst. Brew.* **2018**, *124*, 389–402. [CrossRef]
17. Nyanga, L.K.; Nout, M.J.R.; Gadaga, T.H.; Theelen, B.; Boekhout, T.; Zwietering, M.H. Yeasts and lactic acid bacteria microbiota from masau (Ziziphus mauritiana) fruits and their fermented fruit pulp in Zimbabwe. *Int. J. Food Microbiol.* **2007**, *120*, 159–166. [CrossRef]
18. Spencer, J.F.T.; Spencer, D.M. *Yeasts in Natural and Artificial Habitats*; Springer Science & Business Media: Berlin/Heidelberg, Germany, 1997; Volume 381, pp. 11–32.
19. Hernandez-Lopez, M.J.; Prieto, J.A.; Randez-Gil, F. Osmotolerance and leavening ability in sweet and frozen sweet dough. Comparative analysis between Torulaspora delbrueckii and Saccharomyces cerevisiae baker's yeast strains. *Antonie van Leeuwenhoek Int. J. Gen. Mol. Microbiol.* **2003**, *84*, 125–134. [CrossRef]
20. Tamang, J.P.; Fleet, G.H. Yeasts diversity in fermented foods and beverages. In *Yeast Biotechnology: Diversity and Applications*; Springer: Dordrecht, The Netherlands, 2009; pp. 169–198.
21. Azmuda, N.; Jahan, N.; Khan, A.R. Production and Comparison of Indigenous and Commercial Baker's Yeasts. *Bangladesh J. Microbiol.* **2006**, *23*, 89–92. [CrossRef]
22. Reale, A.; Reale, A.; Di Renzo, T.; Succi, M.; Tremonte, P.; Coppola, R.; Sorrentino, E. Microbiological and Fermentative Properties of Baker's Yeast Starter Used in Breadmaking. *J. Food Sci.* **2013**, *78*, M1224–M1231. [CrossRef]
23. Alfonzo, A.; Gaglio, R.; Barbera, M.; Francesca, N.; Moschetti, G.; Settanni, L. Evaluation of the Fermentation Dynamics of Commercial Baker's Yeast in Presence of Pistachio Powder to Produce Lysine-Enriched Breads. *Fermentation* **2019**, *6*, 2. [CrossRef]
24. Meroth, C.B.; Hammes, W.P.; Hertel, C. Identification and Population Dynamics of Yeasts in Sourdough Fermentation Processes by PCR-Denaturing Gradient Gel Electrophoresis. *Appl. Environ. Microbiol.* **2003**, *69*, 7453–7461. [CrossRef] [PubMed]
25. Mamun-Or-Rashid, A.N.M.; Dash, B.K.; Chowdhury, M.N.A.; Waheed, M.F.; Pramanik, M.K. Exploration of potential baker's yeast from sugarcane juice: Optimization and evaluation. *Pakistan J. Biol. Sci.* **2013**, *16*, 617–623. [CrossRef] [PubMed]
26. Akyüz, G.; Mazı, B.G. Physicochemical and sensory characterization of bread produced from different dough formulations by Kluyveromyces lactis. *J. Food Process. Preserv.* **2020**, *44*, e14498. [CrossRef]
27. Aslankoohi, E.; Herrera-Malaver, B.; Rezaei, M.N.; Steensels, J.; Courtin, C.M.; Verstrepen, K.J. Non-conventional yeast strains increase the aroma complexity of bread. *PLoS ONE* **2016**, *11*, e0165126. [CrossRef] [PubMed]
28. Domizio, P.; House, J.F.; Joseph, C.M.L.; Bisson, L.F.; Bamforth, C.W. Lachancea thermotolerans as an alternative yeast for the production of beer. *J. Inst. Brew.* **2016**, *122*, 599–604. [CrossRef]
29. Urien, C.; Legrand, J.; Montalent, P.; Casaregola, S.; Sicard, D. Fungal species diversity in French bread sourdoughs made of organic wheat flour. *Front. Microbiol.* **2019**, *10*, 201. [CrossRef]
30. Singh Nehra, K.; Jangra, M.R.; Pooja, S.; Minakshi, A.; Pooja, M.; Rama, B.; Hitesh, S.; Pardeep, P.; Sumit, J. Production of Bioethanol from Sugarcane Juice, Molasses and Paddy Straw using Saccharomyces cerevisiae. *Biosci. Biotechnol. Res. Commun.* **2021**, *14*, 581–586. [CrossRef]
31. Albuquerque, M.G.E.; Concas, S.; Bengtsson, S.; Reis, M.A.M. Mixed culture polyhydroxyalkanoates production from sugar molasses: The use of a 2-stage CSTR system for culture selection. *Bioresour. Technol.* **2010**, *101*, 7112–7122. [CrossRef]
32. Rattanapan, A.; Limtong, S.; Phisalaphong, M. Ethanol production by repeated batch and continuous fermentations of blackstrap molasses using immobilized yeast cells on thin-shell silk cocoons. *Appl. Energy* **2011**, *88*, 4400–4404. [CrossRef]
33. Cáceres-Farfán, M.; Lappe, P.; Larqué-Saavedra, A.; Magdub-Méndez, A.; Barahona-Pérez, L. Ethanol production from henequen (Agave fourcroydes Lem.) juice and molasses by a mixture of two yeasts. *Bioresour. Technol.* **2008**, *99*, 9036–9039. [CrossRef] [PubMed]
34. Rández-Gil, F.; Ballester-Tomás, L.; Prieto, J.A. Yeast. In *Bakery Products Science and Technology*, 2nd ed.; John Wiley & Sons: Hoboken, NJ, USA, 2014; pp. 153–174. ISBN 9781119967156.
35. Satyanarayana, T.; Kunze, G. *Yeast Biotechnology: Diversity and Applications*; Springer: Dordrecht, The Netherlands, 2009; ISBN 978-90-481-7833-9.
36. Masneuf-Pomarede, I.; Bely, M.; Marullo, P.; Albertin, W. The genetics of non-conventional wine yeasts: Current knowledge and future challenges. *Front. Microbiol.* **2016**, *6*, 1563. [CrossRef] [PubMed]

37. Naseeb, S.; Visinoni, F.; Hu, Y.; Hinks Roberts, A.J.; Maslowska, A.; Walsh, T.; Smart, K.A.; Louis, E.J.; Delneri, D. Restoring fertility in yeast hybrids: Breeding and quantitative genetics of beneficial traits. *Proc. Natl. Acad. Sci. USA* **2021**, *118*, e2101242118. [CrossRef] [PubMed]
38. Ceccato-Antonini, S.R.; Covre, E.A. From baker's yeast to genetically modified budding yeasts: The scientific evolution of bioethanol industry from sugarcane. *FEMS Yeast Res.* **2020**, *20*, foaa065. [CrossRef] [PubMed]
39. Codón, A.C.; Rincón, A.; Rincon, A.M.; Moreno-Mateos, M.A.; Delgado-Jarana, J.; Rey, M.; Limon, C.; Rosado, I.V.; Cubero, B.; Peñate, X.; et al. New Saccharomyces cerevisiae baker's yeast displaying enhanced resistance to freezing. *J. Agric. Food Chem.* **2003**, *51*, 483–491. [CrossRef]
40. Davis, T.S.; Boundy-Mills, K.; Landolt, P.J. Volatile Emissions from an Epiphytic Fungus are Semiochemicals for Eusocial Wasps. *Microb. Ecol.* **2012**, *64*, 1056–1063. [CrossRef]
41. Bellut, K.; Krogerus, K.; Arendt, E.K. Lachancea fermentati Strains Isolated From Kombucha: Fundamental Insights, and Practical Application in Low Alcohol Beer Brewing. *Front. Microbiol.* **2020**, *11*, 764. [CrossRef]
42. Salomé, S.; Pais, M.; Chaves Das Neves, H.J. Sugar Content of the Nectary Exudate of Epipactis Atropurpurea Rafin. *Apidologie* **1980**, *11*, 39–45. [CrossRef]
43. Kemsawasd, V.; Branco, P.; Almeida, M.G.; Caldeira, J.; Albergaria, H.; Arneborg, N. Cell-to-cell contact and antimicrobial peptides play a combined role in the death of Lachanchea thermotolerans during mixed-culture alcoholic fermentation with Saccharomyces cerevisiae. *FEMS Microbiol. Lett.* **2015**, *362*, fnv103. [CrossRef]
44. Zhou, W.; Hui, Y.H.; De Leyn, I.; Pagani, M.A.; Rosell, C.M.; Selman, J.D.; Therdthai, N. *Bakery Products Science and Technology*, 2nd ed.; John Wiley & Sons: Hoboken, NJ, USA, 2014; ISBN 9781119967156.
45. De Araújo Vicente, M.; Fietto, L.G.; De Miranda Castro, I.; Gonçalves Dos Santos, A.N.; Coutrim, M.X.; Brandão, R.L. Isolation of Saccharomyces cerevisiae strains producing higher levels of flavoring compounds for production of "cachaça" the Brazilian sugarcane spirit. *Int. J. Food Microbiol.* **2006**, *108*, 51–59. [CrossRef]
46. Sanni, A.I.; Lönner, C. Identification of yeasts isolated from Nigerian traditional alcoholic beverages. *Food Microbiol.* **1993**, *10*, 517–523. [CrossRef]
47. Barnett, J.A.; James, A.; Payne, R.W.; Yarrow, D. *Yeasts: Characteristics and Identification*; Cambridge University Press: Cambridge, UK, 1983; p. 811.
48. Boekhout, T.; Robert, V. *Yeasts in Food*; Elsevier: Amsterdam, The Netherlands, 2003; pp. 1–488.
49. Harrigan, W.F.; McCance, M.E. *Laboratory Methods in Microbiology*; Academic Press: Cambridge, MA, USA, 1982; ISBN 1483274349.
50. Palla, M.; Blandino, M.; Grassi, A.; Giordano, D.; Sgherri, C.; Quartacci, M.F.; Reyneri, A.; Agnolucci, M.; Giovannetti, M. Characterization and selection of functional yeast strains during sourdough fermentation of different cereal wholegrain flours. *Sci. Rep.* **2020**, *10*, 12856. [CrossRef]
51. Camargo, J.Z.; Nascimento, V.M.; Stefanello, I.; de Andrade Silva, C.A.; Gonçalves, F.A.; Perdomo, I.C.; Vilela, D.M.; Simionatto, S.; Pereira, R.M.; da Paz, M.F.; et al. Biochemical evaluation, molecular characterization and identification of novel yeast strains isolated from Brazilian savannah fruits, chicken litter and a sugar and alcohol mill with biotechnological potential for biofuel and food industries. *Biocatal. Agric. Biotechnol.* **2018**, *16*, 390–399. [CrossRef]
52. Murali, N.; Srinivas, K.; Ahring, B.K. Biochemical production and separation of carboxylic acids for biorefinery applications. *Fermentation* **2017**, *3*, 22. [CrossRef]
53. Krogerus, K.; Magalhães, F.; Castillo, S.; Peddinti, G.; Vidgren, V.; De Chiara, M.; Yue, J.-X.; Liti, G.; Gibson, B. Lager Yeast Design Through Meiotic Segregation of a *Saccharomyces cerevisiae* × *Saccharomyces eubayanus* Hybrid. *Front. Fungal Biol.* **2021**, *2*, 733655. [CrossRef]
54. Garofalo, C.; Arena, M.P.; Laddomada, B.; Cappello, M.S.; Bleve, G.; Grieco, F.; Beneduce, L.; Berbegal, C.; Spano, G.; Capozzi, V. Starter cultures for sparkling wine. *Fermentation* **2016**, *2*, 21. [CrossRef]
55. Bigey, F.; Segond, D.; Friedrich, A.; Guezenec, S.; Bourgais, A.; Huyghe, L.; Agier, N.; Nidelet, T.; Sicard, D. Evidence for Two Main Domestication Trajectories in Saccharomyces cerevisiae Linked to Distinct Bread-Making Processes. *Curr. Biol.* **2021**, *31*, 722–732.e5. [CrossRef]
56. Hamelman, J. *Bread: A Baker's Book of Techniques and Recipes*; John Wiley & Sons: Hoboken, NJ, USA, 2004; p. 415.
57. Bailey, C.H.; Sherwood, R.C. The Carbohydrate Sequence. Biochemistry of Bread Making. *Ind. Eng. Chem.* **1935**, *27*, 1426–1430. [CrossRef]
58. Jansen, M.L.A.; Bracher, J.M.; Papapetridis, I.; Verhoeven, M.D.; de Bruijn, H.; de Waal, P.P.; van Maris, A.J.A.; Klaassen, P.; Pronk, J.T. Saccharomyces cerevisiae strains for second-generation ethanol production: From academic exploration to industrial implementation. *FEMS Yeast Res.* **2017**, *17*, fox044. [CrossRef]
59. Naumova, E.S.; Sadykova, A.Z.; Martynenko, N.N.; Naumov, G.I. Molecular genetic characteristics of Saccharomyces cerevisiae distillers' yeasts. *Microbiol. Russian Fed.* **2013**, *82*, 175–185. [CrossRef]
60. Naumov, G.I.; Naumova, E.S.; Michels, C.A. Genetic variation of the repeated MAL loci in natural populations of Saccharomyces cerevisiae and Saccharomyces paradoxus. *Genetics* **1994**, *136*, 803–812. [CrossRef] [PubMed]
61. Mishra, S.; Baranwal, R. Yeast genetics and biotechnological applications. In *Yeast Biotechnology: Diversity and Applications*; Springer: Dordrecht, The Netherlands, 2009; pp. 323–355.
62. Hyma, K.E.; Saerens, S.M.; Verstrepen, K.J.; Fay, J.C. Divergence in wine characteristics produced by wild and domesticated strains of Saccharomyces cerevisiae. *FEMS Yeast Res.* **2011**, *11*, 540–551. [CrossRef] [PubMed]

63. Ayoub, M.J.; Legras, J.L.; Saliba, R.; Gaillardin, C. Application of Multi Locus Sequence Typing to the analysis of the biodiversity of indigenous Saccharomyces cerevisiae wine yeasts from Lebanon. *J. Appl. Microbiol.* **2006**, *100*, 699–711. [CrossRef] [PubMed]
64. Azumi, M.; Goto-Yamamoto, N. AFLP analysis of type strains and laboratory and industrial strains of Saccharomyces sensu stricto and its application to phenetic clustering. *Yeast* **2001**, *18*, 1145–1154. [CrossRef]
65. Legras, J.L.; Merdinoglu, D.; Cornuet, J.M.; Karst, F. Bread, beer and wine: Saccharomyces cerevisiae diversity reflects human history. *Mol. Ecol.* **2007**, *16*, 2091–2102. [CrossRef]
66. Alves-Araújo, C.; Almeida, M.J.; Sousa, M.J.; Leão, C. Freeze tolerance of the yeast Torulaspora delbrueckii: Cellular and biochemical basis. *FEMS Microbiol. Lett.* **2004**, *240*, 7–14. [CrossRef]
67. Donalies, U.E.B.; Nguyen, H.T.T.; Stahl, U.; Nevoigt, E. Improvement of Saccharomyces yeast strains used in brewing, wine making and baking. *Adv. Biochem. Eng. Biotechnol.* **2008**, *111*, 67–98.
68. Timouma, S.; Balarezo-Cisneros, L.N.; Pinto, J.; De La Cerda, R.; Bond, U.; Schwartz, J.M.; Delneri, D. Transcriptional Profile of the Industrial Hybrid Saccharomyces pastorianus Reveals Temperature-Dependent Allele Expression Bias and Preferential Orthologous Protein Assemblies. *Mol. Biol. Evol.* **2021**, *38*, 5437–5452. [CrossRef]
69. Bamforth, C.W.; Cook, D.J. *Food, Fermentation, and Micro-Organisms*, 2nd ed.; John Wiley & Sons: Hoboken, NJ, USA, 2019; pp. 1–245.
70. Compagno, C.; Porro, D.; Smeraldi, C.; Ranzi, B.M. Fermentation of whey and starch by transformed Saccharomyces cerevisiae cells. *Appl. Microbiol. Biotechnol.* **1995**, *43*, 822–825. [CrossRef]
71. Moyad, M.A. Brewer's/baker's yeast (Saccharomyces cerevisiae) and preventive medicine: Part I. *Urol. Nurs.* **2007**, *27*, 560–561.
72. Lopez, C.L.F.; Beaufort, S.; Brandam, C.; Taillandier, P. Interactions between Kluyveromyces marxianus and Saccharomyces cerevisiae in tequila must type medium fermentation. *World J. Microbiol. Biotechnol.* **2014**, *30*, 2223–2229. [CrossRef] [PubMed]
73. Díaz-Montaño, D.M.; De Jesús Ramírez Córdova, J. The fermentative and aromatic ability of Kloeckera and Hanseniaspora yeasts. In *Yeast Biotechnology: Diversity and Applications*; Springer: Dordrecht, The Netherlands, 2009; pp. 281–305.
74. Picazo, C.; Gamero-Sandemetrio, E.; Orozco, H.; Albertin, W.; Marullo, P.; Matallana, E.; Aranda, A. Mitochondria inheritance is a key factor for tolerance to dehydration in wine yeast production. *Lett. Appl. Microbiol.* **2015**, *60*, 217–222. [CrossRef] [PubMed]