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RESEARCH ARTICLE

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Production and characterization of bioemulsifier from local isolate of *Saccharomyces cerevisiae* strain JZT351: Antioxidant and antimicrobial effects

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Abstract

The widespread use and circulation of industrial emulsifiers pose significant health risks, compounded by their limited availability and high cost. Consequently, there is growing interest in exploring the potential of natural sources, such as microorganisms like yeast, for emulsifier production. In this study, 25 strains of *Saccharomyces cerevisiae* were isolated from 17 distinct local sources. The yeasts were characterized based on cell shape, size, and colony morphology using yeast malt agar, followed by morphological, microscopic, and biochemical analyzes. Among the isolates, AC1 from a vinegar starter demonstrated superior bioemulsifier production. Emulsification efficiency tests revealed that AC1 exhibited values of 7.1 cm, 0.71 nm, and 34.50%, outperforming other isolates. The biomass yield was approximately 4.35 g/L. This strain was registered in the gene bank as JZT351 (OR115510) after a 100% match with *S. cerevisiae*. Optimal bioemulsifier production conditions for JZT351 were identified using a liquid yeast extract peptone dextrose medium, with date juice replacing 75% of the glucose, at pH 5.5, 30°C, and 72 h. The resulting emulsification activity, index, and oil displacement were 8.69 cm, 0.95 nm, and 39.7%, respectively. Fourier-transform infrared spectroscopy (FTIR) compared the functional groups of the bioemulsifier with those of conventional emulsifiers. Molecular characterization was confirmed by 1H NMR. FTIR spectra revealed bioemulsifier bands at 3443.28, 2929.34, 1656.559, 1534.09, 807.06, and 1656.55 cm^{-1}. At 0.5–5 ppm, signals and spectral frequencies corresponded to a mannoprotein structure. The bioemulsifier exhibited 58.09% antioxidant activity at 10 mg/mL, as assessed by DPPH scavenging. It showed the highest inhibition against *Pseudomonas aeruginosa* and the least against *Candida albicans*.

KEYWORDS

antimicrobial, antioxidant, bioemulsifier, characterization, *Saccharomyces cerevisiae*

1 | INTRODUCTION

An emulsion is a system in which one immiscible liquid is distributed in another. Water-in-oil (W/O) and oil-in-water (O/W) emulsions are two examples of various emulsion systems. Other types include oil-in-water-in-oil (O/W/O) and water-in-oil-in-water (W/O/W) (Tan & McClements, 2021). Emulsifiers are used to homogenize food systems, and their effectiveness is closely related to their chemical composition

(Alizadeh-Sani et al., 2018). As amphiphilic molecules with both hydrophilic and hydrophobic ends, emulsifiers reduce the repulsive forces between different phases, facilitating mixing. This makes them excellent emulsifying, foaming, and dispersing agents (Wang et al., 2023). The extensive use of synthetic emulsifiers, along with their adverse health effects, limited availability, and high costs, has driven researchers to explore the synthesis of emulsifiers from natural sources, particularly microorganisms such as fungi and bacteria (Fenibo et al., 2019).

Bioemulsifiers encompass compounds such as glycolipids, lipopeptides, lipoproteins, lipopolysaccharides, phospholipids, fatty acids, and glycoproteins. Their properties often match or surpass those of chemically produced emulsifiers, featuring nontoxicity, biodegradability, high foaming capacity, efficiency at low concentrations, and stability across various temperatures and pH levels. Additionally, they can be produced in an environmentally controlled manner from natural sources, using a variety of industrial and agricultural wastes. Consequently, bioemulsifiers are suitable for numerous biological, industrial, food, and medicinal applications (Ribeiro et al., 2020a; Thraeib et al., 2022).

Yeasts such as *Saccharomyces cerevisiae*, *Kluyveromyces marxianus*, and *Candida lipolytica* have been shown to produce bioemulsifiers (Hajhosseini et al., 2020; Ribeiro, et al., 2020b; Zaparoli et al., 2020). These yeasts are significant for the production of various biotechnological products (Ethafa & Al-Manhel, 2022) and are classified as Generally Recognized as Safe by the U.S. Food and Drug Administration (FDA). Their products are safe and can be manufactured cost-effectively using locally available media to optimize production conditions (Al-Jumaiee et al., 2019; Fernandes et al., 2023; Ribeiro, et al., 2020b).

S. cerevisiae JZT351 is a particularly noteworthy strain due to its ability to produce a complex bioemulsifier with promising industrial applications. While mannan, a polysaccharide found in yeast cell walls, contributes to emulsification, its capabilities as a sole emulsifier are limited by its simpler structure and fewer functional groups. In contrast, the bioemulsifier produced by *S. cerevisiae* JZT351 is a multifaceted mixture of biomolecules, including proteins, lipids, and polysaccharides. This complexity enhances its emulsifying efficiency and stability across various conditions (Parapouli et al., 2020).

Mannan, naturally occurring in the exterior cell walls of many microbes, including certain plants, molds like *Paecilomyces*, yeasts such as *S. cerevisiae*, and edible fungi like *Ganoderma lucidum*, is a linear, branching chain of β-1,4-mannose units linked to α-1,6-galactose (Al-Manhel & Niamah, 2017). Derived particularly from fungi, especially baker's yeast, mannan comprises approximately 25%–70% of the yeast cell wall and can form mannoproteins (Feldmann, 2012; Kwiatkowski & Edgar, 2012). In addition to its superior emulsifying properties, the bioemulsifier from *S. cerevisiae* also exhibits notable antioxidant and antimicrobial effects. These properties enhance its potential applications in industries such as food preservation, pharmaceuticals, and cosmetics. The antioxidant activity helps prevent oxidative damage, while the antimicrobial properties inhibit the growth of harmful microorganisms, making this bioemulsifier a multifunctional ingredient (Alcantara et al., 2010).

Mannoproteins are widely utilized in the food industry due to their unique structural and molecular characteristics (Hajhosseini et al., 2023; Bzducha Wróbel et al., 2022). They function as natural emulsifiers and stabilizers without altering the sensory properties of the final products, making them valuable assets in various techno-functional applications. Additionally, their ability to promote the growth of *Lacticaseibacillus* species highlights their significance for human health (Alcantara et al., 2013; Alizadeh-Sani et al., 2018; Thraeib et al., 2022). Many of these bioemulsifiers, including mannoproteins, are now approved by the FDA (Paul et al., 2023).

The challenge stems from the widespread use and availability of industrial emulsifiers, which not only negatively impact human health but also face limitations due to their restricted availability and high costs. Consequently, there is increasing interest in exploring the potential of producing emulsifiers from natural sources, such as microorganisms, including yeast. This research aimed to generate an emulsifier from microbial sources using a low-cost medium derived from industrial or agricultural wastes. This is particularly relevant given the limited local studies on the development of bioemulsifiers from indigenous microbial sources like *S. cerevisiae*. The purpose of incorporating this emulsifier into food systems, including dairy products, is to enhance their quality. Therefore, the availability of diverse fresh-like food products for consumers depends on the outcomes of this study.

2 | MATERIALS AND METHODS

Commercial dry baker's yeast from various sources available in local markets in Basra was compared with local isolates to produce bioemulsifiers. Each yeast sample was assigned a unique code, as detailed in Table 1.

2.1 | Isolation of yeast from natural sources

S. cerevisiae yeast was isolated from 17 sources, including soil, various dairy products, and both imported and locally grown fruits and vegetables. Each sample was diluted to 10^{-7} by adding 1 g of the sample to 9 mL of a

TABLE 1 Dry Baker's yeasts, their different brands, and origins used in the current study.

Company name	Code	Origin
Dry baker's yeast		
Super Maya	SM	Iraq
Glorioan	GL	Egypt
Saf-instant	Si	Turkey
Best baker	BK	China
ALDANAMAYA	DM	Russia
SHETAB	SH	Iran

dilution solution. Approximately 0.1 mL of this dilution was then plated onto Yeast Malt Agar (YMA) medium (Himedia). After spreading the sample evenly using a sterile L-shaped glass spreader, the plates were incubated at 28°C for 48 h. Individual yeast colonies with morphological characteristics similar to those of baker's yeast were selected using a sterile inoculating loop and streaked onto fresh YMA plates. This process was repeated three times to obtain pure colonies (Dikit, Maneerat, et al., 2010).

2.2 | Diagnosis of isolated local yeasts

The morphological test was performed by streaking the yeast on Malt Extract Agar medium at 20°C for 30 days. The shape, size, height, color, and colony arrangement were then recorded as morphological characteristics of the yeast. Microscopically, the isolated yeast was grown in a liquid malt extract medium for 72 h at 25°C. Subsequently, a portion of the yeast colony was spread on a slide, stained with methylene blue, and examined under a 40x magnification microscope (Kurtzman & Fell, 1998).

Other biochemical experiments conducted included the Sugar Fermentation Test (Durham tube), the Urea Hydrolysis Test using liquid urea medium, and the Acid Production Test using glucose-calcium carbonate agar media. Growth on solid Sabouraud medium with cycloheximide at concentrations of 100 and 1000 mg/L was assessed. Temperatures at which growth was examined included 25°C, 30°C, 37°C, and 40°C. The development of ascospores was investigated using safranin and malachite green stains. Additionally, experiments were conducted for the assimilation of carbon and nitrogen sources (Kurtzman & Fell, 1998; Lodder, 1970).

2.3 | Estimation of the biomass

The biomass of the local isolates was determined by dry weight. A 10 mL sample of the culture media was centrifuged at 3000 rpm for 10 min at 4°C. The resulting cell pellet was then washed with distilled water and centrifuged again under the same conditions. After drying at 105°C until a constant weight was achieved, the dry weight of the sedimented cells was measured using a sensitive balance (Sartorius) (Alcantara et al., 2012; Hajhosseini et al., 2023).

2.4 | Inoculum preparation

One full loop of active yeast was added to 50 mL of Yeast Malt Extract Broth (YMB) medium in a 125 mL flask to prepare the inoculum. The flask was then incubated overnight at 28°C (Alcantara et al., 2012).

2.5 | Counting live cells in the inoculum

One milliliter of active *S. cerevisiae* was added to 9 mL of a sterilized 0.1% diluent solution to estimate the cell count. Following a series of dilutions, 1 mL from the final dilution was transferred to agar plates supplemented with YMA medium. After incubating the plates at 28°C for 48 h, a count of 10^6 colony-forming units per milliliter (CFU/mL) was obtained.

2.6 | Screening of local and commercial yeast isolates to select the most suitable for producing the bioemulsifier

The best yeast isolates for the production of bioemulsifiers were selected by screening. Two milliliters of active yeast were added to 100 mL of Yeast Extract Peptone Glucose Broth (Himedia) in 250-mL flasks. The flasks were then placed in a shaking incubator (Sartorius) set at 28°C and 150 rpm for 48 h (Cameron et al., 1988).

2.7 | Bioemulsifier extraction

The method described by Cameron et al. (1988) was followed for the extraction of a bioemulsifier from yeast cells. To achieve this, 20 g of the collected pellet were centrifuged and added to 100 mL of a buffer solution containing 0.1 M potassium citrate and 0.02 M potassium metabisulfite at pH 7. The mixture was then autoclaved at 121°C for 3 h. The supernatant was removed by a second centrifugation step at room temperature for 10 min at 5000 rpm. The pellet was resuspended in 3 volumes of 95% ethanol supplemented with 1% (v/v) concentrated acetic acid and allowed to sediment overnight at 4°C. The crude bioemulsifier precipitate was dried, centrifuged at 10,000 rpm, and stored at room temperature until used in emulsifier activity tests to select the optimal isolate for bioemulsifier production.

2.8 | Emulsification effectiveness test

Three methods were followed to test the emulsification effectiveness, which included:

2.8.1 | Oil displacement test

This test was performed according to the method outlined by Alwaely et al. (2019) by adding 50 mL of distilled water to a petri dish with a diameter of 150 mm. Then, add 50 μL of crude oil to it. Subsequently, add 100 μL of the cell-free supernatant to the center of the membrane formed in the dish. Then, measure the diameter of the halo formed after 30 s.

2.8.2 | Estimation of emulsification effectiveness

Three milliliters of the bioemulsifier solution, prepared by dissolving 0.08 g of bioemulsifier in 3 mL of distilled water according to the method described by Torabizadeh et al. (1996), were mixed with 0.5 mL of olive oil in a test tube. The mixture was vortexed for 2 min and then incubated for 1 h at 37°C. A blank was prepared by following the same procedure but without adding the bioemulsifier. The absorbance of the tubes was measured in triplicate using a spectrophotometer set to a wavelength of 400 nm. An increase in absorbance indicates a higher emulsification efficiency (Patil & Chopade, 2001).

2.8.3 | Emulsification index (E24)

The emulsification index was determined using the method described by Alwaely et al. (2019). A test tube containing 2 mL of sunflower oil and 2 mL of yeast-free supernatant was vortexed for 2 min. The mixture was then allowed to rest at room temperature for 24 h. The emulsification index (E24) was calculated using the following formula:

$$\text{Emulsification Index (E24)} = \frac{\text{height of the emulsified layer}}{\text{total height of the mixture}} \times 100.$$

2.9 | Genetic characterization of the most efficient isolate in producing bioemulsifier emulsion

DNA was extracted and PCR products were amplified at the Biovet Laboratory in Basra. Deoxyribonucleic acid (DNA) was isolated from locally obtained yeast samples that produced the bioemulsifier (mannoprotein) using the RiboPure™ Kit Yeast from Life Technologies. The extraction and purification process followed the method described by Legras and Karst (2003), utilizing universal primers as detailed in Table 2.

The PCR products were subjected to electrophoresis according to the method described by Sambrook et al. (1989). DNA bands were visualized using a UV transilluminator at a wavelength of 254 nm. Genetic sequencing was conducted at the National Environmental Management Center's Biotechnology Laboratory in South Korea. The results were analyzed using the Basic Local Alignment Search Tool (BLAST) available on the National Center for Biotechnology Information (NCBI) website and the BioEdit program. PCR products were purified, sequenced, and analyzed to identify the isolate using the BLAST tool available on the NCBI website. The sequences were then submitted to GenBank online at www.ncbi.nlm.nih.gov/nucleotide, obtaining the specific sequences for the sample from the NCBI DNA database.

2.10 | Optimization of production conditions of bioemulsifier from the local isolate of *S. cerevisiae*

The optimal culture medium was one of several factors tested to determine the ideal growing conditions for bioemulsifier production from the local isolate. Six different culture media were used: Yeast Extract Peptone Dextrose Broth (YEPD) (Bicca et al., 2022); Cooper and Paddock's medium, consisting of 0.1% KH_2PO_4, 0.5% $MgSO_4{\cdot}7H_2O$, 0.01% $CaCl_2$, 0.01% NaCl, and 0.5% yeast extract, with 5% sunflower oil cooking waste and 8% glucose (Alcantara et al., 2010); a medium composed of 0.1 g NaCl, 5.0 g NH_4Cl, 7.0 g KH_2PO_4, 0.4 g $MgSO_4{\cdot}7H_2O$, and 1.0 g yeast extract dissolved in 1 L of distilled water, with the addition of 5% (v/v) gas oil (Lima & Alegre, 2009); a medium made by dissolving 0.40 g $NaNO_3$, 0.10 g NaCl, 0.10 g KCl, 0.01 g $CaCl_2$, 0.30 g KH_2PO_4, 0.30 g Na_2HPO_4, 0.02 g $MgSO_4$, 0.10 g $FeSO_4$, 0.08 g $CoCl_2$, 0.07 g $CuSO_4{\cdot}5H_2O$, 0.07 g $MnSO_4$, 0.001 g H_3BO_3, 0.07 g $Fe(SO_4)_3$, and 0.07 g $ZnSO_4$ in 100 mL of distilled water, with 1% olive oil and 1% NH_4NO_3 (Ribeiro, et al., 2020b); YMB, prepared by dissolving 0.3 g malt extract, 0.3 g yeast extract, 0.5 g peptone, and 1 g glucose in distilled water, then adjusting the volume to 100 mL for bioemulsifier production (Malabuyoc et al., 2023); and a mineral salts medium, consisting of 0.3 g $NaNO_3$, 0.3 g KH_2PO_4, 0.3 g $MgSO_4{\cdot}7H_2O$, and 1 g yeast extract dissolved in 100 mL of distilled water, with 4% (v/v) sunflower oil (Alwaely et al., 2019). The most efficient yeast isolates for bioemulsifier production were inoculated by adding 2 mL of the activated culture to each medium individually. The

TABLE 2 Nitrogen bases sequence of the universal primer.

Primer name and type	Nitrogen bases sequence	Primer length
Primer forward	5′-GCATATCAATAAGCGGAGGAAAAG-3′	24 bp
primer Reverse	5′-GGTCCGTGTTTCAAGACGG-3′	19 bp

pH was adjusted to 6.5, and the cultures were incubated in a shaker at 150 rpm and 28°C for 48 h. Emulsification activity tests were then performed on each culture medium to identify the optimal one.

As alternative carbon sources for the ideal production medium, three local substrates were evaluated: sugarcane molasses, grape juice waste, and Zahdi date juice. Using the Lane and Eynon method to determine the total sugar content of each source, the results were as follows: date juice contained 46% sugar, sugarcane molasses contained 62%, and grape juice waste contained 16%. To identify the optimal local substitute for the carbon source, glucose in the YEPD production medium was replaced with each substrate at concentrations of 25%, 50%, 75%, and 100%, under the same conditions used for bioemulsifier production.

The ideal medium was prepared with several pH values (4, 4.5, 5.5, 6, 6.5, 7, 8, 8.5, 9), to investigate the optimum pH. The ideal pH was maintained for the growth medium at, 25°C, 28°C, 30°C, 35°C, and 37°C. Furthermore, looked at were the optimal times of 24, 48, 72, and 96 h for the yeast to create the bioemulsifier. Furthermore, various amounts of activated yeast culture (1, 2, 3, 4, 5, and 6 mL per 100 mL) were added to the best alternative medium for the production of bioemulsifier. The ideal alternative medium was then incubated in a shaker at various speeds (100, 150, 200, 300 rpm) to ascertain the ideal shaking speed for the bioemulsifier synthesis while keeping the previously established ideal conditions. Every growth media underwent tests of emulsification activity.

2.11 | Characterization of the bioemulsifier

Fourier-transform infrared spectroscopy (FTIR) was used to analyze the bioemulsifier at the Polymer Research Center labs, University of Basrah. The standard bioemulsifier (mannoprotein) was compared with effective samples of the bioemulsifier derived from *S. cerevisiae*. Specifically, 1 mg of freeze-dried bioemulsifier was mixed with 100 mg of potassium bromide and pressed for 30 s to produce semi-transparent pellets. Characterization was performed over a spectral range of 400–4000 cm^{-1}. Additionally, the bioemulsifier (mannoprotein) was characterized at the College of Education, Department of Chemistry, University of Basrah, using an American-made Bruker AVII 700 MHz spectrometer. The samples were dissolved in 600 μL of deuterium oxide (D_2O), and proton (1H) spectra were obtained using a 30-degree pulse angle. The nuclear magnetic resonance (NMR) data were processed and analyzed using the Topspin software from Bruker Biospin AG, Switzerland, as well as the Casper program available online.

2.12 | Antioxidant efficacy of the bioemulsifier mannoprotein

The DPPH radical scavenging assay was conducted according to the method described by Dudonné et al. (2009) with minor modifications. A 0.125 mM DPPH solution was prepared in ethanol and used immediately. Various concentrations of the bioemulsifier (10, 8, 6, 4, 2 mg/mL) were tested by adding 1 mL of each concentration to 1 mL of the DPPH solution in a test tube. These were compared to control using the synthetic antioxidant BHT, prepared at a concentration of 5 mg/mL. After 20 min of dark incubation at 30°C, a spectrophotometer was used to detect the absorbance at 517 nm wavelength. A blank was prepared using the same method but the sample was replaced with distilled water. The emulsification activity was calculated using the following equation:

$$I\% = ((Abs_0 - Abs_1))/Abs_0 \times 100,$$

where (Abs_0) represents the absorbance of the blank, and (Abs_1) represents the absorbance of the sample with the DPPH solution.

2.13 | Antimicrobial efficacy of the bioemulsifier mannoprotein

Different concentrations of bioemulsifier (100, 75, 50 mg/mL) were prepared to study its inhibitory effectiveness using Mueller-Hinton Agar against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Candida albicans*. These microorganisms were obtained from the laboratories of the Department of Food Science, College of Agriculture, University of Basrah, and reactivated in Nutrient Broth. The well diffusion method was employed, where 0.1 mL (1×10^8 CFU/mL) of each bacterium and yeast was individually spread on the surface of the solid medium using a sterile L-shaped glass. Wells with a diameter of 6 mm were created in the solid

medium using a cork borer and filled with 50 μL of the bioemulsifier solution. Plates were incubated at 37°C for 24 h for bacteria and at 28°C for 48 h for yeast. The diameters of the clear zones around the wells were measured using a ruler (Perez, 1990; Walencka et al., 2007).

2.14 | Statistical analysis

The statistical analysis of the obtained results was conducted using a Completely Randomized Design. The data were analyzed using an analysis of variance (ANOVA) table through the statistical analysis software SPSS ver. 22. Significant differences between the treatment means were compared using the Least Significant Difference test at a probability level of 0.05.

3 | RESULTS AND DISCUSSION

3.1 | Isolation

Table 3 lists 25 local yeast isolates obtained from 17 distinct isolation sources. *S. cerevisiae* yeast was probably separated from them by purifying their uniform colonies in terms of size, shape, and color many times on YMA medium to produce pure cultures. This is based on previous scientific studies (Hammadi et al., 2018; Lakew, 2022; Thapa et al., 2015) that indicated the possibility of isolating *S. cerevisiae* yeast from natural sources such as soil, fruits, vegetables, and food products. This is also consistent with the findings of Elsaygh et al. (2023) in obtaining 30 yeast isolates from various sources using YMA medium. Thus, choosing the isolation source is a crucial stage to take into account to get the intended local isolates.

TABLE 3 Isolates of yeast from various sources.

#	Code	Source	No. of isolates
1	PD	Imported peach	1
2	SF1 SF2	Soil from the Faculty of Agriculture field	2
3	SG	Soil from the Faculty of Agriculture Garden	1
4	AC1 AC2 AC3	Vinegar (Al-Khayyam)/Basra	3
5	K	Iraqi peach	1
6	H	Honey	1
7	DZ1 DZ2 DZ3	Zahdi date	3
8	G1 G2	Iraqi grapes	2
9	P	Pineapple	1
10	O	Orange	1
11	GR	Iranian grapes	1
12	A	Imported apples	1
13	OT	Iraqi apricot	1
14	M1 M2 M3	Sugarcane molasses	3
15	Y	Local yogurt	1
16	C	Local cheese	1
17	DF	Local fig	1

3.2 | Characterization of isolates

Figure 1A illustrates various colony morphologies grown on 5% solid malt extract agar medium for 30 days at 20°C. The colonies are characterized by their medium size, off-white to creamy white color, raised and smooth edges, regular circular shapes, and viscous texture. These characteristics align with the visual descriptions reported in diagnostic studies of *S. cerevisiae* (Elsaygh et al., 2023; Kreger-van Rij, 1984; Kurtzman, 2011).

3.3 | Microscopic evaluation

When local yeast isolates were stained with methylene blue dye, microscopic analysis of cells grown in 5% liquid malt extract medium at 25°C for 72 h revealed that the cells were spherical to oval in shape. As shown in Figure 1B, these cells appeared in chains, pairs, or clusters resembling honeycomb cells, and were free of fungal filaments. These observations are consistent with the diagnostic and taxonomic criteria for *S. cerevisiae* (Kreger-van Rij, 1984; Kurtzman, 2011).

3.4 | Biochemical evaluation

Table 4 and Figure 2 illustrate the biochemical tests conducted on the local yeast isolates. The isolates' ability to produce acetic acid during glucose fermentation was demonstrated by the acid production test findings, which showed colonies growing on glucose-calcium carbonate agar medium forming distinct halos around them. The urea hydrolysis medium did not

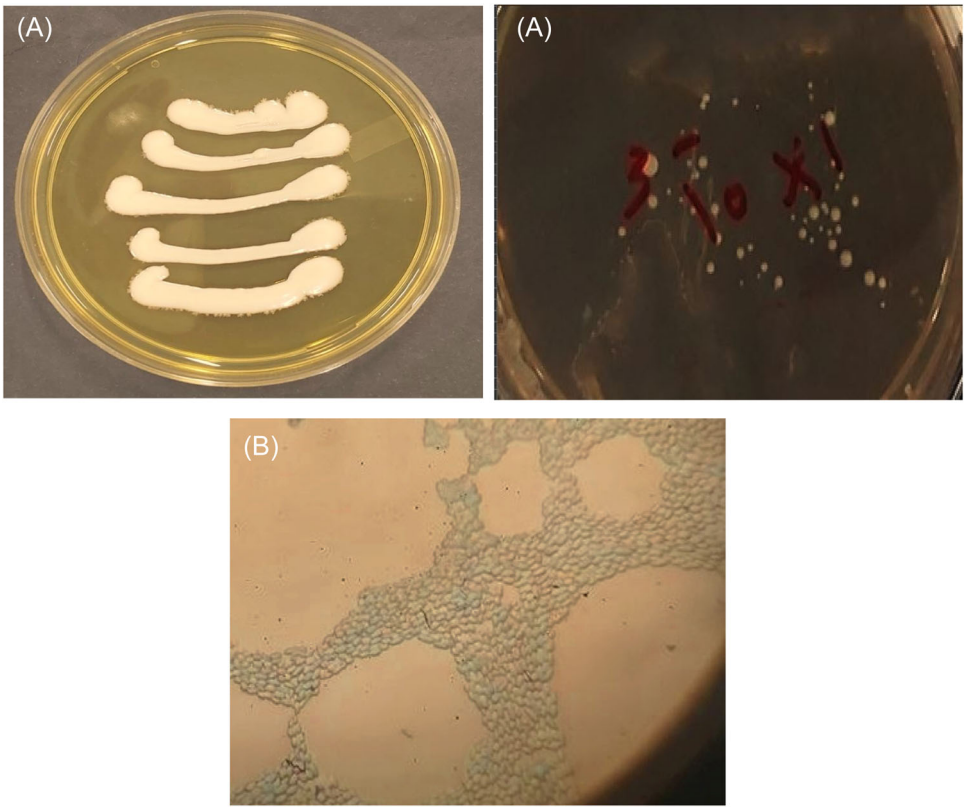

FIGURE 1 (A) Morphological examination of colonies of local yeast isolates (B) Microscopic examination of locally isolated yeast cells stained with methylene blue dye.

change color, indicating that the isolates lacked urease enzyme activity and were therefore unable to produce ammonia for nitrogen absorption. Several isolates exhibited the ability to ferment carbohydrates, as evidenced by the color shift of bromothymol blue dye from green to yellow due to a pH drop toward acidity, and the production of carbon dioxide (CO_2) gas in Durham tubes. However, these isolates were unable to ferment trehalose, maltose, or lactose, likely due to the absence of the necessary enzymes for both fermentation and the utilization of these carbon sources. Additionally, the isolates demonstrated sensitivity to the antifungal agent cycloheximide, as evidenced by the lack of growth at 100 and 1000 parts per million. The isolates showed optimal growth at 25°C and 30°C, with weak or no growth at 37°C and no growth at 40°C. Ascospores were observed under a microscope in isolates grown on potassium acetate-yeast extract glucose medium, using safranin and green malachite stains. The isolates were also capable of hydrolyzing monobasic ammonium phosphate and sulfate, but were unable to produce nitrate reductase enzyme, and could utilize nitrogen sources other than potassium (Table 5). Table 4 shows that most isolates, when cultivated on Yeast Nitrogen Base medium, were able to use carbon sources other than lactose and maltose. Visual, microscopic, and biochemical testing indicated that only 20 out of the 25 isolates collected from various sources exhibited characteristics typical of *S. cerevisiae* yeast, as supported by scientific references (Kreger-van Rij, 1984; Kurtzman, 2011; Lodder, 1970; Rasheed & Haydar, 2023). Isolates with codes Y, C, M2, DF, and P were excluded from this classification.

Screening the yeast isolates and comparing them with six different kinds of commercial dry yeasts from various countries revealed that isolate (AC1), which was obtained from vinegar starter at the Al-Khayam Vinegar Factory in Abu Al-Khasib district, Basra city, produced the bioemulsifier the most effectively (Figure 3). The highest values for oil displacement, emulsification efficiency, and emulsification index (E24) were obtained by isolate (AC1) at 7.1 cm, 0.71 nm, and 34.50%, respectively, after emulsification activity tests; isolate (DZ1) showed the lowest values for these tests at 4 cm, 0.36 nm, and 22.77%, respectively. Further, isolate (AC1) produced the highest amount of biomass, 4.35 g/L, whereas isolates with codes (A, OT) produced the least amount, 2.75 g/L. The means of oil displacement and biomass production did not differ significantly ($p > 0.05$) according to statistical analysis; however, the means of emulsification efficiency and emulsification index (E24) tests did differ significantly ($p < 0.05$). Because of

TABLE 4 Biochemical tests of local isolates obtained from various sources.

Isolate	Acid production	Urea hydrolysis	Sugar fermentation								Growth on a medium containing cycloheximide		Growth at different temperatures				Cystic spores
			Glucose	Galactose	Maltose	Lactose	Trehalose	Sucrose	Raffinose	Melibiose	100 ppm	1000 ppm	25°C	30°C	37°C	40°C	
PD	+	−	+	+	+	−	−	+	+	−	−	−	+	+	− +	−	+
SF1	+	−	+	+	+	−	−	+	+	−	−	−	+	+	−	−	+
SF2	+	−	+	+	+	−	−	+	+	−	−	−	+	+	− +	−	+
SG	+	−	+	+	+	−	−	+	+	−	−	−	+	+	−	−	+
AC1	+	−	+	+	+	−	−	+	+	−	−	−	+	+	− +	−	+
AC2	+	−	+	+	+	−	−	+	+	−	−	−	+	+	− +	−	+
AC3	+	−	+	+	+	−	−	+	+	−	−	−	+	+	− +	−	+
K	+	−	+	+	+	−	−	+	+	−	−	−	+	+	− +	−	+
H	+	−	+	+	+	−	−	+	+	−	−	−	+	+	−	−	+
DZ1	+	−	+	+	+	−	−	+	+	−	−	−	+	+	− +	−	+
DZ2	+	−	+	+	+	−	−	+	+	−	−	−	+	+	−	−	+
DZ3	+	−	+	+	+	−	−	+	+	−	−	−	+	+	−	−	+
G1	+	−	+	+	+	−	−	+	+	−	−	−	+	+	−	−	+
G2	+	−	+	+	+	−	−	+	+	−	−	−	+	+	−	−	+
P	+	−	+	+	−	−	−	+	+	+	−	−	+	+	−	−	+
O	+	−	+	+	+	−	−	+	+	−	−	−	+	+	− +	−	+
GR	+	−	+	+	+	−	−	+	+	−	−	−	+	+	−	−	+
A	+	−	+	+	+	−	−	+	+	−	−	−	+	+	− +	−	+
OT	+	−	+	+	+	−	−	+	+	−	−	−	+	+	−	−	+
M1	+	−	+	+	+	−	−	+	+	−	−	−	+	+	− +	−	+
M2	+	−	+	+	−	−	+	+	+	−	−	−	+	+	−	−	+
M3	+	−	+	+	+	−	−	+	+	−	−	−	+	+	− +	−	+
Y	+	−	+	− +	+	−	−	+	+	− +	−	−	+	+	−	−	+
C	+	−	+	− +	−	−	−	+	+	−	+	+	+	+	−	−	+
DF	+	−	+	+	− +	−	−	+	+	−	−	−	+	+	+	−	+

Note: +: Positive for examination; −: negative for examination; − +: variable for examination.

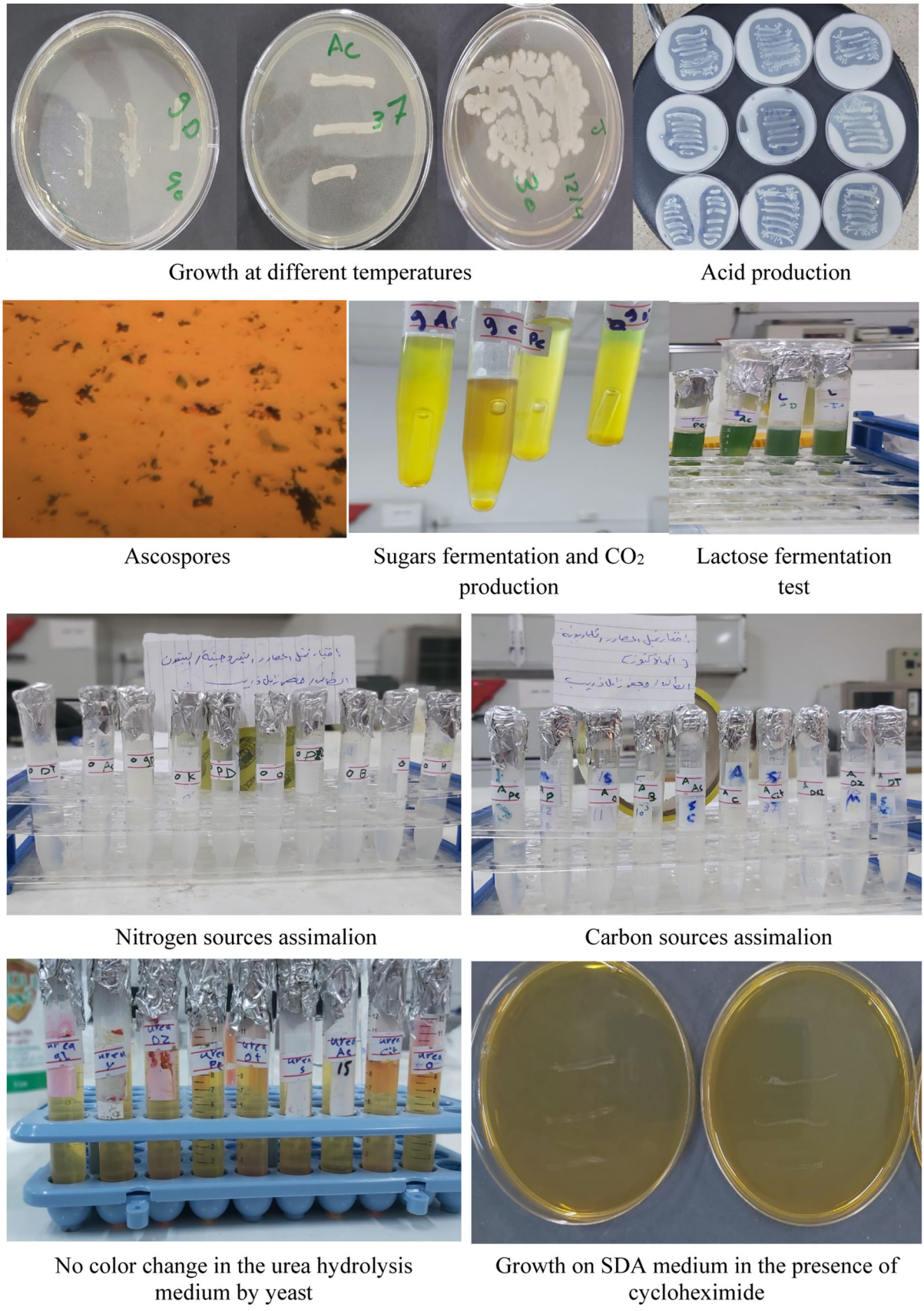

FIGURE 2 Examples of the biochemical test results for local yeast isolates.

TABLE 5 Carbon and nitrogen source assimalion.

	Assimalion of carbon source					Assimalion of nitrogen sources			
Isolate	Glucose	Galactose	Sucrose	Lactose	Melibiose	Ammonium phosphate	Potassium nitrate	Ammonium sulfate	Peptone
PD	+	+	+	−	−	+	−	+	+
SF1	+	+	+	−	−	+	−	+	+
SF2	+	+	+	−	−	+	−	+	+
SG	+	+	+	−	−	+	−	+	+
AC1	+	+	+	−	−	+	−	+	+
AC2	+	+	+	−	−	+	−	+	+
AC3	+	+	+	−	−	+	−	+	+
K	+	+	+	−	−	+	−	+	+
H	+	+	+	−	−	+	−	+	+
DZ1	+	+	+	−	−	+	−	+	+
DZ2	+	+	+	−	−	+	−	+	+
DZ3	+	+	+	−	−	+	−	+	+
G1	+	+	+	−	−	+	−	+	+
G2	+	+	+	−	−	+	−	+	+
P	+	+	+	−	+	+	−	+	+
O	+	+	+	−	−	+	−	+	+
GR	+	+	+	−	−	+	−	+	+
A	+	+	+	−	−	+	−	+	+
OT	+	+	+	−	−	+	−	+	+
M1	+	+	+	−	−	+	−	+	+
M2	+	+	+	−	−	+	−	+	+
M3	+	+	+	−	−	+	−	+	+
Y	+	±	+	−	±	+	−	+	+
C	+	±	−	−	±	+	−	+	+
DF	+	+	+	−	−	+	−	+	+

Note: +: Positive for examination; −: negative for examination; ±: variable for examination.

variations in isolation sources, physiological activities of each isolate, or genetic features influencing enzymatic activity, metabolic activities, and nutrient utilization reflected in biosynthesis, isolates varied in their capacity to produce bioemulsifier emulsifier and biomass. This aligns with the findings of Fernandes et al. (2023) regarding yeast isolates showing varying capacities in bioemulsifier emulsifier production, as differences in the cell wall composition of *S. cerevisiae* yeast can be attributed to several variables or factors influencing its growth, such as nitrogen and carbon sources, temperature, acidity, oxygen, and salts (Aguilar-Uscanga & Francois, 2003; Ilori et al., 2005). Furthermore, as genes related to bioemulsifier production pathways differ, Trindade et al. (2022) proposed that genetic changes across strains might be responsible for variances in emulsification efficiency. Isolate AC1 was selected for the current investigation based on the obtained results.

3.5 | Genetic diagnosis of the local isolate most proficient in bioemulsifier production using 26S ribosomal RNA (rRNA)

3.5.1 | DNA extraction

DNA was extracted from the locally isolated yeast strain AC1 using a repeated procedure. Results from agarose gel electrophoresis confirmed the purity of the extraction. Figure 4A shows clear bands corresponding to the yeast DNA.

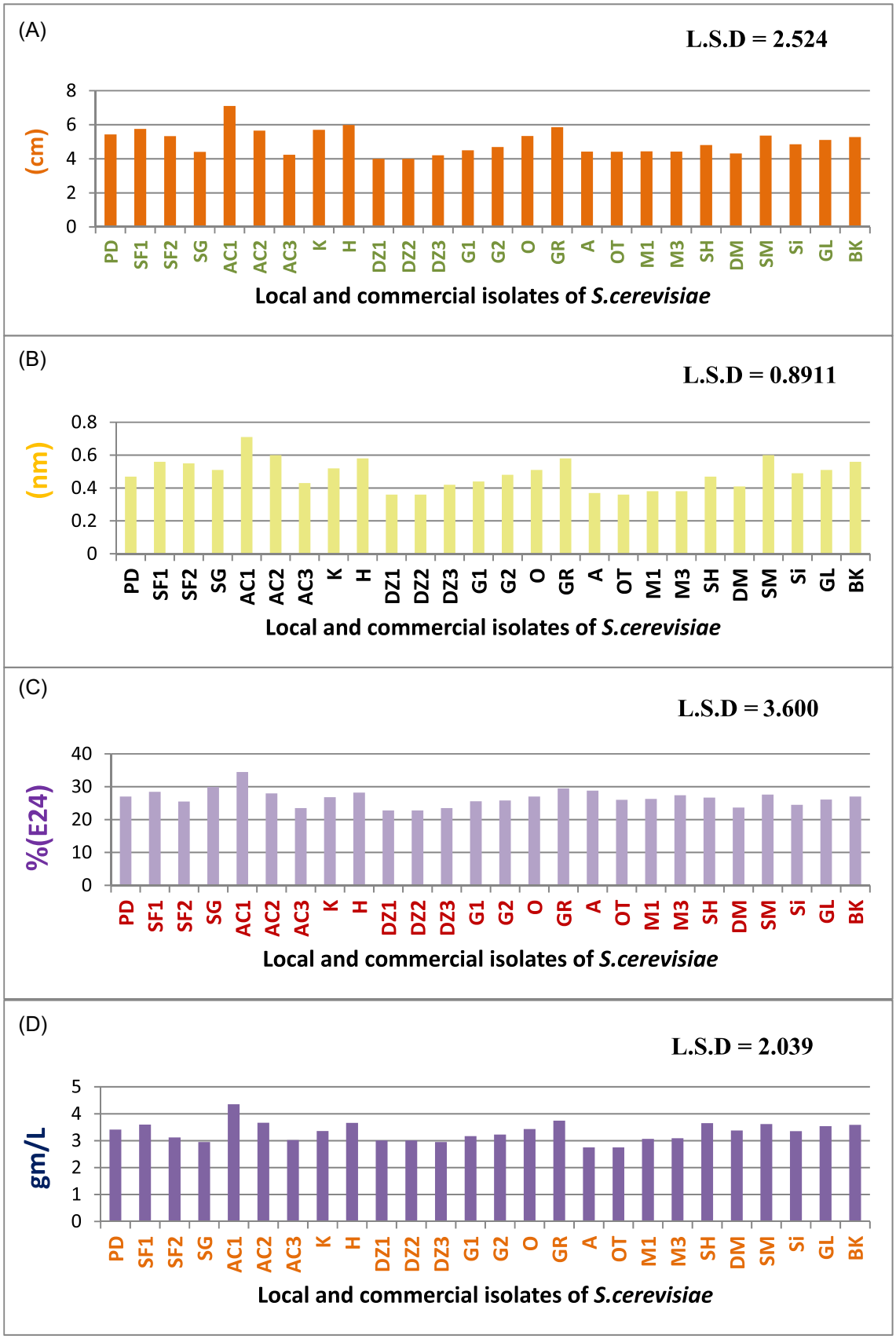

FIGURE 3 Screening of isolates to select the best producer of the bioemulsifier, (A) Oil displacement, (B) Emulsification activity, (C) E24 emulsification index, (D) Biomass yield.

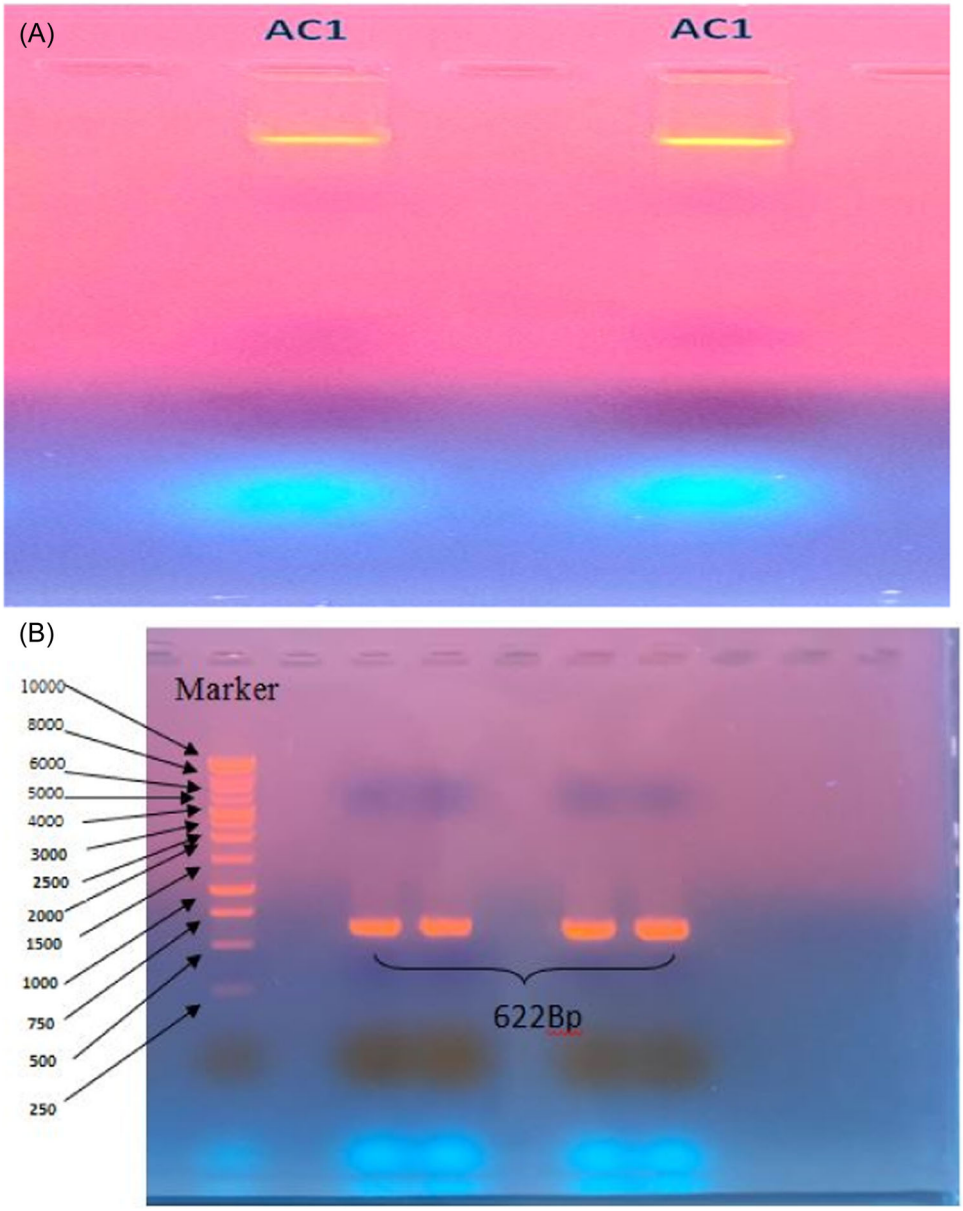

FIGURE 4 (A) Electrophoretic migration of DNA extracted from the local yeast isolate (AC1), the most efficient in bioemulsifier production, with duplicates. (B) Electrophoretic migration of PCR reaction products on 2% agarose gel.

3.5.2 | DNA amplification using the polymerase chain reaction (PCR) technique

Utilizing the 26S rRNA test, the PCR method was used. Amplification was done with primers unique to the gene. When the amplified gene for the yeast isolate was gel electrophoresed with four repeats, as shown in Figure 4B, distinct orange bands showed up, suggesting an amplicon size of 622 base pairs. This shows that the primers bonded to the 26S rRNA target gene exclusively, not to other parts of the extracted DNA from the locally isolated yeast strain. The study is consistent with most research confirming the feasibility of using the 26S rRNA assay in studying the nucleotide sequence of oxygen-deprived DNA to diagnose *Sacharomyces* sp. yeast isolates (Elsaygh et al., 2023; Guimarães et al., 2006; Majeed & Al-jader, 2022).

3.5.3 | Analysis of the amplification products

A Korean company named Macrogen received the amplified gene sequences along with the primers to determine the nucleotide sequences of the yeast strain under investigation. These sequences were used with the BLAST Nucleotide software to determine the species of

the chosen isolate by aligning them with the information on this gene in the NCBI Gene Bank. A 100% match between the local isolate and the *S. cerevisiae* strain JY3-2 registered in the Gene Bank was found by BLAST analysis of the nucleotide sequences of the 26S rRNA gene amplified from the local yeast isolate. As such, this yeast species was identified as the local isolation.

The 26S rRNA gene sequences of the isolates were used to build the genetic tree with the MEAG 11.0.13 program. As depicted in Figure 5, the local isolate was recorded in the NCBI Gene Bank under the name *S. cerevisiae* strain JZT351(OR115510), accessible via the link: https://www.ncbi.nlm.nih.gov/nuccore/2515605893.

3.6 | Optimization of conditions for bioemulsifier (Mannoprotein) production from locally isolated *S. cerevisiae* strain JZT351

3.6.1 | Optimal medium for bioemulsifier production

The *S. cerevisiae* strain JZT351 bioemulsifier was produced using six growth mediums. Oil displacement, emulsification efficiency, and emulsification index (E24) were among the emulsification test findings shown in Figure 8, which were used to choose the best medium for the development of the local isolate and the bioemulsifier production. With values for oil displacement, emulsification efficiency, and E24 of 7.10 cm, 0.71 nm, and 34.50%, respectively, the first medium (YEPD) showed maximum productivity. Conversely, the lowest productivity was noted with the sixth medium (composed of 0.3 g $NaNO_3$, 0.3 g KH_2PO_4, 0.3 g $MgSO_4{\cdot}7H_2O$, and 1 g yeast extract in 100 mL distilled water with 4% sunflower oil v/v), yielding 4.67 cm, 0.41 nm, and 25.59%, respectively. Additionally, the first medium produced more biomass (4.35 g/L) compared to the sixth medium (3.76 g/L). The mean results of the emulsification efficiency test for *yeast* grown on various media showed no statistically significant differences ($p > 0.05$) according to the ANOVA. These outcomes led to the choice of the YEPD medium for more investigation. This variance may be attributed to the influence of yeast growth on the nutritional components of the culture media used, which in turn affects the rate of bioemulsifier production (Nerurkar et al., 2009). The results were consistent with the findings of Slama (2021) and Hajhosseini et al. (2023), who selected the YEPD medium for bioemulsifier production from *S. cerevisiae* and *Kluyveromyces marxianus*, respectively. Additionally, Bzducha Wróbel et al. (2022) utilized the YEPD medium for bioemulsifier production from *Wickerhamomyces anomalus* and *Metschnikowia reukaufii*, while Alcantara et al. (2012) found the second medium (Cooper and Paddock's medium) to be the best for bioemulsifier production. YEPD medium, containing glucose as a simple carbon source, is ideal for the growth of most yeast species. Additionally, yeast extract serves as an excellent medium for bioemulsifier production, as the biomass of yeast grows in this medium (Sherman, 1991). Yeast extract is rich in amino acids, B vitamins, and peptone, which contribute to the effective development of bioemulsifiers (Figure 6).

3.6.2 | Selection of the optimal local carbon source substitute in the production medium

In the standard production medium (YEPD), three local alternative carbon sources spoiled grape juice, sugarcane molasses, and date palm juice were substituted 100% for glucose. Figure 9 presents the results, which indicate that substituting glucose with date palm juice was the most effective medium for synthesizing the bioemulsifier. With this local alternative, the emulsification test results were highest, with oil displacement at 7.39 cm, emulsification efficiency at 0.79 nm, and emulsification index (E24) at 34.71%. In contrast, using sugarcane molasses as a substitute yielded less favorable results: oil displacement at 5.97 cm, emulsification efficiency at 0.47 nm, and emulsification index (E24) at 27.32%.

Date palm juice also demonstrated superior performance in biomass generation, producing 4.42 g/L compared to 3.68 g/L from sugarcane molasses. While the mean values for oil displacement, emulsification efficiency, and biomass production tests showed no statistically significant differences ($p > 0.05$), the mean values for the E24 emulsification efficiency test did exhibit statistically significant differences.

The increased yield or efficiency of various carbon sources has been employed to make biomass or bioemulsifier. The local isolation and the intended output determine the alternative raw material to use. Date palm juice is better than other carbon sources because it contains amino acids, vitamins, and minerals that promote yeast cell growth in addition to simple sugars (glucose and fructose) that S. *cerevisiae* cells can easily absorb and metabolize. On the other hand, molasses is mostly composed of disaccharides, or sucrose, together with other unfavorable elements including heavy metals and contaminants that might interfere with development and the generation of biomass (Acourene et al., 2007; Al-Jasass et al., 2010). This aligns with the findings of Al-Eid et al. (2010), who reported that date syrup was superior to molasses in *S. cerevisiae* production on a laboratory and semi-industrial scale. Additionally, Ghasemi et al. (2018) used low-quality date palm juice in bioemulsifier production from *Lactobacillus rhamnosus*.

3.6.3 | Determination of the best substitution ratio for the local carbon source

With a maximum oil displacement value of 7.80 cm at a 75% replacement rate and the lowest displacement recorded at 100% substitution, which was 7.39 cm, the

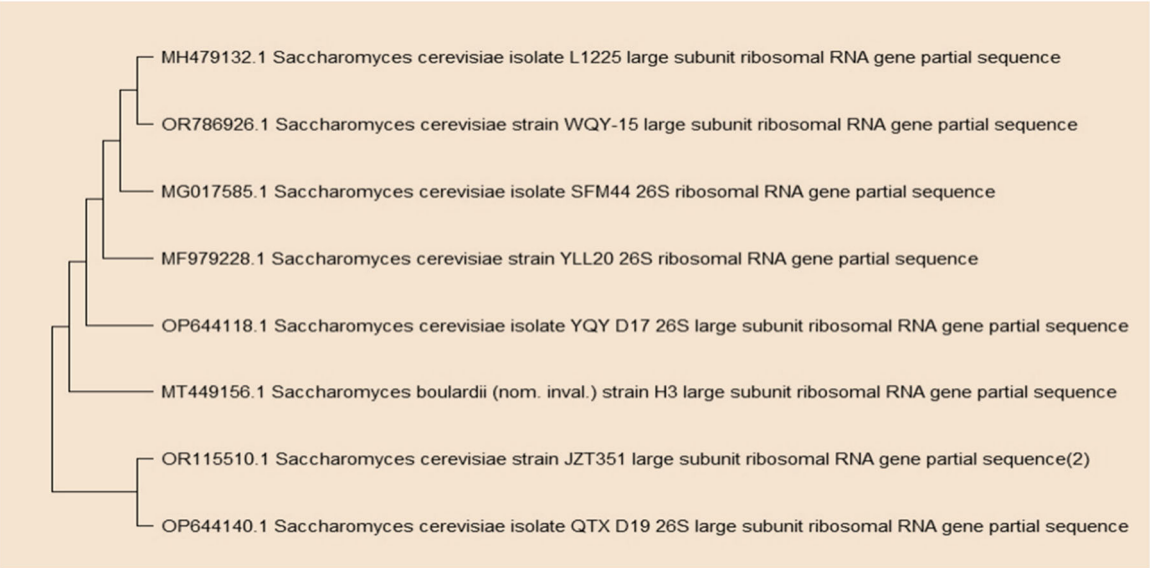

FIGURE 5 Phylogenetic tree of locally isolated yeast strain and its relationship with strains from the same yeast species in the NCBI GenBank.

results shown in Figure 10 confirmed the effectiveness of the emulsification tests. At the 75% replacement rate, the emulsification efficiency test and the emulsification index (E24) reached their peak values of 0.82 nm and 36.57%, respectively. In contrast, the lowest values recorded at 25% and 100% replacement rates were 0.73 nm and 34.71%, respectively. Additionally, the biomass findings, which reached 4.60 g/L, were consistent with the emulsification efficacy results concerning the substitution rate. The mean results of the emulsification efficacy and biomass output tests did not differ statistically significantly ($p > 0.05$).

Up to a certain point, increasing the concentration of date palm juice enhances the growth of *S. cerevisiae* cells. However, beyond this optimal level, the inhibitory effects of sugars—resulting from reduced biomass or the rapid conversion to byproducts such as organic acids, alcohols, and carbon dioxide—cause cell growth to slow down (Al-Jasass et al., 2010). The results varied with different ratios of date palm juice used as a carbon source. Beiroti and Hooseini (2007) found that a 50% substitution ratio was optimal for increasing the biomass of *S. cerevisiae*. Similarly, Mnif et al. (2021) reported that a 25% substitution ratio of date palm juice in the growth medium was optimal for the production of a bioemulsifier from *Bacillus mojavensis* BI2.

3.6.4 | The optimal initial pH of the medium for producing the bioemulsifier

Figure 7A shows that pH 5.5 is the ideal starting pH for bioemulsifier production. The maximum values of 8.01 cm, 0.85 nm, and 37.65%, respectively, were obtained for oil displacement tests, emulsification efficiency, and emulsification index (E24), at this pH level. These experiments produced poorer results at pH 9, however. Furthermore, the average biomass value of 4.76 g/L at pH 5.5 was the greatest biomass output recorded; at pH 9, it was 1.78 g/L. For the tests of emulsification efficacy and biomass output, significant differences ($p < 0.05$) were found. The pH has a big impact on yeast development and, by changing the solubility, ionization, and transfer of nutritional elements in the culture medium, which changes their availability for consumption, biomass and bioemulsifier synthesis (Allaith et al., 2022; Kurtzman, 2011).

This finding aligns with the observations of Hajhosseini et al. (2020), who reported that pH ranging from 5.4 to 6.1 was optimal for bioemulsifier production. It is also consistent with the findings of Aguilar-Uscanga and Francois (2003) and Alwaely et al. (2019), indicating that a pH range of 3–5 was optimal for increasing yeast cell wall components, including bioemulsifier, with a subsequent decrease observed outside this range. Moreover, the results are in agreement with Alcantara et al. (2012), who found that a pH range of 5–8 resulted in the highest biomass production for *S. cerevisiae*, while biomass production decreased at extreme acidic or alkaline pH values. These findings closely resemble those of Elsaygh et al. (2023), who noted that the maximum effectiveness of the bioemulsifier produced from *S. cerevisiae* was achieved at pH 6.5.

3.6.5 | The optimal temperature for producing the bioemulsifier

The impact of temperature on bioemulsifier production from the local isolate of *S. cerevisiae* strain JZT351 was studied at five temperature levels: 37°C, 35°C, 30°C, 28°C, and 25°C. Through Figure 7B, it was observed that the highest bioemulsifier production occurred at 30°C,

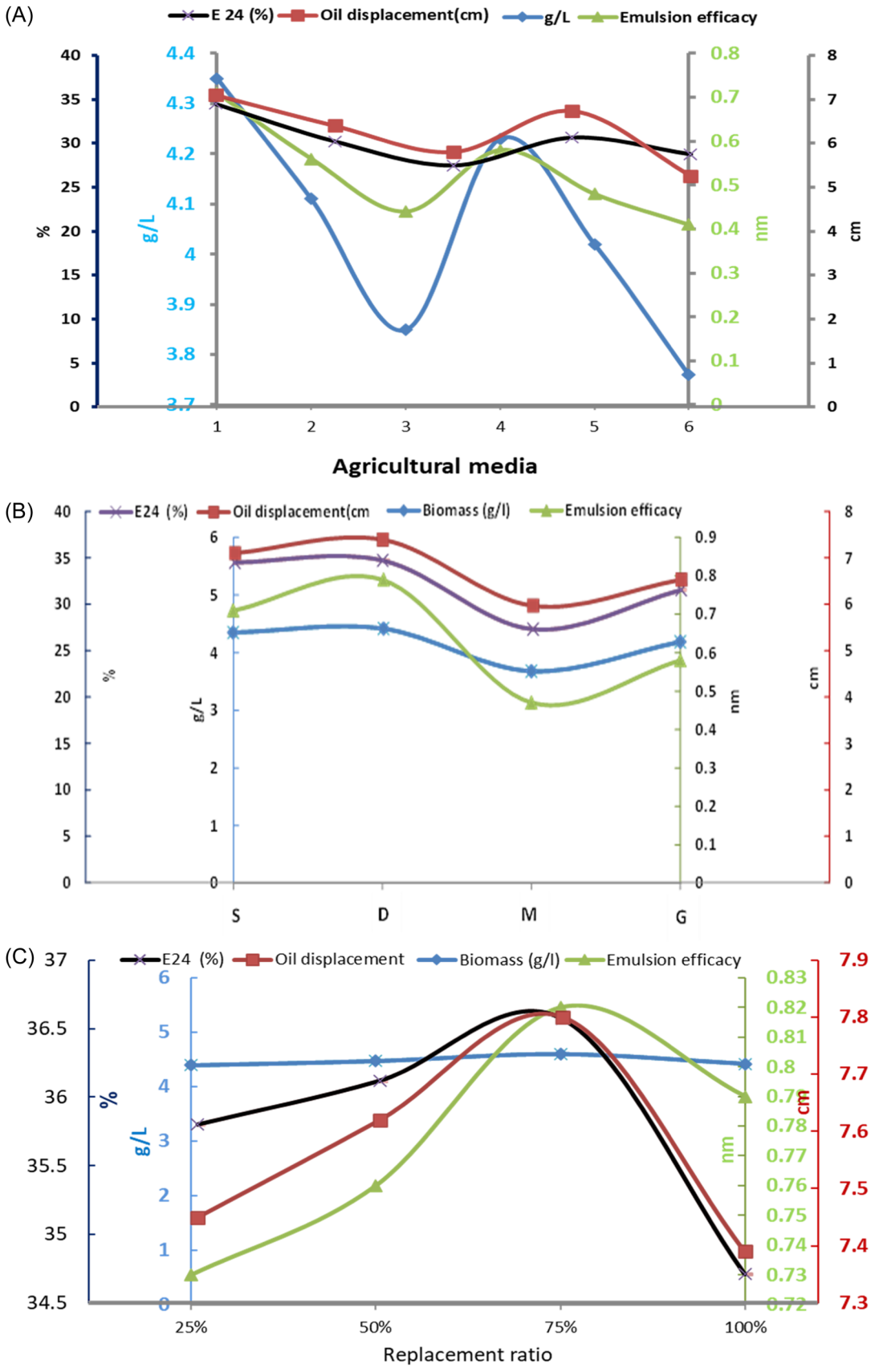

FIGURE 6 (See caption on next page).

where the largest oil displacement was recorded at 8.26 cm, while the lowest displacement was observed at 37°C, measuring 5.48 cm. Similarly, the emulsification efficiency tests and emulsification index (E24) showed parallel results, reaching values of 0.91 nm and 38.19%, respectively, at 30°C. Furthermore, biomass production was maximized at 30°C, averaging 4.92 g/L, compared to the lowest value of 2.13 g/L at 37°C. While there were no significant differences ($p > 0.05$) in emulsification efficiency, significant differences ($p < 0.05$) were observed in the E24 values.

Temperature is a crucial factor influencing yeast growth rate and biomass production due to its effects on enzymatic systems or the consumption and exchange of dissolved components in the culture medium through the yeast cytoplasmic membrane, ultimately impacting its metabolic outcomes and cell wall components, including bioemulsifiers (Kurtzman, 2011). Obtaining the highest bioemulsifier production or biomass from the local isolate at 30°C suggests that this temperature is optimal for the enzymatic activities necessary for bioemulsifier production in the yeast cell wall. Any temperature deviation from this optimal range may affect metabolism and consequently productivity (Xia et al., 2022).

These findings are consistent with those of Hajhosseini et al. (2020), who found 30°C to be the optimal temperature for producing bioemulsifier from *Kluyveromyces marxianus*. They also align with Sudha et al. (2010), who identified 30°C as the optimal temperature for producing sophorolipids bioemulsifier from *Candida tropicalis* yeast. Liu et al. (2009) similarly reported 32°C as the optimal temperature for bioemulsifier protein production. Alwaely et al. (2019) found that the ideal temperature for bioemulsifier production from *Candida* spp. yeast was 30°C, noting a decrease in bioemulsifier effectiveness from 2.285 to 1.007 when the temperature increased to 40°C. Additionally, Ribeiro, et al. (2020b) reported that the highest production of bioemulsifiers was achieved from *S. cerevisiae* at 28°C.

3.6.6 | The optimal incubation period for the production of the bioemulsifier

Four incubation periods (96, 72, 48, and 24 h) were examined to determine the optimal duration for the synthesis of bioemulsifier protein. As shown in Figure 7C, the maximum values of emulsification tests oil displacement, emulsification efficiency, and emulsification index (E24) were reached after a 72-h incubation period: 8.48 cm, 0.93 nm, and 39.25%, respectively. These figure were, however, lower after a 24-h incubation period, measuring 7.19 cm, 0.65 nm, and 34.61%, in that order. The 72-h incubation period was found to be optimal for biomass production, yielding an average of 5.39 g/L, compared to the lowest production of 2.36 g/L observed after 24 h. The averages of the emulsification efficiency tests and biomass generation showed statistically significant differences ($p < 0.05$).

By depleting nutrients in the production medium and accumulating secondary metabolic byproducts, which stunt cell development, prolonging the incubation period may negatively impact biomass and, in turn, bioemulsifier output (Tuite & Oliver, 1991). These results are consistent with those reported by Malabuyoc et al. (2023), who found that a 3-day incubation period was optimal for producing bioemulsifier protein from *S. cerevisiae* strain 2031, and with Alwaely et al. (2019), who identified a 72-h incubation period as optimal for bioemulsifier production from *Candida spp.* yeast. However, Hajhosseini et al. (2020) suggested that a 96-h incubation period was optimal for producing bioemulsifier proteins from *Kluyveromyces marxianus* yeast. Malabuyoc et al. (2023) also noted that the highest biomass and emulsification index for bioemulsifier protein were achieved after a 4-day incubation period. Nevertheless, Elsaygh et al. (2023) observed that a 36-h incubation period was sufficient to produce the highest biomass quantity for *S. cerevisiae* yeast, reaching 3.17 g/L.

3.6.7 | The optimal inoculum size for the production of the bioemulsifier

To find the best inoculum size for the generation of bioemulsifiers, several volumes of activated inoculum of the yeast *S. cerevisiae* strain JZT351 were tested. Each 100 mL of manufacturing media had quantities ranging from 1 to 6 mL. At an inoculum size of 3 mL, the values for the emulsification activity tests of the bioemulsifier—measured by oil displacement, emulsification activity, and the emulsification index E24—were highest, measuring 8.69 cm, 0.95 nm, and 39.7%, respectively, as shown in Figure 8A. At a 6-mL inoculum size, these values fell to 7.68 cm, 0.78 nm, and 37.15%, in that order. At an inoculum size of 3 mL, the biomass output was highest, reaching 5.85 g/L. In contrast, at an inoculum

FIGURE 6 Shows effect of (A) different cultivation media; (B) Replacing the Optimal Carbon Source with Local Alternatives [S: Standard Medium, D: Date Juice, M: Cane Molasses, G: Grape Residue Juice]; (C) the optimal replacement ratio of the carbon source in the optimal medium on the production of bioemulsifier from the local yeast isolate *Saccharomyces cerevisiae* strain JZT351. (A) LSD oil displacement: 3.273, LSD for emulsification activity: 0.4047, LSD for E24: 2.243, and LSD for biomass: 2.145; (B) LSD Oil Displacement: 2.813, LSD Emulsification Activity: 0.1090, LSD E24: 3.996, and LSD Biomass: 1.355; (C) LSD oil displacement: 4.164, LSD emulsification activity: 0.4099, LSD E24: 2.236, and LSD biomass: 2.2.

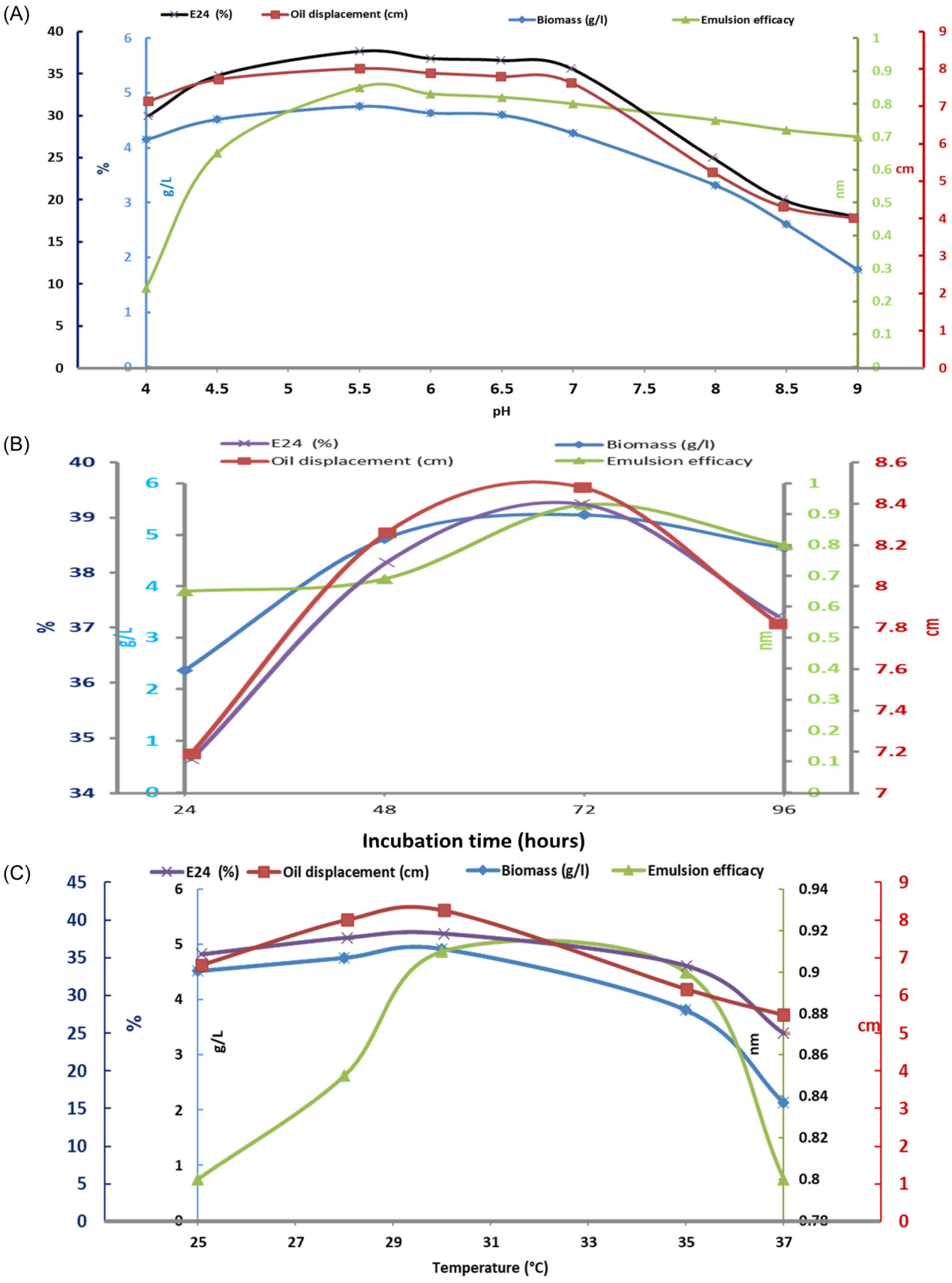

FIGURE 7 (See caption on next page).

size of 6 mL, biomass production was lowest, yielding 4.47 g/L. The averages of the experiments on emulsification and biomass showed no statistically significant differences ($p > 0.05$).

An important element greatly affecting the fermentation process and biomass production is the size of the inoculum. Higher concentrations of the medium components and inoculum size improve emulsification activity or yield (Ribeiro et al., 2023; Sen & Swaminathan, 2004). Production at inoculum sizes of 1 and 2 mL may be reduced because the production medium did not ferment completely in the allotted incubation time. On the other hand, competition among yeast cells for few nutrients in the production medium may be the reason of the lower bioemulsifier yield when inoculum sizes are larger than 3 mL (Elsaygh et al., 2023; Laluce et al., 2009). These findings are consistent with Carrau et al. (2010), who noted that while inoculum size initially increases biomass, production starts to decline beyond a certain level. Similarly, Ding et al. (2009) reported that certain yeast cell wall components decrease with increasing inoculum sizes. Liu et al. (2009) found that an inoculum size of 5 mL was optimal for bioemulsifier production in *S. cerevisiae*. Ribeiro et al. (2019) also found similar results, indicating that the best inoculum size for producing bioemulsifiers from *Candida utilis* was 2%.

3.6.8 | The optimal shaker incubator speed for the production of the bioemulsifier

Emulsification activity tests were most affected by a speed of 150 rpm among the four shaker incubator speeds employed to incubate the bioemulsifier synthesis medium (Figure 8B). The emulsification activity, emulsification index E24, and oil displacement were among these tests; the results were 8.69 cm, 0.95 nm, and 39.7%, in that order. The least effect was observed with 100 rpm, resulting in values of 7.84 cm, 0.68 nm, and 35.13%, respectively. Furthermore, biomass production peaked at 150 rpm, at 5.79 g/L, and fell to 4.87 g/L at 100 rpm. The mean results of the tests showed no statistically significant variations ($p > 0.05$).

Higher speeds may lead to cell disruption, negatively reducing biomass development, whereas moderate speeds enhance the mixing of oxygen and nutrients in the production medium and avoid cell clumping (Liu et al., 2009). This finding aligns with Joaad and Hassan (2015), who reported that a shaker speed of 150 rpm was optimal for bioemulsifier production from *Candida guilliermondii* yeast. Similarly, Elsaygh et al. (2023) found that a shaker speed of 150 rpm was optimal for the production of the bioemulsifier (mannoprotein) from *S. cerevisiae* yeast. Ribeiro et al. (2020a) also found that the highest activity in producing bioemulsifiers or surface-active substances from *S. cerevisiae* occurred at an incubation speed of 150 rpm. In contrast, Malabuyoc et al. (2023) indicated that a shaker speed of 100 rpm was most effective and stable for producing the bioemulsifier. Alwaely et al. (2019) found that a shaker speed of 180 rpm was optimal for producing bioemulsifiers from *Candida spp.* yeast.

3.7 | Characterization of the bioemulsifier

3.7.1 | FTIR analysis

FTIR was employed to qualitatively detect and identify functional groups in bioemulsifiers, which are stable at specific wavelengths. This was achieved through the peaks observed at these wavelengths (see Figure 9A,B). Broad absorption bands were seen at wavelengths of 3410.14 and 3443.28 cm^{-1}, respectively, for the normal bioemulsifier and the bioemulsifier taken from the local isolate of *S. cerevisiae* strain JZT351. These bands are attributed to the vibration of hydroxyl groups (OH) present in sugar molecules (Elsaygh et al., 2023) and the overlapping amine groups (NH) with hydroxyl groups appearing in the same region. A modest quantity of lipids at this absorption was also indicated by the observation of small bands at wavelengths of 2929.87 and 2929.34 cm^{-1}, respectively, which correspond to the stretching vibrations of aliphatic CH and CH_2 groups (Nogueira et al., 2020; Rasheed & Haydar, 2023).

Bands corresponding to the stretching vibrations of the carbonyl group in acetyl groups (C = O) were seen at wavelengths of 1651.07 and 1656.55 cm^{-1}. Glycosidic linkages, the cyclic structure of monosaccharides, and the stretching of (C-O) and (C-C) bonds were also shown by the spectra in an absorption band between 1500 and 2000 cm^{-1}. Moreover, in line with earlier research, the absorption areas at 806.25 and 807.06 cm^{-1} correspond to alpha-type glycosidic linkages, which are typical of mannan sugar (Elsaygh et al., 2023; Liu et al., 2015; Qiao et al., 2022).

Strong stretching vibrations were observed for the amide group in the spectra of both the sample and the reference bioemulsifier, at wavelengths of 1543.05 and 1534.09 cm^{-1}, respectively. They are linked to C-N and

FIGURE 7 Shows the effect of (A) the initial pH; (B) temperature; (C) incubation time on the production of the bioemulsifier, from the local yeast isolate *Saccharomyces cerevisiae* strain JZT351. (A) LSD oil displacement: 2.812, LSD emulsification activity: 0.2627, LSD E24: 3.779, and LSD biomass: 1.547; (B) LSD oil displacement: 2.851, LSD emulsification activity: 0.2308, LSD E24: 3.259, and LSD biomass: 2.038; (C) LSD oil displacement: 1.350, LSD emulsification activity: 0.1617, LSD E24: 3.004, and LSD biomass: 1.223.

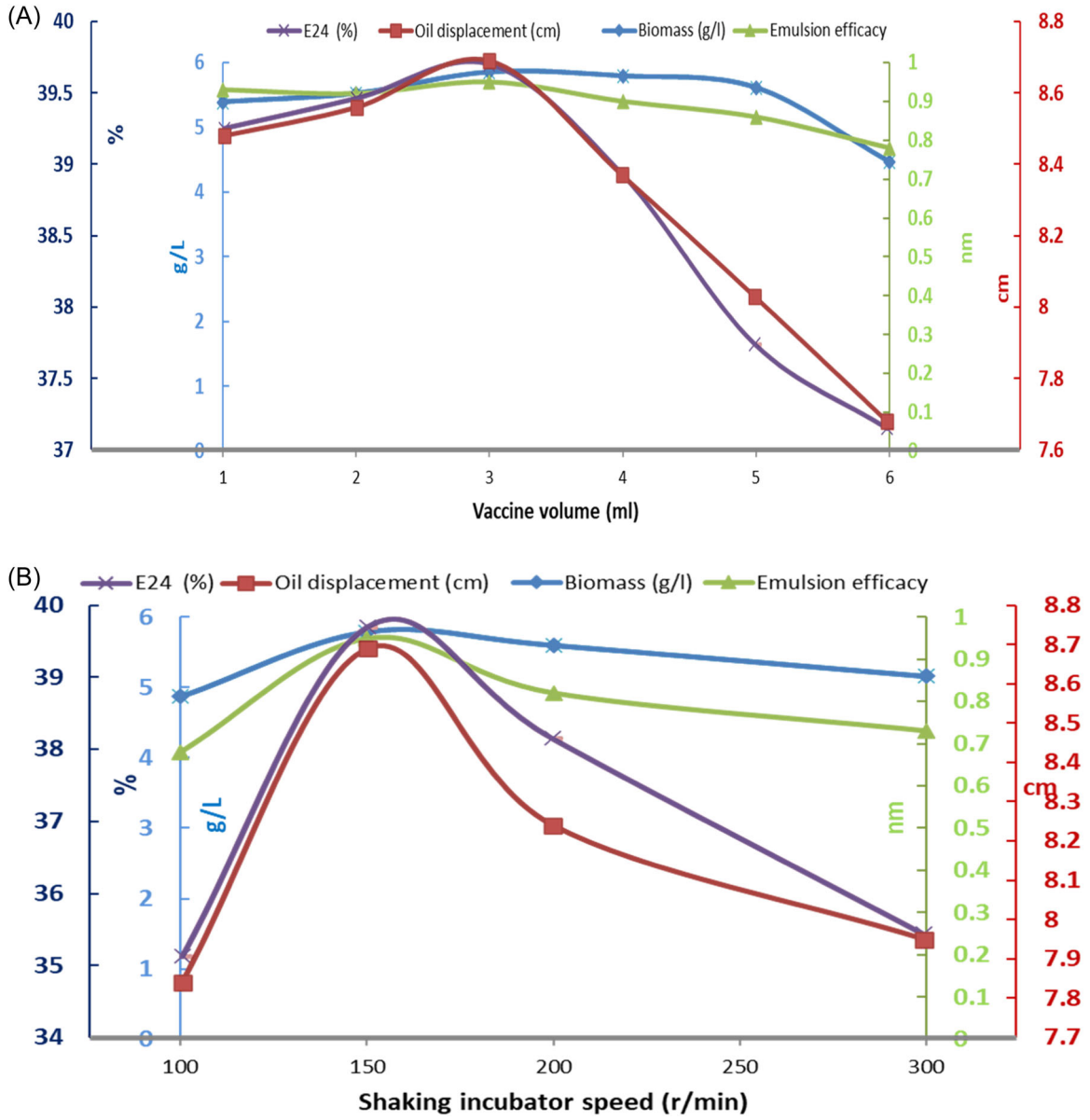

FIGURE 8 Shows the effect of (A) inoculum size; and (B) shaker incubator speed on the production of the bioemulsifier, from the local yeast isolate *Saccharomyces cerevisiae* strain JZT351. (A) LSD oil displacement: 1.828, LSD emulsification activity: 0.1301, LSD E24: 1.945, and LSD biomass: 2.835. (B) LSD biomass: 2.912, LSD oil displacement: 2.657, LSD emulsification activity: 0.2196, and LSD E24: 1961.6.

N-H groups, which suggest the existence of acylated amino sugars and protein. This confirms that the bioemulsifier (Mannoprotein) contains protein and carbohydrates in its structure, forming glycoproteins (Alcantara et al., 2010; Bzducha Wróbel et al., 2022; Nogueira et al., 2020; Qiao et al., 2022).

3.7.2 | NMR (^{1}H NMR) technique

The bioemulsifier obtained from the local isolate of *S. cerevisiae* strain JZT351 is characterized using proton NMR (^{1}H NMR) spectroscopy, as shown in Figure 10A,B. Results indicated proton signals between 0.5 and 5 ppm in the basic components of the bioemulsifier structure. These signals stand for the protons in the CH and CH_2 molecules, which are the fundamental building blocks of mannoproteins. The presence of alkane groups (R-CH_3, R-CH_2, R_3-CH) in the bioemulsifier was observed in the proton range of 0.7–2 ppm, and the alcohol group (R-OH) appeared in the range of 3.4–4 ppm (Elsaygh et al., 2023).

The anomeric region (C1-OH) for the β configuration varied from 4.1 to 4.5 ppm, and for the α configuration from 4.6 to 5 ppm. Carbon atoms C_2 through C_6 of the sugar ring were identified by peaks observed between 3 and 4 ppm. Protein groups associated with glucan-protein structures were found at 1 and 1.8 ppm, attributed to N-CH_3 and N-H,

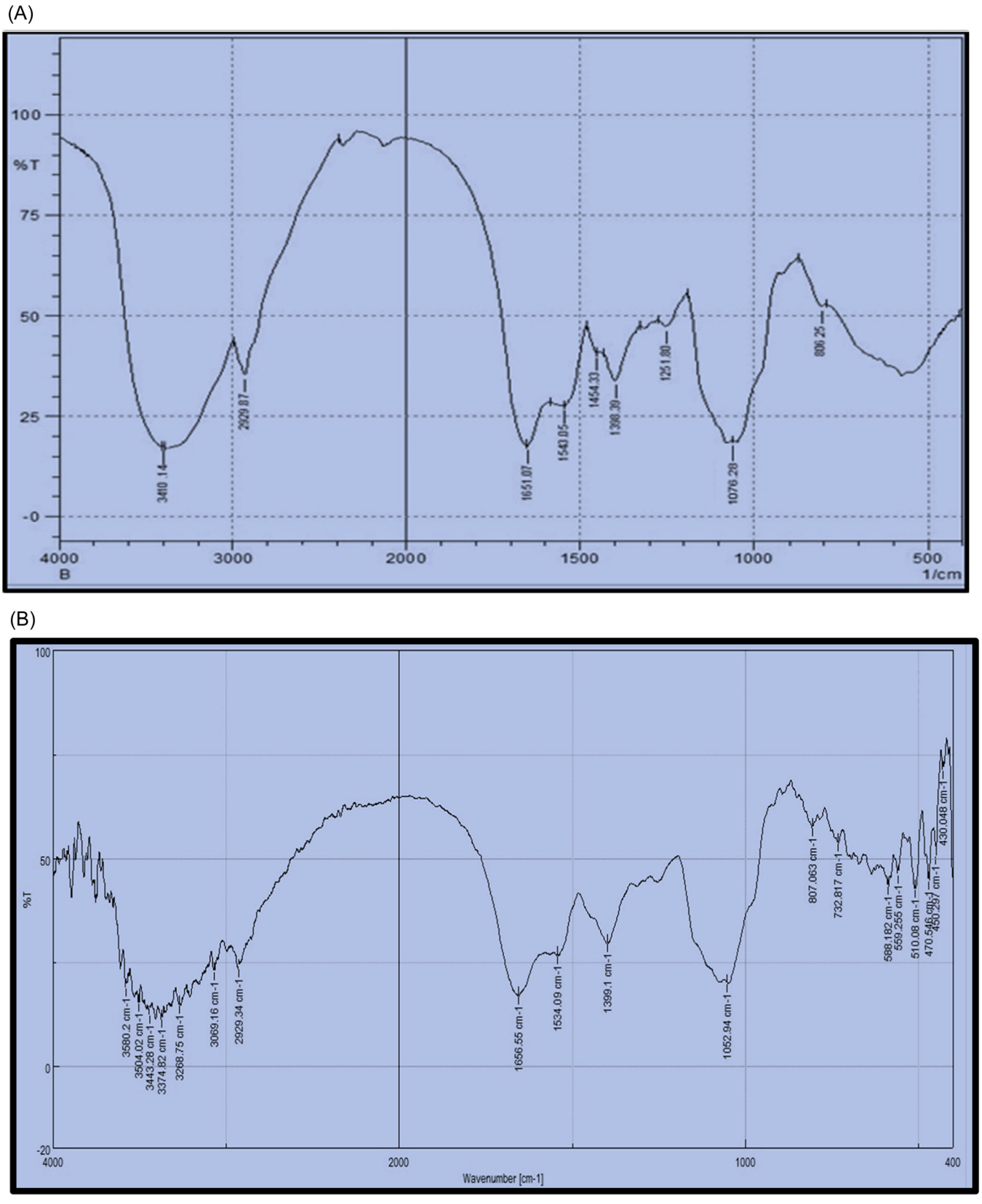

FIGURE 9 (A) Shows the infrared spectrum profile of the standard bioemulsifier (mannoprotein). (B) Shows the infrared spectrum profile of the bioemulsifier (mannoprotein) extracted from the locally isolated yeast *saccharomyces cerevisiae* strain JZT351.

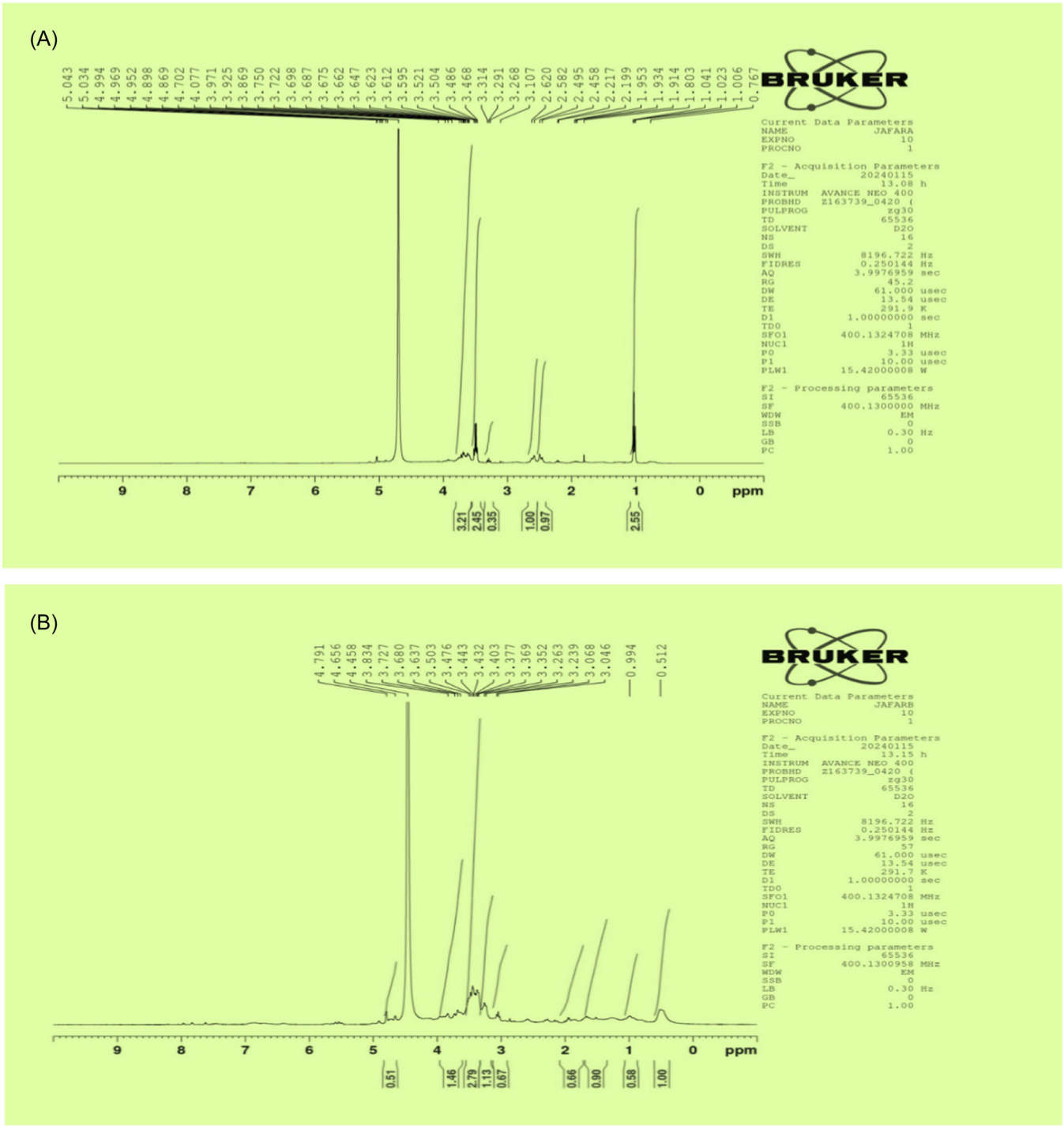

FIGURE 10 (A) Shows the (^{1}H)-NMR spectrum profile of the standard bioemulsifier. (B) Shows the (^{1}H)-NMR spectrum profile of the bioemulsifier produced by the locally isolated yeast *saccharomyces cerevisiae* strain JZT351.

which could also be observed in the range of 0.5–3 ppm (Dikit, 2010; Gonzaga et al., 2005; Qiao et al., 2022).

The figures demonstrated that the observed peaks in the bioemulsifier extracted from *S. cerevisiae* matched those of the standard bioemulsifier. The variation in peak appearance when using ^{1}H NMR for proton identification in the molecular structure produced by microorganisms may be due to the type of microorganism, the nature of the medium, the type of fermentation, and the solvent used in the analysis (Ribeiro, et al., 2020b).

3.7.3 | Antioxidant activity of bioemulsifier

Comparing the commercial antioxidant BHT at a concentration of 5 mg/mL to the bioemulsifier isolated from

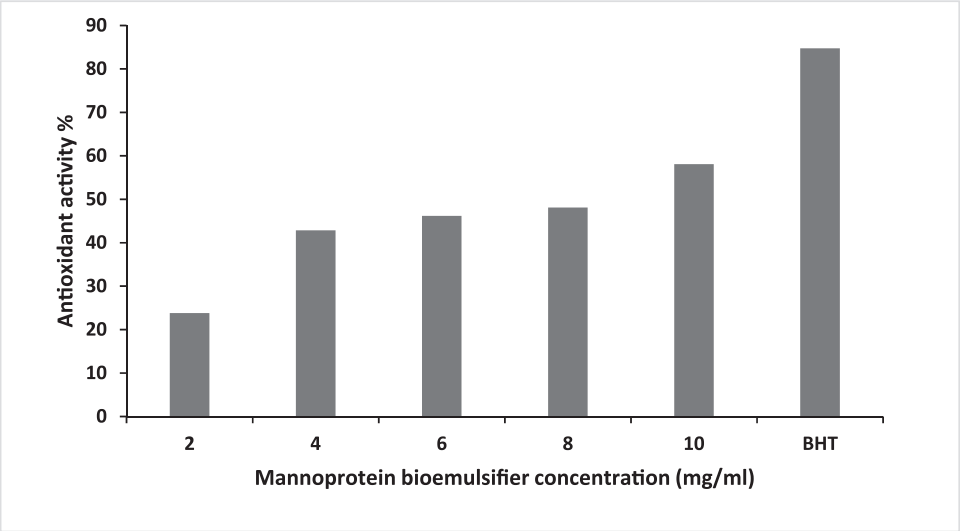

FIGURE 11 Antioxidant activity of bioemulsifier (mannoprotein).

S. cerevisae yeast at different concentrations (10, 8, 6, 4, 2 mg/mL), Figure 11 shows the antioxidant activity of the former. Statistical analysis revealed significant differences at a probability level ($p < 0.01$). The findings showed that the antioxidant activity of the bioemulsifier increased with its concentration. Specifically, a concentration of 10 mg/mL exhibited the highest DPPH scavenging activity at 58.0952%, while the lowest concentration demonstrated a scavenging activity of 23.8095%. These results are consistent with findings by Basit et al. (2018) and Elsaygh et al. (2023), which demonstrate the antioxidant activity of bioemulsifiers.

Furthermore, studies by Križková et al. (2006) confirmed that the antioxidant activity of mannans extracted from S. *cerevisae* yeast is attributed to their ability to donate protons to unstable free radicals, thus interrupting their chain reactions. The binding of mannans with proteins enhances their antioxidant properties, making them effective antioxidants. Additionally, the antioxidant activity depends on the degree of mannans' binding with proteins more than their binding with other carbohydrates. Studies by MacHová and Bystrický (2012) showed that the effective group in bioemulsifier responsible for scavenging free radicals is the hydroxide group, and the antioxidant activity depends on the concentration of the bioemulsifier.

The physical and chemical characteristics of the amino acids particularly those with cyclic structures (tyrosine, tryptophan, and phenylalanine) and thiol groups (cysteine, cystathionine, and methionine) that can donate protons and stop the chain reactions of free radicals also determine the protein components of the bioemulsifier. Additionally, the hydrophobic amino acids (valine, leucine, and tyrosine) in the protein moiety of the bioemulsifier facilitate access to unsaturated fatty acids, from which unstable and oxidized free radicals arise (Chen et al., 1998; Qian et al., 2008).

3.7.4 | Antimicrobial activity of bioemulsifier

Three different kinds of harmful bacteria Gram-positive *Staphylococcus aureus*, Gram-negative *Pseudomonas aeruginosa*, and *Candida albicans* yeast were tested for the antibacterial activity of the bioemulsifier. The bioemulsifier concentrations tested were 50, 75, and 100 mg/mL. The data presented in Figure 12A demonstrated a dose-dependent inhibitory effect. Specifically, the bioemulsifier exhibited increased inhibitory action at higher concentrations. A zone of inhibition of 11 mm was the result of the maximum inhibitory activity against *Pseudomonas aeruginosa* at a dosage of 100 mg/mL. On the other hand, at the same dose, there was the least inhibitory effect against *Candida albicans*, as seen by the 8.5 mm zone of inhibition (Figure 12B). ANOVA indicated no significant differences ($p > 0.05$) in the diameters of the clear zones resulting from microbial growth inhibition.

Most antimicrobial agents, including the bioemulsifier, primarily work by affecting ion flow and the pores of cellular membranes. This interference disrupts membrane function or prevents the synthesis of essential cell membranes, thereby inhibiting microbial growth or causing cell death. It is possible to explain the variation in test microorganisms' reaction to inhibition to variations in their strains, which differ in the number or makeup of receptors on the cell wall.

Previous studies (Saleh et al., 2020) have demonstrated that the bioemulsifier exhibits inhibitory activity against a broad spectrum of both Gram-positive and

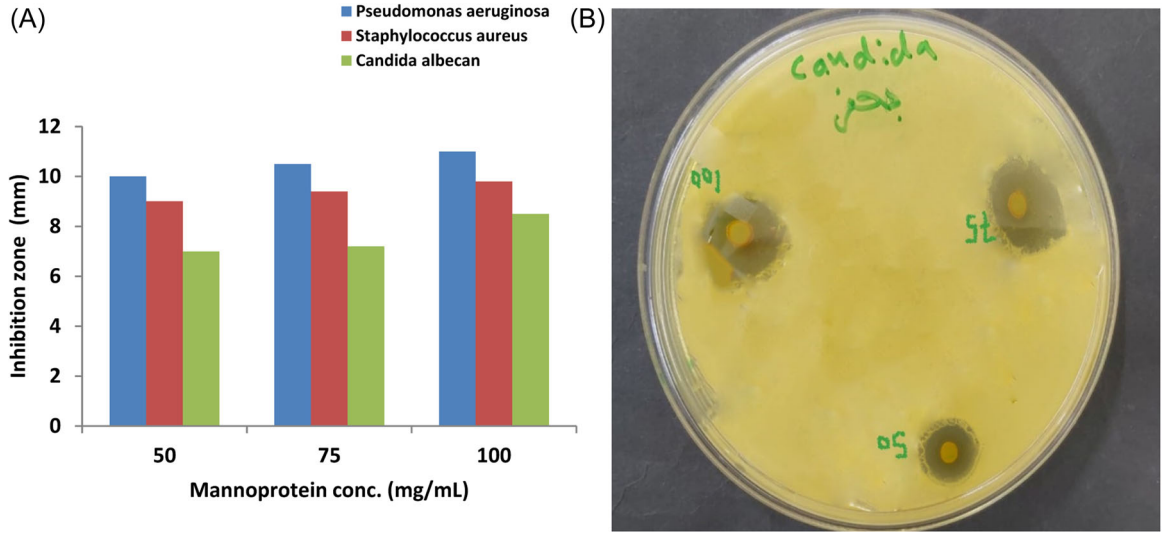

FIGURE 12 (A) Inhibitory effect of the bioemulsifier (mannoprotein) against *Candida albicans* yeast. (B) Inhibitory activity of the bioemulsifier (mannoprotein).

Gram-negative bacteria, and its inhibitory activity is directly proportional to the purity of the bioemulsifier. Various disease-causing microorganisms share common mechanisms, such as their ability to adhere to and invade host cells, leading to cellular damage. For example, *Escherichia coli* and *Staphylococcus aureus* utilize similar strategies to attach to host tissues and evade the immune system, resulting in detrimental effects on host health.

The results are consistent with previous findings by Mahmood (2018), which confirmed the inhibitory effectiveness of bioemulsifier. Additionally, they align with the observations of Rasheed and Haydar (2023), who reported that bioemulsifier exhibits inhibitory activity against *Pseudomonas aeruginosa* and *Staphylococcus aureus* when added at concentrations ranging from 50 to 200 mg/mL. Hyder (2015) also noted that the inhibitory effectiveness of bioemulsifier produced by *Acinetobacter baumanii* AC5 *Acinetobacter* increases with its concentration.

Moreover, Bzducha Wróbel et al. (2022) found that the inhibitory effectiveness of bioemulsifier produced by *S. cerevisiae* depends on the source and concentration of the bioemulsifier. However, they found that the bioemulsifier's inhibitory impact against *Escherichia coli* bacteria varied from 77% to 95%, whereas it was less efficient against *Salmonella* and *Pseudomonas* species, at a rate of 23%–35%. Furthermore, strong inhibitory action was shown against the most vulnerable strains, *Enterococcus* and *Staphylococcus aureus* cocci.

4 | CONCLUSION

The study successfully isolated and characterized 25 *S. cerevisiae* yeast strains from various culinary and environmental sources in Basra and Maysan, identifying the AC1 isolate from the Khayam factory in Basra as the most potent producer of bioemulsifiers. This strain, now registered as JZT351, demonstrated superior emulsification efficiency with the specific values obtained for emulsification efficiency (7.1 cm, 0.71 nm, and 34.50%). Optimization of conditions for bioemulsifier production revealed that using 75% date juice instead of glucose significantly enhanced bioemulsifier yield under specific conditions (pH 5.5, 30°C, 72 h, 3 mL inoculum, 150 rpm). The refined bioemulsifier exhibited improved emulsification activity, index, and oil displacement (8.69 cm, 0.95 nm, and 39.7%, respectively). Characterization using FTIR and ^{1}H NMR confirmed the bioemulsifier structure, identifying functional groups and chemical structure consistency. Additionally, the bioemulsifier displayed significant antioxidant activity (58.0952% at 10 mg/mL in the DPPH assay). Also, the highest inhibition against *Pseudomonas aeruginosa* and the least against *Candida albicans* for bioemulsifier was observed. In conclusion, the local isolate *S. cerevisiae* strain JZT351 is an effective source for producing bioemulsifiers with enhanced emulsification properties, antioxidant activity, and antibacterial effects, particularly against *Pseudomonas aeruginosa*. The use of date juice as a substrate significantly boosts bioemulsifier production, making this strain a promising candidate for industrial and biotechnological applications.

AUTHOR CONTRIBUTIONS

Jaffar Z. Thraeib: Formal analysis; Investigation; Methodology; Resources; Writing—original draft; Writing—review and editing. **Ammar B. Altemimi**: Formal analysis; Investigation; Methodology; Resources; Supervision; Writing—original draft; Writing—review and editing. **Alaa Jabbar Abd Al-Manhel**: Formal analysis; Investigation; Methodology; Resources; Writing—

original draft; Writing—review and editing. **Rawaa H. Tlay**: Conceptualization; Data curation; Formal analysis; Investigation; Resources; Software; Visualization; Writing—original draft; Writing—review and editing. **Mohamed Ibrahim Younis**: Conceptualization; Data curation; Formal analysis; Investigation; Resources; Software; Visualization; Writing—original draft; Writing—review and editing. **Tarek Gamal Abedelmaksoud**: Conceptualization; Data curation; Formal analysis; Investigation; Resources; Software; Visualization; Writing—original draft; Writing—review and editing.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

ETHICS STATEMENT

None declared.

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REFERENCES

Acourene, S., Khalid, A. K., Bacha, A., Tama, M., & Taleb, B. (2007). Optimization of bakery yeast production cultivated on musts of dates. *Journal of Applied Sciences Research*, *3*(10), 964–971.

Aguilar-Uscanga, B., & Francois, J. M. (2003). A study of the yeast cell wall composition and structure in response to growth conditions and mode of cultivation. *Letters in Applied Microbiology*, *37*(3), 268–274.

Alcantara, V. A., Pajares, I. G., Simbahan, J. F., & Edding, S. N. (2013). Downstream recovery and purification of a bioemulsifier from *saccharomyces cerevisiae* 2031. *Philippine Agricultural Scientist*, *96*(4), 349–358.

Alcantara, V. A., Pajares, I. G., Simbahan, J. F., & Rubio, M. D. (2012). Substrate dependent production and isolation of an extracellular bioemulsifier from *saccharomyces cerevisiae* 2031. *Philippine Journal of Science*, *141*, 13–24.

Alcantara, V. A., Pajares, I. G., Simbahan, J. F., Villarante, N. R., & Rubio, M. L. D. (2010). Characterization of bioemulsifier from *saccharomyces cerevisiae* 2031 and evaluation of emulsification activity for potential application in bioremediation. *Philippine Agricultural Scientist*, *93*(1), 22–30.

Al-Eid, S. M., Al-Jasass, F. M., & Hamad, S. H. (2010). Performance of baker's yeast produced using date syrup substrate on Arabic bread quality. *African journal of Biotechnology*, *9*(21), 3167–3174.

Alizadeh-Sani, M., Hamishehkar, H., Khezerlou, A., Azizi-Lalabadi, M., Azadi, Y., Nattagh-Eshtivani, E., Fasihi, M., Ghavami, A., Aynehchi, A., & Ehsani, A. (2018). Bioemulsifiers derived from microorganisms: Applications in the drug and food industry. *Advanced Pharmaceutical Bulletin*, *8*(2), 191–199.

Al-Jasass, F. M., Al-Eid, S. M., & Ali, S. H. H. (2010). A comparative study on date syrup (dips) as substrate for the production of baker's yeast (*saccharomyces cerevisiae*). *Acta Horticulturae*, *882*, 699–704.

Al-Jumaiee, S. A., Al-Hussainy, K. S., & Al-Manhel, A. J. (2019). Effect of different level of β-glucan extracted from baker's yeast Saccharomyces cerevisiae and barley bran in the physicochemical properties of fish patties at cooling storage periods. *Basrah Journal of Agricultural Sciences*, *32*(1), 79–87.

Allaith, S. A., Abdel-aziz, M. E., Thabit, Z. A., Altemimi, A. B., Abd El-Ghany, K., Giuffrè, A. M., Al-Manhel, A. J. A., Ebrahim, H. S., Mohamed, R. M., & Abedelmaksoud, T. G. (2022). Screening and molecular identification of lactic acid bacteria producing β-glucan in boza and cider. *Fermentation*, *8*(8), 350.

Al-Manhel, A. J., & Niamah, A. K. (2017). Mannan extract from *saccharomyces cerevisiae* used as prebiotic in bio-yogurt production from buffalo milk. *International Food Research Journal*, *24*(5), 2259–2264.

Alwaely, W. A. S., Ghadban, A. K., & Alrubayae, I. M. N. (2019). Production and properties of bioemulsifier from the local isolation of candida spp. *Drug Invention Today*, *12*(5), 948–953.

Basit, M., Rasool, M. H., Naqvi, S. A. R., Waseem, M., & Aslam, B. (2018). Bioemulsifiers production potential of native strains of Bacillus cereus and their antimicrobial, cytotoxic and antioxidant activities. *Pakistan Journal of Pharmaceutical Sciences*, *31*, 251–256.

Beiroti, A., & Hosseini, S. N. (2007). Production of Baker's yeast using date juice. *Chinese Journal of Biotechnology*, *1*, 6–10.

Bicca, S. A., Poncet-Legrand, C., Williams, P., Mekoue Nguela, J., Doco, T., & Vernhet, A. (2022). Structural characteristics of *saccharomyces cerevisiae* mannoproteins: Impact of their polysaccharide part. *Carbohydrate Polymers*, *277*, 118758.

Bzducha Wróbel, A., Farkaš, P., Chraniuk, P., Popielarz, D., Synowiec, A., Pobiega, K., & Janowicz, M. (2022). Antimicrobial and prebiotic activity of mannoproteins isolated from conventional and nonconventional yeast species—the study on selected microorganisms. *World Journal of Microbiology and Biotechnology*, *38*(12), 256.

Cameron, D. R., Cooper, D. G., & Neufeld, R. J. (1988). The mannoprotein of *saccharomyces cerevisiae* is an effective bioemulsifier. *Applied and Environmental Microbiology*, *54*(6), 1420–1425. https://doi.org/10.1128/aem.54.6.1420-1425.1988

Carrau, F., Medina, K., Fariña, L., Boido, E., & Dellacassa, E. (2010). Effect of *saccharomyces cerevisiae* inoculum size on wine fermentation aroma compounds and its relation with assimilable nitrogen content. *International Journal of Food Microbiology*, *143*(1–2), 81–85. https://doi.org/10.1016/j.ijfoodmicro.2010.07.024

Chen, H.M., Muramoto, K., Yamauchi, F., Fujimoto, K., & Nokihara, K. (1998). Antioxidative properties of histidine-containing peptides designed from peptide fragments found in the digests of a soybean protein. *Journal of Agricultural and Food Chemistry*, *46*(1), 49–53.

Dikit, P. (2010). Extraction, Purification and Composition of Bioemulsifier from Spent Yeast Obtained from Thai Traditional Liquor Distillation and Its Application Paweena Dikit A Thesis Submitted in Partial Fulfillment of the Requirements for the Degree of Doctor of Phil.

Dikit, P., Maneerat, S., Musikasang, H., & H-kittikun, A. (2010). Emulsifier properties of the mannoprotein extract from yeast isolated from sugar palm wine. *Science Asia*, *36*(4), 312–318.

Ding, M. Z., Tian, H. C., Cheng, J. S., & Yuan, Y. J. (2009). Inoculum size-dependent interactive regulation of metabolism and stress response of *saccharomyces cerevisiae* revealed by comparative metabolomics. *Journal of Biotechnology*, *144*(4), 279–286. https://doi.org/10.1016/j.jbiotec.2009.09.020

Dudonné, S., Vitrac, X., Coutière, P., Woillez, M., & Mérillon, J.-M. (2009). Comparative study of antioxidant properties and total phenolic content of 30 plant extracts of industrial interest using DPPH, ABTS, FRAP, SOD, and ORAC assays. *Journal of Agricultural and Food Chemistry*, *57*(5), 1768–1774.

Elsaygh, Y. A., Gouda, M. K., Elbahloul, Y., Hakim, M. A., & El Halfawy, N. M. (2023). Production and structural characterization of eco - friendly bioemulsifier SC04 from *saccharomyces cerevisiae* strain MYN04 with potential applications. *Microbial Cell Factories*, *22*, 176. https://doi.org/10.1186/s12934-023-02186-z

Ethafa, E. S., & Al-Manhel, A. J. (2022). Determination of optimum conditions for ergosterol production by Saccharomyces cerevisiae. *Basrah Journal of Agricultural Sciences*, *35*(2), 34–48.

Feldmann, H. (2012). Yeast cell architecture and functions. *Yeast: Molecular and Cell Biology: Second Edition*, 5–24. https://doi.org/10.1002/9783527659180.ch2

Fenibo, E. O., Ijoma, G. N., Selvarajan, R., & Chikere, C. B. (2019). Microbial surfactants: The next generation multifunctional biomolecules for applications in the petroleum industry and its associated environmental remediation. *Microorganisms*, *7*(11), 581.

Fernandes, N. A. T., Simões, L. A., & Dias, D. R. (2023). Biosurfactants produced by yeasts: Fermentation, screening, recovery, purification, characterization, and applications. *Fermentation*, *9*(3), 207. https://doi.org/10.3390/fermentation9030207

Ghasemi, A., Moosavi-Nasab, M., Behzadnia, A., & Rezaei, M. (2018). Enhanced biosurfactant production with low-quality date syrup by lactobacillus rhamnosus using a fed-batch fermentation. *Food science and biotechnology*, *27*(4), 1137–1144. https://doi.org/10.1007/s10068-018-0366-5

Gonzaga, M. L. C., Ricardo, N. M. P. S., Heatley, F., & Soares, S. A. (2005). Isolation and characterization of polysaccharides from Agaricus blazei Murill. *Carbohydrate Polymers*, *60*(1), 43–49.

Guimarães, T. M., Moriel, D. G., Machado, I. P., Picheth, C. M. T. F., & Bonfim, T. M. B. (2006). Isolation and characterization of *saccharomyces cerevisiae* strains of winery interest. *Journal of Pharmaceutical Sciences*, *42*(1), 119–126. https://doi.org/10.1590/S1516-93322006000100013

Hajhosseini, A., Doroud, D., Sharifan, A., & Eftekhari, Z. (2020). Optimizing growth conditions of kluyveromyces marxianus for mannan production as a bioemulsifier. *Applied Food Biotechnology*, *7*(2), 115–126. https://doi.org/10.22037/afb.v7i2.28055

Hajhosseini, A., Sharifan, A., Eftekhari, Z., Alavi, A., & Doroud, D. (2023). Optimal extraction and deproteinization method for mannoprotein purification from kluyveromyces marxianus. *Iranian Biomedical Journal*, *27*(5), 320–325.

Hammadi, S. Y., Hussein, A. S., Majeed, D. M., Dheeb, B. I., & Ismail, E. N. (2018). RAPD and ISSR analyses of *saccharomyces cerevisiae* isolates from different sources. *Biotechnology Research Center*, *12*(2). https://doi.org/10.24126/jobrc.2018.12.2.537

Hyder, N. H. (2015). Production, characterization and antimicrobial activity of a bioemulsifier produced by acinetobacter baumanii AC5 utilizing edible oils. *Iraqi Journal of Biotechnology*, *14*(2), 55–70. https://www.iasj.net/iasj/article/105935

Ilori, M. O., Amobi, C. J., & Odocha, A. C. (2005). Factors affecting biosurfactant production by oil degrading aeromonas spp. isolated from a tropical environment. *Chemosphere*, *61*(7), 985–992.

Joaad, A. I. M., & Hassan, S. S. (2015). Effect of different environmental and nutritional factors on bioemulsifier production from *Candida guilliermondii*. *Iraqi Journal of Science*, *56*(1B), 329–336.

Kreger-van Rij, N. J. W. (1984). *Yeasts*. Elsevier.

Križková, L., Žitňanová, I., Mislovičová, D., Masárová, J., Sasinková, V., Ďuračková, Z., & Krajčovič, J. (2006). Antioxidant and antimutagenic activity of mannan neoglycoconjugates: Mannan-human serum albumine and mannan-penicillin G acylase. *Mutation Research/Genetic Toxicology and Environmental Mutagenesis*, *606*(1–2), 72–79. https://doi.org/10.1016/j.mrgentox.2006.03.003

Kurtzman, C. P., & Fell, J. W. (1998). Definition, classification and nomenclature of the yeasts. In *The yeasts* (vol. *1*, 3–5).

Kurtzman, C. P., Fell, J. W., & Boekhout, T. (Eds.). (2011). *The yeasts: a taxonomic study*. Elsevier.

Kwiatkowski, S., & Edgar, S. (2012). Yeast (*saccharomyces cerevisiae*) glucan polysaccharides – Occurrence, separation and application in food, feed and health industries. *The Complex World of Polysaccharides*. https://doi.org/10.5772/48100

Lakew, E. (2022). *Characterization of Indigenous Yeasts isolated from some Fruits collected from Fruit Vendors*. 1–13.

Laluce, C., Tognolli, J. O., De Oliveira, K. F., Souza, C. S., & Morais, M. R. (2009). Optimization of temperature, sugar concentration, and inoculum size to maximize ethanol production without significant decrease in yeast cell viability. *Applied Microbiology and Biotechnology*, *83*(4), 627–637. https://doi.org/10.1007/s00253-009-1885-z

Legras, J.-L., & Karst, F. (2003). Optimisation of interdelta analysis for *saccharomyces cerevisiae* strain characterisation. *FEMS Microbiology Letters*, *221*(2), 249–255.

Lima, Á. S., & Alegre, R. M. (2009). Evaluation of emulsifier stability of biosurfactant produced by saccharomyces lipolytica CCT-0913. *Brazilian Archives of Biology and Technology*, *52*, 285–290.

Liu, H., Wang, Q., Liu, Y., & Fang, F. (2009). Statistical Optimization of Culture Media and Conditions for Production of Mannan by *saccharomyces cerevisiae*. 577–583. https://doi.org/10.1007/s12257-008-0248-4

Liu, H. Z., Liu, L., Hui, H., & Wang, Q. (2015). Structural characterization and antineoplastic activity of *saccharomyces cerevisiae* mannoprotein. *International Journal of Food Properties*, *18*(2), 359–371. https://doi.org/10.1080/10942912.2013.819364

Lodder, J. (1970). *A taxonomic study*. North-Holland Publishing Company.

MacHová, E., & Bystrický, S. (2012). Yeast mannans protect liposomes against peroxidation but do not scavenge free radicals. *Carbohydrate Polymers*, *88*(2), 793–797. https://doi.org/10.1016/j.carbpol.2012.01.008

Mahmood, N. N. (2018). Effect of bioemulsifiers purified from *saccharomyces cerevisiae* against corynebacterium urealyticum. *Journal of Pharmaceutical Sciences and Research*, *10*(3), 481–486.

Majeed, H. A., & Al-jader, Z. W. (2022). *Isolation and Diagnosis of Different Local Isolates of Saccharomyces Cerevisiae using DNA Sequencing*. *3*(3).

Malabuyoc, J. A. S., Alcantara, V. A., Arocena, R. E., & Elegado, F. B. (2023). Substrate optimization for bioemulsification using *saccharomyces cerevisiae* 2031 by response surface methodology. *Agro Bali: Agricultural Journal*, *6*(1), 1–11.

Mnif, I., Bouallegue, A., Mekki, S., & Ghribi, D. (2021). Valorization of date juice by the production of lipopeptide biosurfactants by a bacillus mojavensis BI2 strain: Bioprocess optimization by response surface methodology and study of surface activities. *Bioprocess and Biosystems Engineering*, *44*(11), 2315–2330. https://doi.org/10.1007/s00449-021-02606-7

Nerurkar, A. S., Hingurao, K. S., & Suthar, H. G. (2009). *Bioemulsifiers from marine microorganisms*.

Nogueira, I. B., Rodríguez, D. M., da Silva Andradade, R. F., Lins, A. B., Bione, A. P., da Silva, I. G. S., Franco, L. O., & De campos-Takaki, G. M. (2020). Bioconversion of agroindustrial waste in the production of bioemulsifier by stenotrophomonas maltophilia UCP 1601 and application in bioremediation process. *International Journal of Chemical Engineering*, *2020*, 1–9.

Parapouli, M., Vasileiadi, A., Afendra, A. S., & Hatziloukas, E. (2020). *Saccharomyces cerevisiae* and its industrial applications. *AIMS Microbiology*, *6*(1), 1–32.

Patil, J. R., & Chopade, B. A. (2001). Studies on bioemulsifier production by acinetobacter strains isolated from healthy human skin. *Journal of Applied Microbiology*, *91*(2), 290–298. https://doi.org/10.1046/j.1365-2672.2001.01382.x

Paul, S., Nadda, A. K., & Goel, G. (2023). Microbial food additives: Types, functions, and challenges, In *Microbes for Natural Food Additives* (pp. 1–12). Springer.

Perez, C. (1990). Antibiotic assay by agar-well diffusion method. *Acta Biologiae et Medicinae Experimentalis*, *15*, 113–115.

Qian, Z.-J., Jung, W.-K., & Kim, S.-K. (2008). Free radical scavenging activity of a novel antioxidative peptide purified from hydrolysate of bullfrog skin, Rana catesbeiana Shaw. *Bioresource Technology*, *99*(6), 1690–1698.

Qiao, Y., Xia, C., Liu, L., Tang, L., Wang, J., Xu, C., Wang, J., Zhang, L., Ye, X., Huang, Y., Mao, D., Liang, Y., Zhoukun, L., & Cui, Z. (2022). Structural characterization and emulsifier property of yeast mannoprotein enzymatically prepared with a $β $-1, 6-glucanase. *LWT*, *168*, 113898.

Rasheed, H. G. H., & Haydar, N. H. (2023). Purification, characterization and evaluation of biological activity of mannoprotein produced from *saccharomyces cerevisiae* by. *Iraqi Journal of Agricultural Sciences*, *54*(2), 347–359. https://doi.org/10.36103/ijas.v54i2.1709

Ribeiro, B. G., Campos Guerra, J. M., & Sarubbo, L. A. (2023). Bioemulsifiers: Promising Biomolecules in the Food Industry, In *Microbes for Natural Food Additives* (pp. 147–168). Springer.

Ribeiro, B. G., Guerra, J. M. C., & Sarubbo, L. A. (2020a). Bioemulsifiers: Production and application prospects in the food industry. *Biotechnology Progress*, *36*(5), e3030.

Ribeiro, B. G., dos Santosb, M. M., Pintoc, M. I. S., Meirac, H. M., Durvald, I. J. B., Guerrab, J. M. C., & Sarubboc, L. A. (2019). Production and optimization of the extraction conditions of the bioemulsifier from *Candida utilis* UFPEDA1009 with potential application in the food industry. *CET Journal-Chemical Engineering Transactions 74*, 14771482.

Ribeiro, B. G., de Veras, B. O., dos Santos Aguiar, J., Medeiros Campos Guerra, J., & Sarubbo, L. A. (2020b). Biosurfactant produced by *Candida utilis* UFPEDA1009 with potential application in cookie formulation. *Electronic Journal of Biotechnology*, *46*, 14–21.

Saleh, A. Y. A., Salman, J. A. S., & Aziz, R. A. (2020). Study of the effect of mannoprotein extracted from *saccharomyces cerevisiae* on some pathogenic bacteria. *EurAsian Journal of BioSciences*, *14*(2), 7297–7300.

Sambrook, J., Fritsch, E. F., & Maniatis, T. (1989). *Molecular cloning: A laboratory manual* (2nd ed.). Cold Spring Harbor Laboratory Press.

Sen, R., & Swaminathan, T. (2004). Response surface modeling and optimization to elucidate and analyze the effects of inoculum age and size on surfactin production. *Biochemical Engineering Journal*, *21*(2), 141–148.

Sherman, F. (1991). Getting started with yeast. *Methods in Enzymology*, *194*(C), 3–21. https://doi.org/10.1016/0076-6879(91)94004-V

Slama, R. (2021). *Study of Saccharomyces cerevisiae growth: Screen the best conditions and evaluate the effects of wine and histamine*.

Sudha, S., Kumanan, R., & Muthusamy, K. (2010). Optimization of cultural conditions for the production of sophorolipids from *Candida tropicalis*. *Der Pharm Lett*, *2*(2), 155–163.

Tan, C., & McClements, D. J. (2021). Application of advanced emulsion technology in the food industry: A review and critical evaluation. *Foods*, *10*(4), 812.

Thapa, S., Shrestha, R., Tirewal, A., Sharma, A., & K.C., Y. (2015). Isolation of yeast from soil and different food samples and its characterization based on fermentation. *Nepal Journal of Biotechnology*, *3*(1), 29–34. https://doi.org/10.3126/njb.v3i1.14226

Thraeib, J. Z., Altemimi, A. B., Jabbar Abd Al-Manhel, A., Abedelmaksoud, T. G., El-Maksoud, A. A. A., Madankar, C. S., & Cacciola, F. (2022). Production and characterization of a bioemulsifier derived from microorganisms with potential application in the food industry. *Life*, *12*(6), 924.

Torabizadeh, H., Shojaosadati, S. A., & Tehrani, H. A. (1996). Preparation and characterisation of bioemulsifier from and its application in food products. *LWT - Food Science and Technology*, *29*(8), 734–737. https://doi.org/10.1006/fstl.1996.0114

Trindade, M., Sithole, N., Kubicki, S., Thies, S., & Burger, A. (2022). Screening strategies for bioemulsifier discovery. *Advances in Biochemical Engineering/Biotechnology*, *181*, 17–52. https://doi.org/10.1007/10_2021_174

Tuite, M. F., & Oliver, S. G. (1991). Saccharomyces. In: Biotechnology Handbooks.4. In *Springer Science+Business Media, LLC* (Vol. 5, Issue 1).

Walencka, E., Wieckowska-Szakiel, M., Rozalska, S., Sadowska, B., & Rozalska, B. (2007). A surface-active agent from *Saccharomyces cerevisiae* influences staphylococcal adhesion and biofilm development. *Zeitschrift Für Naturforschung C*, *62*(5–6), 433–438.

Wang, Y., Ai, C., Wang, H., Chen, C., Teng, H., Xiao, J., & Chen, L. (2023). Emulsion and its application in the food field: An update review. *EFood*, *4*(4), e102.

Xia, J., Sánchez, B. J., Chen, Y., Campbell, K., Kasvandik, S., & Nielsen, J. (2022). Proteome allocations change linearly with the specific growth rate of *saccharomyces cerevisiae* under glucose limitation. *Nature Communications*, *13*(1), 2819.

Zaparoli, M., Kreling, N. E., & Colla, L. M. (2020). Increasing the production of extracellular biosurfactants fromSaccharomyces cerevisiae. *Environmental Quality Management*, *29*(4), 51–58.

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