

Probiotic Potential of Indigenous Yeast Isolated from Chao, a Traditional Indonesian Fermented Fish

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Abstract

Indigenous yeasts from traditional fermented foods represent a promising yet underexplored source of probiotic candidates. Chao, a traditional fermented fish from Pangkajene Kepulauan Regency, South Sulawesi, is characterized by yeast-associated fermentation, suggesting its potential as a promising source of functional microorganisms. This study aimed to evaluate the probiotic potential of yeast isolates from Chao through integrated physiological, functional, and molecular analyses. Yeasts were isolated using yeast peptone dextrose (YPD) medium and screened for tolerance to acidic pH, bile salts, and osmotic stress, as well as for hydrophobicity and co-aggregation with pathogenic bacteria. Out of 40 isolates, 3 strains (C32B.1, C35B.1, and C37B.1) exhibited strong tolerance to gastrointestinal-like conditions and favorable adhesion-related properties. The selected isolates demonstrated high cell surface hydrophobicity (>65%) and moderate to strong co-aggregation ability with pathogenic bacteria. Functional characterization demonstrated that these isolates possessed notable bioactivities, including antidiabetic activity (up to 70.20%), antioxidant activity (>73%), and anti-inflammatory potential (>56%), with activities increasing in a concentration-dependent manner. Molecular identification based on 18S rRNA gene sequence analysis revealed that the selected isolates were affiliated to *Pichia kudriavzevii* (99.74% similarity). These findings highlight the potential of indigenous yeasts from Chao as promising probiotic candidates and support their application in functional food development and related biotechnological industries from traditional fermented foods.

Keywords: Chao, Fermented fish, Probiotic yeast, *Pichia kudriavzevii*

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Introduction

Traditional fermented foods are increasingly recognized as valuable sources of indigenous microorganisms with potential applications in functional foods. This relevance is supported by the rapid growth of the functional food sector, which is driven by consumer demand for products that provide health benefits beyond basic nutrition (The Global Wellness Institute, 2021). Among these, fermented foods are particularly important due to their rich microbial diversity and their capacity to generate bioactive compounds during fermentation (Hernández-Velázquez et al., 2024; Lara-Hidalgo et al., 2019). Importantly, many of these microorganisms are associated with probiotic functionality.

Probiotics represent a functionally important group of microorganisms that, when administered in adequate amounts, exert beneficial effects on the host. These microorganisms act through several key mechanisms, including modulation of gut microbiota, inhibition of pathogenic microorganisms, and enhancement of immune responses. These microorganisms act through several key mechanisms, including modulation of gut microbiota, inhibition of pathogenic microorganisms, and enhancement of immune responses (Pais et al., 2020; Leeuwendaal et al., 2022). Beyond these established roles, probiotics are increasingly recognized for their antioxidant capacity, which plays a crucial role in neutralizing reactive oxygen species and reducing oxidative stress associated with cellular damage and chronic diseases (Lara-

Hidalgo et al., 2019; Xu et al., 2011). In addition, probiotics exhibit anti-inflammatory effects by modulating immune responses and regulating pro-inflammatory pathways, thereby contributing to the prevention and management of inflammation-related disorders (Kelesidis & Pothoulakis, 2012; Maccaferri et al., 2012; Pothoulakis, 2009). Furthermore, certain probiotics have demonstrated antidiabetic potential through mechanisms such as inhibition of carbohydrate-hydrolyzing enzymes and improvement of glucose metabolism, highlighting their relevance in addressing metabolic disorders (Ragavan & Das, 2020; Raygan et al., 2018). Collectively, these functional attributes underscore the importance of evaluating probiotic microorganisms not only for their survival and safety but also for their potential health-promoting bioactivities.

While lactic acid bacteria have been extensively explored as probiotic agents, yeasts are emerging as promising alternatives with distinct physiological and functional advantages. Yeasts generally exhibit higher tolerance to environmental stress, including acidic conditions, bile salts, and osmotic pressure, enabling their survival during gastrointestinal transit (Arévalo-Villena et al., 2018; de Miranda et al., 2023; Imre & Crook, 2025; Pais et al., 2020). In addition, yeasts are capable of producing a wide range of bioactive metabolites, including enzymes and polysaccharides, which contribute to their functional properties such as antioxidant and anti-inflammatory activities (Arévalo-Villena et al., 2018; Maccaferri et al., 2012). Importantly, yeasts can act synergistically with lactic acid bacteria through complex interactions such as mutualism and co-aggregation, enhancing bacterial survival, adhesion, and stability under gastrointestinal conditions. These effects are often attributed to yeast-derived metabolites, including amino acids, peptides, and vitamins, as well as cell wall components such as mannoproteins and polysaccharides that facilitate microbial interactions and colonization (Golowczyc et al., 2009; Katakura et al., 2010; Stadie et al., 2013; Xie et al., 2011). Consequently, yeasts can complement bacterial probiotics by providing unique metabolic and functional contributions, making them attractive candidates for multifunctional probiotic applications.

Traditional fermented foods represent valuable ecological niches for diverse microorganisms, including yeasts with potential probiotic properties. Indonesia is recognized for its rich diversity of such fermented products, which serve as important reservoirs of indigenous microorganisms (Wicaksono et al., 2026). One such product is Chao, a traditional fermented fish originating from Pangkajene Kepulauan Regency, South Sulawesi, characterized by yeast-driven fermentation involving complex microbial interactions (Matti et al., 2021; Matti & Kumalasari, 2017). Despite its traditional significance, the yeast community associated with Chao remains largely underexplored, particularly in relation to their probiotic potential and functional bioactivities. This is notable given the growing evidence that yeasts can exhibit stress tolerance, functional bioactivities, and synergistic interactions with lactic acid bacteria, making them promising candidates for multifunctional probiotic applications. Thus, Chao represents a promising yet largely untapped source of novel yeast-based probiotic candidates.

Therefore, this study aimed to isolate and evaluate indigenous yeast strains from Chao as potential probiotics, with particular emphasis on their functional bioactivities. The isolates were assessed for tolerance to gastrointestinal-like conditions, adhesion-related properties, and safety aspects, followed by the evaluation of key functional activities, including antioxidant, anti-inflammatory, and antidiabetic properties. Molecular identification was subsequently performed based on 18S rRNA gene sequence analysis to determine the taxonomic affiliation of selected isolates.

Materials and methods

1. Sample collection and yeast isolation

Chao samples, a traditional Indonesian fermented fish, were collected from household-scale producers in Pangkajene Kepulauan Regency, South Sulawesi, Indonesia. Four independent batches were collected directly from local producers to account for production variability among household producers. Each sample was aseptically transferred into sterile polyethylene containers, labeled, and placed in an insulated ice box (4°C) during transport. All samples were delivered to the laboratory within 24 h of collection and processed immediately for microbial isolation. Portions intended for molecular analysis were stored at -20°C until further use.

Following sample collection, 10 g of Chao was homogenized in 90 mL of 0.85% NaCl to prepare a 10^{-1} suspension. Serial dilutions were made up to 10^{-10} , and 100 µL aliquots of each dilution were spread onto yeast peptone dextrose (YPD) (M1363, HiMedia Laboratories Pvt. Ltd., India) agar plates supplemented with chloramphenicol (100 mg/L) to suppress bacterial growth. Plates were incubated at 37°C for 48–72 h, and distinct yeast colonies were selected based on morphology. Pure cultures were obtained through repeated streaking on fresh YPD agar and preserved in YPD broth containing 20% (v/v) glycerol at -80°C for further

characterization. The isolates were morphologically characterized by examining their colony traits, including shape, color, margin, and surface texture.

2. Preparation and standardization of yeast inoculum

Yeast isolates were pre-cultured in yeast peptone dextrose (YPD) broth for 24 h at 30°C with shaking at 180 rpm to obtain actively growing cells. Following incubation, the cultures were harvested and the cell suspensions were adjusted to a final concentration of approximately 10^6 – 10^7 CFU/mL using sterile 0.85% (w/v) NaCl solution. This standardized inoculum was subsequently used for all subsequent assays.

3. Acid and bile salt tolerance test

For the acid tolerance assay, 1% (v/v) of the standardized inoculum was transferred into fresh YPD broth adjusted to pH 2.0, 3.0, and 5.0 following previously reported methods with slight modifications (Kumari et al., 2023; Somashekaraiah et al., 2021). Each pH value was achieved by adding the appropriate buffer solution to adjust the pH of the medium. After inoculation, the cultures were incubated at 30°C for 24 h with shaking at 180 rpm to ensure uniform growth.

For bile salt tolerance assay, 1% (v/v) of the standardized inoculum was inoculated into YPD broth supplemented with bile salts at concentrations of 0.10, 0.30, and 0.50% (Divyashree et al., 2021; Somashekaraiah et al., 2021), followed by incubation under the same condition.

Following incubation, yeast viability was determined using the Total Plate Count (TPC) method. Briefly, 100 μ L of each culture was serially diluted in 0.85% (w/v) NaCl solution up to 10^{-7} . Aliquots (100 μ L) from appropriate dilutions were spread onto YPD agar plates and incubated at 30°C for 16 h. Colony-forming units were enumerated, and the results were expressed as log CFU/mL for both acid and bile salt cultures.

4. Sugar tolerance assay at different concentrations

Sugar tolerance was evaluated by inoculating 1% (v/v) of the inoculum into fresh YPD broth supplemented with glucose at final concentrations of 4, 6, 8 and 10% (w/v). The cultures were incubated at 30°C for 24, 36, and 48 h with shaking at 180 rpm. To obtain quantifiable viable counts, 100 μ L of the culture was diluted in 0.85% (w/v) NaCl to prepare a 10^{-1} suspension, followed by a series of tenfold dilutions up to 10^{-7} . Aliquots of 100 μ L from each dilution were spread onto YPD agar plates and incubated at 30°C for 16 h, and colony counts were recorded and expressed as log CFU/mL.

5. NaCl tolerance assay at gradual concentrations

Salt tolerance was evaluated by inoculating 1% (v/v) the inoculum into YPD broth supplemented with 2, 4, and 6% (w/v) NaCl. Each treatment was performed in triplicate, while YPD broth without NaCl served as the control. The cultures were incubated at 30°C with agitation at 180 rpm. Yeast growth was monitored by measuring optical density at 600 nm (OD_{600}) using a UV-Vis spectrophotometer (UV-8000T, Shanghai Metash Instruments Co., Ltd., China) at 18 h intervals throughout the incubation period. Salt tolerance was evaluated by comparing the OD_{600} values obtained under different NaCl concentrations.

6. Temperature-dependent assay

Temperature tolerance was evaluated by inoculating 1% (v/v) of the inoculum was transferred into 30 mL of YPD broth, following by incubation at 30, 37, and 42°C for 24 h, as described by Manguntungi et al. (2025). All cultures were incubated with agitation at 180 rpm. Yeast growth was evaluated by measuring optical density at 600 nm (OD_{600}) using a UV-Vis spectrophotometer. The selected temperatures represent optimal, moderate stress, and high-stress conditions for yeast growth, respectively.

7. Antibiotic susceptibility testing of yeast isolates

The antibiotic resistance profile of yeast isolates was determined using the disk diffusion method according to the Kirby–Bauer protocol with slight modifications (Adithi et al., 2022). The standardized suspension was spread uniformly onto the surface of YPD agar plates using sterile cotton swabs (OneMed, Inti Medicom Retailindo Co., Ltd., Indonesia). Commercial antibiotic disks containing ampicillin (10 μ g), streptomycin (10 μ g), tetracycline (30 μ g), and kanamycin (30 μ g) (Oxoid, Oxoid Co., Ltd., UK) were aseptically placed on the agar surface. The plates were incubated at 30°C for 48 h, and the diameter of the inhibition zones around each disk was measured in mm. Antibiotic susceptibility was interpreted based on the presence or absence of inhibition zones. All experiments were performed in duplicate.

9. Co-aggregation assay

The co-aggregation ability of yeast isolates with pathogenic bacteria was quantified as described by Paul et al. (2023) with modifications. Yeast isolates were pre-cultured in YPD broth at 30°C for 24 h, harvested by centrifugation (5000 $\times$ g, for 10 min), washed twice with sterile phosphate-buffered saline (PBS, pH 7.2), and resuspended in PBS. The cell density was adjusted to $OD_{600}\approx 0.5$, which was considered as the stock suspension (100%). Serial dilutions were prepared using PBS to obtain concentrations of 20, 40, 60, and 80%. Pathogenic bacteria (*Escherichia coli*, *Staphylococcus aureus*, *Salmonella typhi*, and *Listeria monocytogenes*) were cultured in appropriate broth media at 37°C for 18–24 h, harvested, washed twice, and standardized to

OD₆₀₀≈0.5.

Equal volumes (2 mL each) of yeast and bacterial suspension were mixed and incubated at 37°C without agitation. The absorbance of individual yeast (A_x) and bacterial (A_y) suspensions, as well as their mixture (A(x+y)), was measured at 600 nm. The percentage of co-aggregation was calculated using the following formula:

$$\text{Co-aggregation \%} = \frac{\frac{(A_x + A_y)}{2} - A(x+y)}{\frac{(A_x + A_y)}{2}} \times 100$$

10. Cell surface hydrophobicity measurement

The cell surface hydrophobicity of yeast isolates was determined using the microbial adhesion to hydrocarbons (MATH) method with slight modifications Rosenberg et al. (1980). Yeast isolates (C32B.1, C35B.1, and C37B.1) were pre-cultured in YPD broth at 30°C for 24 h with shaking at 180 rpm. The cultures were harvested by centrifugation (5000×g, 10 min), washed twice with phosphate-buffered saline (PBS, pH 7.2), and resuspended in the same buffer to obtain a standardized cell suspension with an initial absorbance (A₀) of approximately 0.5 at 600 nm. Subsequently, 2 mL of the yeast suspension was mixed with 1 mL of xylene (Merck, Merck KGaA, Germany) in a test tube. The mixture was vortexed vigorously for 1 min to allow interaction between the cells and the hydrocarbon phase, followed by incubation at room temperature for 15 min to facilitate phase separation. After separation, the aqueous phase was carefully collected, and its absorbance (A) was measured at 600 nm using a spectrophotometer.

The percentage of cell surface hydrophobicity was calculated using the following formula:

$$\text{Hydrophobicity \%} = \frac{A_0 - A}{A_0} \times 100$$

11. Functional bioactivity assay

The antidiabetic potential of the yeast isolates was assessed by determining their ability to inhibit α-amylase and α-glucosidase activities, following modified procedures commonly applied in probiotic bioactivity studies (Goyal & Shukla, 2013; Kumari et al., 2023). For the α-amylase inhibition assay, 100 μL of the yeast cell-free supernatant was mixed with 100 μL of α-amylase solution and incubated at 37°C for 10 min, followed by the addition of soluble starch as the reaction substrate. After further incubation, DNS reagent was added to terminate the reaction, and the mixture was heated at 90°C for color development before absorbance was read at 540 nm. For α-glucosidase inhibition, 50 μL of supernatant was combined with 100 μL of α-glucosidase enzyme and preincubated at 37°C, after which p-nitrophenyl-α-D-glucopyranoside (pNPG) (Sigma-Aldrich, Merck KGaA, Germany) was added as the substrate. The release of p-nitrophenol was quantified at 517 nm after incubation. Appropriate controls were included: Enzyme and substrate mixtures without supernatant served as negative controls, whereas acarbose was used as the positive control for both assays.

Antioxidant potential was determined based on the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay following modified procedures described by Gulcin (2020) and Yasin et al. (2022). For each reaction, 100 μL of freshly prepared 0.2 mM DPPH solution was mixed with 100 μL of yeast supernatant in a 96-well microplate. The plate was incubated in complete darkness for 30 min to prevent photodegradation of the DPPH reagent. A blank consisting of DPPH without sample was used for baseline correction, while vitamin C (100 ppm) served as the positive antioxidant control. After incubation, absorbance was measured at 660 nm using a Multiskan Go Microplate Spectrophotometer (A5111960DPC, Thermo Fisher Scientific Inc., USA). Antioxidant activity was calculated using the unified formula:

$$\% \text{ Inhibition} = \frac{A_0 - A}{A_0} \times 100$$

where A₀ represents the absorbance of the control reaction and A₁ the absorbance of the sample.

The anti-inflammatory activity of the yeast supernatant was evaluated using the protein denaturation inhibition method with bovine serum albumin (BSA) (HiMedia, HiMedia Laboratories Pvt. Ltd., India), adapted from established protocols (Garbi et al., 2017; Khan et al., 2021). In this assay, 500 μL of BSA solution was transferred into a microtube and combined with 100 μL of the yeast cell-free supernatant. The mixture was allowed to stand at room temperature for 10 min to facilitate initial interaction between

the BSA and bioactive metabolites. The tubes were then incubated at 51°C for 20 min to induce controlled protein denaturation. A negative control consisting of BSA mixed with uninoculated growth medium was included, while diclofenac-treated BSA served as the positive control. After incubation, absorbance was recorded at 660 nm using a UV-Vis spectrophotometer (UV-8000T, Shanghai Metash Instruments Co., Ltd., China). The percentage inhibition of protein denaturation was calculated using the following formula:

$$\% \text{ Inhibition} = \frac{A_1 - A_2}{A_0} \times 100$$

where A_1 represent the absorbance of the sample, A_2 is the absorbance of the product control and A_0 is the absorbance of the positive control.

12. Molecular identification and phylogenetic analysis

A total of 12 yeast isolates were subjected to molecular identification through PCR amplification of the 18S rRNA gene region. Genomic DNA was extracted and purified using the DNeasy UltraClean Microbial Kit (QIAGEN, QIAGEN GmbH, Germany) following the manufacturer's instructions, and PCR reactions were prepared using a commercial PCR kit. Amplification of the 18S rRNA region was performed using the universal primers NS1 (5'-GTAGTCATATGCTTGTCTC-3') and NS8 (5'-TCCGCAGGTTACCTACGGA-3'), as previously described in standard yeast molecular taxonomy protocols. The purified PCR products were sequenced at Genetika Science (Jakarta, Indonesia). The resulting sequences were edited and aligned using MUSCLE, then compared with reference sequences in the NCBI GenBank database via BLAST to determine the closest taxonomic match. Phylogenetic analysis was performed using MEGA 12 software (United States) with the neighbor-joining method and 10,000 bootstrap replications to assess the robustness of the inferred evolutionary relationships (Tamura et al., 2021). The final phylogenetic tree was used to confirm species-level identification based on sequence clustering with known yeast taxa.

13. Statistical analysis

All analyses were conducted in duplicate with a minimum of 2 replicates. The mean values and standard deviations were computed and displayed in tables.

Results and discussion

1. Morphological characteristics of selected yeast isolates

Yeasts were successfully isolated from Chao, a traditional fermented food, collected from 3 different local markets in Pangkep Regency, South Sulawesi. A total of 40 isolates were initially obtained and cultured on yeast peptone dextrose (YPD) agar. Preliminary screening based on colony morphology revealed phenotypic variation among the isolates, which served as the basis for selecting representative strains for further characterization. Based on their tolerance to gastrointestinal-like conditions and functional screening, three isolates (C32B.1, C35B.1, and C37B.1) were selected for subsequent analyses. The colony morphology of these selected isolates is presented in Table 1.

Table 1 Colony morphology of selected yeast isolates

Isolate	Shape	Margin	Elevation	Size	Texture	Pigmentation
C32B.1	Circular	Curled	Flat	Punctiform	Butyrous	White
C35B.1	Circular	Undulate	Flat	Moderate	Butyrous	White
C37B.1	Circular	Undulate	Raised	Moderate	Butyrous	White

Isolates C35B.1 and C37B.1 displayed undulate margins, while C32B.1 showed a curled margin, accompanied by differences in elevation ranging from raised to flat (Fig. 1). Colony size varied from punctiform to moderate, indicating phenotypic variation among the isolates. All isolates exhibited butyrous texture and white pigmentation, indicating similar macroscopic colony characteristics. These subtle morphological differences, particularly in colony edge and elevation, may reflect variations in cell surface structure and growth behavior, which are often associated with microbial adaptability to environmental conditions (Hu et al., 2025).

Although colony morphology alone is insufficient for definitive identification, it provides important

preliminary insights into phenotypic diversity and potential functional traits of the isolates. Variations in morphological characteristics have been reported to correlate with differences in stress tolerance, adhesion properties, and metabolic activity, which are key attributes of probiotic candidates. Therefore, to further elucidate their functional potential, the selected isolates were subjected to a series of physiological assays, including tolerance to acidic pH, bile salts, osmotic stress, and temperature, to evaluate their ability to survive and perform under gastrointestinal-like conditions.

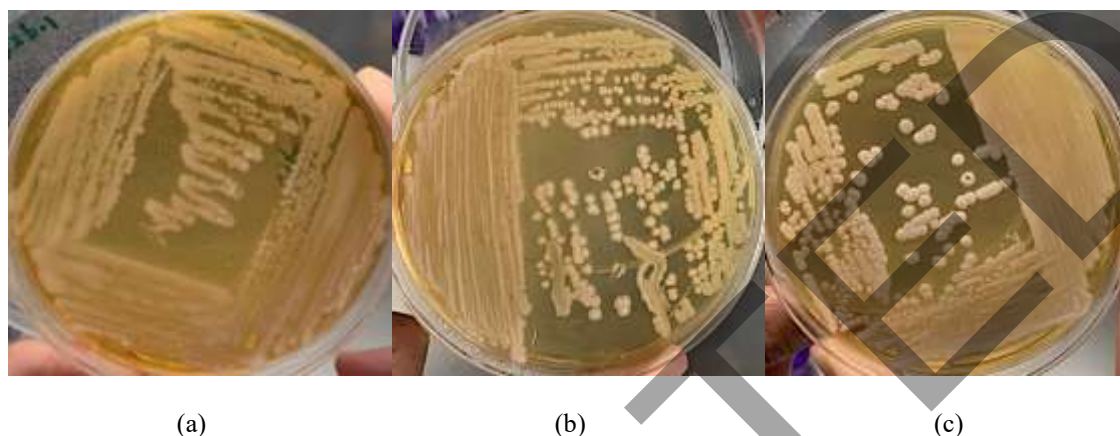


Fig. 1 Morphology of probiotic candidate isolates: (a) Isolate C32B.1, (b) Isolate C35B.1, and (c) Isolate C37B.1

2. Tolerance of the isolates to bile salt and acidic conditions

The majority of isolates showed a marked reduction in viability under these stress conditions, while several exhibited no detectable growth after incubation. However, three isolates (C32B.1, and C35B.1, C37B.1) remained viable under both acidic and bile salt conditions. Survival at low pH and high bile concentrations is widely recognized as an essential prerequisite for selecting probiotic microorganisms, given that only strains with specific stress-adaptive mechanisms can withstand these harsh environments (Papadimitriou et al., 2016). Tolerance to bile salts varied among the isolates, with C32B.1 and C37B.1 showing decreased viability at higher concentrations, while C35B.1 maintained optimal growth up to 0.50%, indicating superior resistance (Table 2). Bile tolerance at concentrations ≥ 0.30 –0.60% (w/v) is considered a key indicator of a microorganism's ability to survive passage through the small intestine, as bile can destabilize membranes and disrupt cellular integrity (Helmy et al, 2019; Pais et al., 2020). The strong performance of C35B.1 suggests the presence of adaptive mechanisms such as membrane remodeling or bile detoxification pathways commonly reported in probiotic yeasts.

Table 2 Bile salt tolerance of selected yeast isolates

Isolate	Bile salt concentration	Log CFU/mL*
C32B.1	0.10%	7.61 $\pm$ 0.04
	0.30%	7.71 $\pm$ 0.38
	0.50%	7.02 $\pm$ 0.23
C35B.1	0.10%	7.93 $\pm$ 0.27
	0.30%	7.82 $\pm$ 0.11
	0.50%	8.43 $\pm$ 0.03
C37B.1	0.10%	7.23 $\pm$ 0.00
	0.30%	7.67 $\pm$ 0.06
	0.50%	7.15 $\pm$ 0.00

Remark: *Values are presented as mean $\pm$ standard deviation (SD) of 3 replicates.

The isolates were also subjected to acid tolerance assays at different pH levels (pH 2, 3, and 5) (Table 3). Isolate C32B.1 was highly sensitive to acidic stress, failing to survive at pH 2 and declining at pH 3,

while remaining stable at pH 5. In contrast, C35B.1 demonstrated strong acid tolerance, maintaining high viability across all conditions tested. Isolate C37B.1 exhibited intermediate acid tolerance, with limited survival at pH 2 but improved viability at pH 3. The ability to withstand acidic environments (pH 1.5–3.0) and to tolerate bile salts (0.3% w/v) is considered a fundamental criterion for selecting potential probiotic yeast strains, as these traits are crucial for ensuring their survival throughout the gastrointestinal tract (Zahoor et al, 2021).

Table 3 Acidic tolerance of selected yeast isolates

Isolate	pH	Log CFU/mL*
C32B.1	pH 2	0.00±0.00
	pH 3	4.83±4.20
	pH 5	7.83±0.34
C35B.1	pH 2	7.73±1.10
	pH 3	7.57±0.53
	pH 5	7.71±0.54
C37B.1	pH 2	7.25±0.85
	pH 3	8.10±0.70
	pH 5	7.94±0.47

Remark: *Values are presented as mean±standard deviation (SD) of 3 replicates.

3. Growth response across different sugar concentrations

The growth performance of the three yeast isolates across different glucose concentrations (4–10%) is presented in Table 4. All isolates maintained viable cell counts in the range of approximately 1.8–2.2 log CFU/mL throughout the 48-h incubation period, indicating their ability to tolerate elevated sugar concentrations. A gradual increase in cell counts was generally observed from 24 to 36 h, followed by stabilization or slight fluctuation at 48 h, suggesting active adaptation and sustained metabolic activity under osmotic stress conditions.

Table 4 Growth of selected yeast isolates in different glucose concentrations at 24, 36, and 48 h

Isolate	Concentration of glucose	Log CFU/mL*		
		24 h	36 h	48 h
C32B.1	4%	1.80±0.09	2.08±0.16	2.14±0.18
	6%	1.81±0.03	1.91±0.23	1.89±0.17
	8%	1.83±0.05	2.04±0.12	2.07±0.15
	10%	1.91±0.03	2.02±0.12	2.19±0.11
C35B.1	4%	1.91±0.14	2.02±0.18	2.19±0.08
	6%	1.94±0.10	2.06±0.14	1.99±0.11
	8%	1.84±0.12	2.13±0.10	2.09±0.17
	10%	1.86±0.12	2.00±0.08	2.10±0.18
C37B.1	4%	1.93±0.07	2.10±0.06	2.08±0.18
	6%	1.82±0.10	1.85±0.03	1.97±0.20
	8%	1.82±0.09	2.07±0.13	2.05±0.24
	10%	1.90±0.03	2.14±0.17	2.17±0.17

Remark: *Values are presented as mean±standard deviation (SD) of 3 replicates.

Differences in growth patterns among isolates were evident. Isolate C37B.1 exhibited relatively stable growth across all glucose concentrations, with slightly higher counts at 10% glucose after 48 h. Similarly, C35B.1 showed consistent growth with a gradual increase over time, particularly at 4% and 10% glucose. In contrast, C32B.1 demonstrated more variable responses at intermediate concentrations (6%), although

it maintained comparable final cell densities. These variations may reflect strain-specific differences in osmotic stress tolerance and metabolic regulation.

The ability of all isolates to sustain growth at glucose concentrations up to 10% suggests a considerable level of osmotolerance, as high sugar environments typically impose hyperosmotic pressure that can limit microbial proliferation. Yeasts adapted to such conditions are known to employ protective mechanisms, including the accumulation of compatible solutes (e.g., glycerol) and modulation of membrane fluidity to maintain cellular integrity (Bauer & Pretorius, 2000; Hohmann, 2002). Collectively, the ability of these isolates to sustain growth under hyperosmotic conditions supports their functional potential for fermentation applications and high-sugar food environments.

4. Osmoadaptive response to increasing NaCl concentrations

Based on Table 5, the yeast isolates C32B.1, C35B.1, and C37B.1 were able to survive NaCl concentrations from 2 to 6% after 16 h of incubation. All isolates showed a slight decrease in growth at 4% NaCl, followed by partial recovery at 6%, indicating that although salt stress reduced viability, the cells maintained sufficient tolerance to remain viable. Among the three, C37B.1 showed the most stable growth trend, while C32B.1 was slightly more affected at 4% NaCl. These findings confirm that the isolates possess osmotolerance, a key attribute for probiotic candidates exposed to osmotic stress during food processing or gastrointestinal transit. A similar pattern was reported by Divyashree et al. (2021), where probiotic isolates tolerated 2–10% NaCl with decreasing viability at higher concentrations, but still maintained growth. This similarity suggests that moderate reductions in cell numbers under salt stress are common but do not eliminate probiotic potential. Therefore, the ability of C32B.1, C35B.1, and C37B.1 to remain viable at up to 6% NaCl further supports their suitability as promising probiotic candidates.

Table 5 Growth of selected yeast isolates at different NaCl concentrations

Isolate	Concentration of NaCl	Absorbance (600 nm)*
C32B1	2%	8.01±0.17
	4%	7.05±0.17
	6%	7.57±0.91
C35B1	2%	7.87±0.10
	4%	7.34±0.51
	6%	7.84±0.59
C37B1	2%	8.08±0.67
	4%	7.22±0.40
	6%	7.84±0.47

Remark: *Values are presented as mean±standard deviation (SD) of 3 replicates.

5. Growth patterns at various incubation temperatures

The temperature tolerance assay revealed that all three yeast isolates were able to survive within the temperature range of 30, 37, and 42°C (Table 6). At 37°C, the average absorbance values ranged between 1.72–1.83, which were slightly lower compared to those observed at 30°C (1.79–1.91). Interestingly, at 42°C, isolates C32B.1 and C35B.1 exhibited a recovery in absorbance, approaching values similar to those recorded at 30°C, while isolate C37B.1 maintained relatively stable growth across all tested temperatures. These results suggest that the reduction in viability at 37°C reflects an adaptive response to the host's physiological condition, whereas the ability to withstand 42°C highlights their resilience to extreme thermal stress.

Table 6 Growth of selected yeast isolates at different incubation temperatures

Isolate	Absorbance (600 nm)*		
	30°C	37°C	42°C
C32B.1	1.80±0.01	1.72±0.03	1.85±0.00
C35B.1	1.88±0.01	1.71±0.04	1.82±0.03
C37B.1	1.91±0.02	1.83±0.11	1.80±0.04

Remark: *Values are presented as mean±standard deviation (SD) of 2 replicates.

Comparable findings were reported by Zahoor et al. (2021), who observed that most yeast isolates could grow at 30 and 37°C, while only a small subset (12 out of 104 isolates) tolerated 42°C. Since mammalian body temperature typically ranges from 37°C to an upper physiological limit of approximately 42°C, evaluating microbial performance within this range is considered essential for probiotic characterization (Alkalbani et al., 2022; Staniszewski & Kordowska-Wiater, 2021). Previous studies on *Saccharomyces cerevisiae* and *S. cerevisiae* var. *boulardii* have documented significant reductions in growth when exposed to elevated temperatures of 42–48°C (Alkalbani et al., 2022). In contrast, the isolates in our study maintained stable viability at 42°C, suggesting that C32B.1, C35B.1, and C37B.1 may possess stronger thermotolerance mechanisms, thereby enhancing their suitability for potential application in high-temperature food processing environments.

6. Antibiotic susceptibility profile of the yeast isolates

The antibiotic susceptibility assay revealed that the yeast isolates C32B.1, C35B.1, and C37B.1 exhibited no inhibition zones against ampicillin, streptomycin, tetracycline, or kanamycin, indicating strong resistance to all tested antibiotics (Fig. 2). This intrinsic resistance is consistent with the structural properties of yeast cell walls, which contain chitin and β -glucans that impede antibiotic penetration, as well as the fundamental differences in cellular targets between eukaryotic yeasts and prokaryotic bacteria (Goel & Halami, 2024). Similar findings were reported in previous studies, where *S. cerevisiae*, *T. delbrueckii*, and *S. boulardii* strains demonstrated resistance to antibacterial agents while remaining susceptible only to certain antifungal compounds, confirming their safety and stability during antibiotic co-administration (Alkalbani et al., 2022; Vrinceanu et al., 2025). Collectively, the antibiotic resistance profile of the Chao-derived isolates supports their potential as robust probiotic candidates capable of maintaining viability and functional activity even under antibiotic treatment conditions.

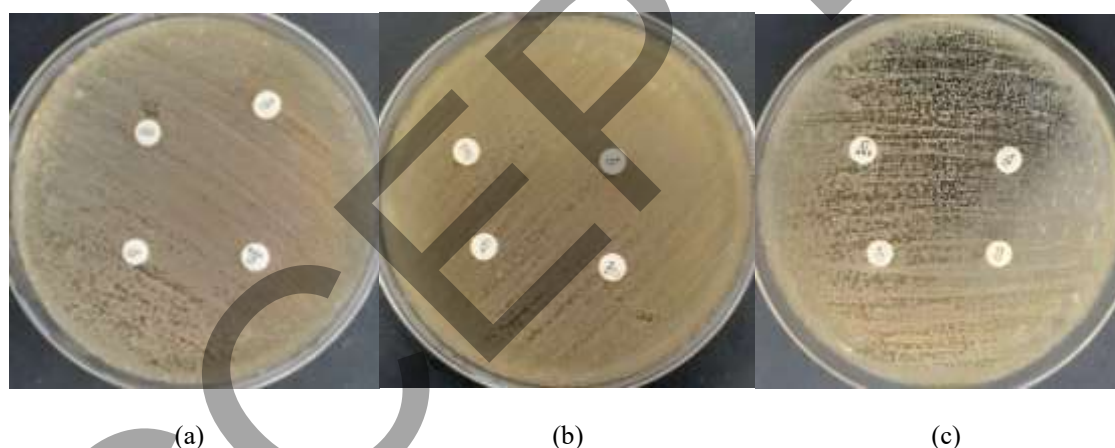


Fig. 2 Antibiotic susceptibility of selected yeast isolates determined by the disk diffusion method. The absence of inhibition zones indicates resistance to the tested antibiotics. (a) C32B.1, (b) C35B.1, and (c) C37B.1.

7. Coaggregation ability with other microorganisms

All three isolates exhibited a dose-dependent co-aggregation profile across the tested cell suspension concentrations (Fig. 3). At the lowest concentration (20%), co-aggregation values ranged between 17–24% across isolates. A gradual increase was observed at 40% (26–34%) and 60% (40–48%), followed by a more pronounced rise at 80% (56–64%). The highest co-aggregation levels were consistently recorded at 100%, ranging from 66–74%. Among the isolates, C32B.1 exhibited the strongest co-aggregation capacity, with the highest value (73–74%) against *L. monocytogenes*. C35B.1 demonstrated intermediate activity, while C37B.1 showed slightly lower values but followed a similar trend.

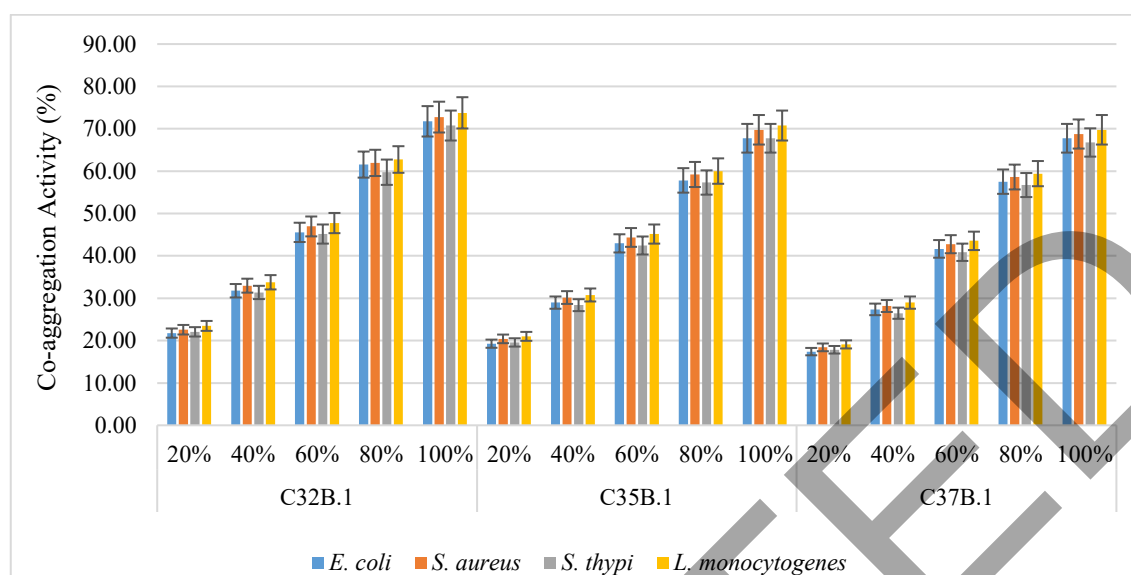


Fig. 3 Co-aggregation ability of selected yeast isolates with pathogenic bacteria

These findings are consistent with previous studies reporting that probiotic yeasts can effectively co-aggregate with both Gram-positive and Gram-negative pathogens, thereby enhancing their ability to prevent pathogen adhesion and colonization in the host gastrointestinal tract (Hati et al., 2023; Ogunremi et al., 2015; Yildiran et al., 2019). Such co-aggregation ability is considered an important probiotic trait because physical association between probiotic and pathogenic cells can entrap pathogens and block their adhesion sites in the intestinal epithelium, thereby reducing colonization (Ogunremi et al., 2015). Overall, the co-aggregation patterns observed in this study suggest that these isolates possess promising adhesion-related probiotic characteristics that may contribute to pathogen inhibition in the gastrointestinal tract.

8. Cell surface hydrophobicity levels

The hydrophobicity assay (Fig. 4) demonstrated that all three probiotic candidate yeast isolates (C32B.1, C35B.1, and C37B.1) exhibited a concentration-dependent increase in cell surface hydrophobicity. At the lowest concentration (20%), hydrophobicity values were relatively low, ranging from approximately 17–24%. However, these values increased markedly with increasing cell concentration, reaching approximately 65–75% at 100%, indicating enhanced interaction with the hydrophobic phase (xylene). This observation supports the well-established role of cell surface hydrophobicity as a key determinant of microbial adhesion and colonization ability in probiotic organisms (Chelliah et al., 2016; Suvarna et al., 2018).

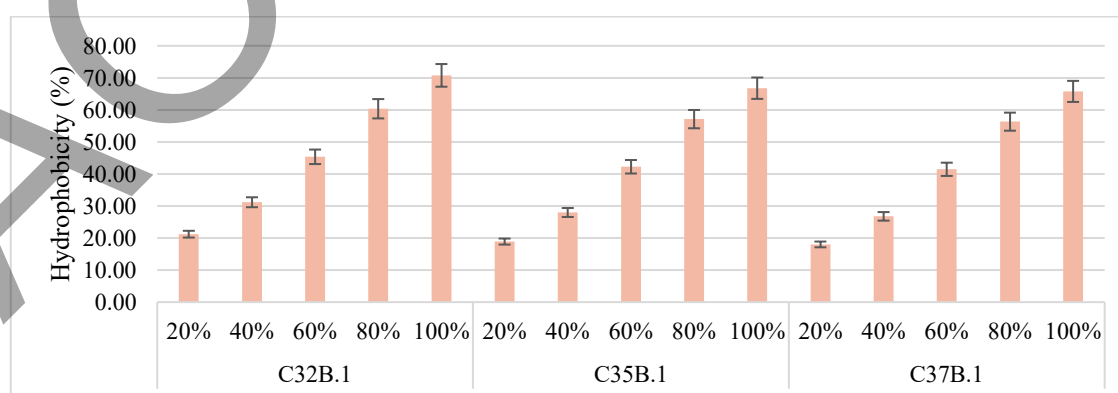


Fig. 4 Cell surface hydrophobicity of selected yeast isolates at different cell suspension concentrations

Among the isolates, C32B.1 consistently exhibited the highest hydrophobicity values across all tested concentrations, reaching approximately 70–75% at 100% concentration. In contrast, C35B.1 and C37B.1

showed slightly lower but comparable values. These differences indicate strain-specific variations in cell surface composition, which may influence their adhesion potential and ecological fitness in the gastrointestinal environment. In general, hydrophobicity values exceeding 40% are considered indicative of strong surface hydrophobicity, which is associated with enhanced interaction with host epithelial surfaces (Lara-Hidalgo et al., 2019). This observation supports the well-established role of cell surface hydrophobicity as a key determinant of microbial adhesion and colonization ability in probiotic organisms (Chelliah et al., 2016; Suvarna et al., 2018).

9. Functional bioactivities of the yeast isolates

The bioprospecting assay of the three probiotic candidate isolates (C32B.1, and C35B.1, C37B.1) revealed measurable antidiabetic, antioxidant, and anti-inflammatory activities, all of which increased progressively with extract concentrations ranging from 20 to 100% (Fig. 5). At lower concentrations (20–40%), the isolates exhibited modest activity, indicating a basal level of functional potential. As concentrations increased to 60 and 80%, a significant rise in activity was observed, particularly in antioxidant and antidiabetic assays, reflecting the presence of bioactive metabolites capable of scavenging free radicals and inhibiting carbohydrate-hydrolyzing enzymes.

At the highest concentration (100%), all isolates achieved their peak activity across the three assays. C35B.1 showed the strongest antidiabetic effect (70.20%), slightly higher than C37B.1 (69.87%) and C32B.1 (67.87%). This trend is consistent with previous studies showing that yeasts possess notable antidiabetic potential, primarily attributed to their ability to inhibit α -amylase and α -glucosidase enzymes, produce bioactive metabolites, and accumulate functional polysaccharides such as β -glucans (Ragavan & Das, 2020). Furthermore, several indigenous fermented-food isolates have been shown to outperform commercial probiotic strains in antidiabetic activity, highlighting their potential for use in functional foods and therapeutic applications.

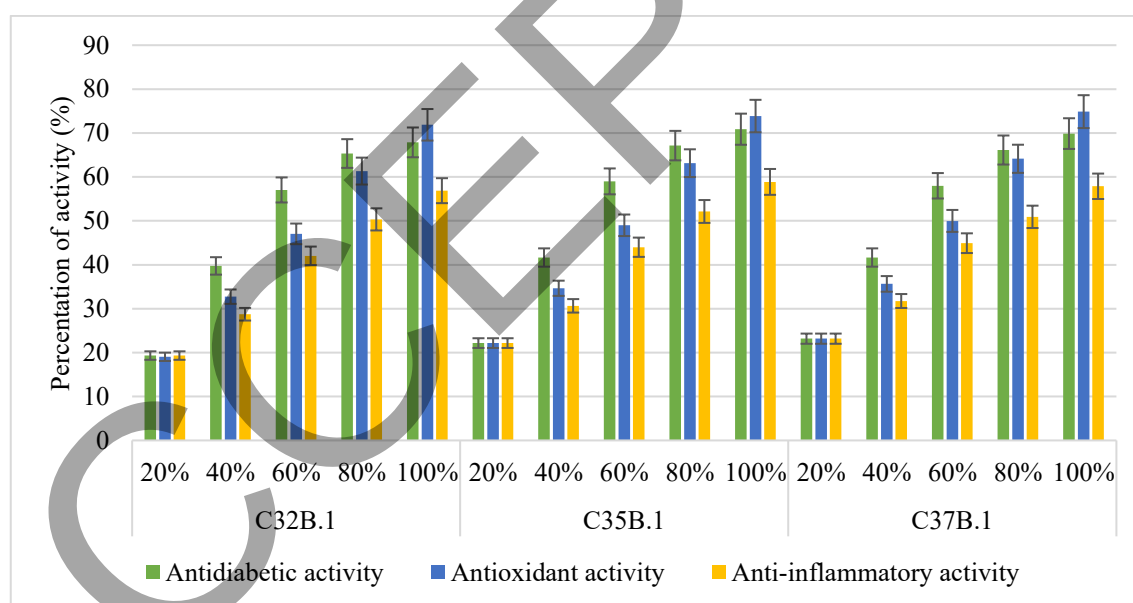


Fig. 5 Functional bioactivities of selected yeast isolates, including antidiabetic, antioxidant, and anti-inflammatory at different concentrations

In antioxidant assays, isolates C37B.1 and C35B.1 demonstrated relatively higher activity (>73%) compared to C32B.1 (71.87%). These values indicate strong free-radical-scavenging capacity and are consistent with previous findings showing that yeasts generally exhibit high antioxidant activity, often exceeding 80% in DPPH assays (Weerabaddhanage et al., 2025). The variation observed among isolates in this study may be attributed to the presence of antioxidant-related enzymes such as superoxide dismutase, glutathione peroxidase, and catalase, as well as cellular components, including exopolysaccharides and lipids, that contribute to free radical neutralization (Ampemohotti et al., 2025; Bastos et al., 2025; Xu et al., 2011). This aligns with earlier reports demonstrating that yeast isolates from diverse food sources typically show strong but variable antioxidant activity depending on species, strain, and their associated bioactive compounds (Lara-Hidalgo et al., 2019; 2017).

In the anti-inflammatory assay, isolate C35B.1 exhibited the highest activity (58.87%), followed

by C37B.1 (57.87%) and C32B.1 (56.87%), indicating a moderate ability to suppress inflammatory responses. These findings are consistent with previous studies reporting that indigenous yeasts also possess promising anti-inflammatory potential, particularly strains with high β -glucan content and strong antioxidant capacity (Hati et al., 2023; Siesto et al., 2022). Overall, C35B.1 emerged as the most potent isolate with consistently strong antidiabetic and anti-inflammatory activities, while C37B.1 demonstrated superior antioxidant potential, and C32B.1 showed slightly lower but still significant activities. Similar results were reported by Khan et al. (2021), who demonstrated that probiotic candidates derived from natural environments and fermented foods are capable of producing bioactive compounds with significant functional effects, thereby supporting the functional potential observed in the isolates of the present study. The clear dose-dependent increase in bioactivity underscores the functional relevance of metabolite concentration, reinforcing the potential of these isolates as multifunctional probiotic candidates for further development and characterization.

10. Molecular identification and phylogenetic confirmation

Molecular identification through 18S rRNA gene sequencing analysis revealed that the three yeast isolates (C32B.1, C35B.1, and C37B.1) showed high sequence similarity to *Pichia kudriavzevii* (99.74–99.75%) when compared with the reference sequence in GenBank (NG_063274.1) (Table 7). These sequence similarities support the assignment of the isolates to *P. kudriavzevii*. This identification was further supported by the phylogenetic tree, with all three isolates clustered together within the *P. kudriavzevii* clade with strong bootstrap support, clearly separating them from other closely related *Pichia* species and demonstrating their evolutionary proximity to the reference strain (Fig. 6). These findings are consistent with previous studies that identified *Pichia* species, particularly *P. kudriavzevii*, as promising probiotic yeast isolated from various traditional fermented foods, including fermented fish, fermented cereals, and fermented dairy products (Chelliah et al., 2016; Ogunremi et al., 2015). These studies highlight the recurrent association of *Pichia* spp. with probiotic-related properties, strengthening the relevance of the present results.

Table 7 Comparative sequence analysis of selected yeast isolates to reference sequence based on 18S rRNA gene analysis

Isolate	Closest relative species	GenBank accession number	Similarity level
C32B.1	<i>Pichia kudriavzevii</i> NRRL Y-5396 18S rRNA gene, partial sequence; from TYPE material	NG_063274.1	99,74%
C35B.1	<i>Pichia kudriavzevii</i> NRRL Y-5396 18S rRNA gene, partial sequence; from TYPE material	NG_063274.1	99,74%
C37B.1	<i>Pichia kudriavzevii</i> NRRL Y-5396 18S rRNA gene, partial sequence; from TYPE material	NG_063274.1	99.75%

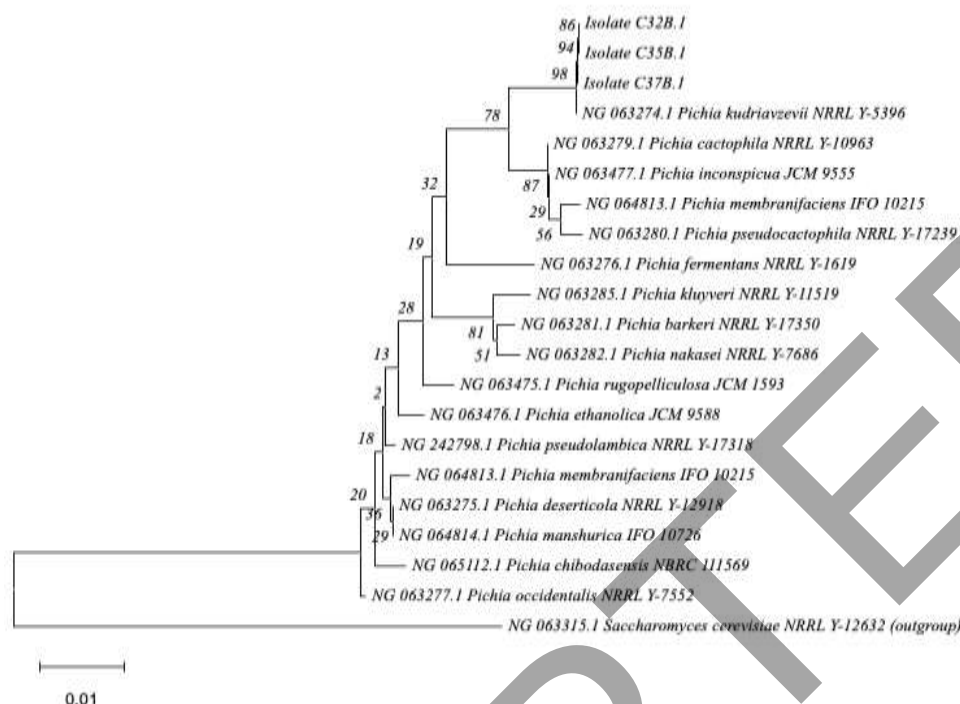


Fig. 6 Neighbour-joining phylogenetic tree based on partial 18S rRNA gene sequences of the selected yeast isolates and related reference strains. Evolutionary distance was computed using the Maximum Composite Likelihood method, and bootstrap values (10,000 replicates) are indicated at the branch nodes.

The finding that *P. kudriavzevii* is the dominant species among the yeast isolates passing probiotic screening provides important insights into its biotechnological potential. This species has also been previously reported in traditional fermented vegetable foods (Chelliah et al., 2016; Ogunremi et al., 2015). Known for its adaptive capacity to extreme environmental conditions, *P. kudriavzevii* has been documented to exhibit functional probiotic properties, including tolerance to low pH and bile salts, alongside antagonistic activity against intestinal pathogens (Chelliah et al., 2016; Ganapathiwar & Bhukya, 2023; Lata et al., 2022). Thus, the identification of *P. kudriavzevii* in these isolates underscores its role as a promising yeast species for the development of probiotics derived from traditional fermented foods. These results also strengthen the evidence that local microbial resources can contribute significantly to the exploration of biological diversity for health-related applications.

Conclusion

Yeast isolates recovered from traditionally fermented Chao in Pangkep, South Sulawesi, were screened for probiotic potential, resulting in the selection of three promising candidates (C32B.1, C35B.1, and C37B.1). Based on partial 18S rRNA gene sequencing, these isolates showed high sequence similarity to *Pichia kudriavzevii* and exhibited tolerance to acidic conditions, bile salts, elevated NaCl concentrations, and temperature stress, as well as resistance to multiple antibiotics. The isolates also displayed favorable adhesion-related properties, including high cell surface hydrophobicity and moderate to strong co-aggregation ability, suggesting their potential for intestinal adhesion and interactions with resident microbiota. Furthermore, their ability to grow under high-glucose conditions indicates good physiological adaptability to osmotic stress. Functional characterization demonstrated multiple biofunctional properties, including α -glucosidase inhibitory, antioxidant, and anti-inflammatory activities, all of which increased in a concentration-dependent manner. Collectively, these findings support the potential of *P. kudriavzevii* isolates from Chao as promising probiotic candidates for future application in functional food development.

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Conflict of interest

The authors would like to declare no conflict of interest.

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this manuscript, the authors declare that no generative AI or AI-assisted technologies were used in the writing process of this manuscript.

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