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Isolation and Characterization of Yeast from the Digestive Tract of Chicken as a Probiotic Candidate

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Abstract

The use of yeast in poultry farming in Indonesia remains underexplored, with applications limited primarily to the genus *Saccharomyces*. Yeast, recognized as part of the gut microbiota, holds considerable promise as a probiotic. This study evaluated the probiotic properties of yeast isolated from the gastrointestinal tract of native chickens and assessed their potential health-promoting effects for both egg-laying and meat breeds. Six isolates were tested for pathogenicity, bile salt and acid tolerance, autoaggregation, and coaggregation. Antibacterial activity against *Salmonella enterica* serovar Typhimurium ATCC14028 and molecular identification were also performed. Results showed that six isolates were resilient to gastrointestinal stress and exhibited strong aggregation ability. But, only UBB1 demonstrated notable antibacterial activity, with an inhibition index of 2.09 against *S.* Typhimurium ATCC14028. The combination of acid and bile tolerance, robust aggregation, and antibacterial activity in UBB1 underscores its potential as a poultry probiotic to enhance gut health and disease resistance. Molecular characterization using ITS sequencing identified UBB1 as *Pichia kudriavzevii*, a non-pathogenic yeast widely associated with fermented foods. These findings support the development of yeast-based probiotic feed additives as a promising strategy to reduce antibiotic reliance and promote sustainable poultry production, such an approach could ultimately improve poultry health.

Keywords: *Pichia kudriavzevii;* antibacterial; chicken.

INTRODUCTION

Poultry is among the most popular types of animal protein today, specifically chicken. (Wessel *et al*. 2021). Data from the Central Statistics Agency (BPS) (2023) from 2016 to 2022 showed that chicken production in Indonesia continued to increase for both egg-laying and meat breeds. However, improper husbandry and high population density enhance susceptibility to infections from pathogenic microorganisms (Sibinelli *et al*. 2022). Among the most common pathogens is *Salmonella*, a bacterium frequently linked to poultry infections. The resulting disease, salmonellosis, can cause digestive disorders, reduced productivity, and in severe cases, death in chickens (Wessel *et al*. 2021).

Salmonella infections in chickens are typically treated with antibiotics. The medication is capable of triggering resistance when used long-term (Ariyanti *et al*. 2023). Therefore, the Indonesian government has banned the addition of growth promoters (AGPs) through Law No. 18/2009 in conjunction with Regulation No. 41/2014. The law prohibits certain hormones and antibiotics in animal feed to prevent drug residues and resistance. Ministry of Agriculture Regulation (PERMENTAN) No. 14/Permentan/PK.350/5/2017 stated that antibiotics may only be used for specific therapies, not AGPs. Probiotics are used as an alternative due to their non-pathogenic nature and ability to stimulate the growth of gut microbiota, thereby improving host health (Abid *et al*. 2022). Microorganisms commonly discovered as probiotic starter cultures include lactic acid bacteria (LAB) from the genera *Lactobacillus* and *Bifidobacterium* (Borody 2016).

In addition to lactic acid bacteria, yeast exhibits considerable potential as a probiotic. Bidura (2019) reported that supplementing chickens with probiotic yeast has the potential to enhance egg mass, feed efficiency, and egg yolk percentage, while reducing cholesterol levels. Currently, the use of probiotics in poultry is limited mainly to the genus *Saccharomyces.* To explore yeast species with probiotic potential, Wulan *et al*. (2021) demonstrated that *Pichia kudriavzevii,* derived from cocoa fermentation, exhibits high antioxidant activity and is capable of growth at temperatures up to 41°C, making it a promising candidate for use as a probiotic in poultry. The discovery of probiotic yeast

from cocoa opens up opportunities for exploring more specific probiotic yeasts derived from the host that could be more effective in supporting poultry health. This study aims to evaluate the probiotic properties of yeast isolated from the chicken digestive tract in Indonesia, as well as its potential to support poultry health.

MATERIALS AND METHODS

Materials

The material used in this study included the gastrointestinal tract of native chicken (large intestine, small intestine, and cecum), yeast isolates isolated from the gastrointestinal tract and feces of native chicken, the pathogenic microorganism used was *Salmonella enterica* serovar Typhimurium ATCC14028, YPD (Yeast Peptone Dextrose) media, NA (Nutrient Agar), NB (Nutrient Broth), bacto agar, blood agar (sheep blood 5%, NaCl, peptone 0.5%, yeast extract 0.3%, agar 1.5%), PBS (Phosphate-buffered saline), bile salt (Himedia), NaOH, HCl, YeaStar Genomic DNA Kit™ (zymoreseach, USA), ITS5 and ITS4 primers.

Isolation and Morphological Observation of Yeast

Yeast isolation was conducted using digestive tract samples from native chicken sold at Ciampea and Anyar Markets in Bogor, West Java, Indonesia. The samples included the lumen of the small intestine, cecum, large intestine, and feces. The procedure followed a modified method described by Torshizi *et al*. (2008). Each sample was cleaned, chopped, and weighed to 1 g, then serial dilutions were prepared using sterile physiological saline (0.85% NaCl) as the diluent. A series of test tubes containing 9 mL of saline were arranged and labeled up to 10^2. From each dilution, 100 µl was plated onto Yeast Peptone Dextrose (YPD) agar and incubated at 41 °C for 24 hours. After incubation, colonies with typical yeast morphology were selected and purified by repeated quadrant plating on YPD agar until single colonies were obtained.

Colony morphology was observed after 3 days of incubation using the quadrant streaking method on YPD agar. The observed characteristics included shape, color, elevation, margin, and opacity. Yeast cell morphology was examined from 24-hour-old cultures grown on YPD agar using an Olympus CX23 binocular LED microscope equipped with the Indomicro application, and no staining was applied during observation. A single colony was suspended in sterile distilled water on a glass slide, while observations focused on cell size, shape, and budding.

Yeast Pathogenicity Test (Hemolysis)

Pathogenicity testing of the previously isolated yeast was conducted using blood agar, following the method described by Gut *et al.* (2019). The isolates were streaked onto blood agar medium composed of 5% sheep blood, 0.5% NaCl, 0.3% peptone, 0.3% yeast extract, and 1.5% agar, then incubated at 41 °C for 48 hours. After incubation, hemolytic activity was evaluated based on clear zones around the colonies. Yeast isolates that did not produce clear zones were considered non-pathogenic and were used for further observations.

Bile Salt Resistance Assay

Non-hemolytic yeast isolates were tested for resistance to bile salts using a modified method from Gut *et al.* (2019). A total of 0.1 ml of 18-hour-old starter culture was inoculated into 10 ml of liquid YPD medium containing 0.5% bile salts (Himedia) and incubated at 41 °C for 5 hours in a shaking incubator. Cell density (optical density, OD) was measured at 0 and 5 hours of incubation using a spectrophotometer at 600 nm.

Acidic pH Resistance Assay

Yeast isolates that were able to grow under bile salt stress were subsequently tested for acid resistance using a modified method from Huynh *et al.* (2017). Liquid YPD medium was adjusted to pH 3 using 1 M HCl and 1 M NaOH, then inoculated with 1% yeast culture and incubated at 41 °C for 5 hours. Acid tolerance was assessed by calculating the difference in log colony-forming units (CFU) between the initial time point (T0) and after 5 hours of incubation (T5).

Autoaggregation and Coaggregation Assay

Yeast isolates capable of growing under acidic pH conditions were further tested for autoaggregation using the method described by Panjaitan *et al.* (2018). For the autoaggregation test, the isolates were cultured in liquid YPD medium at 41 °C for 24 hours. Cells were harvested by centrifugation at 3500 rpm for 20 minutes, and the resulting pellets were washed twice with phosphate-buffered saline (PBS) and adjusted to a final concentration of 10^8 CFU/ml. Autoaggregation was evaluated by measuring absorbance at 0 and 4 hours. A 0.1 ml aliquot of the upper suspension was transferred to 3.9 ml of PBS, and the absorbance was measured at 600 nm using a spectrophotometer. The percentage of autoaggregation was calculated by applying the following formula:

$$Autoaggregation\ (\%) = \left(1 - \left(\frac{A_t}{A_0}\right)\right) x\ 100\%$$

At = Absorbance after incubation for 5 hours
A0 = Initial absorbance (0 hours)

In parallel with the autoaggregation test, a coaggregation assay was conducted using the pathogenic isolate *Salmonella* Typhimurium ATCC14028, following the method of Ladha and Jeevaratnam (2018). A 0.5% yeast starter culture was inoculated into liquid YPD medium and incubated at 41 °C for 24 hours. Similarly, a 0.5% bacterial starter culture of *S. Typhimurium* ATCC14028 was inoculated into Nutrient Broth (NB) and incubated at

room temperature for 24 hours. After incubation, both cultures were centrifuged, and the pellets were washed twice and resuspended in phosphate-buffered saline (PBS) at pH 7.2. The test mixture consisted of 2 mL each of yeast and *S.* Typhimurium ATCC14028 suspensions, which were vortexed and incubated at 41 °C for 4 hours. Optical density (OD_{600}) was measured at 0 and 4 hours, and the percentage of coaggregation was calculated by applying the following formula:

$$Coaggregation\ (\%) = \frac{(\frac{Ax+Ay}{2}) - A(x+y)}{(\frac{Ax+Ay}{2})} \times 100$$

$A_{(x+y)}$ = Absorbance of yeast isolate and pathogen suspension mixtures
A_x = Absorbance of yeast isolate suspension
A_y = Absorbance of pathogen isolate

Antibacterial Activity against Pathogenic Bacteria

Yeast isolates with high coaggregation values against *S.* Typhimurium ATCC14028 were tested for antibacterial activity using the well diffusion method. A 14-hour-old culture of *S.* Typhimurium ATCC14028 grown in NB was mixed into warm Nutrient Agar (NA) to a final bacterial concentration of 10^7 CFU/mL. After the medium solidified, wells with a diameter of 0.5 cm were created through a sterile cork borer. Yeast cultures aged 72 hours in liquid YPD medium were centrifuged at 5000 rpm for 15 minutes at 41 °C, and the supernatants were used in the assay. The test was conducted in triplicate, and the zones of inhibition (clear zones) around the well were observed and their diameters were measured using the ImageJ application to calculate the inhibition index. The inhibition index (IP) is calculated using the following formula:

$$\text{Inhibition index} = \frac{\text{Clear zone diameter} - \text{well diameter}}{\text{well diameter}}$$

Identification of Yeast Based on Internal Transcribed Spacer (ITS) Sequence

Genomic DNA was isolated from selected yeast isolates grown for 18 hours in liquid YPD medium using the YeaStar™ Genomic DNA Kit. The concentration and purity were measured through a NanoDrop spectrophotometer (MaestroGen, Taiwan). The extracted DNA was then adopted as a template to amplify the ITS region. Amplification was conducted using GoTaq® Green Master Mix 2X (Promega, USA) in a polymerase chain reaction (PCR) machine (Applied Biosystems™ 2720 Thermal Cycler, USA), following the manufacturer's protocol. The primers applied were ITS5 forward (5′-GGAAGTAAAGTCGTAACAAGG-3′) and ITS4 reverse (5′-TCCTCCGCTTATTGATATGC-3′) (White *et al*. 1990). PCR conditions include pre-denaturation at 94 °C for 4 minutes, 30 cycles of denaturation at 94 °C for 30 seconds, annealing at 49 °C for 30 seconds, and extension at 68 °C for 1 minute. This was followed by a final extension at 68 °C for 20 seconds and cooling at 15 °C for 5 minutes. The PCR products (amplicons) were visualized by electrophoresis on a 0.8% agarose gel at 75 V for 30 minutes. Amplicon sequencing was performed through a commercial sequencing service, and analysis of the obtained DNA sequences began with contig assembly using SeqTrace software. The resulting sequences were aligned with reference sequences in GenBank using the Basic Local Alignment Search Tool for nucleotides (BLASTn), available at https://blast.ncbi.nlm.nih.gov. The BLAST results were used to identify species with the highest sequence similarity. Furthermore, sequences with the highest percentage of identity were selected for further analysis. Phylogenetic analysis was conducted using Molecular Evolutionary Genetics Analysis (MEGA) software version 6.0. The phylogenetic tree was constructed by adopting the Neighbor-Joining method with the Kimura 2-parameter + Gamma Distribution model (complete deletion option), and the reliability was evaluated through a bootstrap test at 1000 replicates (Tamura *et al.* 2013).

Data analysis

The data obtained from this study were analyzed using R Studio 4.5.1

RESULTS AND DISCUSSION

Isolation and Observation of Yeast

A total of six yeast isolates were obtained from the digestive tracts of native chickens and subsequently subjected to experimental evaluation. Morphological examination demonstrated distinct variations among the isolates, characterized by cell size and shape differences. The cells appeared as small or large spherical forms, ovoid or cylindrical structures, and in some cases exhibited pseudohyphal-like formations, suggesting a notable degree of morphological plasticity that may be associated with their ecological adaptation and potential functional roles in the gastrointestinal tract (Figure 1).

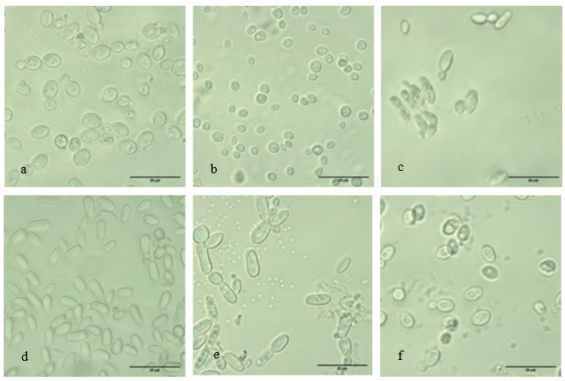


Figure 1. The results of observations of yeast cells under a microscope show that yeast has cell shapes of (a) large round, (b) small round, (c) oval, (d) cylindrical forms, (e) structures resembling pseudohyphae, and (f) egg-shaped.

Yeast Pathogenicity Test (Hemolysis)

All yeast isolates were tested for the ability to hemolyze red blood cells on blood agar to assess potential safety concerns associated with their probiotic application. The results indicated that all six isolates showed no evidence of hemolysis and were therefore categorized as ϒ hemolysis

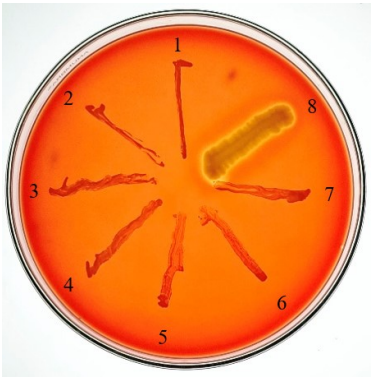


Figure 2. Yeast growth on blood agar after 48 hours incubation at 41 °C (1) FJ1, (2) FJ3, (3) SKJB, (4) SKJK, (5) UBB1, (6) UBK1. (7) Negative Control (*Saccharomyces cerevisiae*), (7) Positive Control (*Candida albicans*).

Bile Salt Resistance Assay

The results showed that six isolates tested could grow well on YPD medium supplemented with 0.5% bile salt media after 5 hours of incubation, indicating their capacity to tolerate bile stress, an essential characteristic for survival in the gastrointestinal tract. These isolates were subsequently subjected to additional testing to determine their tolerance to acidic pH conditions, thereby providing further insight into their potential as robust probiotic strains capable of withstanding multiple gastrointestinal challenges (Figure 3).

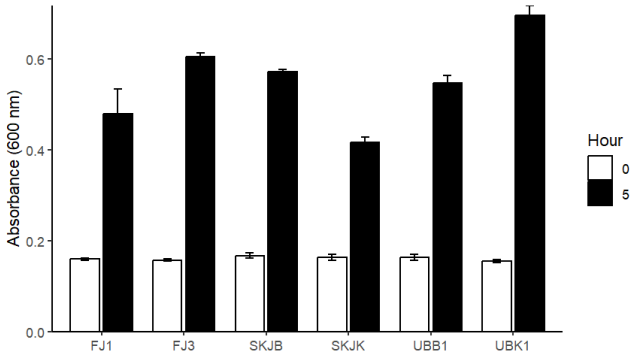


Figure 3. Growth of yeast isolates in liquid yeast peptone dextrose (YPD)+0.5% bile salt media after 5 hours. UBK1 showed the best results, while SKJK showed the lowest value among other isolates.

Acidic pH Resistance Assay

The test showed that all isolates could grow at pH 3 after 5 hours of incubation, indicating their potential to withstand the acidic environment of the stomach. This acid tolerance is a critical prerequisite for probiotic functionality, as it determines the likelihood of the isolates reaching the intestine in a viable state. These six isolates were selected for autoaggregation and coaggregation tests against *S.* Typhimurium ATCC14028 (Table 1)

Table 1. The acidic pH stress test results showed that the SKJK isolate had the best survival ability compared to other isolates.

Isolate	Dilution Factor	0 Hour (± SE)	5 Hours (± SE)
SKJB	10^{-3} CFU/g	142 ± 3,3	180 ± 2,3
FJ1	10^{3} CFU/g	53 ± 4,09	116 ± 2,9
UBB1	10^{-3} CFU/g	53 ± 2,08	117 ± 3,1
FJ3	10^{-3} CFU/g	45 ± 2,7	129 ± 3,05
UBK1	10^{-3} CFU/g	29 ± 2,4	95 ± 22,3
SKJK	10^{-3} CFU/g	111 ± 2,3	190 ± 1,2

Autoaggregation and Coaggregation Assay

The test results showed that all yeast isolates had varying autoaggregation abilities and experienced an increase in the 4th hour of incubation (Figure 4). Furthermore, the coaggregation ability against *S.* Typhimurium ATCC14028 showed differences among the isolates, with UBB1 displaying the highest coaggregation percentage after 4 hours of incubation compared to the other strains. This strong coaggregation capacity, which indicates the potential of UBB1 to interact with pathogenic bacteria competitively, provides reassurance about its candidacy as a promising probiotic isolate (Figure 5).

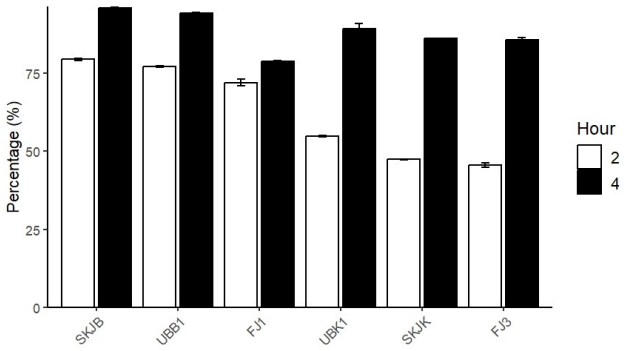


Figure 4. The autoaggregation assay revealed that all isolates exhibited a substantial percentage of autoaggregation, as evidenced by the progressive increase observed between the 2nd and 4th hours of incubation.

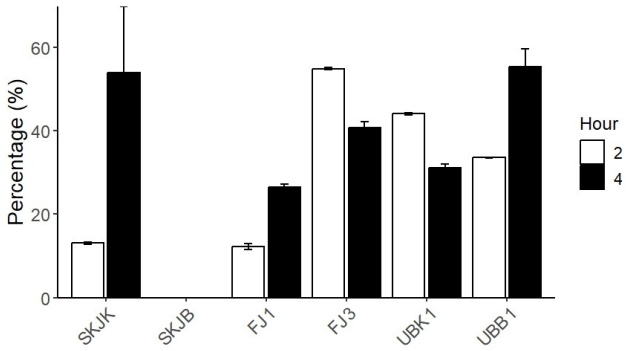


Figure 5. Coaggregation assay results showed that the SKJB isolate could not aggregate with *S.* Typhimurium ATCC14028. The UBB1 isolate showed the best aggregation percentage.

Antibacterial Activity against Pathogenic Bacteria

The antibacterial test results against *S.* Typhimurium ATCC14028 bacteria showed that the UBB1 isolate was capable of inhibiting bacterial growth with an inhibition index of 2.09 (Table 2). The yeast culture supernatants were used without prior concentration. Following the incubation period, inhibition zones were evaluated, and among all isolates examined, only UBB1 produced a distinct and measurable clear zone, further confirming its antagonistic activity against the pathogen (Figure 6).

Table 2. Yeast antibacterial assay results against *S.* Typhimurium ATCC14028. Only one (UBB1) demonstrated measurable antibacterial activity, as indicated by the presence of a clear inhibition zone, while the remaining five isolates showed no inhibitory effect.

No.	Isolate	*S.* Typhimurium ATCC14028 Diameter of clear zone (mm)	Inhibition Index (IP)
1	SKJB	-	-
2	SKJK	-	-
3	UBB1	15.45	2.09
4	UBK1	-	-
5	FJ1	-	-
6	FJ3	-	-
7	Positive	21.25	3.25
8	Negative	-	-

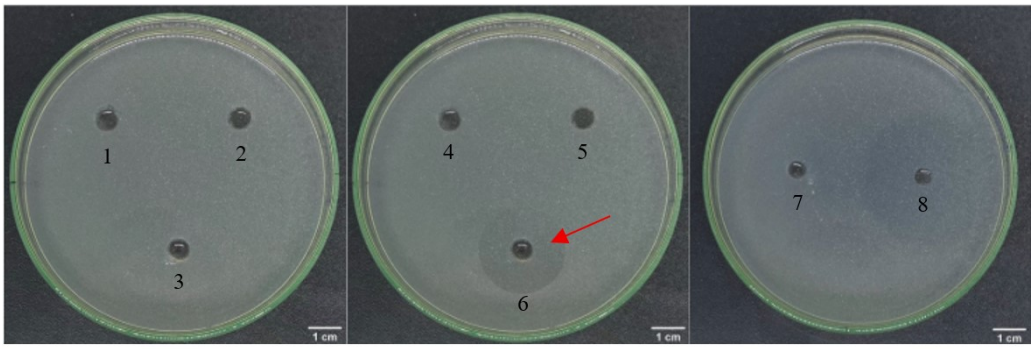


Figure 6. Antibacterial activity of yeast supernatant against *Salmonella* Typhimurium ATCC14048. UBK1 (1), SKJK (2), SKJB (3), FJ1(4), FJ3 (5), UBB1 (6), Negative control (7), Positive control (8). The UBB1 isolate showed antibacterial activity (arrow).

Identification of Yeast Based on ITS Sequence

The obtained and arranged sequence was registered in GenBank (https://www.ncbi.nlm.nih.gov/) with the ITS number PV960659. The blast results displayed that the yeast had 100.00% similarity to *P. kudriavzevii* ATCC 6258 as the top hits. The phylogenetic tree (Figure 7) shows that PV960659 is in the same clade as *P. kudriavzevii* ATCC 6858 with a bootstrap value of 100%, which can strengthen the BLAST results. This phylogenetic tree also shows the division of the genus *Pichia* into several clades, including *P. bovicola, P. exigua, P. cephaloecera,* and *P. eremophila,* with a bootstrap value of 99%.

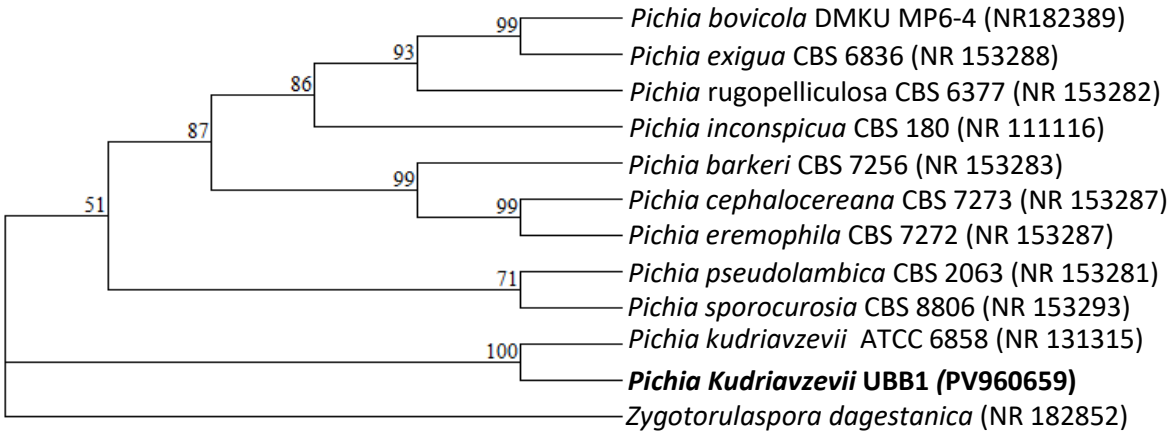


Figure 7. The phylogenetic tree of the yeast ITS region was compared with the ITS region sequences available from the NCBI data collection. *Zygotorulaspora dagestanica* was used as the outgroup. The tree was constructed using the Neighbor Joining (NJ) method with the p-value model.

Discussion

The selection of a probiotic candidate includes ensuring that the tested microorganisms are non-pathogenic and safe for living organisms. Pathogenicity in yeast can be assessed using blood agar medium, and it is signified by a clear zone around colonies or a greenish-brown discoloration of the medium (Almwafy, 2020). In the current study, a total of six isolates were tested, and the results showed were non-pathogenic. The non-pathogenic isolates were subsequently used in resistance testing of bile salt, which acts as a stressor in the small intestine (Alkabani *et al*. 2022).

Probiotic agents are expected to colonize and survive under bile salt stress conditions, which is known to damage cell membranes and lead to cell death. (Shao *et al.* 2021; Azhar and Munaim. 2023). Based on the result, six yeast cultures showed vigorous growth during the assay, as indicated by their higher OD compared to the other isolates, demonstrating their ability to tolerate the membrane-disrupting effects of bile salt, particularly under 0.5% bile salt conditions after 5 hours of incubation. The increase in cell density within liquid media from 0 to 5 hours varied, with the highest value observed in UBK1, which had an OD value greater than 0.8. The results show a favorable comparison with a previous study by Hsu and Jui (2021), who showed that yeast densities of *S. cerevisiae*, *S. fibuligera,* and *P. kudriavzevii* had OD values of less than 0.5 after exposure to 0.3% bile salts for 5 hours. As a result, the eight isolates might survive in the intestinal environment and function effectively when applied to poultry.

To survive and colonize the digestive tract, probiotics must withstand the stomach's acidic environment of pH 2.5–4.0 (Pane *et al*. 2018). In this study, most yeast isolates demonstrated the ability to survive at pH 3.0, with SKJB exhibiting a higher survival rate. These findings are consistent with those of Hu *et al*. (2018), which showed that most yeast isolates from the duck gastrointestinal tract could tolerate acidic environments. This indicates that acidic tolerance is an adaptive trait of gastrointestinal yeasts, although differences exist among isolates in maintaining cell viability. Microscopic observation further revealed that yeasts differ in their ability to support cell morphology under acidic conditions, with three isolates showing changes in cell size when exposed to acidic pH. Isolate FJ1 showed a smaller cell size than its standard size, while isolates FJ3 and UBK1 showed a larger cell size than their standard size. This could have occurred due to the yeast's mechanism for maintaining its viability (Samagaci *et al.* 2014).

An essential criterion for selecting probiotic agents is the ability to autoaggregate, which refers to the capacity of yeast cells to aggregate or adhere to each other (Wang *et al.* 2024). The test results showed that the isolates exhibited an increase in autoaggregation percentage from the second to the fourth hour. The autoaggregation percentage in all tested samples was high, exceeding 60%, indicating that the obtained yeast has considerable potential as a probiotic agent. According to Dessalegn and Andualem (2023), values above 60% are classified as high. The autoaggregation values obtained in the present study were higher than those reported by Kanak and Suzan (2025), who documented maximum and minimum values of 71.9% and 13.8%, respectively, after 4 hours of incubation. Furthermore, five cultures in this study exceeded 90%, and only one isolate was below 90%. This finding indicates that the yeast has strong potential as a probiotic agent.

In addition to the autoaggregation test, a coaggregation test was conducted to evaluate the ability of yeast cells to aggregate or bind with pathogenic bacteria, a property considered in preventing bacterial adhesion to the surface of the digestive tract (Dessalegn and Andualem 2023). The result showed that five out of six isolates could aggregate with *S.* Typhimurium

ATCC14028, showing coaggregation percentages ranging from 13-54% after two hours of incubation, while one isolate did not show aggregation. After four hours, two isolates (FJ3 and UBK1) showed a decrease in aggregation with *S.* Typhimurium ATCC14028, whereas three isolates showed an increase, with the highest value observed in isolate UBB1 (55.24%). Kanak and Suzan (2025) reported that *P. kudriavzevii* had the highest coaggregation value at 79%, while *Kloeckera apiculata* recorded the lowest at 49%. According to Panjaitan *et al* (2018), yeasts with coaggregation values between 30% and 100% remain promising probiotic candidates, supporting the potential of five isolates in this study for further development as probiotic agents.

The antibacterial potential of yeast culture supernatants was rigorously evaluated against *S.* Typhimurium ATCC14028 to identify an isolate capable of inhibiting bacterial growth. Among the six supernatants aged 72 hours, only UBB1 showed antibacterial activity. Based on observation, the supernatant used was not concentrated. The inhibition index around the UBB1 well was reached 2.09, which is classified as vigorous activity according to the criteria of Nostro *et al.* (2000). This antibacterial activity may be attributed to the yeast producing volatile compounds, organic acids, and killer toxins (Younis *et al*. 2017). UBB1 has better antibacterial activity than yeast isolated by Cai *et al* (2022), which showed an inhibition index of less than 0.5, and is different from previous research conducted by Wulan *et al* (2021), which did not report any antibacterial activity in yeast isolates isolated from cocoa fermentation. This finding indicates that UBB1, isolated from the digestive tract of native chickens, has excellent potential as a probiotic candidate with superior antibacterial activity, despite the tests being limited to in vitro tests and a small number of isolates.

Based on a series of probiotic candidate selection tests conducted, one potential isolate, UBB1, was identified and showed good antibacterial activity. Molecular identification results using the BLAST program on the NCBI website signified that UBB1 had 100% homology with *P. kudriavzevii* (access number NR_131315.1). This relationship is further supported by phylogenetic tree analysis, which grouped UBB1 in the same clade as *P. kudriavzevii*, reflecting evolutionary proximity. This species has an oval or elongated elliptical shape, and the budding process has dimorphism (Chu *et al*. 2022). *P. kudriavzevii* is commonly discovered in fermented foods such as cheese, kimchi, and cocoa fermentation, making it a strong candidate as a probiotic agent (Lata *et al.* 2022).

CONCLUSIONS

Based on the in vitro evaluation of probiotic properties, *Pichia kudriavzevii* UBB1 isolated from the digestive tract of native chickens was identified as a potential probiotic candidate. The isolate exhibited non-pathogenic characteristics, the ability to survive at 41 °C, resistance to bile salts, tolerance to acidic pH conditions, as well as autoaggregation and coaggregation abilities against *Salmonella Typhimurium* ATCC14028. In addition, the UBB1 isolate demonstrated antibacterial activity with a high inhibition index (2.09), indicating its potential to support poultry health through the suppression of pathogenic bacteria.

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Authors' Contributions: Anja Meryandini & Ivan Permana Putra designed the study. Saroci Dorratul Zahira carried out the laboratory work. Saroci Dorratul Zahira analyzed the data. Saroci Dorratul Zahira wrote the manuscript. All authors read and approved the final version of the manuscript

Competing Interests: The authors declare that there are no competing interests.

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REFERENCES

Abid, R., Waseem, H., Ali, J., Ghazanfar, S., Ali, G.M., Elasbali, A.M., Alharethi SH. 2022. Probiotic yeast *Saccharomyces*: back to nature to improve human health. *J Fungi*. 8(5) 444. https://doi.org/10.3390/jof8050444

Alkabani, N.S., Tareq, M.O., Anas, A.A.N., Amin, N.O., Shao, Q. L., Nagendra, P.S., Vasso, A., Mutamed, M.A., 2022. Assessment of yeast as potential probiotics: A review of gastrointestinal tract condition and investigation method. *J Fungi.* 8(4): 365. https://doi.org/10.3390/jof8040365

Almwafy, A., 2020. Preliminary characterization and identification of gram positive hemolysis bacteria. *Al-Azhar J Pharm Sci*. 62(2):96–109. https://doi.org/10.21608/ajps.2020.118378

Ariyanti, T., Sukatma., Suhaemi., Mulyati, S., Rachmawati, F., Noor, S.M., 2023. Determination of antimicrobial resistance profile of *Salmonella enteritidis* isolated from chicken and human in Indonesia. *IOP Conf Ser Earth Environ Sci*. 1253(1). https://doi.org/10.1088/1755-1315/1253/1/012089

Bidura IGNG. 2019. Suplementasi probiotik *Saccaromyces* spp. Kb-13 dalam ransum terhadap produksi karakteristik kualitas telur, dan kolesterol kuning telur ayam lohmann brown. *Makalah Ilmiah Peternakan.* 22(3) :140-148. https://doi.org/10.24843/MIP.2019.v22.i03.p08

Borody TJ. 2016. Probiotic recolonisation therapy. United State Patent Application.

Cai, J., Lujuan, X., Wangang, Z., Lijuan, F., Jian, Z., 2022. Selection of potential probiotic yeast from dry cured xuanwei ham and identification of yeast-derived antioxidant peptides.

Antioxidants. 11 : 1970. https://doi.org/10.3390/antiox11101970

Chu, Y., Li, M., Jin, J., Dong, X., Xu, K., Jin, L., Qiao, Y., 2023. Advances in the application of the non-conventional yeast *Pichia kudriavzevii* in food and biotechnology industries. *Journal of Fungi*. 9(2):170. https://doi.org/10.3390/jof9020170

Dessalegn, M., Andualem, B., 2023. In vitro evaluation of probiotic properties of yeasts and lactic acid bacteria isolated from ersho samples collected from different parts of Ethiopia. *Int J Food Prop*. 26(2):3048–3064. https://doi.org/10.1080/10942912.2023.2270177

Fadda, M.E., Mossa, V., Deplano, M., Pisano, M.B., Cosentino, S., 2017. In vitro screening of Kluyveromyces strains isolated from Fiore Sardo cheese for potential use as probiotics. *Food Sci Technol.* 75:100–106. https://doi.org/10.1016/j.lwt.2016.08.020

Gut, A.M., Vasiljevic, T., Yeager, T., Donkor, O.N., 2019. Characterization of yeasts isolated from traditional kefir grains for potential probiotic properties. *J Funct Foods*. 58:56–66. https://doi.org/10.1016/j.jff.2019.04.046

Hsu, S.A., Jui, Y.C., 2021. Yeast in fermented food and kefir: in vitro characterization of probiotic traits. *Journal of Animal and Plant Science.* 31(2): 2021. https://doi.org/10.36899/JAPS.2021.2.0245

Hu, X.Q., Liu, Q., Hu, P., Zhou, J.J., Zhang, X., Peng, S.Y., Peng, L.J., Wang, X.D., 2018. Identification and characterization of probiotic yeast isolated from digestive tract of ducks. *Poultry Science* 9(8): 2902-2908. https://doi.org/10.3382/ps/pey152

Huynh, P., Ngọc., Thạnh, N., Bui, L., Xuan., Phong, H., Quyen, M., Ngoc, T.N., Hoang, D. L. B., Thi P.D.N., Thi, P.N., 2017. Selection of high acid-producing lactic acid bacteria and potential application in pineapple juice fermentation. *Bioprocess Eng*. 1(2):58–64. https://doi:10.11648/j.be.20170102.15.

Kanak, E.K., Öztürk, Y.S., 2025. Determination of the probiotic and functional properties of yeasts isolated from different dairy products. *Fermentation*. 11(2):1–18. https://doi:10.3390/fermentation11020104.

Ladha, G., Jeevaratnam, K., 2018. Probiotic potential of *Pediococcus pentosaceus* ljr1, a bacteriocinogenic strain isolated from rumen liquor of goat (*Capra aegagrus hircus*). *Food Biotechnol*. 32(1):60–77. https://doi:10.1080/08905436.2017.1414700.

Lata, P., Kumari, R., Sharma, K.B., Rangra, S., Savitri., 2022. In vitro evaluation of probiotic potential and enzymatic profiling of *Pichia kudriavzevii* Y33 isolated from traditional home-made mango pickle. *J Genet Eng Biotechnol*. 20(1):132. https://doi:10.1186/s43141-022-00416-2.

Nostro, A., Germano, M. P., D'angelo, V., Marino, A., & Cannatelli, M. A., (2000). Activity of plant extracts and plant-derived compounds against *Aeromonas hydrophila*. *Letters in Applied Microbiology, 31*(2), 174–177. https://doi.org/10.1046/j.1472-765x.2000.00792.x

Pane, K., Valeria, C., Angela, A., Marcelo, D.T.T., Adriana, V., Eliana, D.G, Maria, R.C., Anrimo, D.M., Andrea, B., Giovanni, G., 2018. Identification of novel crytic multifunctional antimicrobial peptides from the human stomach enable by a computational experimental platform. *Syntetyc Biology.* 7 (9): 2105-2115. https://doi.org/10.1021/acssynbio.8b00084

Panjaitan, R., Nuraida, L., Dewanti, H,R., 2018. Seleksi isolat bakteri asam laktat asal tempe dan tape sebagai kandidat probiotik. *J Teknol dan Ind Pangan*. 29(2):175–184. https://doi:10.6066/jtip.2018.29.2.175.

Republic of Indonesia, Ministry of Agriculture. (2017). Available at : https://simrek.ditjenpkh.pertanian.go.id/fileinfo/Regulasi-3-Permentan142017.pdf?utm_[Date accesed : 12 July 2025]

Republic of Indonesia. (2009). Available at : https://peraturan.bpk.go.id/Details/38801?utm_[Date accesed : 12 July 2025]

Samagaci, L., Ouattara, H.G., Goualie, B.G., Niamke, S.L., 2014. Growth capacity of yeasts potential starter strains under cocoa fermentation stress conditions in Ivory Coast. *Emirates J Food Agric*. 26(10) : 861-870. https://doi:10.9755/ejfa.v26i10.18114.

Shao, J.W., Tian, G., Seng, Z.C., Gang, W., Qin, Y., Chun, H.H., Li, C.X., Zhi, C., 2021. Role of bile acids in liver diseases mediated by the gut microbiome. *World J Gastroenterol.* 27: 3010–3021. https://doi:10.3748/wjg.v27.i22.3010

Sibinelli, S.S., Araújo, S.A.L., Hespanhol, J.T., Bayer, S.E., 2022. Revisiting the steps of Salmonella gut infection with a focus on antagonistic interbacterial interactions. *FEBS J*. 289(14):4192–4211. https://doi:10.1111/febs.16211.

Statistics Indonesia. (2023). Available at : https://www.bps.go.id/id/statistics-table/3/ZFdobU5GbDZXWFl5UjA5M1IyTmpUR00zTkc5aWR6MDkjMw%3D%3D/produksi-daging-unggas-menurut-provinsi-dan-jenis-unggas--kg---2023.html?year=2023&utm. [Date accesed : 12 July 2025]

Tamura, K., Stecher, G., Peterson, D., Filipski, A., & Kumar, S. (2013). MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. *Molecular Biology and Evolution*, 30(12), 2725–2729. https://doi.org/10.1093/molbev/mst197

Torshizi, M.A.K., Rahimi, S., Mojgani, N., Esmaeilkhanian, S., Grimes, J.L., 2008. Screening of indigenous strains of lactic acid bacteria for development of a probiotic for poultry. *Asian-Australasian J Anim Sci*. 21(10):1495–1500. https://doi:10.5713/ajas.2008.80081.

Wang, B., Rutherfurd, M.K., Liu, N., Zhang, X.X., Mutukumira, A.N., 2024. Evaluation of the probiotic potential of yeast isolated from kombucha in New Zealand. *Curr Res Food Sci*. 8 March 2024. https://doi:10.1016/j.crfs.2024.100711.

Wessels, K., Rip, D., Gouws, P., 2021. *Salmonella* in chicken meat: Consumption, outbreaks, characteristics, current control methods and the potential of bacteriophage use. *Foods*. 10(8). https://doi:10.3390/foods10081742.

White, T.J., Bruns, T., Lee, S., Taylor, J., 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White T, editor. *PCR Protocols: A Guide to Methods and Applications*. New York (US): Academic Press. hlm. 315–322.

Wulan, R., Astuti, R.I., Rukayadi, Y., Meryandini, A., 2021. Evaluation of indigenous *Pichia kudriavzevii* from cocoa fermentation for a probiotic candidate. *Biodiversitas*. 22(3):1317–1325. https://doi:10.13057/biodiv/d220331.

Xueqinn, L.V., Ke, J., Yu, Y. Lingang, S., Xiang, X., Jianghua, L., Guocheng, D., Jian, C., Long, L., 2023. Analysis of acid tolerance mechanism based on membrane microdomains in Saccharomyces cerevisiae. *Microbial Cell Vactory*. 22: 180. https://doi:10.1186/s12934-023-02195-y

Younis, G., Awad, A., Dawod, R.E., Yousef, N.E., 2017. Antimicrobial activity of yeasts against some pathogenic bacteria. *Vet World*. 10(8):979–983. https://doi:10.14202/vetworld.2017.979-983.