BIODIVERSITAS
Volume 24, Number 2, February 2023
Pages: 958-965

ISSN: 1412-033X
E-ISSN: 2085-4722
DOI: 10.13057/biodiv/d240235

Inhibitor α-glucosidase activity of *Pediococcus acidilactici* DNH16 isolated from Dali ni Horbo, a traditional food from North Sumatra, Indonesia

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Manuscript received: 17 November 2022. Revision accepted: 8 February 2023.

Abstract. *Fachrial E, Anggraini S, Harmileni, Saryono, Nugroho TT. 2023. Inhibitor α-glucosidase activity of* Pediococcus acidilactici *DNH16 isolated from Dali ni Hhorbo, a traditional food from North Sumatra, Indonesia. Biodiversitas 24: 958-965.* Traditional foods, specifically those made from milk, are a potential source of probiotics. These components positively impact digestion and inhibit α-glucosidase, implying that they can be used as supplements for type 2 diabetes patients. Therefore, this study aims to isolate and identify probiotics from Dali ni Horbo, which have α-glucosidase inhibitory activity. Lactic Acid Bacteria (LAB) isolates from Dali ni Horbo were characterized morphologically and biochemically using Gram staining, Catalase test, and type of fermentation. They were further analyzed for their probiotic properties, namely antimicrobial activity, using agar diffusion, acid resistance, and bile salt with a spectrophotometer. Isolates that showed the highest antimicrobial activity, acid resistance, and bile salt were then further analyzed for their α-glucosidase inhibitor activity. The samples were then identified molecularly using PCR amplifying the 16S rRNA gene. The PCR products were sequenced and submitted to the NCBI GenBank to determine the homology of the microorganism. The results showed that 1 out of 23 isolates, namely DNH16, had the highest antimicrobial activity against *Staphylococcus aureus* with an inhibition zone of 4.6 mm. It also has a high tolerance to acidic environments and bile salt, indicated by the percentage growth of 91.6% and 66.5% in an MRS medium containing 0.3% bile salt with a pH of 3. DNH16 isolate showed α-glucosidase inhibitor activity of 27% and was identified as *Pediococcus acidilactici*. The results show DNH16 has the potential to be used as a probiotic as well as an α-glucosidase inhibitor.

Keywords: Antimicrobial, lactic acid bacteria, molecular identification, probiotic

INTRODUCTION

Diabetes mellitus (DM) is one of the oldest diseases in the world, and a manuscript in Egypt revealed that it was first reported approximately 3,000 years ago. In 1936, a distinction was made between type 1 and 2 DM, where type 2 was the most common and characterized by hyperglycemia, insulin resistance, and relative insulin deficiency (Olokoba et al. 2015). The global prevalence of the disease also continued to increase throughout the year. In 2019, more than 463 million people had diabetes, which was predicted to increase to 700 million by 2045. Furthermore, more than 90% of people who suffer from DM are type 2 patients (Thrasher 2017). In 2019, the disease was the direct cause of death among 1.5 million people worldwide. Furthermore, 48% of these mortalities were caused by diabetes before age 70. DM caused 460,000 deaths from kidney failure, and elevated blood glucose levels accounted for 20% of mortality from cardiovascular disease (WHO 2022). The cost of treating, preventing, or managing diabetes and its complications was reported to be 548 billion USD, but none of the drugs/therapy can completely cure the disease (Hu et al. 2017). Some of the common medications used for type 2 DM include biguanides (metformin), sulfonylureas (glyburide and glipizide), meglitinides (repaglinide and nateglinides), thiazolidinediones (pioglitazone), and α-glucosidase inhibitors (acarbose). These classes of medicine are the first-line drugs to prevent the decline or severity of DM status (Kalsi et al. 2015). α-glucosidase inhibitors (AGI) are often prescribed for type 2 DM, which works by inhibiting the action of the α-glucosidase enzyme found in the brush border of the small intestine, thereby interfering with postprandial glucose and postprandial insulin (Laar et al. 2006). They have also been reported to be associated with several hepatic disorders and elevated liver enzymes, although the causative mechanism is unknown. It was written in a meta-analysis of 2,881 articles that there was more than a threefold increase in the normal limits of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) in patients treated with the drugs compared to the controls. The results showed that AGI

could increase the risk of hepatotoxicity, and higher doses were associated with increased risk (Zhang et al. 2016). Intestinal dysbiosis was related to inflammatory bowel disease, celiac disease, obesity, and metabolic disorders. It was also associated with increased oxidative stress, which plays a role in the complexity of various conditions (Carding et al. 2015). Several studies revealed that the host and its microbiota have a beneficial symbiosis and cooperative interaction.

Probiotics are defined as live microorganisms that have a positive effect on the health of the host. Lactic acid bacteria (LAB) are commonly used as probiotics and have been recommended as complementary therapeutic agents (Jeong et al. 2021). They have also been reported to act as α-glucosidase inhibitors, indicating that these microbes can potentially complement the therapy of type 2 DM. A previous study stated that LAB isolated from traditional Korean fermented foods, namely *Lactobacillus sakei* MBEL1397, had α-glucosidase inhibitor activity of 3.91±0.25%, 2.3 times greater than acarbose (Kwun et al. 2020). In another study, it was reported that 20 isolates were screened for inhibitory effect from the intestines of rats, and nine strains had this activity, of which four were tolerant to gastric and intestinal fluids, namely *Lactobacillus acidophilus* CCFM6, *Lactobacillus plantarum* CCFM47, CCFM232, and *Lactobacillus rhamnosus* GG (Muganga et al. 2015). Previous studies revealed that traditional fermented foods are a potential source of LAB. Several studies also explored the antimicrobial activity and characterization of bacteriocins from curd (Syukur et al. 2014) as well as LAB isolated from naniura (Aloysius et al. 2019), and Dali ni Horbo from buffalo milk (Nasution et al. 2020).

There have been many studies on the ability of probiotics from traditional foods to inhibit α-glucosidase, but most of them have yet to be identified molecularly. That is the first study to report that *Pediococcus acidilactici* isolates from the traditional food from North Sumatra, Dali ni Horbo, has acted as an α-glucosidase inhibitor. This study aimed to isolate lactic acid bacteria from Dali ni Horbo, characterize isolates that have potential as probiotics, and molecularly identify probiotic isolates that have activity in inhibiting α-glucosidase.

MATERIALS AND METHODS

Bacterial isolation and characterization

Dali ni Horbo was obtained from local markets around Medan city, Indonesia. A total of 1 g of the sample was added to 9 mL of sterile deMan Rogosa Sharpe (MRS) broth in a test tube and incubated at 37°C for 24 h. Furthermore, 0.1 mL aliquots were diluted successively into 0.9 mL physiological NaCl and homogenized by vortexing, which was referred to as the first dilution and continued until the 7^{th}. In the last stage, 0.1 mL aliquots were spread onto the surface of the MRS agar supplemented with 1% $CaCO_3$, followed by incubation for 48 h at 37°C. Bacterial colonies with a clear zone around the agar surface were purified by streaking four quadrants on a new MRS agar medium. Characterization was carried out morphologically and biochemically, namely, Gram staining, catalase test, and fermentation type. Gram-positive samples could retain crystal violet and safranin in the cell membrane. In comparison, Gram-negative bacteria cannot retain those stains. The catalase test was observed by placing a loopful of isolate on a glass object, adding 1-2 drops of 3% H_2O_2, and observing the production of a bubble or gas. Furthermore, gas production was observed by inoculating 1 mL of LAB in a test tube containing sterile MRS broth and an inverted Durham tube, followed by incubation for 48 h at 37°C (Chen et al. 2005).

Antibacterial activity

Antibacterial activity was determined using the disc diffusion method with a few modifications. First, LAB isolates were inoculated into MRS broth for 18 h on a test tube, then centrifugated at 8,000 rpm, 20 minutes at 4°C, to obtain the supernatant. Next, the indicator bacteria, *Escherichia coli* and *Staphylococcus aureus* were standardized with a spectrophotometer at 600 nm using physiological NaCl until the absorbance was 0.1. Next, the indicator bacteria were swabbed onto nutrient agar media in a Petri dish using a cotton swab. Furthermore, the sterile disc paper was dipped in the supernatant of the lactic acid bacteria and placed onto the nutrient agar surface, which had been previously swabbed with indicator bacteria. The Petri plates were then incubated for 48 h at 37°C. Finally, the diameter of the inhibition zone around the disc was measured. Chloramphenicol of 30µg was used as the positive control (Yamato et al. 2003).

Acid and bile salt resistance assays

Furthermore, to evaluate the capacity to tolerate acidic conditions, the pH of the MRS broth was adjusted to 3.0 and 6.5 using 1 M HCl. A total of 100 µL LAB isolates were incubated for 18 h and added to 5 mL of MRS medium at pH of 3.0 and 6.5, and the mixtures were then re-incubated for 4 hours at 37°C. The optical density was determined at a wavelength of 600 nm, and the growth rate was calculated using the formula: growth in MRS pH 3.0/growth in MRS pH 6.5 x 100.

The tolerance capacity of LAB to bile salt was determined by inoculating the isolates into an MRS broth medium supplemented with and without 0.3% bile salt. The two media were then incubated for four hours at 37°C, and the optical density was determined at a wavelength of 600 nm. Finally, the growth rate was calculated using percentage (%) of growth = growth in bile salt medium/growth in control medium x 100 (Dowarah et al. 2018; Nami et al. 2019).

Inhibition activity of α-glucosidase

Selected LAB isolates were cultured into sterile MRS broth media at 37°C for 24 h. The bacterial culture was then centrifuged at 3,500 rpm for 15 min at 4°C to obtain the supernatant, which was further tested to inhibit α-glucosidase activity. The reaction mixture consisting of 2 µL of supernatant, 48 µL of phosphate buffer (100 mM, pH 7), and 25 µL of α-glucosidase enzyme of 10 units/mL was

incubated at 37°C for 5 min. After incubation, 25 µL of the p-nitrophenyl α-D-glucopyranoside substrate was added to the mixture and further set for 15 min at 37°C. A total of 100 µL of 200 mM Na_2CO_3 were added to stop the reaction. The absorbance was read using a microplate reader at a wavelength of 415 nm, and a 1% acarbose solution was used for comparison. The enzymatic reaction design for one sample was with a total volume of 200 µL (Table 1).

The inhibitory activity of supernatant and acarbose on α-glycosidase can be calculated using the following formula:

% inhibition = ($A1_{absorbance}$ - $A0_{absorbance}$)-($AI1_{absorbance}$ - $AI0_{absorbance}$)/($AI_{absorbance}$-$A0_{absorbance}$) x 100% (Susilowati et al. 2019).

Molecular identification of bacteria

Genomic DNA isolation was performed using a Quick-DNA Fungal / Bacterial miniprep kit (Zymo Research, D6005), and the DNA concentration was quantified using Nanodrop. The procedure for isolating DNA was as follows: one loopful of the bacterial isolate was cultured for 18 h in nutrient broth. The ZRBashingbead™ lysis tube was filled with 750 µL of Bashingbead™ buffer after the pellet had been resuspended in phosphate buffer saline with a pH of 7. Subsequently, it was centrifuged for one minute at 10,000 rpm. A total of 400 µL of the supernatant was centrifuged at 8,000 rpm for one minute, followed by adding 1200 µL lysis buffer into the collection tube. Eight hundred microliters of the solution were transferred to the Zymo-Spin™ IICR column in a new collection tube and centrifuged at 10,000 rpm for one minute. After adding 500 µL of d-DNA Wash Buffer to the Zymo-Spin™ IICR Column, it was centrifuged for one minute at 10,000 rpm. The column was then transferred to a clean 1.5 mL microcentrifuge tube, followed by adding 100 µL DNA Elution Buffer directly to the matrix. The mixture was centrifuged at 10,000 rpm for 30 sec to elute the DNA. The 27F/1492R primer was then used to amplify the 16SrRNA gene. Pre-denaturation was performed at 95°C for 90 sec, followed by 30 cycles of denaturation for 30 sec at 95°C, annealing for 30 sec at 55°C, extension for 90 sec at 72°C, and final extension for 3 min at 72°C. A total of 1 µL PCR products were assessed by electrophoresis using 0.8% TBE agarose, and 1 Kb bp DNA was used as a marker. The PCR products were then sequenced using the Bi-directional sequencing method. The sequence result was trimmed and assembled using the BioEdit program, which was submitted to http://www.ncbi.nlm.nih.gov/BLAST to determine the homology of the microorganism. The neighbor-joining method was used to reconstruct the phylogenetic tree from evolutionary distance data (Stackebrandt and Goebel 1994).

RESULTS AND DISCUSSION

Bacterial isolation and characterization

A total of 3.3×10^7 CFU/mL of LAB have been isolated from 1 gram of Dali no Horbo. A total of 23 isolates were randomly selected for further characterization. The results of morphological and biochemical characterization were illustrated (Table 2). From the table, it can be concluded that all isolates (25 isolates) were Gram-positive, 2 isolates showed a positive reaction in the catalase test, 4 isolates were classified as homofermentative, and 21 isolates were classified as heterofermentative. Based on these data, only Gram-positive and catalase-negative samples were further analyzed, indicating that DNH1 and DNH9 were excluded in the next test.

Antibacterial activity

All isolates did not show antibacterial activity against *Escherichia coli* with an inhibition zone diameter of < 1 mm, but they had an effect against *Staphylococcus aureus*. The diameter of the inhibition zone against *Staphylococcus aureus* was illustrated (Figure 1).

Table 1. Reaction test design of enzymatic inhibitory activity of α-glucosidase

	Mixture			
	A_0 (µL)	**A_1 (µL)**	**AI_0 (µL)**	**AI_1 (µL)**
Supernatant	-	-	2	2
MRS broth	2	2	-	-
Buffer pH 7	48	48	48	48
Enzyme	-	25	-	25
Incubated at 37°C for 5 minutes				
Buffer pH 7	25	-	25	-
Substrate	25	25	25	25
Incubated at 37°C for 5 minutes				
Na_2CO_3	100	100	100	100

Table 2. Characterization of isolates

Isolates	Characteristics		
	Gram staining	**Catalase test**	**Fermentation type**
DNH1	Positive	Positive	Heterofermentative
DNH2	Positive	Negative	Heterofermentative
DNH3	Positive	Negative	Homofermentative
DNH4	Positive	Negative	Heterofermentative
DNH5	Positive	Negative	Homofermentative
DNH6	Positive	Negative	Heterofermentative
DNH7	Positive	Negative	Heterofermentative
DNH8	Positive	Negative	Heterofermentative
DNH9	Positive	Positive	Homofermentative
DNH10	Positive	Negative	Heterofermentative
DNH11	Positive	Negative	Heterofermentative
DNH12	Positive	Negative	Heterofermentative
DNH13	Positive	Negative	Heterofermentative
DNH14	Positive	Negative	Heterofermentative
DNH15	Positive	Negative	Heterofermentative
DNH16	Positive	Negative	Homofermentative
DNH17	Positive	Negative	Heterofermentative
DNH18	Positive	Negative	Heterofermentative
DNH19	Positive	Negative	Heterofermentative
DNH20	Positive	Negative	Heterofermentative
DNH21	Positive	Negative	Heterofermentative
DNH22	Positive	Negative	Heterofermentative
DNH23	Positive	Negative	Heterofermentative
DNH24	Positive	Negative	Heterofermentative
DNH25	Positive	Negative	Heterofermentative

Figure 1 shows that of the 22 isolates, six did not show antibacterial activity. In contrast, isolate DNH16 showed the most potent antibacterial activity with an inhibition zone of 4.6 mm. The antibacterial activity of isolate DNH16 in the Petri dish against *Staphylococcus aureus* is shown in Figure 2.

Acid and bile salt resistance assays

All isolates were tested for their ability to grow in an acidic environment, where the pH of the MRS broth medium is 3 (Figure 3). The figure shows variations in the ability of bacteria to grow in an acidic environment, with a growth percentage ranging from 2.38% shown by isolates DNH4 and DNH22. The highest growth percentage is DNH16, with a value of 91.6%. This value indicates that isolate DNH16 has excellent tolerance to acidic environments.

All isolates were further tested for tolerance to bile salts. The percentage growth of isolates on MRS broth media supplemented with 3% bile salts is shown in Figure 4. The figure shows the percentage growth of isolates on media with a bile salt, with a percentage range between 4.13% shown by isolate DNH13, to 66.5% shown by isolate DNH16.

Inhibition activity of α-glucosidase

Based on antimicrobial activity and tolerance to acid and bile salt, DNH16 isolate was selected to be tested for its α-glucosidase inhibitory activity. This activity was indicated by a decrease in the activity of α-glucosidase in catalyzing the hydrolysis of p-nitrophenol α-D-glucopyranoside into p-nitrophenol (yellow) and glucose after the addition of bacterial culture supernatant containing an inhibitor. This activity was then compared with 1% acarbose as a positive control. This study showed that the inhibition activity of α-glucosidase of isolate DNH 16 and acarbose was 27% and 90%, respectively.

Molecular identification of DNH 16

The genomic DNA of DNH16 was isolated with the Quick-DNA fungal/bacteria Miniprep Kit. After the concentration quantification, the isolated DNA was 82.7 ng/µL. Electrophoresis results of the DNA isolate PCR product showed a molecular weight of 1459 bp. BLAST results of DNH16 isolates against the NCBI database showed that it was identified as *Pediococcus acidilactici* without uncultured sample sequences. That result was confirmed by phylogenetic analysis, which showed that the DNH 16 isolate was a bacterium identified as *Pediococcus acidilactici* (Figure 5).

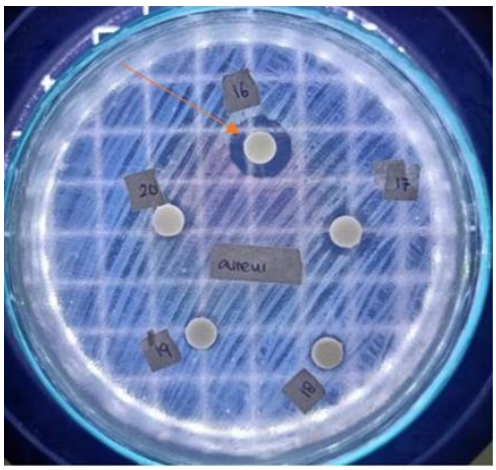

Figure 2. An arrow pointed the antibacterial activity of DNH 16 against Staphylococcus aureus

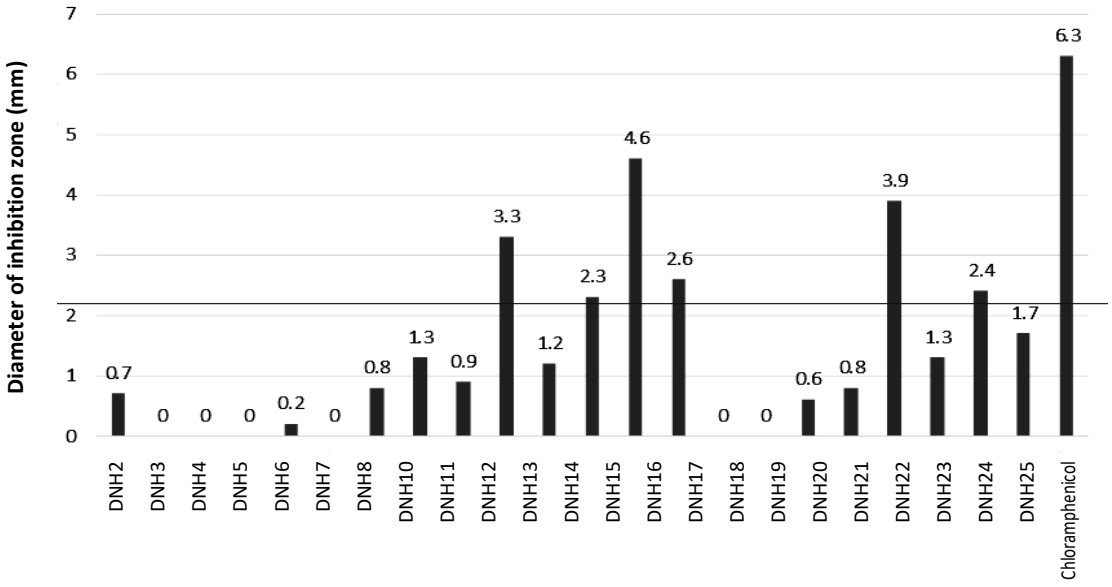

Figure 1. Antibacterial activity of LAB isolates against *Staphylococcus aureus*

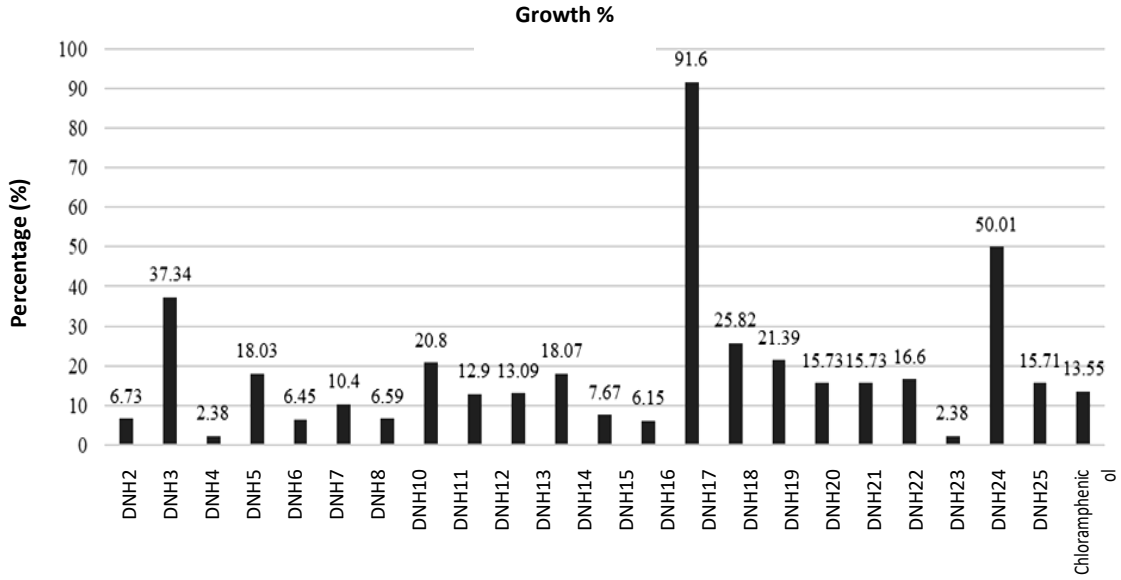

Figure 3. Growth percentage of LAB isolates in MRS broth medium pH 3

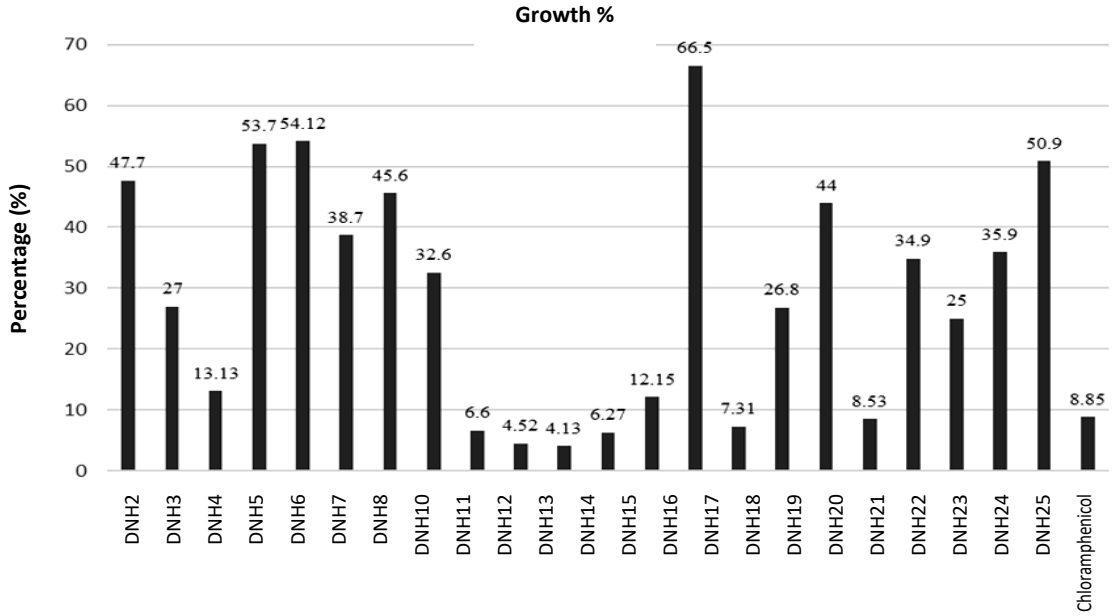

Figure 4. Bile salt tolerance of LAB isolates in MRS broth containing 3% bile salt

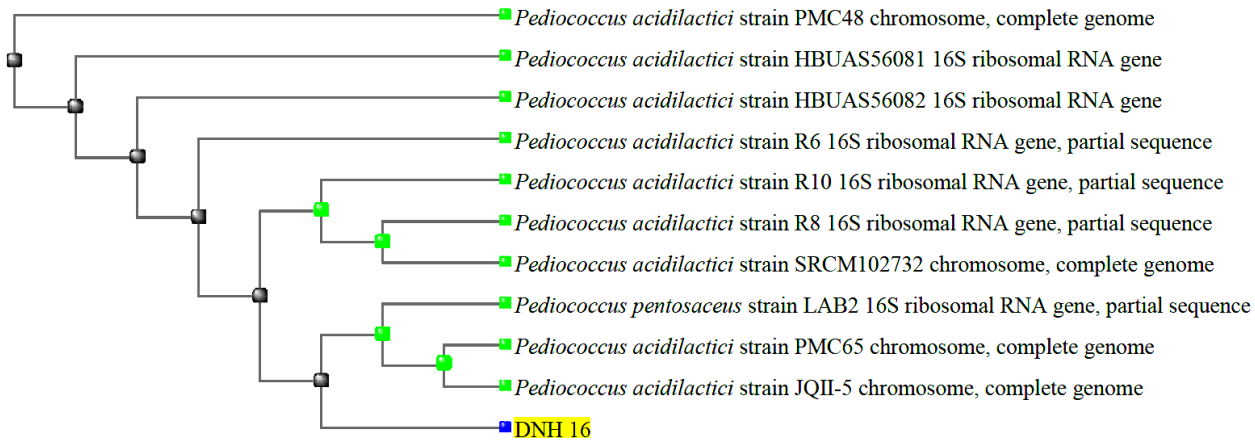

Figure 5. Phylogenetic analysis of DNH 16

Discussion

Dali ni Horbo was produced by mixing buffalo milk with juice from papaya or pineapple leaves to form a coagulant, which changes its color to greenish-white. The traditional cheese is not publicly known because only the local communities utilize them. One of its benefits is the safety of the coagulation process, which does not involve chemicals and preservatives (Luthfi and Sanggramasari 2018). The result of the isolation of lactic acid bacteria from Dali ni Horbo showed a yield of 3.3×10^7 CFU/mL. The number of LAB contained in the sample still met the minimum requirement for probiotics in fermented products based on the Indonesian National Standard and FAO regulations, namely 10^7 CFU/mL (Parasthi et al. 2020). LAB are typically found in environments rich in nutrients, such as milk, meat, and vegetables. However, some are also found in the mammals' mouths, intestines, and vaginas. Therefore, Buffalo milk is important to fulfill human nutritional needs, specifically in developing countries, making it a potential source of probiotics. A previous study revealed that *Lactobacillus fermentum* strain L23 is a species of LAB isolated from Buffalo milk obtained in the Agam district, West Sumatra, Indonesia (Melia et al. 2018).

Lactic acid bacteria (LAB) is a Gram-positive, non-spore-forming, non-respiring, fastidious, cocci, cocci-bacilli, acid-tolerant, and catalase-negative devoid of cytochrome, but is aerotolerant. They also produce lactic acid as one of the essential products by utilizing carbohydrates during fermentation (Ayivi et al. 2020; Gupta et al. 2018). Based on the type of fermentation, LAB can be classified into homofermentative and heterofermentative (Blajman et al. 2020). They can also be classified into three groups: obligate homofermentative facultative and obligate heterofermentative. The homofermentative LAB produces lactic acid, the primary product of glucose fermentation using an aldolase enzyme. The homofermentative LAB includes *Streptococcus* and *Pediococcus* (Carret al. 2002). Furthermore, obligate heterofermentative LAB can produce lactic and organic acids, such as acetic and formic acids. They also produce carbon dioxide and ethanol, which are effective as antifungal and prolong aerobic stability (Irawan et al. 2021).

In this study, all isolates except DNH1 and DNH 9 did not show antimicrobial activity against *Escherichia coli*. Pato et al. (2022) reported that LAB isolated from "Dadih" had a more substantial effect against Gram-positive bacteria compared to Gram-negative. The most significant antimicrobial activity against *Staphylococcus aureus* was found in R-55, which showed a high effect against *Listeria monocytogenes* but relatively low toxicity on *Escherichia coli* (Pato et al. 2022). Bacteriocins are ribosomally integrated antimicrobial peptides created by microorganisms living in various eubacterial-ordered branches, specifically LAB. Most were small, cationic, and membrane-active compounds that cause membrane potentials to be disrupted and death in the target cells (Oscáriz and Pisabarro 2001). Gram-negative bacteria have an outer membrane, an efficient barrier permeability to exclude macromolecules. It excludes macromolecules, such as bacteriocins and enzymes, and hydrophobic compounds, such as antibiotics. The permeability barrier property of the outer membrane is primarily due to the presence of a specific lipopolysaccharide (LPS) layer on the membrane surface. LPS molecules consist of a lipid part, termed lipid A, and a heteropolysaccharide chain protruding outward and providing the cell with a hydrophilic surface (Alakomi et al. 2000). Among the 23 isolates tested for antibacterial activity, DNH16 had the inhibition zone of 4.6 mm against Gram-positive *Staphylococcus aureus*. LAB also has a high antibacterial effect against this bacterium. Similar results were obtained by Pato et al. (2022), where 9 out of 12 isolates from curd had toxic effects against *Staphylococcus aureus* with an inhibition zone of > 3.5 mm (Pato et al. 2022).

The primary antimicrobial effect of LAB was due to the production of organic acids, which reduce the pH of the surrounding environment. A decrease in the pH of the environment will make it difficult for various Gram-positive bacteria to grow. The charge of biological molecules is influenced by protonation at low pH, which affects their structure and functions. Lipid bilayers are typically very impermeable to protons, and this feature allows the proton gradient across a membrane to be used for energy generation. Weak organic acids, such as lactate or acetic, are protonated at low pH and are more lipophilic. Those organic acids can penetrate the lipid bilayer and release protons in a neutral intracellular environment. Therefore, organic acids have toxic effects on microbial cells because they can trigger cytoplasmic acidification and collapse the proton gradient (Lund et al. 2020). The primary antimicrobial substance of LAB is bacteriocin, which is sensitive to proteases compared to antibiotics due to its peptide backbone. However, they can affect target cells, such as cell wall synthesis, membrane integrity, nucleic acid replication, translation, and protein synthesis. Bacteriocins are mainly active against Gram-positive bacteria compared to Gram-negative with an outer membrane (Lund et al. 2020).

A previous study stated that LAB isolates from "Bekasam," a fermented product of bog fish, were identified as *Pediococcus acidilactici* strain PB22, which had an antimicrobial activity of 18.23 mm against *Staphylococcus aureus* (Melia et al. 2019). Another study reported that *Pediococcus acidilactici* PFC69 isolates from "Tarhana," a traditional fermented food from Anatolia, had an effect of 12,800 AU/mL. These bacteria also produce bacteriocins with a molecular weight of 4.5 kDa (Kaya and Şimşek 2020). DNH16 isolate had excellent tolerance to acidic environments with a pH of 3 and bile salt, with growth percentages of 91.5% and 66.5%. Resistance to gastric acidity and bile salt is one of the criteria for probiotics. A study reported that *Pediococcus acidilactici* B14 showed probiotic characteristics: survival rates of 45.9% at a pH of 2.5 and 72.4% at 0.3% bile salt. The tolerance to the acidity of LAB has been associated with the induction of the activity of H^+-ATPase. The resistance mechanisms were strongly correlated to the presence of hydrolase activity, which probably exerts a

detoxification effect by catalyzing the hydrolysis of glycine or taurine-conjugated bile salt into amino acid residues and unconjugated molecules (Ribeiro et al. 2014).

Several strains of lactic acid bacteria have been reported to have α-glucosidase inhibitor activity. In this study, DNH16 isolate had α-glucosidase inhibitor activity of 27%. Inhibition of this enzyme can prevent the absorption of carbohydrates and reduce hyperglycemia. It was reported in a previous study that LAB isolated from kimchi and identified as *Lactobacillus sakei* showed α-glucosidase inhibitor activity of 3.91 ± 0.25% (Kwun et al. 2020). Jeon et al. (2021) revealed that the probiotic found in Yuzu was identified as *Lactobacillus sakei* NY 518. Its oligosaccharides were glucooligosaccharides and had the α-glucosidase inhibitory activity of 67% (Jeon et al. 2022).

Isolate DNH16 was identified as *Pediococcus acidilactici* strain DNH16. In several studies, it was reported that *Pediococcus acidilactici* is a probiotic that has the potential as a supplement in overcoming type 2 DM. It revealed that its administration in a murine model of high-fat diet (HFD)-induced type 2 DM significantly attenuated body weight gain and mitigated glucose dysregulation by reducing fasting blood glucose levels, glucose tolerance test, leptin levels, and insulin resistance. It also increased C-peptide and GLP-1 levels, enhanced pancreatic functions, and improved intestinal histology (Cabello-Olmo et al. 2022). A previous study stated that *Pediococcus acidilactici* 004 and *Lactobacillus plantarum* 152 had better antidiabetic potential than *Lactobacillus rhamnosus* GG. *Pediococcus acidilactici* 004 was reported to have α-glucosidase inhibitor activity of 30.79±0.1, hydroxyl radical scavenging with IC value of 2.18±0.03, and high antioxidant enzyme activity, specifically superoxide dismutase (SOD) activity (Cai et al. 2019).

In conclusion, a total of 23 isolates of LAB were isolated from traditional food, Dali ni Horbo. Furthermore, one of them, DNH 16, had the most potent antimicrobial activity against *Staphylococcus aureus* with an inhibition zone of 4.6 mm. The highest growth percentage for acid pH and bile salt was 91.5% and 66.5%, respectively. Based on the amplified 16S rRNA gene sequence, the isolate was identified as *Pedicoccus acidilactici* strain DNH16, which had α-glucosidase inhibitor activity of 27%. The results showed that in the future, *Lactobacillus acidilactici* strain DNH16 has the potential as a candidate for natural medicine, namely as a probiotic for digestive health and a supplement for people with type 2 DM. Further research is needed on the antidiabetic activity of *Pediococcus acidilactici* strain DNH16 in vivo.

ACKNOWLEDGEMENTS

The authors are grateful to the Directorate of Research and Community Service, the Ministry of Education, Culture and Higher Education for the Higher Education Decentralization Research grant (PDUPT) in 2022 with contract number 125/LL1/LT/K/2022.

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