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OPEN ACCESS Pakistan Journal of Nutrition ISSN 1680-5194 DOI: 10.3923/pjn.2017.618.628 Research Article [Isolation and Identification of Lactic Acid Bacteria from Okara and Evaluation of Their Potential as Probiotics](#) 1Salam N. Aritonang, 1Elly Roza, 2Evy Rossi, 1Endang Purwati and 1Husmaini 1Department of [Animal Production, Faculty of Animal Science, Universitas Andalas, Padang, Indonesia](#) 2Department of [Agricultural Technology, Faculty of Agriculture, Universitas Riau, Indonesia](#) Abstract Background: Okara is an industrial waste product resulting from the manufacture of soy milk or tofu. This waste is a pulp consisting of the insoluble parts of the soybean that remain after pureed soybeans are filtered during the production of soy milk and tofu. Okara is not widely used and is dumped into the environment, causing pollution. However, this waste contains nutrients and may be a good medium for the growth and development of microorganisms such as Lactic Acid Bacteria (LAB). Objective: The purpose of this study was to isolate and identify probiotic LAB from okara and determine their antimicrobial activity against pathogenic bacteria (*Escherichia coli* 0157, *Staphylococcus aureus* ATCC 25923 and *Listeria monocytogenes* EP01). Methodology: Isolation and purification of LAB were carried out on MRS agar supplemented with 0.2% CaCO₃. Isolates were identified by morphological and physiological tests. Antibacterial activity was tested using the well diffusion method and the obtained data were analyzed descriptively. Twenty-four isolates were grown on MRS agar. Results: Sixteen isolates that exhibited growth at the clear zone were identified as gram positive, catalase-negative homofermentative rod and coccus cells. The results showed that the LAB isolated from okara had the ability to inhibit pathogenic bacteria, causing various clear zone diameters. The survival rates of these isolates under acidic conditions and their tolerance to 0.5% bile salt were varied. Based on the antimicrobial activity and probiotic properties of the LAB isolated from okara, these isolates belong to the species *Lactobacillus spentosus* and *Lactobacillus plantarum*. Conclusion: The results showed that the LAB isolated from okara belonged to the species *Lactobacillus pentosus* and *Lactobacillus plantarum*. Both species exhibited properties attributed to probiotics. Key words: Okara, lactic acid bacteria, antibacterial activity,

pathogenic bacteria, bile salt Received: April 26, 2017 Accepted: June 03, 2017 Published: July 15, 2017 Citation: [Salam N. Aritonang, Elly Roza, Evy Rossi, Endang Purwati and Husmaini, 2017. Isolation and identification of lactic acid bacteria from okara and evaluation of their potential as candidate probiotics.](#) Pak. J. Nutr., 16: 618-628.

Corresponding Author: Salam N. Aritonang, [Department of Animal Production, Faculty of Animal Science, Universitas Andalas, Padang, Indonesia](#) Copyright: © 2017 Salam N. Aritonang et al. This is an open access article distributed [under the terms of the creative commons attribution](#) License, which permits unrestricted use, distribution and reproduction in any medium, provided the original author and source are credited. Competing Interest: The authors have declared that no competing interest exists. Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION Soy milk is a popular Indonesian beverage made by grinding soy beans that have been soaked in water, filtered, cooked until boiling and then supplemented with sugar and essence to enhance the flavor. The process of making soy milk produces solid waste such as soybean pulp (okara)¹ that is rarely consumed by humans and therefore has low economic value². Okara is made up of 16.1% crude proteins, 52.6% carbohydrates and 2.2% crude lipids as well as vitamin B and the amino acids lysine and methionine³. The high nutrient content of okara supports the growth of microorganisms such as Lactic Acid Bacteria (LAB)². Okara also contains isoflavones (22%) is a potential source of antioxidants, has prebiotic characteristics and can lower cholesterol and blood sugar levels⁴. The LAB have been isolated and identified as probiotics from many sources in nature, including Turkish pastirma, a dry-cured meat product⁵, the heaps and boxes used in the cocoa fermentation process⁶, human milk⁷, Brazilian kefir grains⁸, forage paddy rice silage⁹ and some Nigerian cereal-based-fermented foods¹⁰. For LAB to be considered probiotics, they must be proven safe for the consumer, exhibit tolerance to gastric acid and bile¹¹ and display antibacterial activity¹². Lactic acid bacteria are beneficial microorganisms used as starter cultures for the processing of functional food^{13,14}. These LAB are not toxic to the host and have the ability to kill pathogenic bacteria¹⁵. Researchers have developed LAB that can be used for food fermentation, for probiotic effects and for food preservation¹⁶. The primary metabolic product of LAB is lactic acid¹⁷; however, LAB also produce secondary metabolites that can inhibit the growth of spoilage and pathogenic microbes because these metabolites function as antimicrobial compounds¹⁸. One of the antimicrobial compounds produced by LAB is bacteriocin. This compound is effective, safe and able to prevent or inhibit the growth of pathogenic bacteria compared to other antimicrobial compounds¹⁹. The antimicrobial activity of LAB is a subject of intense study. Ammor et al.²⁰ studied the antibacterial activity of LAB against spoilage and pathogenic bacteria isolated from the same small-scale meat facility. Arques et al.²¹ evaluated the antimicrobial activity of LAB in dairy products and the gut. Rosa et al.²² characterized the antimicrobial activity of LAB isolated from cassava processing waste water. Previously, Siroli et al.²³ found that combining selected strains with natural antimicrobials further increased in the shelf-life of these products without detrimental effects on their organoleptic qualities. Additionally, Li²⁴ evaluated [the antimicrobial activity of lactic acid bacteria isolated from](#) fermented taro skins against the pathogenic bacteria *Salmonella typhimurium* and *Listeria monocytogenes*. Antimicrobial compounds produced by LAB provide several advantages. These compounds can extend food storage time, can suppress the growth of undesirable microorganisms and are safe for humans to consume because they are not toxic. The [activity of lactic acid bacteria isolated from](#) fermented taro skins against *Salmonella typhimurium* and *Listeria monocytogenes* has been demonstrated²⁴. Developing a natural food preservative is a focus of current research. This natural preservative can replace the synthetic additives that are currently

used often as food preservatives^{16 25}. Developing LAB as food preservatives is of great interest as they can be an alternative to synthetic (non-food) food additives, which are often misused. The purpose of this study was to isolate and identify the LAB of okara and evaluate their antimicrobial activity against pathogenic bacteria such as *Escherichia coli* 0157, *Staphylococcus aureus* ATCC 25923 and *Listeria monocytogenes* EP01.

MATERIALS AND METHODS

Isolation of lactic acid bacteria from okara: Okara, the material used in this study, was taken from three locations of soybean milk production in Taman Karya District, Pekanbaru, Indonesia. Each sample (1 mL) of spontaneously fermented (48 h) okara was homogenized with 9 mL of 0.85% sterile NaCl. The mixture was then serially diluted (10G1 to 10G5) for bacterial enumeration. One milliliter samples of each dilution were spread directly on the surface of MRS agar. To differentiate acid-producing bacteria from other bacteria, 1% CaCO₃ was added to the MRS agar. Plates were incubated at 37EC for 48 h and colonies exhibiting a surrounding clear zone were collected. The colonies that formed a clear zone on MRS agar were randomly selected as acid-producing bacteria. The selected colonies were purified using the streak plate technique. Lactic Acid Bacteria (LAB) isolation was carried out according to the method described by Chen et al.²⁶. The purified LAB isolates were stored at -20EC in 15% glycerol and 85% MRS broth.

Morphological and physiological identification of lactic acid bacteria: The selected LAB isolates were examined using the following methods as described by Felten et al.²⁷.

(a) Gram staining was performed, where Gram-positive bacteria retain crystal violet and safranin in the cell membrane and Gram-negative bacteria are unable to retain the crystal violet stain.

(b) Cell shape was also determined using Gram staining followed by microscopic observation. Holzapfel and Wood²⁸, previously determined that LAB consist of two families, Lactobacillaceae and Streptococcaceae. The genera *Lactobacillus* and *Bifidobacterium* are found within the rod-shaped Lactobacillaceae family and the round-shaped Streptococcaceae cells include the genera *Streptococcus*, *Leuconostoc* and *Pediococcus*.

(c) Catalase production was observed by placing one dose of the bacterial suspension on a glass object, adding 1-2 drops of 3% hydrogen peroxide and then observing the production of a bubble or gas. Roberts et al.²⁹ suggested that a positive reaction in the catalase test is indicated by the appearance of bubbles (gas) caused by the enzymatic activity of catalase, which is an enzyme known to break down H₂O₂ into H₂O and CO₂. If this reaction does not generate gas, it can be concluded that the bacteria do not have the catalase enzyme and the bacteria are then categorized as catalase negative.

(d) Gas production by LAB was observed by inoculating 1 mL suspension of bacterial cells in a test tube containing MRS broth (Merck) and an inverted Durham tube. After incubation at 37EC for 48 h, production of gas in the Durham tube indicates that the bacteria ferment glucose to CO₂ and acid. Salminen et al.³⁰ stated that homofermentative LAB only produce lactic acid through the glycolysis pathway, whereas heterofermentative LAB produce not only lactic acid but also acetic acid, ethanol and carbon dioxide (CO₂).

(e) Growth observation at various temperatures was performed to determine the optimal temperature conditions for LAB isolates. One milliliter of LAB cell suspension was inoculated into a test tube containing MRS broth medium and incubated at 10, 60 and 70EC for 48 h. The sample was then poured into MRS agar and incubated at 37EC for 48 h before colony growth was observed.

Potential evaluation of lab isolates as probiotics

Antimicrobial activity Bacterial strains and media: Sixteen LAB isolates were preserved in MRS broth (Merck) with 20% (v/v) glycerol at 20EC. All isolates were propagated in MRS broth and incubated at 37EC for 24 h to obtain active cultures. The pathogenic indicator strains, *Escherichia coli* 0157, *Staphylococcus aureus* ATCC 25923 and *Listeria monocytogenes* EP01 were inoculated into a test tube containing 5 mL nutrient broth (Merck) and incubated at 37EC for 24 h to obtain active cultures. Active

cultures were marked by media turbidity. The pathogenic bacteria were obtained from the collection of the Animal Food Technology Laboratory, Universitas Andalas, Padang, Indonesia. Preparation of LAB cell-free supernatant: Each LAB isolate from okara was inoculated into MRS broth and then incubated at 37EC for 24 h. Afterwards, these isolates were centrifuged at a speed of 8,000 rpm at 4EC for 10 min to separate the cells and supernatant. The cell-free supernatant was then harvested by centrifugation. Antimicrobial activity of lab against pathogen bacteria: The antimicrobial activity of LAB was determined by the well diffusion method³¹. Using a sterile pipette, 0.1 mL of indicator bacteria culture was placed on Nutrient Agar (NA) media³² and swabbed using a hockey stick. To create 5 mm wells, the agar was drilled using the bottom of a sterile blue pipette tip and the bottom of the wellbore was covered with sterile agar. Furthermore, 50 μ L of each LAB isolate (an overnight culture of each LAB isolate grown in MRS broth at 37EC that had been standardized to an optical density of 0.5 at 600 nm) was added to the previously made wells and incubated at 37EC for 24 h. After incubation, the clear zone diameter was measured three times at different positions and averaged. Isolates that displayed antimicrobial activity (a clear zone larger than 5 mm) were selected for survival assays, including survival under conditions of pH 3 for 3 h and of 0.3% bile salt (w/v) for 5 h. Total viable colonies were enumerated for 0 and 5 h using the pour plate technique. Assay of the antimicrobial activity of the lab cell-free supernatant against various bacteria: This test was previously described by Bromberg et al.³¹ and used 50 mL of the supernatant in previously prepared wells. Each petri dish and its contents were placed in the refrigerator for 2 h to allow the agar to adsorb the supernatant. Then, the plates were incubated at 37EC for 24 h. The formation of a clear zone demonstrates that the supernatant could prevent the growth of bacteria. The diameter of the clear zone was measured using calipers three times at different positions and averaged. Survival rate under low pH and bile salt conditions: Lactic acid bacteria that exhibited a clear zone diameter of more than 6 mm against pathogenic bacteria were used for survival tests. Three samples of each isolate were assayed as replicates. The initial population of the isolate was determined. The responses of LAB isolates to pH 3, the pH of the stomach was used to evaluate their survival rates using a modified method described by Oluwajoba et al.³³. The MRS broth solutions at pH 6.5 (control), 2.5 and 3 were inoculated with a 1% (v/v) LAB isolate and then incubated at 37EC for 1.5 h. The surviving LAB were plated onto MRS agar. The number of CFU mLG1 was calculated and compared to the CFU mLG1 at time 0. The effect of bile salt on the growth of LAB isolates was evaluated by inoculating 1 mL of LAB isolate into MRS broth containing 0.3% bile salts (Sigma). After incubation at 37EC for 5 h, the surviving LAB were counted by plating onto MRS agar and incubating for 24 h at 37EC. The number of CFU mLG1 was calculated and compared to the CFU mLG1 at time 0. Initial identification of lactic acid bacteria strains using an api 50 CHL kit: The selected isolates were identified using an API 50 CHL Kit (bioMeriux, France), which evaluates LAB based on their carbohydrate fermentation activity. During anaerobic incubation at 30EC, LAB fermented the 49 carbohydrates on the API 50 CHL strip, which produced a decrease in pH that was detected by the change in the color as an indicator. The results of biochemical tests and carbohydrate fermentation were determined after 24 and 48 h of incubation. All of the results were confirmed by observing the biochemical profile using API webTM identification software to identify the strain. Statistical analysis: All experiments were carried out in quadruplicate. Statistical analysis was carried out using SPSS Ver. 15 for Windows. Antibacterial activities and survival Table 1: Morphological, biochemical and physiological profiles of LAB isolates rates under low pH and bile salt exposure were analyzed for statistical significance using one way analysis of variance (ANOVA). Duncan's multiple range test was used to determine significant ($p < 0.05$)

differences among the mean values. RESULTS AND DISCUSSION Isolation and identification of lactic acid bacteria: Isolation of LAB from okara in MRS agar medium supplemented with 0.2% CaCO₃ yielded 24 isolates. Sixteen isolates exhibited a clear zone in the medium, while the remaining eight isolates did not produce a clear zone. The CaCO₃ served as a buffer and was used for the initial selection of lactic acid-producing bacteria. Seeley et al.³⁴ suggested that lactic acid production would cause the formation of a clear zone around the colony and it was used as an early marker of LAB colony formation. Candidate LAB can be found in okara due to the retained nutritional content of the dregs. According to Lu et al.³⁵ okara still retains nutritional components such as protein, fat, dietary fiber, minerals, monosaccharides and oligosaccharides. This nutrition allows the growth of microorganisms such as LAB. The genera of the candidate probiotic LAB isolated from okara were identified by tests such as Gram staining, cell morphology, gas production and catalase production. The identification results are shown in Table 1. Table 1 indicates that all isolates have either round (cocci) or rod (Bacillus) cell shapes. Holzapfel dan Wood³⁶ stated that based on the shape of LAB cells, they consist of two families: (a) Lactobacillaceae, which are rod shaped and consist of the genera Lactobacillus and Bifidobacterium and (b) Streptococcaceae, which are round and consist of the Isolate code Gram stain Cell shape Catalase test Gas production Growth at -----

Isolate code	Gram stain	Cell shape	Catalase test	Gas production	Growth at
15EC	+	Coccus	+	+	+
60EC	+	Coccus	+	+	+
70EC	+	Coccus	+	+	+
A.11.3	+	Coccus	+	+	+
A.11.4	+	Coccus	+	+	+
A.12.4	+	Coccus	+	+	+
A.13.1	+	Coccus	+	+	+
A.13.2	+	Bacillus	+	+	+
A.13.3	+	Bacillus	+	+	+
A.13.4	+	Bacillus	+	+	+
A.21.1	+	Bacillus	+	+	+
A.21.2	+	Bacillus	+	+	+
A.21.3	+	Bacillus	+	+	+
A.22.1	+	Bacillus	+	+	+
A.22.2	+	Coccus	+	+	+
A.22.3	+	Coccus	+	+	+
A.22.4	+	Coccus	+	+	+
A.23.2	+	Bacillus	+	+	+
A.23.4	+	Bacillus	+	+	+
A.23.5	-	Bacillus	-	-	-
A.23.6	-	Bacillus	-	-	-
A.23.7	-	Bacillus	-	-	-
A.23.8	-	Bacillus	-	-	-
A.23.9	-	Bacillus	-	-	-
A.23.10	-	Bacillus	-	-	-
A.23.11	-	Bacillus	-	-	-
A.23.12	-	Bacillus	-	-	-
A.23.13	-	Bacillus	-	-	-
A.23.14	-	Bacillus	-	-	-
A.23.15	-	Bacillus	-	-	-
A.23.16	-	Bacillus	-	-	-
A.23.17	-	Bacillus	-	-	-
A.23.18	-	Bacillus	-	-	-
A.23.19	-	Bacillus	-	-	-
A.23.20	-	Bacillus	-	-	-
A.23.21	-	Bacillus	-	-	-
A.23.22	-	Bacillus	-	-	-
A.23.23	-	Bacillus	-	-	-
A.23.24	-	Bacillus	-	-	-

----- Escherichia coli Staphylococcus aureus Listeria monocytogenes A.11.3 6.25abc A.11.4 5.68ab A.12.4 7.58 cde A.13.1 7.27 cde A.13.2 9.28 fg A.13.3 6.34bc A.13.4 7.14cd A.21.1 7.32cde A.21.2 6.54bc A.21.3 8.74ef A.22.1 8.57ef A.22.2 6.73bc A.22.3 9.90g A.22.4 8.48def A.23.2 7.12cd A.23.4 9.11g 5.06a 5.13a 7.15defg 6.43bcde 8.00fgh 5.49abcd 7.02cdef 6.93cdef 5.37ab 7.44efg 7.90fgh 6.26abcde 6.43bcde 5.77abc 8.33gh 9.10h 7.90abcd 8.49de 8.41de 9.44e 7.42abc 8.45bcd 7.20a 8.15abcd 8.37cd 8.35abcd 12.85f 8.42cd 7.31ab 14.49g 13.56g 8.22abcd Values in the same columns followed by different letters (a-h) are significantly different (p<0.05), genera Streptococcus, Leuconostoc and Pediococcus. None of the isolates demonstrated catalase activity, indicating that all observed isolates did not produce the enzyme catalase. Roberts and Greenwood³⁷ stated that positive reactions in catalase test are indicated by the appearance of bubbles (gas) due to the activity of the enzyme catalase, which breaks down H₂O₂ into H₂O and CO₂. Taken together, these results indicate that all the isolates were LAB. The ability of the LAB isolates to produce gas from glucose was then assayed to determine the types of fermentation that the LAB isolates could perform. The results in Table 1 show that all the LAB isolates are homofermentative, i.e., glycolysis only produced lactic acid³⁸. The LAB demonstrating homofermentative include Streptococcus, Pediococcus and Lactobacillus, while some LAB are known to be heterofermentative, namely, Leuconostoc and some species of Lactobacillus. Further identification of LAB isolates was performed through growth tests at 15, 60 and 70EC for 48 h. All LAB isolates demonstrated growth at 15 and 60EC,

whereas only eight LAB isolates showed growth at 70EC. Bacteria that can grow at 60EC are defined as thermophilic bacteria. Garbutt³⁹ stated that thermophilic bacteria can grow at temperatures up to 70EC. Antibacterial activity of lactic acid bacteria: Antibacterial compounds produced by microorganisms can interfere with the growth and metabolism of other microorganisms. Antibacterial-producing LAB have the potential to be used as probiotics. Probiotics are live microorganisms that when consumed, can provide health benefits. A probiotic microorganism is capable of producing antimicrobial compounds that may inhibit the growth of pathogenic microbes. Allen et al.⁴⁰ stated that the main requirement for strains to be used as probiotic agents are resistance to acid and bile and the production of antimicrobial compounds. The presence of antibacterial compounds produced by LAB can be identified by inhibition of the growth of pathogenic bacteria. The LAB isolated from okara demonstrate various abilities to inhibit the growth of pathogenic bacteria such as *Escherichia coli* 0157, *Staphylococcus aureus* ATCC 25923 and *Listeria monocytogenes* EP01; this inhibitory ability is characterized by the formation of a clear zone around the wells. Table 2 shows that the average antimicrobial activity of LAB isolates against *Listeria monocytogenes* EP01 was higher than those of *Escherichia coli* 0157 and *Staphylococcus aureus* ATCC 25923. The highest antimicrobial activity of LAB was against *Listeria monocytogenes* EP01 as demonstrated by the formation of a greater clear zone around the wells that were filled with LAB isolate cells from okara. However, the antibacterial activity of LAB isolates on *Escherichia coli* was similar to their antimicrobial activity against *Staphylococcus aureus* ATCC 25923. The average inhibition zone diameter of LAB isolates against *Escherichia coli* 0157 ranged from 5.68-9.90 mm, *Staphylococcus aureus* ATCC 25923, from 5.06-9.10 mm and *Listeria monocytogenes* EP01, from 7.31-12.85 mm (Table 2). Most antimicrobial activities were in the range from 5.06-9.90 mm, indicating moderate antimicrobial activity; only a few isolates have demonstrated high antimicrobial activity⁴¹. Table 3: Diameter of the inhibition zone of lactic acid bacteria cell-free supernatant against bacterial pathogens Diameter of the inhibition zone of LAB cell-free supernatant (mm) -----

----- LAB cell-free supernatant *Escherichia coli*
Staphylococcus aureus *Listeria monocytogenes* A.11.3 6.30g A.11.4 2.40a
 A.12.4 7.77fgh A.13.1 6.79def A.13.2 5.00bc A.13.3 6.37bcde A.13.4
 6.37bcde A.21.1 5.64bcd A.21.2 7.15ef A.21.3 5.39bc A.22.1 7.67fgh
 A.22.2 3.79a A.22.3 4.97bc A.22.4 7.41efg A.23.2 7.11ef A.23.4 7.11ef
 4.94b 7.93f 7.87f 6.80de 3.43a 9.65g 5.83c 9.91g 7.46ef 7.62ef 5.75c
 3.67a 7.61ef 5.05c 6.91e 6.07e 7.97d 7.46de 7.53de 7.63de 11.74e
 5.60abc 6.03bc 12.26e 6.59cd 6.16c 5.65abc 4.90ab 7.48de 4.91ab 4.64a
 7.65de Values in the same columns followed by different letters (a-f) are significantly different (p<0.05). Differences in the sensitivity of pathogenic bacteria to LAB antibacterial activity were caused by the differences in strains, cell wall structure of pathogens and the different antimicrobial substances produced by LAB isolates⁴². Gram-negative bacteria contain cell wall structures that are much thinner than those of Gram-positive bacteria, such that antibacterial compounds accumulate more easily and damage the cell membrane. According to Jawetz et al.⁴³, differences in cell wall structure determine the penetration, binding and antibacterial activity of the compounds. Inhibition of Gram-negative bacteria is due to antibacterial compounds, including organic acids such as lactic acid. Lactic acid can disrupt the permeability of Gram-negative bacteria by damaging their outer membrane. Antibacterial activity of lactic acid bacteria cell-free supernatant: Table 3 indicates that the cell-free supernatant isolated from okara also retains antibacterial activity. The greatest antibacterial activity was found against *Listeria monocytogenes* EP01. The diameter of the clear zone that formed around the wells was 7.14 mm, whereas the diameters of the clear zones inhibiting *Escherichia coli* 0157 and *Staphylococcus*

aureus ATCC 25923 growth were 6.23 and 6.66 mm, respectively. Inhibition of the growth of gram-negative bacteria such as *Escherichia coli* 0157, *Staphylococcus aureus* ATCC 25923 and *Listeria monocytogenes* EP01 is primarily due to antibacterial compounds, including organic acids such as LAB-produced lactic acid. Lactic acid can disrupt the permeability of gram-negative bacteria by damaging the bacterial outer membrane. According to Alakomi et al.⁴⁴, lactic acid is a water soluble molecule that can penetrate the periplasm of gram-negative bacteria through the outer membrane. Lipopolysaccharide (LPS), located on the membrane surface, can be marred by lactic acid. Damage to LPS on the exterior of the cell can allow other antimicrobial compounds such as diacetyl, hydrogen peroxide and bacteriocins to invade the cytoplasmic membrane and disrupt intracellular activity, resulting in cell death. The diameters of the inhibitory zones generated by LAB isolates were greater than those of the cell-free supernatants. Figure 1-3 show a comparison of the antimicrobial activity of LAB isolates and of the cell-free supernatant against *Escherichia coli* 0157, *Staphylococcus aureus* ATCC 25923 and *Listeria monocytogenes* EP01, respectively. These figures show that all LAB isolates displayed greater antimicrobial activity than that of the cell-free supernatants against each pathogenic bacterial species. This finding is likely due to the antimicrobial activity of the LAB isolates, which still produce primary and secondary metabolites, such as organic acids, bacteriocin, diacetyl, bactericidal proteins and hydrogen peroxide⁴⁵, whereas cell-free supernatants only contain secondary metabolites of LAB, such as bacteriocin and hydrogen peroxide. As defined previously by Schillinger and Lucke⁴⁶, the antimicrobial activities of the LAB in this study were considered positive when the LAB had a clear zone diameter of 0.5 mm or larger. Survival rates of LAB isolates under conditions of low pH and bile salt exposure: For LAB to be successful probiotics, they must be able to tolerate gut conditions⁴⁷. To reach the 10 E. coli (cell) E. coli (supernatant) Diameter clear zone (mm) 9 8 7 6 5 4 3 2 1 0 A.11.3 A.11.4 A.12.4 A.13.1 A.13.2 A.13.3 A.13.4 A.21.1 A.21.3 A.21.3 A.22.1 A.22.2 A.22.3 A.22.4 A.23.2 A.23.4 LAB isolates Fig. 1: Comparison of the antimicrobial activity of LAB isolates and CFS against *Escherichia coli* 0157 12 S. aureus (cell) S. aureus (supernatant) 10 Diameter clear zone (mm) 8 6 4 2 0 A .11.3 .11.4 .12.4 .13.1 .13.2 .13.3 .13.4 .21.1 .21.3 .21.3 .22.1 .22.2 .22.3 .22.4 .23.2 .23.4 A A A A A A A A A A A A A A A A A A LAB isolates Fig. 2: Comparison of the antimicrobial activity of LAB isolates and CFS against *Staphylococcus aureus* ATCC 25923 intestine, LAB must be able to live in very extreme conditions found in the stomach (low pH 2-3); the time from initial consumption to passage through the stomach was estimated to be 90 min. These conditions can lead to a significant decrease ($p < 0.05$) in LAB viability due to low pH. Table 4 shows that not all isolated LAB can survive at pH 2.5 and 3 for 90 min. Isolates A21.1 and A21.3 displayed very low tolerance to acidic conditions (pH 2.5 and 3) and two isolates (A22.4 and A23.2) displayed relatively high resistance at pH 3, with a survival rate of more than 90%. Food is known to stay in the stomach for an average of 3 h⁴⁸. Bile salt tolerance is also required for probiotics to survive in the small intestine⁴⁹. The survival rate of LAB isolates on 0.5% oxgall as shown in Table 4, ranged from 77-139.80% after incubation for 5 h. Isolate A21.3 demonstrated the highest survival rate compared with other isolate survival rates. Three isolates showed survival rates of more than 50% (A23.4, A13.2 and A23.2) and three other isolates had very low survival rates, at less than 10%. This decrease in the survival rate of LAB is likely caused by changes in the cell membrane permeability of 16 L. monocytogenes (cell) L. monocytogenes (supernatant) 14 Diameter clear zone (mm) 12 10 8 6 4 2 0 A.11.3 .11.4 .12.4 A.13.1 A.13.2 A.13.3 .13.4 A.21.1 A.21.3 A.21.3 A.22.1 A.22.2 A.22.3 .22.4 A.23.2 .23.4 A A A A A LAB isolates Fig. 3: Comparison of the antimicrobial activity of LAB isolates and CFS against *Listeria monocytogenes* EP01 Table 4: Survival rate of lactic acid bacteria isolates under conditions of low pH and bile salt

exposure Isolated strains Survival rate (%) (90 min) -----
 ----- pH 2.5 pH 3 Survival
 rate (%) (5 h) ----- Oxgall (0.5%) A13.2 58.54b
 62.11c 116.67c A21.1 4.07a 7.59b 124.10c A21.3 1.29a 20.00b 139.80d
 A22.4 59.71b 90.53d 77.00a A23.2 69.99c 95.21e 90.00b A23.4 62.50b
 89.9d 134.00d Values in the same columns followed by different
 (a-e) are significantly different (p<0.05). Table 5: Identification of isolated
 LAB using the standard API 50 CHL kit Isolated LAB Significant LAB taxa
 Accuracy (%) Remarks A13.2 Lactobacillus pentosus 99.9 Excellent
 identification A21.1 Lactobacillus pentosus 86.1 Good identification A21.3
 Lactobacillus plantarum L. 99.9 Excellent identification A22.4 Lactobacillus
 pentosus 99.4 Very good identification A23.2 Lactobacillus pentosus 99.4
 Very good identification A23.4 Lactobacillus plantarum L. 91.7 Good
 identification Gram-positive bacteria, resulting in leakage of intracellular
 material due to cell lysis, resulting in cell death. Identification of candidate
 probiotics using the standard API 50 CHL kit: The LAB isolates were
 selected based on their antibacterial activity and their survival rates under
 conditions of low pH and 0.5% bile salt. Isolates were then identified by
 their carbohydrate fermentation patterns using an API 50 CHL kit (Table
 5). The API 50 CHL kit results showed that the LAB isolated from okara
 consisted of Lactobacillus pentosus for isolates A13.2, A21.1, A22.4 and
 A23.2 and Lactobacillus plantarum for isolates A1.3 and A23.4. According
 to Delgado et al.⁵⁰ the species Lactobacillus pentosus, isolated from
 plants, produces high bacteriocin titers. Probiotics with properties of
 Lactobacillus plantarum have been used in food fermentation^{51,52}. These
 species are both used in food fermentation because they have
 antimicrobial activity against pathogenic bacteria and are able to tolerate
 low pH and bile acid exposure^{53,54}. Lactic acid bacteria contribute greatly
 to the environment and have health benefits⁵⁵. Another study found that
 Lactobacillus pentosus and Lactobacillus plantarum isolated from
 fermented olives has probiotic potential⁵⁶. The identified LAB isolated
 from okara displayed various probiotic properties. These LAB showed
 antimicrobial activities against pathogenic bacteria (Escherichia coli 0157,
 Staphylococcus aureus ATCC 25923 and Listeria monocytogenes EP01) and
 displayed survival rates of more than 50% under conditions of low pH and
 bile salt exposure. These findings require further studies, especially with
 regard to the molecular identification of LAB, identification of their
 antimicrobial compounds and their applications in food as probiotics and
 food biopreservatives. CONCLUSION The antimicrobial activity of LAB
 isolated from okara varied among individual isolates and the antibacterial
 activity of the LAB isolates was higher than that of the cell-free
 supernatant. Based on the antimicrobial activity and probiotic properties of
 the LAB isolated from okara, these isolates belong to the species
 Lactobacillus pentosus and Lactobacillus plantarum. SIGNIFICANCE
 STATEMENTS This study isolated LAB from okara and identified them as
 the species Lactobacillus pentosus and Lactobacillus plantarum. This study
 will help the researchers to uncover the benefits of indigenous LAB isolated
 from okara, that was a waste product of soymilk production. Using these
 LAB for producing functional food products from soybean and other
 foodstuffs and for biopreserving foods will be proven new explorations.
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