Molecular Identification and Antimicrobial Potency of Probiotic Lactic Acid Bacteria Pado (Fish Fermentation) Nagari Balingka IV Koto District-West Sumatra as a Functional Food

by Rinita Amelia

Submission date: 22-Jul-2023 03:45PM (UTC+0800)
Submission ID: 2134907193
File name: -balingka-iv-koto-district-west-sumatra-as-a-functional-food.pdf (740.84K)
Word count: 4332
Character count: 23397

Molecular Identification and Antimicrobial Potency of Probiotic Lactic Acid Bacteria *Pado* (Fish Fermentation) Nagari Balingka IV Koto District-West Sumatra as a Functional Food

Molecular Identification and Antimicrobial Potency of Probiotic Lactic Acid Bacteria *Pado* (Fish Fermentation) Nagari Balingka IV Koto District-West Sumatra as a Functional Food

Anisah[1,2], H Harun[*1,2], H Jannah[3], R Amelia[4], E Purwati[5,6]
[1]Biotechnology-School of Postgraduate, Andalas University, Padang, Indonesia
[2]Internal Medicine Departement, Medical Faculty, Andalas University, Padang, Indonesia
[3]Magister program of Animal Science, Andalas University, Padang, Indonesia
[4]Medical Faculty, Baiturrahmah University, Padang, Indonesia.
[5]Professor of Biotechnology-School of Postgraduate, Andalas University, Padang, Indonesia
[6]Head of Senator, STIKES Prima Indonesia, Bekasi, Indonesia

E-mail: harnavi@med.unand.ac.id, Orcid ID: 0000-0002-0795-7943

Abstract. *Pado*, indigenous cuisine from the region of West Sumatera, is a mixture of fish with the meat seed of *Simuang* (*Pangium edule* Reinw) and grated coconut fermented for 4-8 days. *Pado* is thought to contain lactic acid bacteria (LAB) activity used as a probiotic-producing functional food which is good nutritional value. Based on it, *Pado* had the potency to improve the economy which had a good impact on regional development. This aims to decide the molecular identifications and antimicrobial activity of LAB as probiotics contain in *Pado* that are useful as functional foods. The sample used as material for this research is *Pado* from Nagari Balingka IV Koto District in Agam Regency-West Sumatera. The research methods are bacterial isolation from *Pado*, determinate LAB using laboratory analysis, and LAB identification using the 16S rRNA method. The result of Gram staining showed Gram-positive that the purple rod-shaped. The other characteristics of *Pado* isolate were homofermentative, catalase-negative, resistant to acidic pH, and bile salt. The colony of *Pado* LAB has a white-beige smooth-convex surface. *Pado* Nagari Balingka IV Koto is a functional food that contains the probiotic *Lactobacillus plantarum* strain SRCM 102737 and it had antimicrobial activity against pathogen bacteria.

1. Introduction

Culinary heritage could be one of the commodities to improve a region's economy so that it had a positive impact on regional development. Besides Rendang, which is famous food worldwide, one of the indigenous culinary from West Sumatra is *Pado*. *Pado* is a mixture of raw fish with meat seed of *Simuang* (*Pangium edule* Reinw) and grated coconut fermented for 4-8 days [1]. Other such local fermented foods are Tempoyak (a fermentation of durian pulp) and *Bekasam* (fermented fish with *Tempoyak*). Those local foods were thought to contain lactic acid bacterial activity and used as a probiotic-producing functional food that good nutritional value.

Probiotics are live microorganisms that could maintain the balance of the gastrointestinal system and provide health effects for the human body [2]. The probiotics within the human body must be able resistant to high temperature, corrosive and bile salt, the expanded concentration of particular particles or supplement consumption, introduction to osmotic stretch, and oxidative in product frameworks in conjunction with section through the gastrointestinal transit that might affect their viability and functionality.

Lactic acid bacteria could rebuild advanced compounds into straightforward compounds to produce lactic acid from glucose [3]. Fermented foods could inhibit most microorganisms such as mould that

produced mycotoxin and pathogenic bacteria, therefore fermented foods have a long shelf life [4]. *Pado* had a shelf life of up to two months [1]. Several lactic acid bacteria are found in a curd that is produced by spontaneous fermentation in West Sumatera such as *Lactobacillus plantarum ssp. plantarum, Lactobacillus pentosus, L. lactis ssp. Lactis, L. lactis ssp. cremoris,* and *Pediococcus pentosaceus* [5]. *Lactobacillus fermentum* strain CAU6337 was isolated in *tempoyak* [6].

This aims to recognize molecularly and decide the antimicrobial activity of LAB as a probiotic contained in *Pado* Nagari Balingka IV Koto. It could be useful to know *Pado* as a probiotic-producing functional food.

2. Materials and methods

2.1Materials

The materials utilized in this study comprise *Pado* from Nagari Balingka IV Koto Area in Agam Regency, West Sumatera-Indonesia.

2.2Methods

2.2.1. Total colonies of LAB. One gram of *Pado* sample was included in a test tube containing 9 ml of MRS broth and after that made suspension. This suspension's 10^{-1} dilution was brooded at 37ºC for 48 h. The 100 μl 10^{-1} dilution was exchanged to the first Eppendorf containing 900 μl of MRS Broth called the 10^{-2} dilution. This preparation was carried out 8 times. The 100 μl 10^{-8} dilution was taken and then planted on MRS media in a petri dish and levelled with an L stick. The inoculum was brooded at 37°C in an anaerobic compartment for 48 h. The total LAB colonies could be counted after 48 h [7].

2.2.2. Isolation and purification of bal pado. The enriched bacterial culture suspension was 100 μl and exchanged to Eppendorf containing 900 μl of MRS Broth. The dilution was carried out until it reached 10^{-8} dilution. The 100 μl 10^{-8} dilution of bacterial culture was planted on a petri dish that already contained MRS media and after that levelled with an L stick. The inoculum was put away in an anaerobic compartment and was brooded at 37°C for 48 h. After that, a single colony that characterized LAB (circular yellowish-white slippery) was exchanged to MRS Agar media for colony refinement by utilizing the streak method with a circular needle (Osse needle). After that, it brooded at 37°C for 24 h [7].

2.2.3. Gram staining. The bacterial culture of *Pado* was taken and spread on a cleaned glass object and dried using a bunsen or dryer. After dripping with crystal violet dye for one minute to allow the stain to be absorbed by the bacteria, it was rinsed under running water then dabbed with iodine complex solution for 1 minute and rinsed again with running water. Afterwards, it was washed with alcohol by dipping it into diluted alcohol and dripped with safranin dye for 30 seconds. After that, it was dried and examined under a microscope [7].

2.2.4. Biochemical test. Fermentation selection tests were performed by embeddings LAB isolates in 5 ml of MRS broth MERCK, embedding Durham tubes upside down and brooding at 37°C for 48 h. Test tube perception was performed by observing the appearance of air bubbles in the Durham tube [8].

A catalase assay was performed by removing its LAB isolate utilizing a circular needle. The isolate is scratched onto the objective glass with 3% Hydrogen peroxide (H_2O_2) drops. The perception was made to decide whether or not gas bubbles were shaped [8].

2.2.5. Acid resistance test. A 500 µl LAB culture that had been enhanced for 24 h was placed in a test tube containing 5 ml of MRS broth. A drop of HCl drop was embedded in the solution until it appears a pH of 3, which is the stomach acid's pH—then brooded for 90 minutes. The solution was diluted to

10^{-6} dilutions. The result of 10^{-6} dilutions was planted 100 µl into MRS agar media on a petri dish—brooded for 48 h before calculating colony and LAB's viability [9].

2.2.6. Bile salt resistance test. 1 ml of bacterial culture was added to 9 ml MRS broth media and brooded at 37 ºC for 24 h. Additionally, 1 ml of the bacterial culture was embedded in a test tube containing 9 ml of MRS broth without ox bile initiation (control) and 0.3% cultured ox bile initiation was applied to the MRS broth for 5 h. The dilution ratio at this time was 10^{-6}, planted on MRS agar medium by the spread method and brooded at 37ºC for 48 h. The number of potentially viable bacteria was counted by the colony-forming unit (CFU) plate counting method, and then the LAB viability was counted [8].

2.2.7. Antimicrobial resistance and antibiotic activity assay. 1 ml of LAB culture enriched was taken by employing a micropipette and was put into Eppendorf. At this time, the supernatant for the antibacterial resistance test was centrifuged at 10,000 rpm for 5 to 7 minutes to prepare 0.4 g. After the medium has cooled slightly (± 45ºC), it was poured into a petri dish of ± 20 ml and was included 0.2% of the test bacteria have been enhanced for 24 hours. After the order hardens a well was made and 50 µl of LAB supernatant was embedded therein. After 24 hours of incubation at 37ºC aerobically, observations were made of the clear zone formed by measuring the diameter using a caliper [8].

2.2.8. Isolation of genome DNA from lactic acid bacteria and 16S rRNA. The isolated LAB was refined in MRS broth at 37°C for 24 h. Genomic DNA isolation was performed utilizing the Promega kit (USA). A 1000 µL colony LAB lock was removed from the MRS broth, placed in an Eppendorf tube and centrifuged at 14000 rpm for 2 minutes. The supernatant and pellets were expelled, and 480 µL of 50 mM EDTA and 120 µL of lysozyme were included. At this point, it was hatched in a 37ºC water chamber for 60 minutes. It was then centrifuged at 14000 rpm for 2 minutes. The supernatant and the pellets were drained and a 600 µL of nuclear lysis solution was included. The solution was brooded at 80ºC for 5 minutes and left at room temperature. After including 3µL of RNAse solution, the plate was hatched in a 37ºC water bath for 60 minutes. It contained 200 μL of protein precipitation solution and was vortexed after including 600 µL of isopropanol. The solution was centrifuged at 14,000 rpm for 2 minutes and the pellets and supernatant were discarded. 600 µL of 70% ethanol was included and the solution was homogenized. Pellets and supernatant were expelled after being centrifuged at 14000 rpm for 2 minutes. Pellet DNA was rehydrated by including 10-100 µL of rehydration solution at a temperature of 65ºC for 30 minutes.

Primer R (16S-1492R, Tm 47°C, 5'-GTT TAC CTT GTT ACT ACT-3') and Primer F (16S-27F, Tm 54.3°C, 5'-AGA GTT TGA TCC TGG CTC AG-3') at 10 μm concentration. 90µL ddH_2O + 10µL (primary R and F) were withdrawn. R and F primers in TE buffer (100 µM concentration) Eppendorf tube in one PCR cocktail (12.5 µL master mix, one µL F primer, one µL R primer, one µL DNA template, 9.5 µL ddH_2O), at 95°C denaturing PCR and tempering at 56°C for 45 sec each, 72°C expansion for 1 minute 40 seconds, 72°C last expansion for 10 minutes. Electrophoresis 10 µL sample into agar well, four µL DNA step was embedded and was set to 100 Volt for 45 minutes. The gel was placed in a container of TBE until submerged. The gel could be viewed with a UV light. 16S rRNA gene sequences from the isolates were submitted to NCBI for BLAST searches. The phylogenetic tree was made by MEGA version 7.0.

3. Results and dicussion

3.1. Macroscopic identification and biochemical quality

Table 1. Macroscopic identification and biochemical of LAB isolates.

Sample	Total LAB Count (CFU/gr)	Colour	Shape	Surface	Catalase Test	Type of Fermentation
Pado Balingka	$2.57x10^{11}$	White-beige	Round	Smooth-Convex	Negative (−)	Homofermentative

Table 1 showed macroscopic observations (shape, size, and colour) of LAB and found that the colony was White-beige, circular in shape, and had a convex elevation with smooth edges. The total calculation showed LAB *Pado* was 2.57 $x10^{11}$ CFU/g. The result of the catalase test was negative and its fermentative type was homofermentative.

The LAB isolate colonies had a circular shape, convex, rough, shiny, and smooth surface [10]. According to the Food and Agriculture Organization, for food to contain probiotics that there must be at least 10^6 CFU/g of LAB. LAB *Pado* was 2.57 $x10^{11}$ CFU/g, *so Pado* contains probiotics [11].

Lactic acid bacteria have negative catalases indicating that no air bubbles were formed due to the presence of O_2 gas. The catalase reaction is obvious within the fast arrangement of bubbles. A few bacteria produced hydrogen peroxide as an oxidative end product of aerobic glucose degradation [12].

It is profoundly harmful to bacteria on the off chance that permitted to construct up and can result in cell death. LABs metabolize carbohydrates for energy, utilizing endogenous carbon sources instead of oxygen as the ultimate electron acceptor. They are protected from oxygen byproducts such as hydrogen peroxide by peroxidases [13].

Lactic acid bacteria are categorized into two sorts of fermentation, they are homofermentative and heterofermentative [14]. LAB, which is predominantly homofermentative, supplies lactic acid from sugars. Heterofermentative produces lactic acid and acetic acid, ethanol and CO_2. LAB may be a group of gram-positive, inclining toward anaerobic conditions, catalase-negative, oxidase-negative, and entirely fermentative bacteria that produce lactic acid as a major or sole item of fermentative metabolism [15].

3.2. Morphological identification

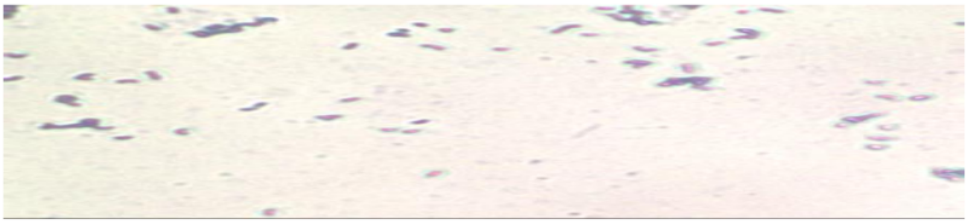

Figure 1. Gram staining *Pado* balingka.

Based on the morphology identification, Fig. 1 the Gram stain result of Pado Balingka showed Gram-positive (purple) and circular-shaped/basil. LAB is a group of gram-positive, cocci or circulars, catalase-negative, and particular organisms [16]. The characteristics of gram-positive bacteria are that they have a thick and homogeneous cell wall consisting of peptidoglycan. Gram-positive bacteria will absorb the purple colour from crystal violet when staining the gram, so it will be purple when viewed under a microscope [3].

3.3. Resistance test of LAB against acids

Table 2. LAB resistance to gastric pH.

Isolate Samples	Number of bacterial cells (CFU/ml)		Viability of LAB (%)
	pH control	pH 3	
Pado Balingka	91x10^7	85 x10^7	93.40

Table 2 showed the result of *Pado* Balingka isolates having high viability resistance to acidic pH which was 93.40%. The results showed that each confine had diverse viability. This was because each confinement had distinctive capacities to survive at a low acidity. This occurs because the differences in cytoplasmic membranes are diverse. The characteristics and membrane permeability influence this diversity.

Resistance to stomach acid is also essential for an isolate that can become a probiotic. This is because, if the isolate enters the human digestive tract, it must withstand stomach acid [17]. LAB can be said to be probiotic if they can be safe for gastric pH (pH 2 to 3), which is caused by the emission of gastric juices [18]. LAB isolates from *Pado* fish can be categorized as probiotics because they can survive gastric pH.

3.4. Bile salt resistance test of LAB

Table 3 showed the results of the LAB resistance to bile salt having 80% viability. This indicated that *Pado* Balingka isolates were bile salt resistant.

Table 3. Bile salt resistance of LAB.

LAB Isolate Samples	Number of Bacterial Cells (CFU/ml)		Viability of LAB (%)
	Control (KOx)	*Ox gall* 0,3% (Ox)	
Pado Balingka	150 x10^7	135 x10^7	80

Lactic acid bacteria can be categorized as probiotics and must survive at an alkaline pH of 7.8-8.4, containing bile salt as much as 0.3% -2% [18]. Bile salt-resistant LAB is connected to the enzyme bile salt hydrolase (BSH) because BSH contributes to the survival of LAB within the stomach-related tract [19]. LAB isolates from Pado fish can be categorized as probiotics because they can survive bile salt.

3.5. Antimicrobial and antibiotic activity

Table 4. Measurement of clear zone diameter for LAB's antimicrobial activity.

Inhibitory source	The diameter of the clear zone (mm)			
	E. coli 0157	*Propionibacterium acnes*	*Acinetobacter baumannii*	*Listeria monocytogenes*
Pado Balingka	5,10	12,6	15,6	9,14
Ampicillin	22,27	-	-	-
Kanamycin	17,22	14,7	12,2	6,11
Penicillin	-	-	-	-

Table 4 showed that *Pado*'s LAB shaped clear zones against *E. coli 0157, Propionibacterium acnes, Acinetobacter baumannii*, and *Listeria monocytogenes* test bacteria. Based on the antimicrobial activity test, *Pado*'s LAB had a clear zone against the pathogenic bacteria.

It exhibits antimicrobial activity against *E. coli O157* (5.10 mm), *Propionibacterium acnes* (12.6 mm), *Acinetobacter baummannii* (15.6 mm), and *Listeria monocytogenes* (9.14 mm). The diameter of the retention zone against pathogens showed low antimicrobial activity when the clear zone was 0-3 mm, moderate antimicrobial activity >3-6 mm, and high antimicrobial activity when >6 mm [20].

Based on Table 4, the activity of LAB in inhibiting the growth of pathogenic bacteria was moderately active againts *E. coli* 0157, with high antimicrobial activity againts *Propionibacterium acnes, Acinetobacter baumanniiand Listeria monocytogenes*.

Lactic acid bacteria can inhibit *Listeria monocytogenes* [21]. *Lactobacillus plantarum* strain 8m-21 which had inhibitory zones on *Escherichia coli* 0157 were 20.25 mm [22]. The clear zone formed indicated a LAB isolate's antimicrobial activity against tested bacteria [23]. The more extensive the clear zone shaped, the greater the lactic acid bacteria in inhibiting pathogenic bacteria's growth. LAB are acid-producing (lactic acid) and acid-tolerant which makes a difference in the LAB to outcompete other bacteria in a natural fermentation in this way hindering the growth of decay as well as pathogenic microorganisms [24]. Pathogenic microorganisms such as *Staphylococcus aureus* and *Salmonella sp* will be inhibited if there were lactic acid bacteria [20].

The antimicrobials activity of BAL can be caused by the production of lactic, acetic, formic, caproate, propionic, butyric, and valerate acid, H_2O_2 compounds as well as bacteriocin [20]. In penicillin antibiotics, no clear zone is formed because test bacteria *Listeria monocytogenes, Escherichia coli O157, Propionibacterium acnes,* and *Acinetobacter baumannii* are resistant to penicillin antibiotics.

3.6. Analysis of 16S rRNA gene sequence isolate from Pado Balingka

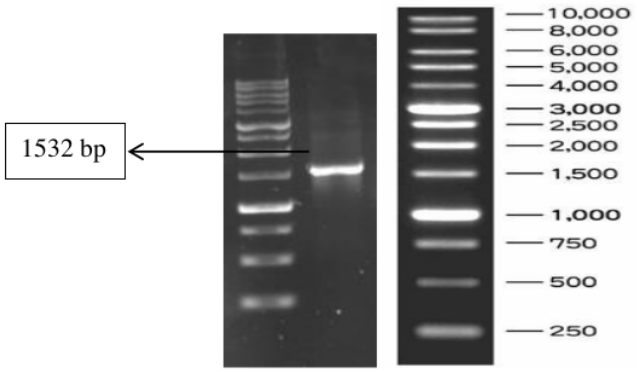

Figure 2. PCR results of BAL *Pado* Balingka isolation sequence.

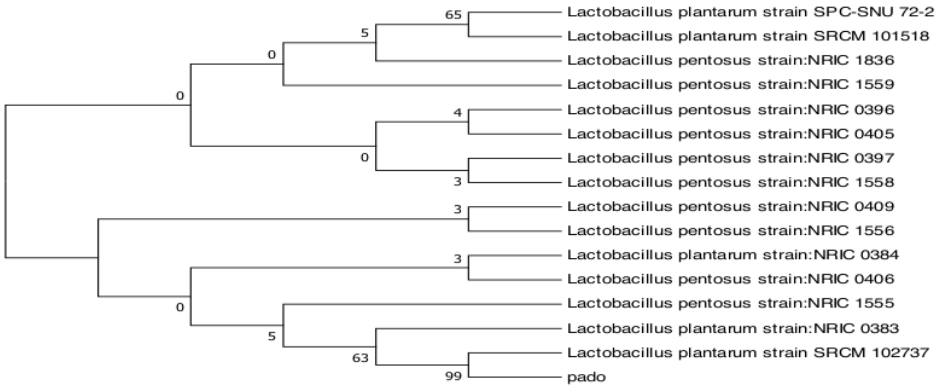

Figure 3. Phylogenetic tree of BAL *Pado* Balingka isolate.

Figure 2 showed the result of *Pado* Balingka PCR sequencing was 1532 bp. Based on sequencing and BLAST analysis, fig. 3 the LAB isolate bacteria from *Pado* were similar to the *Lactobacillus plantarum* strain SRCM 102737. The phylogenetic tree shows that the closest distance to *Pado*

isolates is *Lactobacillus plantarum* strain SRCM 102737. the formation of a phylogenetic tree. This is done by looking at the similarities in the genetics of the organisms being compared [25]. The higher the level of genetic similarity, the closer the evolutionary and related relationships [26]. The organisms that form the phylogenetic tree must have similar genetics.

4. Conclusion

The sequencing results of the LAB *Pado* PBY isolate from the Nagari Koto Tuo area, IV Koto District, Agam Regency were *Lactobacillus plantarum* strains SRCM 10273 which is had antimicrobial activity against pathogens. It approved *Pado* as a probiotic-producing functional food.

Acknowledgments

This research is upheld by the Research Facility Livestock Products Technology, Faculty of Animal Husbandry - Andalas University. The authors are thankful to the Institute for Community Service Research (LPPM) for the substantial investigation of the remarkable research cluster for the professor's publication under contract no. T/15/UN.16.17/PT.01.03/KO-PDU-KRPIGB/2020.

References

[1] Hasbullah, Kasim A, Novelina and Nurdin H 2016 Characterization of traditional food *Pado* from West Sumatera *Scholars Research Library* **8** 26-31
[2] Terpou A, Papadaki A, Lappa IK, Kachrimanidou V, Bosnea LA and Kopsahelis N 2019 Probiotics in food systems: significance and emerging strategies towards improved viability and delivery of enhanced beneficial value *Nutrient* **11** 1591 2-32
[3] Fevria R and Hartanto I 2019 Isolation and characterization of lactic acid bacteria (*Lactobacillus* sp.) from Sauerkraut *Advances in Biological Sciences Research* **10** 74-77
[4] Moller CODA, Freire L, Rickhard-Forget F 2021 Effect of lactic acid bacteria strains on the growth and aflatoxin production potential of *Aspergillus parasiticus* and their ability to bind Aflatoxin B1, Ochratoxin A and Zearalenone in vitro *J. Food Control* **21** 370-380
[5] Wirawati CU, Sudarwanto MB, Lukman DW, Wientarsih I and Srihanto EA 2019 The diversity of lactic acid bacteria in curd produced by either back-slopping or spontaneous fermentation from two West Sumatera regions, Indonesia *Vet World* **12** (6) 823-829
[6] Juliyarsi I, Purwati E, Hartini P, Yuherman, Djamaan A, Arief, Purwanto H, Aritonang SN and Hellyward J 2018 Characterization of lactic acid bacteria and determination of antimicrobial activity in tempoyak from Padang Pariaman District, West Sumatera, Indonesia *Pakistan Journal of Nutrition* **17** 506 - 511
[7] Purwati E, Melia S, Kurnia YF and Pratama DR 2019 Antimicrobial potential of Pediococcus acidilactici from Bekasam, fermentation of sepat rawa fish (Tricopodus trichopterus) from Banyuasin, South Sumatra, Indonesia *Biodiversitas Journal of Biological Diversity* **12** 3532-38
[8] Amelia R, Philip K, Pratama YE and Purwati E 2021 Characterization and probiotic potential of lactic acid bacteria isolated from dadiah sampled in West Sumatra *Food Science and Technology* **41**(2) pp.746-752
[9] Diza YH, Asben A dan Anggraini T 2020 Isolasi, identifikasi dan penyiapan sediaan kering bakteri asam laktat yang berpotensi sebagai probiotik dari dadih asal Sijunjung Sumatera Barat. *Jurnal Litbang Industri Dan Teknologi Indonesia*, **10** 2 155-164
[10] Bagheri F, Azari AA, and Koohsari H 2022 Antibacterial activity of Lactobacilli from buffalo milk and yoghurt in Bandar-E Gaz, North-West Iran *Bulgarian Journal of Veterinary Medicine* **25** 3 379-386
[11] Food and Agriculture Organization. 2013. Guidelines for the Evaluation of Probiotics in Food: Report of a Joint FAO. Rome, Italy
[12] Public Health England 2019 Catalase test *UK Standards for Microbiology Investigations* 2 - 14

[13] Wang Y, Wu J, Lv M, Shao Z *et al* 2021 Metabolism Characteristics of Lactic Acid Bacteria and the Expanding Applications in Food Industry *Front Bioeng Biotechnol* **12** (9) 612285
[14] Meena KK, Taneja NK, Jain D, Ojha A, Kumawat D and Mishra V 2022 In vitro assessment of probiotic and technological properties of lactic acid bacteria isolated from indigenously fermented cereal-based food products. *Fermentation* **8** 529
[15] Ngasotter S, Waikhom D, Mukherjee S, Devi MS and Singh AS 2020 Diversity of lactic acid bacteria (LAB) in fermented fish products: a review *International Journal of Current Microbiology and Applied Sciences* **9** 2238-49
[16] Mokoena MP 2017 Lactic acid bacteria and their bacteriocins: classification, biosynthesis, and applications against uropathogens: a mini-review *Molecules* **22** 2-13
[17] Guan X, Xu Q, Zheng Y, Lei Q and Bin L 2017 Screening and characterization of lactic acid bacterial strains that produce fermented milk and reduce cholesterol levels *Brazilian journal of microbiology* **48** 730–39
[18] Ramadhanti N, Melia S, Hellyward J and Purwati E 2021 Characteristics of lactic acid bacteria isolated from palm sugar from West Sumatera, Indonesia, and their potential as a probiotic *Biodiversitas* **22** 2610-16
[19] Allain T, Chaouch S, Thomas M, Vellee I, *et al* 2018 Bile-salt-hydrolases from the probiotic strain *Lactobacillus johnsonii* La1 mediate anti-giardial activity *in vitro* and *in vivo Front Microbiol* **8** 2707
[20] Chidre P and Revanasiddappa KC 2017 Probiotic potential of *Lactobacilli* with antagonistic activity against pathogenic strains: an in vitro validation for the production of inhibitory substances *Biomedical Journal* **40** 5 270-283
[21] Melia S, Yuherman, Jaswandi, Purwati E, Aritonang S and Silaen M 2017 Characterization of the antimicrobial activity of lactic acid bacteria isolated from buffalo milk in west Sumatra (Indonesia) against *Listeria monocytogenes Pakistan Journal of Nutrition* **16** 645-50
[22] Harun H, Wirasti Y, Purwanto B and Purwati E 2020 Characterization of lactic acid bacteria and determination of antimicrobial activity in dadih from Air Dingin Alahan Panjang District, Solok Regency-West Sumatera *Sys Rev Pharm* **11**(3) 583-86
[23] Afriani N, Yusmarini and Pato U 2017 Aktivitas antimikroba *Lactobacillus plantarum* 1 yang diisolasi dari industri pengolahan pati sagu terhadap bakteri patogen *Escherichia coli* FNCC-19 dan *Staphylococcus aureus* FNCC-15 *Jom Faperta* **4** 1-12
[24] Yang E, Fan L, Yan J, *et al* 2018 Influence of culture media, pH and temperature on growth and bacteriocin production of bacteriocinogenic lactic acid bacteria *AMB Expr* **8** 10-16
[25] Seprianto, Feliatra and Nugroho TT 2017 Isolasi dan identifikasi bakteri probiotik dari usus udang Windu (*Penaeus monodon*) berdasarkan sekuens gen 16S rRNA *Jurnal Ilmiah Biologi* **5** 83-92
[26] Ludwig W, KH Schleifer and WB Whitman 2015 Lactobacillales ord. nov. Bergey Systematic bacteriology vol. 3 London Springer 465-511

Molecular Identification and Antimicrobial Potency of Probiotic Lactic Acid Bacteria Pado (Fish Fermentation) Nagari Balingka IV Koto District-West Sumatra as a Functional Food

ORIGINALITY REPORT

9%	%	9%	%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	A Dalimunthe, M Muhammad, S.B Waruwu, M Rafi, V.E Kaban, D Satria. "Phytochemicals and Proximate Analysis of Litsea Cubeba Lour. Barks", IOP Conference Series: Earth and Environmental Science, 2023 Publication	5%
2	Rinita AMELIA, Koshy PHILIP, Yudha Endra PRATAMA, Endang PURWATI. "Characterization and probiotic potential of lactic acid bacteria isolated from dadiah sampled in West Sumatra", Food Science and Technology, 2020 Publication	4%

Exclude quotes	On	Exclude matches	< 3%
Exclude bibliography	On		