BIODIVERSITAS
Volume 25, Number 4, April 2024
Pages: 1521-1527

ISSN: 1412-033X
E-ISSN: 2085-4722
DOI: 10.13057/biodiv/d250420

Characteristics of probiotic lactic acid bacteria isolated from edible birds nests as antimicrobials potential

ARI HARMAYANI[1,♥♥], MARLINA[1,2,♥], ENDANG PURWATI[1,3]

[1]Graduate School of Biotechnology, Universitas Andalas. Jl. Kampus Unad, Limau Manis, Padang 25171, West Sumatra, Indonesia. Tel. +62-751-71686, ♥email: marlina@phar.unand.ac.id; ♥♥arienzzta@gmail.com

[2]Faculty of Pharmacy, Universitas Andalas. Jl. Kampus Unad, Limau Manis, Padang 25171, West Sumatra, Indonesia

[3]Stikes Prima Indonesia. Jl. Raya Babelan, Bekasi District 17610, West Java, Indonesia

Manuscript received: 27 August 2023. Revision accepted: 13 April 2024.

Abstract. *Harmayani A, Marlina, Purwati E. 2024. Characteristics of probiotic lactic acid bacteria isolated from edible birds nests as antimicrobials potential. Biodiversitas 25: 1521-1527.* Indonesia's export commodities include Edible Bird Nests (EBN), which possess significant economic worth. EBN is a nutrient-dense functional food that offers substantial health benefits. This study assessed the antibacterial capabilities of Lactic Acid Bacteria (LAB) extracted from EBN. This study utilized EBN sourced from three samples in West Sumatra, Indonesia, specifically in the Agam District. Use the method of experimental and collection sample EBN and identification of LAB in vitro. The isolates were examined for their morphological and biochemical characteristics. In addition, an antibacterial test was conducted along with molecular identification of the 16S rRNA gene. The findings indicated that the LAB exhibited gram-positive features, with bacilli morphology. They were also catalase-negative and classified in the homofermentative category. Antimicrobial activity was seen in all three EBN samples. BK3 edible birds' nest isolate exhibited the highest antimicrobial activity against *Listeria monocytogenes* CFSN004330, with an inhibition zone diameter of 19.53 mm. It also demonstrated significant activity against *Staphylococcus aureus* ATCC 25923 (18.71 mm), *Propionibacterium acne* (18.20 mm), and *Escherichia coli* 0157 (14.60 mm). The molecular identification using 16S rRNA revealed that the LAB obtained from the BK3 edible birds' nest isolate had similarities with *Lacticaseibacillus paracasei* strain HBUAS6004, which possesses antibacterial properties against pathogenic bacteria. There was an expectation that LAB isolates derived from EBN may serve as natural antimicrobials, providing benefits to animal and human health benefits.

Keywords: 16S rRNA, antimicrobial, edible bird nest, LAB, lactic acid bacteria, probiotics

Abbrevations: EBN: edible bird's nest; H_2O_2: hydrogen peroxide; LAB: lactic acid bacteria

INTRODUCTION

Indonesia is the world's largest Edible Bird Nest (EBN) producer. EBN is one of Indonesia's export commodities with high economic value because it is known for its health benefits. Based on 2017 data from the Indonesian Trade Promotion Center (ITPC) of the Ministry of Trade, it was noted that as much as 78% of the world's EBN supply comes from Indonesia. The main market for EBN is China (Kementan 2021). One of the provinces in Indonesia that produces EBN is West Sumatra Province. There are many EBN cultivars in West Sumatra. However, most of the EBN is sent between areas to Java, Medan, Kalimantan, and other regions, where it is then exported abroad, especially to China. According to the IQFast system from the Padang Class I Agricultural Quarantine Center, in 2020, 23.6 tons, and in 2021, as many as 19 tons of EBN were trafficked out of West Sumatra.

EBN is a nest made from the dried saliva of male swallows of the genus *Aerodramus* or *Colocalia* when the female swallows lay their eggs. EBN is often consumed as a healthy and luxurious food, especially by Chinese people, because of its nutritional health benefits (water-soluble protein, carbohydrates, iron, inorganic salts, and fiber) and its medical benefits (anti-aging, anti-cancer, and immune-boosting) (Elfita et al. 2020). EBN components have been studied as functional food bioactive ingredients in peptides (Wong et al. 2017) and glycoproteins (Shim et al. 2016), which are safe for human consumption.

Probiotics are food supplements in the form of non-pathogenic live microorganisms, resistant to stomach acid, can colonize the large intestine (colon), and are beneficial to health (FAO and WHO 2006). The most well-known type of probiotic bacteria is the lactic acid (LAB) group. Lactic acid bacteria are generally known as harmless microorganisms and have a good history of safety in food fermentation techniques to obtain a specific taste and preserve food products naturally. Food preservatives of natural origin are considered a potential and safe source of antimicrobials, but their effective use in practice is limited. This possibility is supported by many studies conducted by researchers and professionals in the food industry (Smid and Gorris 2020).

The use of synthetic antibiotics in animals and humans today causes antibiotic resistance to bacteria in medicine, food preservatives, and livestock production. Antibiotic application in livestock and humans can only be done if natural antimicrobial alternatives are available. Natural

antimicrobial activity can be obtained from LAB according to research on bekasam, budu, and palm sap (Melia et al. 2019; Pratama et al. 2021; Efendi et al. 2023) Therefore, more research needs to be done to isolate, identify, and test the effectiveness of natural antimicrobials in various natural products LAB.

Research on LAB in EBN, a functional food, has never been done. However, many studies have been conducted to identify LAB isolated from other living natural resources. *Lactobacillus fermentum* was isolated from buffalo, cow, and goat milk (Melia et al. 2017), *Pediococcus acidilactici* PB22 from bekasam (Melia et al. 2019), *Lactobacillus fermentum* 1743 from palm sugar (Ramadhanti et al. 2021), and *Lactobacillus plantarum* SN13T from gallo-galo honey (Melia et al. 2022). Overall, these studies showed the presence of antimicrobial activity.

This probiotic LAB can be a potential innovation in the development of probiotic products, including as a natural antimicrobial that can be used as an alternative to antibiotics because it can inhibit gram-positive and gram-negative bacteria. Besides that, it can also be developed as food biopreservation. Isolation and identification of LAB from EBN are important because, in addition to EBN as a functional food, it is hoped that they can be used as natural antimicrobials that are beneficial to animal and human health so that they can be developed for further research and become good business prospects in the world. Health and food safety, as well as improving the quality of public health and the world of animal husbandry. Furthermore, this work aimed to acquire strains of LAB found in edible bird's nests using molecular identification utilizing the 16S rRNA gene sequence.

MATERIALS AND METHODS

Sampling

This study used samples of EBN in Ampang Gadang Village, Ampek Angkek Sub-district, Agam District, West Sumatra, Indonesia (00^0 01' 34" - 00^0 28' 43" S, 99^0 46' 39" - 100^0 32' 50" E) (Figure 1). Three samples of EBN from were used to isolate and identify LAB. EBN samples were taken directly from farmers in the field and brought to the laboratory for observation and testing (Figure 2).

Figure 2. Edible birds nest

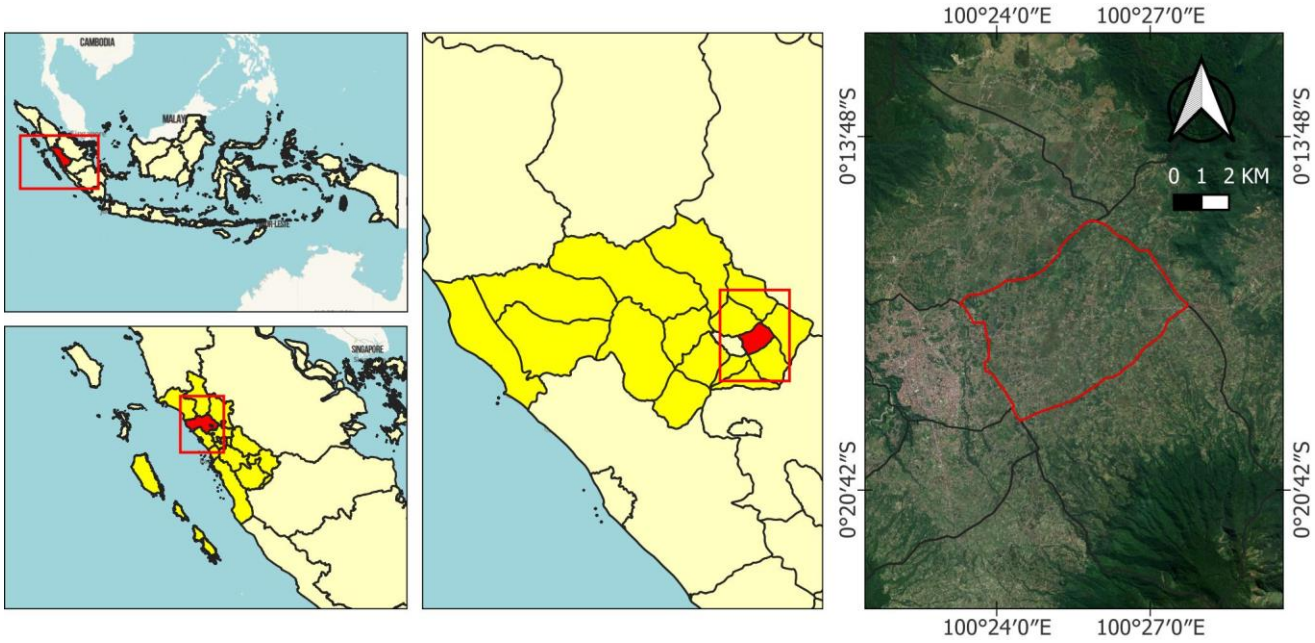

Figure 1. Collecting sample edible bird's nests in Ampek Angkek Sub-district, Agam District, West Sumatra, Indonesia

Isolation and identification of Lactic Acid Bacteria

Lactic Acid Bacteria (LAB) were isolated from three samples of EBN. Each sample was weighed at 1 g, then added with 9 ml of MRS Broth (Neogen, USA)), vortexed, and homogenized, after dilution was carried out to 10^{-8}, then isolated in a petri dish containing MRS agar media, incubated at 37°C for 48 hours in the incubator after growing single colonies were purified and subcultured in MRS Broth (Kopermsub and Yunchalard 2010; Melia et al. 2022).

Macroscopic and microscopic identification

Media of de Man Ragosa Sharpe (MRS) broth was used for dilution. The LAB sample was dispersed using spreading techniques, inoculated, and then placed in an anaerobic jar for incubation at 37°C for 48 hours. A colony exhibiting round morphology, smooth texture, and white-yellowish hue, characteristic of LAB, was subsequently transferred to MRS media to purify the colony using streak techniques. The incubation period lasted for 24 hours at a temperature of 37°C. Furthermore, for microscopic identification, we used gram staining and microscopy observation.

Biochemical test

The examination of catalase activity and fermentation type analyzed the features of LAB. The assessment of the fermentation method by employing a Durham tube. The Durham tube was put in an inverted position, followed by incubation for 48 hours at a temperature of 37°C. Subsequently, the presence or absence of air bubbles in the Durham tube was examined. In addition, the catalase test was conducted by gently scraping the isolated substance onto a glass slide and adding a 3% solution of hydrogen peroxide (H_2O_2). The catalyst test involves isolating lactic acid bacteria using an inoculating loop. The isolation is applied over the object glass, and 3% hydrogen peroxide (H_2O_2) is dispensed using a 50 µL pipette. Bacterial dispersion was seen to result in the generation of gas (Public Health England 2014).

Antimicrobial activity test

The antibacterial activity of LAB isolates was carried out on four indicator strains: *Escherichia coli O157, Listeria monocytogenes* CFSN004330, *Staphylococcus aureus* ATCC 25923, *and Propionibacterium acne*. The LAB culture was grown in MRS broth for 24 hours at 37°C under anaerobic conditions in an incubator, likewise, for the enrichment of the test bacteria or pathogenic microbes at 37°C for 24 hours. The LAB isolate culture was centrifuged at 10,000 rpm for 5 minutes at 4°C. 0.2% of the indicator strain was put into 20 ml of Nutrient Agar (Neogen, USA), cooled to 50°C, and allowed to stand until it solidified. Then a well was made in the compacted media with a diameter of 4 mm using a cock borer. Then 50 µL of LAB supernatant was added to each well in the indicator strain media and allowed to stand for 1 hour at room temperature. Antibiotic dishes, namely kanamycin (30 µg) and ampicillin (10 µg) as controls. After that, it was incubated aerobically at 37°C for 24 hours, and the diameter of the clear zone formed around the well was measured (Yang et al. 2012; Rossi et al. 2021).

Molecular identification using 16S rRNA

LAB cultured in MRS broth at 37°C for 24 hours were then isolated from genomic DNA using the GeneJET Genomic DNA Purification Kit (Thermo Fisher Scientific, USA). Identification by PCR of 16S rRNA gene amplification using primer pairs 16SrRNA_27F (5' AGA GTT TGA TCM TGG CTC AG 3') and 16SrRNA_1525R (5' AAG GAG GTG WTC CAR CC 3'), which are primers for 16SrRNA gene amplification with an estimated product size of 1,498 bp. The PCR amplification results were examined by electrophoresis in 1% agarose and observed in the Gel Doc. The nucleotide sequencing process will follow the PCR product of the 16S rRNA gene of the bacterial sample. The nucleotide sequences were then processed using the BioEdit software. LAB consanguinity was determined using BLASTN in an order adapted from NCBI (https://www.ncbi.nlm.nih.gov/). Phylogenetic trees were constructed using the MEGA X program. Alignment was performed using the Clustal W algorithm. Phylogenetic trees were constructed using the Neighbor-Joining method, and evolutionary distances were analyzed using the Kimura 2-parameter method. The bootstrap value used is 1,000. Genetic distances were analyzed using the Pairwise Distances method.

RESULTS AND DISCUSSION

The observations showed that EBN contained LAB with the results of 10^8 CFU/gr, related to the identification and characteristics of LAB that have the potential as probiotics from EBN for macroscopic observations can be seen in Table 1. This table reveals that the EBN obtained from Ampek Angkek Sub-district was subjected to observation, where three distinct colonies were chosen. It can be seen that the three isolates have a uniform macroscopic appearance with a cream color, spherical shape, with entire margins and raised elevation. LAB isolates from EBN continued to be observed for their morphological and biochemical properties. All isolates were catalase-negative and homofermentative (Table 2).

Table 1. Macroscopic identification of LAB from EBN

LAB isolate	Color	Colony form	Margin	Elevation
BK1	Cream	Spherical	Entire	Raised
BK2	Cream	Spherical	Entire	Raised
BK3	Cream	Spherical	Entire	Raised

Table 2. Biochemical test of lactic acid bacteria isolates from EBN

LAB isolate	Catalase test	Fermentation type
BK1	Negative (-)	Homofermentative
BK2	Negative (-)	Homofermentative
BK3	Negative (-)	Homofermentative

Observations found that all isolates from EBN got catalase-negative results and were homo-fermentation types, so the three LABs would be carried out further testing related to their ability as probiotic candidates. Furthermore, these isolates were tested for antimicrobial activity against pathogenic bacteria, namely *Escherichia coli* O157, *Staphylococcus aureus*, *Propionibacterium acne*, and *Listeria monocytogenes* (Table 3). All of these pathogenic bacteria were compared with the antibiotics kanamycin and ampicillin. All isolates have antimicrobial activity against the above pathogenic bacteria.

From the testing of antimicrobial activity, it can be seen that the three isolates can produce a clear zone, whereas *E. coli* O157 bacteria obtained a clear zone diameter of 11.50-14.60 mm with the best results from isolate BK3, whose 14.60 results are better if compared with commercial antibiotics ampicillin 9.50 mm and Kanamycin 12.10 mm. In bacteria *S. aureus*, the best results in isolate BK3 is with a clear zone area of 18.71 mm, better than the other two isolates and antibiotics ampicillin and kanamycin, which are 10.01 and 15.12 mm, respectively. Likewise, in the bacteria *L. monocytogenes* isolate, the best results are in BK3 with a clear zone area of 19.53 mm higher than the Ampicillin and Kanamycin antibiotics, which are 18.07 and 11.23 mm, and finally, in the observation of *Propionibacterium acne* bacteria, it is known that the range of clear zones from LAB from EBN is 10.30-18.20 mm, with the best results from BK3 isolate being better than the Ampicillin and Kanamycin antibiotics. BK3 isolate exhibited the highest antimicrobial activity against pathogenic bacteria (Table 3). The results of the 16S rRNA gene amplification of BK3 isolate can be seen in Figure 3.

Figure 3 shows isolate BK3, which was PCR to get a DNA base sequence of 1498bp. The phylogenetic tree based on the 16S rRNA gene sequence can be seen in Figure 4. After amplification by PCR using the 16S rRNA gene, sequencing was then carried out to determine the genus and strain of LAB isolated from EBN based on the DNA base sequence of EBN, which had been carried out similarity from NCBI and analyzed using bioinformatics tools. The obtained phylogenetic of LAB can be seen in Figure 4, which shows that isolate BK3 has a similarity with *Lacticaseibacillus paracasei*.

Macroscopic and microscopic identification

Three isolates of LAB isolated from EBN showed a spherical, cream-colored morphology macroscopically. Microscopically, it includes gram-positive, purple, and rod-shaped. According to Salminen et al. (2004), LAB had a gram-positive staining pattern with either rod-shaped or round morphologies. They are characterized by their ability to thrive in aerobic and anaerobic conditions, and they do not produce spores. Lactic acid production is the principal outcome of their carbohydrate fermentation, mainly glucose. Similarly, Ramadhanti et al. (2021), also depicted that LAB exhibit a gram-positive characteristic and a rod-shaped morphology.

Biochemical test

The biochemical tests revealed that the LAB isolate obtained from EBN exhibited a negative catalase reaction, as evidenced by the lack of gas bubbles when H_2O_2 was added to the bacterial sample. This occurs due to the absence of the catalase enzyme in LAB, which converts hydrogen peroxide (H_2O_2) into water and oxygen (Widodo et al. 2017). Furthermore, the LAB present in the EBN exhibits homofermentative characteristics, evident from the lack of gas bubbles observed in the Durham tube. The result aligns with the research of (Abdullah et al. 2021), which showed negative and homofermentative catalase in LAB isolates from palm sugar.

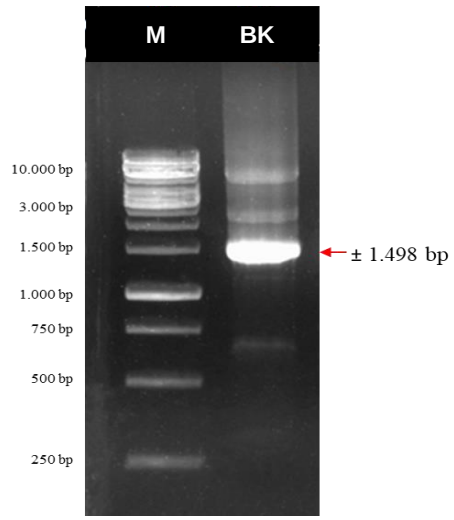

Figure 3. PCR amplification of the ribosomal RNA gene using *16SrRNA_27F* and *16SrRNA_1525R*. BK is an isolated lactic acid bacteria edible bird's nest (M=1 kB DNA Ladder)

Table 3. Antimicrobial activity of edible bird's nest isolates and antibiotic tests

Sample code	Clear zone (mm)			
	***E. coli* 0157**	***S. aureus***	***L. monocytogenes***	***Propionibacterium acne***
BK1	11.50	13.41	13.53	15.17
BK2	10.92	16.40	16.80	10.30
BK3	14.60	18.71	19.53	18.20
Ampicillin 10 µg	9.50	10.01	18.07	9.80
Kanamycin 30 µg	12.10	15.12	11.23	8.73

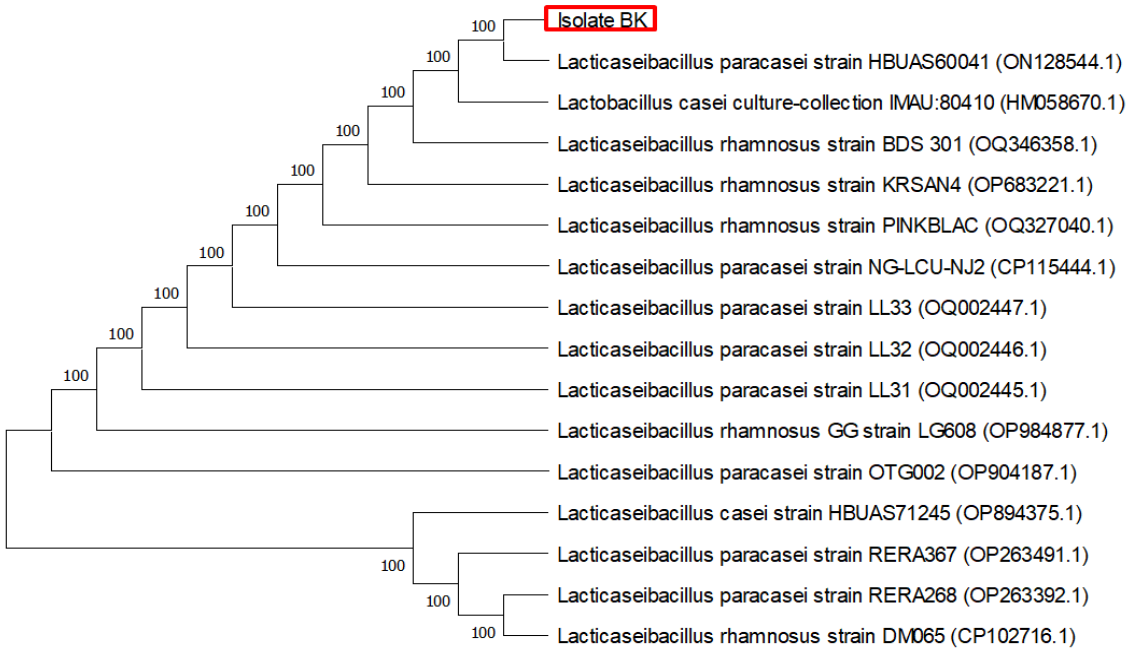

Figure 4. Phylogenetic tree of lactic acid bacteria BK from edible bird's nest

Likewise, research conducted by (Roza et al. 2022) showed that LAB isolates from *dadiah* were also known to produce negative catalase and are included in homofermentative bacteria. Lactic acid is the primary substance produced from glucose in certain types of LAB (*Streptococcus, Lactococcus, Enterococcus, Pediococcus*, and some *Lactobacillus* species) formed via the Embden-Meyerhof (EM) pathway that yields 2 mol pyruvate/mol glucose, which acts as an electron acceptor and was reduced to 2 mol lactic acid in a reaction catalyzed by lactate dehydrogenase (LDH) (Hatti-Kaul et al. 2018).

Homofermentative LAB, such as *Lactococcus* and *Streptococcus*, produces two lactate molecules from one glucose molecule. On the other hand, heterofermentative LAB, like *Leuconostoc, Wiessella*, and *Lactobacillus*, generates lactate, ethanol, and carbon dioxide from one glucose molecule (Salminen et al. 2004; Vinderola et al. 2019). Traditionally, LAB was classified using physiological and biochemical traits. However, in recent times, molecular characterization has emerged as a crucial method for classifying and identifying LAB (Hatti-Kaul et al. 2018).

The antimicrobial activity of EBN isolate on pathogenic bacteria tested *Escherichia coli* O157, *Listeria monocytogenes* CFSN004330, *Staphylococcus aureus* ATCC 25923, and *Propionibacterium acne*, which can be seen in Table 2. The antibiotics used as controls were kanamycin (30 µg) and ampicillin (10 µg) in Table 2. The most significant antimicrobial activity was shown in BK3 edible bird's nest isolate against *L. monocytogenes* with a clear zone diameter of 19.53 mm, followed by *S. aureus* 18.71 mm, *Propionibacterium acne* 18.20 mm, and *E. coli* 0157 14.60 mm. Furthermore, when compared with the controls, namely the antibiotics kanamycin and ampicillin, BK3 isolates had better activity than other isolates (Table 2). But overall, LAB isolates had antimicrobial activity against all pathogenic bacteria. Inhibition zone activity can be grouped into four categories: weak (<5mm), moderate (5-10mm), strong (>10-20mm), and very strong (>20-30mm) activity (Morales et al. 2003). Therefore, BK3 isolates from EBN had clear zones categorized as strong against pathogenic bacteria. The study by Melia et al. (2019) reported that the *Pediococcus acidilactici* BK01 isolated from bekasam could inhibit *E. coli* O157:H7, *S. aureus*, and *L. monocytogenes*. Then, in the research by Ramadhanti et al. (2021), *Lactobacillus fermentum* strain 1743, isolated from palm sugar, was able to inhibit *E. coli* O157, *Propionibacterium acne*, *Acinetobacter baumannii*, and *L. monocytogenes*. This result is also better than *Pediococcus acidilactici* isolated from dadiah (Dewi et al. 2023) and *Lactobacillus fermentum* isolated from *dadiah* against bacteria that cause skin infections (Amelia et al. 2021; Amelia et al. 2021). It can be concluded that BK3 LAB isolated from EBN has the potential to be an antimicrobial. This showed that there was the highest antimicrobial activity against *L. monocytogenes* with an inhibition zone diameter of 19.53 mm, followed by *S. aureus* at 18.71 mm, *Propionibacterium acne* at 18.20 mm, and *E. coli* 0157 at 14.60 mm. We continued to the next step for analysis molecular using 16S rRNA gene.

PCR was generally considered a rapid, sensitive, and time-saving method for detecting bacterial species (Youn et al. 2017). The specificity of the primer pair used determined the accuracy of PCR. The 16S rRNA gene was considered a marker gene for bacterial genotype analysis and is useful for accurately identifying bacteria (Kim et al. 2020). Studies on identifying *Lactobacillus* have mainly utilized PCR-based molecular analysis with primer pairs

targeting variable regions of the 16S rRNA gene sequence (Karapetsas et al. 2010). Identification of LAB from NEB by PCR of 16S rRNA gene amplification using primer pairs 16SrRNA_27F (5' AGA GTT TGA TCM TGG CTC AG 3') and 16SrRNA_1525R (5' AAG GAG GTG WTC CAR CC 3'), which are primers for 16S rRNA gene amplification with an estimated product size of 1,498 bp. Molecular characterization includes random amplified polymorphic DNA profiling, 16S rRNA gene sequencing, PCR-based fingerprinting, and soluble protein patterns (Sharma et al. 2020) and the differentiation of species by multiplex PCR assays using specific recA-derived primers (Ni et al. 2015).

The phylogenetic tree based on the 16S rRNA gene sequence can be seen in Figure 4. Bacterial sequencing of BK3 isolates was compared to GenBank data using the BLAST program on the NCBI website, having the closest kinship with the bacterium *Lacticaseibacillus paracasei* strain HBUAS60041 (ON128544.1). As evidenced by the percent identity value when BLAST is 100%. Therefore, it can be concluded that BK3 lactic acid bacteria isolated from EBN are *Lacticaseibacillus paracasei* strain HBUAS6004. The construction of phylogenetic trees highlights that samples from the same species can have different positions in the tree conformation. This dynamic can be explained by the limitation of the 16S ribosomal region that does not allow identification of subspecies (Forouhandeh et al. 2021) and the fact that the primers used are only efficient for identification up to species level (Hou et al. 2018). In addition, sequences of the same species that differ in position can mean mutations, which result in genetic variation, and these substitutions reflect mutations that can increase or decrease metabolic functions (Hershberg 2015), a group or taxon of LAB, such as antimicrobial capacity and the capacity for the development of sensory characteristics of a product (Carvalho et al. 2023).

The classification system was linked to certain factors: glucose fermentation characteristics, cell morphology, capacity to utilize sugars, and optimum growth temperature range. This classification system thus recognized only four lactic acid bacteria genera: *Lactobacillus*, *Pediococcus*, *Leucononostoc*, and *Streptococcus* (Quinto et al. 2014). The results of the phylogenetic tree based on the 16S rRNA gene sequence, BK3 isolates from EBN have similarities with *Lacticaseibacillus paracasei* strain HBUAS6004. In the research that has been conducted, the results of the analysis of probiotic characteristics of EBN, it was found that isolate EBN from Agam District has potential as a probiotic candidate because it does not produce a catalase enzyme and is included in the homofermentative fermentation type. It also has the ability to have the best antimicrobial activity against *Escherichia coli* O157, *Listeria monocytogenes* CFSN004330, *Staphylococcus aureus* ATCC 25923, and *Propionibacterium acne* on isolate BK3. Besides that, based on the phylogenetic similarity analysis of this BK3 isolate, it is closely related to *Lacticaseibacillus paracasei*. So that further testing and research can be carried out related to the use of BK3 isolates from EBN in the future.

ACKNOWLEDGEMENTS

This work received assistance from the Laboratory of Animal Products Technology, Faculty of Animal Science, Universitas Andalas, Padang, Indonesia. The author acknowledges the Institute for Community Service Research (LPPM) at Universitas Andalas for their support in the form of the prestigious basic research strategy for the professor publication research clusters, under contract number 94/UN.16.19/PT.01.03/2023, particularly to Prof. Marlina.

REFERENCES

Abdullah D, Poddar S, Rai RP, Purwati E, Dewi NP, Pratama YE. 2021. Molecular identification of lactic acid bacteria an approach to sustainable food security. J Public Health Res 10 (2_suppl): 23-29. DOI: 10.4081/jphr.2021.2508.

Amelia R, Abdullah D, Pratama YE, Purwati E. 2021. Antimicrobial activity of lactic acid bacteria found in dadiah on disease-causing skin infections. Indian J Forensic Med Toxicol 15 (2): 3314-3318. DOI: 10.37506/ijfmt.v15i2.14887.

Amelia R, Philip K, Pratama YE, Purwati E. 2021. Characterization and probiotic potential of lactic acid bacteria isolated from dadiah sampled in West Sumatra. Food Sci Technol 41: 746-752. DOI: 10.1590/fst.30020.

Carvalho L, Fernandes N, Silva BN, Gonzales-Barron U, Cadavez V. 2023. Molecular identification and phylogenetic analysis of lactic acid bacteria isolated from goat's raw milk cheese. In: Cristina A, Fente (eds). Biology and Life Science Forum. MDPI. DOI: 10.33899/ijvs.2019.125896.1176.

Dewi NP, Poddar S, Rai RP, Purwati E, Abdullah D, Yudha. 2023. The Potential Lactic Acid Bacteria from Dadiah Sianok bukittinggi City West Sumatera as Probiotic. Res J Pharm Technol 16 (2): 627-634. DOI: 10.52711/0974-360X.2023.00107.

Efendi R, Rahmayuni, Rossi E, Zalfiatri Y. 2023. Antimicrobial properties of lactic acid bacteria from fermented palm sap against *E. coli* and *S. aureus* growth. IOP Conf Ser: Earth Environ Sci 1241 (1): 012092. DOI: 10.1088/1755-1315/1241/1/012092.

Elfita L, Wientarsih I, Sajuthi D, Bachtiar I, Darusman H. S. 2020. The diversity in nutritional profile of farmed edible bird's nests from several regions in Indonesia. Biodiversitas 21 (6): 2362-2368. DOI: 10.13057/biodiv/d210604.

FAO and WHO. 2006. Probiotics in food Health and Nutritional properties and guidelines for evaluation. In Chemical and Functional Properties of Food Components Third Edition. DOI: 10.1201/9781420009613.ch16.

Forouhandeh H, Vahed SZ, Ahangari H, Tarhriz V, Hejazi MS. 2021. Phenotypic and phylogenetic characterization of *Lactobacillus* species isolated from traditional Lighvan cheese. Food Prod Process Nutr 3 (1): 1-9. DOI: 10.1186/s43014-021-00065-x.

Hatti-Kaul R, Chen L, Dishisha T, Enshasy HEl. 2018. Lactic acid bacteria: From starter cultures to producers of chemicals. FEMS Microbiol Lett 365 (20): 1-20. DOI: 10.1093/femsle/fny213.

Hershberg R. 2015. Mutation—the engine of evolution: Studying mutation and its role in the evolution of bacteria. Cold Spring Harbor Perspect Biol 7 (9): 1-13. DOI: 10.1101/cshperspect.a018077.

Hou Q, Bai X, Li W, Gao X, Zhang F, Sun Z, Zhang H. 2018. Design of primers for evaluation of lactic acid bacteria populations in complex biological samples. Front Microbiol 9 (AUG): 1-10. DOI: 10.3389/fmicb.2018.02045.

Karapetsas A, Vavoulidis E, Galanis A, Sandaltzopoulos R, Kourkoutas Y. 2010. Rapid detection and identification of probiotic *Lactobacillus casei* ATCC 393 by multiplex PCR. J Mol Microbiol Biotechnol 18 (3): 156-161. DOI: 10.1159/000308518.

Kementan [Kementerian Pertanian]. 2021. Decree of the head of the Indonesian agricultural quarantine agency number 6291/Kpts/HK.140/K/7/2021 concerning guidelines for animal quarantine actions on the export of edible bird nests from the territory of the Republic of Indonesia to Countries Other. [Indonesia]

Kim E, Yang SM, Lim B, Park SH, Rackerby B, Kim HY. 2020. Design of PCR assays to specifically detect and identify 37 *Lactobacillus* species in a single 96 well plate. BMC Microbiol 20 (1): 96. DOI: 10.1186/s12866-020-01781-z.

Kopermsub P, Yunchalard S. 2010. Identification of lactic acid bacteria associated with the production of plaa-som a traditional fermented fish product of Thailand. Intl J Food Microbiol 138 (3): 200-204. DOI: 10.1016/j.ijfoodmicro.2010.01.024.

Melia S, Juliyarsi I, Kurnia YF, Rossi E, Alzahra H. 2022. Selection of potential lactic acid bacteria as a candidate probiotic on cheese making by product whey. Adv Anim Vet Sci 10 (7): 1633-40. DOI: 10.17582/journal.aavs/2022/10.7.1633.1640.

Melia S, Aritonang SN, Juliyarsi I, Kurnia YF, Rusdimansyah, Hernita VO. 2022. The screening of probiotic lactic acid bacteria from honey of stingless bee from West Sumatra Indonesia and using as starter culture. Biodiversitas 23 (12): 6379-6385. DOI: 10.13057/biodiv/d231235.

Melia S, Endang P, Kurnia YF, Pratama DR. 2019. Antimicrobial potential of Pediococcus acidilactici from Bekasam fermentation of sepat rawa fish (*Tricopodus trichopterus*) from Banyuasin South Sumatra Indonesia. Biodiversitas 20 (12): 3532-3538. DOI: 10.13057/biodiv/d201210.

Melia S, Purwati EYJ, Aritonang SN, Silaen M. 2017. Characterization of the antimicrobial activity of lactic acid bacteria isolated from buffalo milk in West Sumatera (Indonesia) against *Listeria monocytogenes*. Pak J Nutr 16 (8): 645-650. DOI: 10.3923/pjn.2017.645.650.

Morales G, Sierra P, Mancilla A, Paredes A, Loyola LA, Gallardo O, Borquez J. 2003. Secondary metabolites from four medicinal plants from northern Chile: Antimicrobial activity and biotoxicity against Artemia salina. J Chilean Chem Soc 48 (2): 13-18. DOI: 10.4067/s0717-97072003000200002.

Ni K, Wang Y, Li D, Cai Y, Pang H. 2015. Characterization identification and application of lactic acid bacteria isolated from forage paddy rice silage. PLoS ONE 10 (3): e0121967. DOI: 10.1371/journal.pone.0121967.

Pratama YE, Jamsari, Yuherman, Melia S, Purwati E. 2021. Molecular identification of lactic acid bacteria from "budu" of west sumatera indonesia as a potential probiotic. AACL Bioflux 14(5): 2837-2844.

Public Health England. 2014. Introduction to the Preliminary Identification of Medically Important Bacteria. In UK Standads Unit.

Quinto EJ, Jiménez P, Caro I, Tejero J, Mateo J, Girbés T. 2014. Probiotic lactic acid bacteria: A review. Food Nutr Sci 05 (18): 1765-1775. DOI: 10.4236/fns.2014.518190.

Ramadhanti N, Melia S, Helliward J, Purwati E. 2021. Characteristics of lactic acid bacteria isolated from palm sugar from West Sumatra Indonesia and their potential as a probiotic. Biodiversitas 22 (5): 2610-2616. DOI: 10.13057/biodiv/d220520.

Rossi E, Ali A, Efendi R, Restuhadi F, Zalfiatri Y, Sofyan Y, Aritonang SN, Purwati E. 2021. Characterization of bacteriocin produced by lactic acid bacteria isolated from solid waste of soymilk production. In IOP Conf Ser: Earth Environ Sci 709 (1): 012020. DOI: 10.1088/1755-1315/709/1/012020.

Roza E, Aritonang SN, Yellita Y, Susanty H, Rizqan, Pratama YE. 2022. Potential of dadiah kapau from Agam District West Sumatra Indonesia as a source of probiotics for health. Biodiversitas 23 (1): 564-571. DOI: 10.13057/biodiv/d230161.

Salminen S, Gibson GR, McCartney AL, Isolauri E. 2004. Influence of mode of delivery on gut microbiota composition in seven year old children. Gut 53 (9): 1388-1389. DOI: 10.1136/gut.2004.041640.

Sharma A, Kaur J, Lee S, Park YS. 2020. Tracking of intentionally inoculated lactic acid bacteria strains in yogurt and probiotic powder. Microorganisms 8 (1): 1-15. DOI: 10.3390/microorganisms8010005.

Shim EKS, Chandra GF, Pedireddy S, Lee SY. 2016. Characterization of swiftlet edible bird nest a mucin glycoprotein and its adulterants by Raman microspectroscopy. J Food Sci Technol 53 (9): 3602-3608. DOI: 10.1007/s13197-016-2344-3.

Smid EJ, Gorris LGM. 2020. Handbook of Food Preservation. In: Rahman MS (eds). 3rd Edition. CRC Press. DOI: DOI: 10.1201/9780429091483.

Vinderola G, Ouwehand AC, Salminen S, von Wright A. 2019. Lactic Acid Bacteria: Microbiological and Functional Aspects. DOI: 10.1201/9780429057465.

Widodo, Wahyuningsih TD, Nurrochmad A, Wahyuni E, Taufiq TT, Anindita NS, Lestari S, Harsita PA, Sukarno AS, Handaka. 2017. Bakteri Asam Laktat Starin Lokal: Isolasi sampai Aplikasi sebagai Probiotik dan Starter Fermentasi Susu. Gadjah Mada University Press, Yogyakarta. [Indonesian]

Wong C-F, Chan G K-L, Zhang M-L, Yao P, Lin H-Q, Dong T T-X, Li G, Lai X-P, Tsim K W-K. 2017. Characterization of edible bird's nest by peptide fingerprinting with principal component analysis. Food Qual Saf 1 (1): 83-92. DOI: 10.1093/fqs/fyx002.

Yang E, Fan L, Jiang Y, Doucette C, Fillmore S. 2012. Antimicrobial activity of bacteriocin-producing lactic acid bacteria isolated from cheeses and yogurts. Amb Expr 2 (1): 1-12. DOI: 10.1186/2191-0855-2-48.

Youn SY, Ji GE, Han YR, Park MS. 2017. Development of strain-specific primers for identification of *Bifidobacterium bifidum* BGN4. J Microbiol Biotechnol 27 (5): 909-915. DOI: 10.4014/jmb.1610.10018.