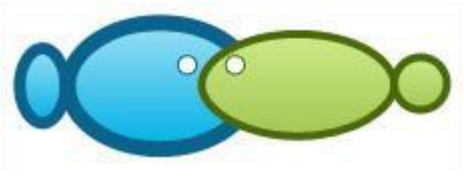

Antimicrobial activities of lactic acid bacteria *Lactobacillus casei* isolated from "bekasam" fermented fish from "siak"

[1,2]Dinna Devi, [1]Nuzulia Irawati, [1]Yanwirasti, [3]Imam B. Putra, [4]Yudha E. Pratama

[1] Department of Biomedical, Faculty of Medicine, Andalas University, Padang, Indonesia; [2] Faculty of Medical, Abdurrab University, Pekanbaru, Riau, Indonesia; [3] Department of Dermatology and Venerology, Faculty of Medicine, Universitas Sumatera Utara, Medan, Indonesia; [4] Faculty of Animal Science, Andalas University, Padang Indonesia.
Corresponding author: N. Irawati, nuzuliairawati@med.unand.ac.id

Abstract. Bekasam is a local food from Indonesia, especially from the island of Sumatra, made from freshwater fish fermented for 5 days with a mixture of rice. This fermentation product produces lactic acid bacteria, which have potential as probiotics, being able to inhibit the growth of pathogenic bacteria. This study aims to determine the potential of lactic acid bacteria from bekasam, which has potential as a probiotic based on its ability as an antimicrobial agent. The method used was testing using agar diffusion with *Lactobacillus casei* isolate from bekasam siak, with the test bacteria being *Listeria monocytogenes*, *Staphylococcus aureus*, *Salmonella* sp., *Propionibacterium acnes*, *Pseudomonas* sp., and *Escherichia coli* O157. The results of this study showed a total mass of *L. casei* of $67x10^7$ CFU mL^{-1}, catalase-negative, and belongs to the homofermentative type. A molecular approach using the 16S sRNA gene shows that the lactic acid bacteria are similar to *L. casei*. The highest antimicrobial activity in the tested bacteria was against *Salmonella* sp. and *S. aureus*.
Key Words: isolation, molecular identification, probiotics, traditional food.

Introduction. Bekasam is a traditional food originating from several regions in Indonesia, such as Java, Sumatra, and South Kalimantan. Bekasam is the result of the spontaneous fermentation of fish. This food is represented by fermented fish. Bekasam contains lactic acid bacteria (LAB), which have many benefits for human health. According to Desniar et al (2013), bekasam is a fish product processed by fermentation, which tastes sour. Freshwater can be used for bekasam. The raw materials are snakehead fish (*Channa striata*), beam fish (*Stystomus rubripinnis*), siam (*Trichopodus pectoralis*), and swamp nails (*Ceratopteris thalictroides*), with the addition of 15-20% salt and 15% sangria rice. The mix is fermented for one week to produce characteristic aroma and taste. Bekasam siak, one of the traditional foods commonly found in traditional markets, has a distinctive aroma and taste. It is generally made from *C. striata*, baung fish (*Mystus nemurus*), and selais (*Kryptopterus lais*).

It is known that fermented foods are often associated with special benefits of fermented bacteria such as LAB. LAB produces antibacterial substances, which are important as probiotic agents and food preservatives. This is related to producing metabolites such as organic acids, hydrogen peroxide, diacetyl, and bacteriocins (Morales et al 2003). These metabolites can inhibit growth and kill pathogenic bacteria. Previous research found that there was antimicrobial activity in fermented foods from Banyuasin, namely the *Pediococcus acidilactici* bacterium, which had the best results on the *Escherichia coli* O157 test bacteria with an inhibition zone of 21.26 mm (Melia et al 2019), and on *Staphylococcus aureus* ATCC 25923 with an inhibition zone of 13.10 mm (Sari et al 2018). According to Desniar et al (2013), LAB have diverse inhibitory abilities against pathogenic bacteria *Listeria monocytogenes, Salmonella typhimurium, Escherichia coli, Bacillus cereus*, and *Staphylococcus aureus*. This study aims to

determine the ability of *Lactobacillus casei* isolated from Siak Regency as an antimicrobial agent.

Material and Method

Description of the study sites. Samples were collected from bekasam manufacturing houses from Siak Regency, Province Riau, Indonesia. Bekasam was prepared by mixing freshwater fish, rice and salt, and fermenting it at room temperature in a closed and light-proof container for 5-7 days. This research was conducted in the Laboratory of Technology of Animal Product, Faculty of Animal Science, Andalas University, Padang, Indonesia, in February 2021.

Isolation of bacteria. The isolation was conducted using the method used by Purwati et al (2005). MRS broth and MRS agar (Merck, Germany) were utilized. 1 g of the sample (bekasam) was dissolved in 9 mL of MRS broth and incubated for 24 h at 37°C. 100 µL of the sample was planted on MRS agar using the spread plate method and incubated at 37^{O}C for 48 h. The number of bacterial colonies was counted, and several single colonies were selected for further testing. The shape, color, Gram coloring, catalyst testing, and fermentation properties were observed.

Acid resistance test. 5N HCL was added to 9 mL of MRS broth until it reached a pH of 3. MRS broth was also used as a control. 1 mL of lactic acid bacteria culture was added to the prepared MRS broth and incubated at 37°C for 90 min. It was then grown on MRS agar media using the spread plate method and incubated for 48 h at 37°C. The viability was calculated with the formula (Rashid & Hassanshahian 2014).

$$Decrease\ in\ colony\ number\ (\%) = \frac{\text{Total colony of LAB} - \text{total colony of LAB teatment}}{\text{Total LAB control}} x\ 100$$

Viability (%) = 100 – decrease in number of colonies (%)

Bile salt resistance test. 1 mL of lactic acid bacterial culture was placed into a test tube with 9 mL MRS broth with ox gall 0.3% and 0.5% and incubated for 5 h. The LAB isolates were placed on agar media with a dilution of 10^{-6} and incubated for 48 h at 37^{O}C. The viability values of the growing colonies were calculated with the next formula (Walker & Gililand 1993).

$$Decrease\ in\ colony\ number\ (\%) = \frac{\text{Total colony of LAB} - \text{total colony of LAB teatment}}{\text{Total LAB control}} x\ 100$$

Viability (%) = 100 – decrease in number of colonies (%)

Antimicrobial activity. 1 mL of culture was incubated for 24 h within 9 mL of MRS broth at 37°C. The mix was then centrifuged at 14000 rpm for 5 min. The supernatant was filtered with a filtration membrane of 0.22 µL. The pH of the cell-free supernatant was corrected to 6.5 with 1N NaOH to avoid the inhibition effect due to the presence of organic acid (Yang & Rannala 2012). Pathogenic bacteria grew in anaerobic conditions at 37°C for 24 h. 20 mL of cultured pathogenic bacteria (0.2%) was inserted in Mueller-Hinton agar (MHA) at the temperature of 50°C. Pathogenic bacteria used in this test consisted of *E. coli, L. monocytogenes*, *S. aureus*, *Propionibacterium acne, Pseudomonas aeruginosa*, and *Salmonella* sp. After the agar solidified, a 6 mm hole was made with a cork borer. The supernatant was collected (50 µL), inserted in each Petri dish, and set aside for 15-20 min. Antimicrobial activity tests were compared using penicillin, ampicillin, and kanamycin antibiotics before incubating for 24 h at 37°C in aerobic conditions. The reading of the results represents the diameter of the clear zone on the MHA media, measured with a caliper.

Genomic DNA isolation of lactic acid bacteria and 16S rRNA. 1000 µL of sample from the single-colony LAB isolates from the MRS broth was pipetted and inserted in a new Eppendorf and centrifuged at 14000 rpm for 2 min. Genomic DNA isolation was done with the Kit Promega (USA).

The supernatant was discarded, the pellet was retrieved, and 480 µL 50 mM EDTA and 120 µL lysozyme were added. The mix was incubated in a water bath at 37°C for 60 min and centrifuged at 14000 rpm for 2 min. The supernatant was discarded, and the pellet was collected. 600 µL nuclei lysis solution was added. The mix was homogenized with a micropipette and incubated at 80°C for 5 min. The mix was set at room temperature. 3 µL RNase solution were added, and the mix was homogenized and incubated in a water bath at 37°C for 60 min. 200 µL protein precipitation solution was added, and the new mix was vortexed, incubated in ice for 5 min, and centrifuged at 14000 rpm for 3 min. Afterward, the supernatant pipette was moved to a new Eppendorf. The pellet was discarded. 600 µL isopropanol was added, homogenized, and centrifuged at 14000 rpm for 2 min. The pellet was retrieved, the supernatant discarded, and 600 µL 70% ethanol was added before homogenizing and centrifuged at 14000 rpm for 2 min. The pellet was collected, and the supernatant was discarded. The Eppendorf containing pellet was aerated for 15 min. The DNA pellet was rehydrated by adding 50 µL rehydration solution for 30 min at 65°C. The DNA pellet was rehydrated by adding 50 µL of rehydration solution, followed by incubation at 65°C for 30 minutes. The primers R (16S-1492R, melting temperature 47°C, 5'GTT TAC CTT GTT ACG ACTT-3) and F (16S-27F, melting temperature 54.3°C, 5'AGA GTT TGA TCC TGG CTC AG-3) were produced at a concentration of 10 pM. 90 µL of distilled water was combined with 10 µL of the R and F primers. The DNA ladder was introduced into a volume of 5 microliters, and an electric potential of 100 volts was applied for 40 minutes. The agarose gel was introduced into a receptacle, followed by tris-acetate-EDTA (TAE) buffer until complete immersion. The gel was examined using ultraviolet (UV) illumination. Following the UV reading, the PCR-derived sample underwent purification in preparation for sequencing.

Phylogenetic analysis. The sequence alignment analysis was performed by comparing the obtained sequences (query) with those in the Gene Bank with database searches in NCBI (http//:www.ncbi.nlm.nih.gov) by using BLAST (Basic Local Alignment Search Tool). Phylogenetic analysis was performed with MEGA v7.0.

Results and Discussion

Isolation and identification of lactic acid bacteria. From the bekasam, total LAB colonies of $67x10^7$ CFU g^{-1} were obtained. The results of the properties observations can be seen in Table 1.

Acid tolerance test. LAB, as a probiotic, should have resistance to acid. LAB acid resistance of bekasam can be seen in Table 2.

Bile salt tolerance. The LABs with resistance to bile salts can be seen in Table 3.

Antimicrobial activity. The area of clear zone of each isolate varies due to the differences in their ability (Figure 1).

Table 1

Characteristics of lactic acid bacteria from bekasam siak

Isolate	*Cell shape*	*Gram*	*Catalase*	*Fermentative type*
BK1	Bacilli	+	-	Homofermentative
BK2	Bacilli	+	-	Homofermentative
BK3	Bacilli	+	-	Homofermentative
BK4	Bacilli	+	-	Homofermentative
BK5	Bacilli	+	-	Homofermentative
BK6	Coccus	+	+	Homofermentative
BK7	Bacilli	+	+	Heterofermentative
BK8	Coccus	+	-	Homofermentative
BK9	Coccus	+	+	Homofermentative
BK10	Bacilli	+	-	Homofermentative
BK11	Coccus	+	-	Heterofermentative
BK12	Bacilli	+	-	Homofermentative
BK13	Bacilli	+	-	Heterofermentative
BK14	Coccus	+	+	Homofermentative
BK15	Coccus	+	+	Homofermentative
BK16	Coccus	+	+	Heterofermentative

Table 2

Lactic acid bacteria viability of bekasam siak at pH 3 and in the control

Sample	*pH 3*	*Control*	
	($x10^6$ CFU mL^{-1})	*($x10^6$ CFU mL^{-1})*	*Viability (%)*
BK1	12	87	13.8
BK2	67	123	54.5
BK3	29	100	29
BK4	15	86	17.4
BK5	36	152	23.7
BK8	58	151	38.4
BK10	49	74	66.2
BK11	7	101	6.9
BK12	65	128	50.8
BK13	23	64	35.9

Table 3

Resistance of bekasam siak isolates against bile salts

Sample	*Oxgall 5% ($x10^6$ CFU mL^{-1})*	*Control ($x10^6$ CFU mL^{-1})*	*Viability (%) 5%*
BK2	26	180	14.4
BK5	13	112	11.6
BK8	31	98	31.6
BK10	8	142	5.6
BK12	62	104	59.6
BK13	31	70	44.3

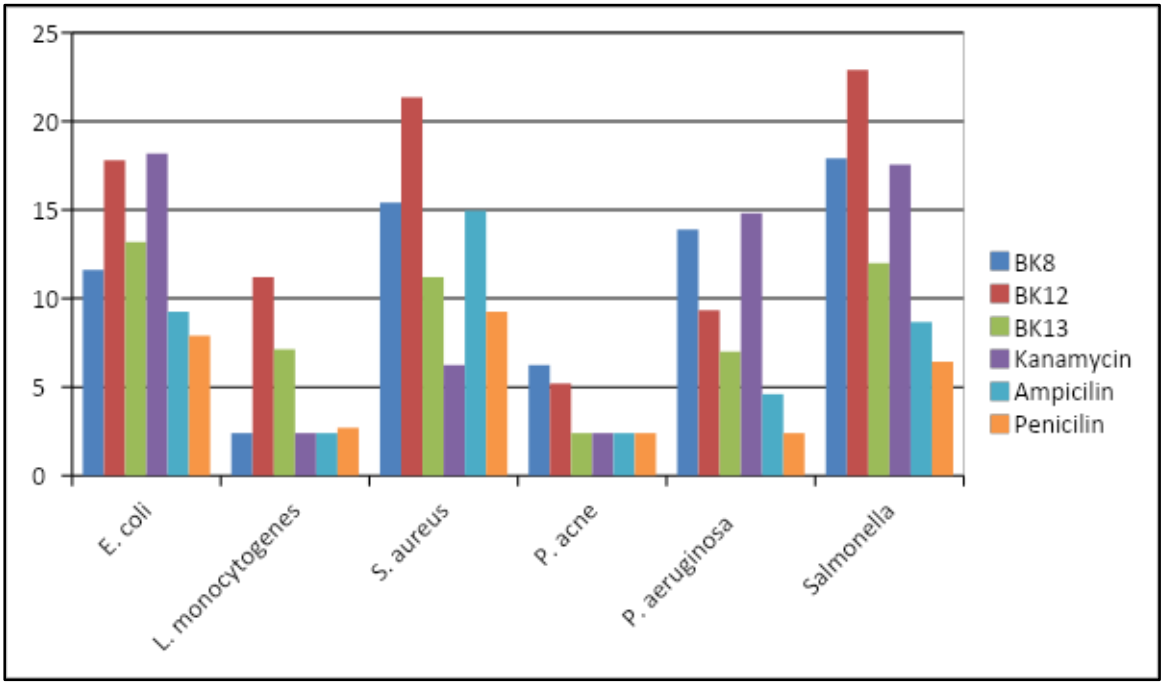

Figure 1. Diameter clear zone (mm) of antimicrobial lactic acid bacteria.

Genome isolation and 16S rRNA reaction of lactic acid bacteria. The electrophoresis results in Figure 2 show that LAB was successfully amplified with 16S rRNA. The display of the PCR 1.5 kB product indicates successful amplification.

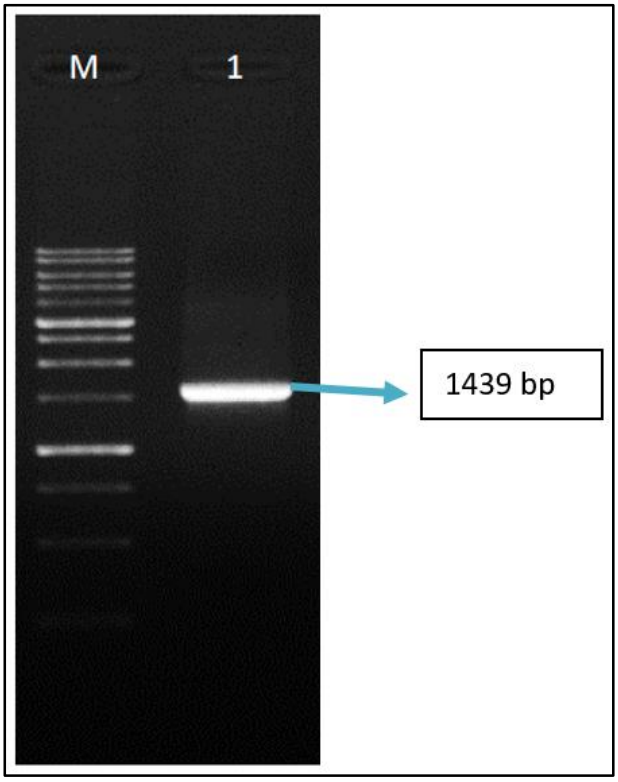

Figure 2. The result of the amplification of the ribosome RNA gene.

Phylogenetic tree analysis. Based on the result of the BLAST analysis, the BK12 isolate bacteria found in bekasam siak has a 99% similarity with *L. casei* HDS-01, showing a close kinship with it. Figure 3 presents the phylogenetic tree.

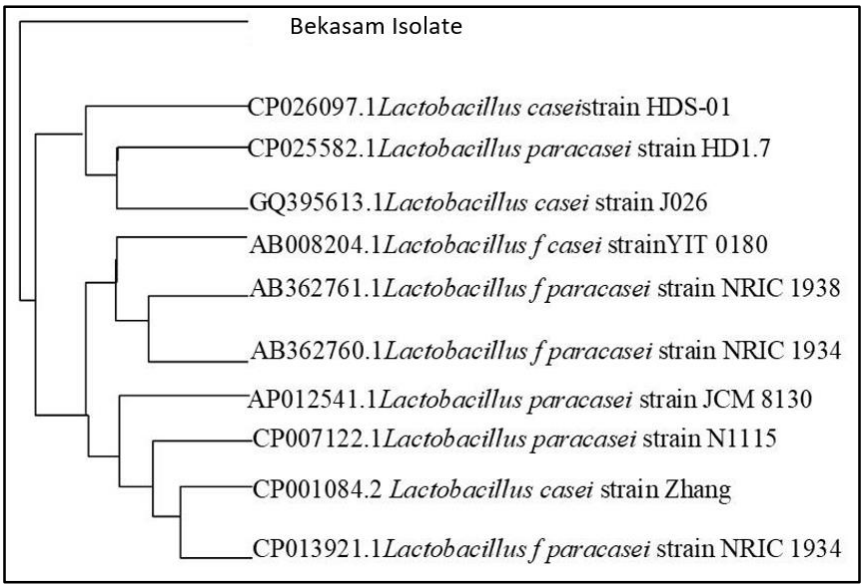

Figure 3. Phylogenetic tree of bekasam sample (BK12).

From the results of the study, all isolates from the bekasam siak were Gram-positive bacteria. Of the 16 isolates tested, 10 had negative catalase results, and 12 isolates observed using a Durham tube were categorized as homofermentative, where, during the fermentation process, no alcohol was produced. The end products of LAB metabolism are lactic acid (homofermentative) and acetic acid, ethanol, and Co2 (heterofermentative) (Shah 2000).

Research conducted by Pratama et al (2021) and Melia et al (2019) found that LAB isolates generally belong to the Gram-positive category, with catalase negative and homofermentative types, where the fermentation does not produce alcohol.

The resistance of LAB in the isolates of the bekasam siak was found to be 6 to 66.2%. The viability of LAB to acid conditions with pH 3 was different for each genus and strain of bacteria. Cotter & Hill (2003) stated that differences in the resistance to the acid of several LAB species are related to the permeability of cell walls to protons (H^+). The permeability of the cell to H^+ is determined by the outflow of protons activated by membrane ATPase for translocation H. The difference in the activity of the ATPase enzyme from each bacteria will determine the permeability of H^+. In addition, the same authors explained that several LAB species could induce a response to acid (acid tolerance response). This response will induce a homeostatic pH system, protection, and repair mechanisms. High acidic conditions will cause damage to cell membranes and the release of intracellular components, resulting in cell death (Cotter & Hill 2003). Previous research found that *L. casei* is resistant to low pH with a viability of 9.39±0.04 log CFU mL^{-1} at pH 2 with incubation for 90 minutes (Orlowski & Bielecka 2006). *Lactobacillus* strain L12 has a viability of 92.42% at pH 2 for 1 hour (Dos Santos Pozza et al 2011) and *Lactobacillus plantarum* has a viability of 65.98 at pH 3 for 90 min (Abdullah et al 2021).

The isolates with the best results of resistance to acid selection were then tested for resistance to bile salts. The resistance to bile salts was in the range of 5.6-59.6%, with only three isolates having a resistance to bile salts of more than 30%. In addition, bile salts can also function as an antibacterial agent through the destruction of cell membranes (Begley et al 2005). This causes the LAB strain to experience a decrease in population in media containing 0.5% bile salts. Bron et al (2004) described the morphological damage of *L. plantarum* under exposure to 0.05-0.15% bile salts for 4 h. The differences in the resistance to bile salts in the various tested strains indicate that

the resistance to bile salts is strain-dependent. Kimoto-Nira et al (2007) reported that there was a relationship between the fatty acid composition of the bacterial cell wall and its ability to withstand bile salts.

The three isolates of bekasam siak that were tested for antimicrobial activity on the test bacteria *E. coli, L. monocytogenes*, *S. aureus*, *P. acne, P. aeruginosa*, and *Salmonella* sp. had various activities depending on the type of isolate because each bacterial strain has a different ability to inhibit pathogenic bacteria. The best results were found in isolate BK12 for the test bacteria *E. coli* (17.8 mm), *L. monocytogenes* (11.2 mm), *S. aureus* (21.36 mm), and *Salmonella* (22.9 mm). Compared to antibiotics, the antimicrobial activity of the bekasam siak isolate is close to or even better than the antibiotics used, so this bekasam siak isolate can be recommended for study as a treatment for infections caused by pathogenic bacteria. A previous study of LAB from bekasam by Desniar et al (2013) produced a clear zone against the *S. aureus* test bacteria of 44 mm and against *S. typhimurium* at 38 mm.

In bekasam from Banyuasin, it is known that the bacteria isolate can inhibit *E. coli* with a clear zone area of 21.26 mm, *S aureus* with a clear area of 18.23 mm, and *L. monocytogenes* with a clear area of 5.10 mm (Melia et al 2019). The results of this study were better when compared to those of Roza et al (2021), where LAB was only able to inhibit *S. aureus* by 8.4 mm and *Salmonella* sp. by 7.5 mm. LAB produces natural antimicrobial compounds that inhibit the growth of pathogenic microbes. For example, LAB produces organic acids, such as lactic, acetic, and formic acids, which cause a decrease in pH and thereby inhibit the growth of pathogens. LAB also produces several antimicrobial compounds, such as bacteriocins (Ibrahim et al 2021).

The relationship between the isolates from the bekasam siak in this study with several partial sequences of *L. casei* and *L. paracasei* can be described using a dendrogram (Figure 3). Figure 3 shows the kinship of several comparator strains with *L. casei* isolates from bekasam siak. Based on the phylogenetic analysis, the isolate of bekasam siak BK12 has a close kinship with *L. casei*. The results of Wikandari et al (2012) differ from ours, the authors identifying in bekasam *L. plants*, *L. pentosus,* and *Pediococcus pentosaceus*. *Lactobacillus brevis* was isolated from fermented mackerel (Pratama et al 2021), and *L. plantarum* from bekasam (Wikandari et al 2012). The differences in bacterial species found were due to the types of fermented fish, the ingredients used to ferment fish, or the method used to ferment it. Organic acids and hydrogen peroxide produced by lactobacilli are reported to inhibit the growth of Gram-positive and Gram-negative bacteria, while bacteriocins influence Gram-positive bacteria (Pan et al 2009). Damage to the cell membrane of pathogenic bacteria, which inhibits metabolic processes and growth, is caused by the diffusion of lactic acid into the bacterial cell and can disrupt the integrity of the cell membrane (Reuben et al 2019). Pan et al (2009) stated that the diameter of the inhibition zone against pathogenic bacteria showed low antimicrobial activity (a clear zone of 0-3 mm), moderate antimicrobial activity (>3-6 mm), and high antimicrobial activity (>6 mm).

Conclusions. The ability of LAB to inhibit pathogenic bacteria characterizes it as a potential probiotic. The results of the sequencing showed that the base length of the BK12 sample bacteria was 1439 bp. Phylogenetic analysis was carried out, showing its proximity to *Lactobacillus casei*. It is necessary to conduct further research on the application to medicine to determine their status as a source of probiotics.

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Conflict of Interest. The authors declare that there is no conflict of interest.

References

Abdullah D., Poddar S., Rai R. P., Purwati E., Dewi N. P., Pratama Y. E., 2021 Molecular identification of lactic acid bacteria approach to sustainable food security. Journal of Public Health Research 10(s2).

Begley M., Gahan C. G. M., Hill C., 2005 The interaction between bacteria and bile. FEMS Microbiology Reviews 29(4):625-651.

Bron P. A., Marco M., Hoffer S. M., Van Mullekom E., de Vos W. M., Kleerebezem M., 2004 Genetic characterization of the bile salt response in *Lactobacillus plantarum* and analysis of responsive promoters *in vitro* and *in situ* in the gastrointestinal tract. Journal of Bacteriology 186(23):7829-7835.

Cotter P. D., Hill C., 2003 Surviving the acid test: responses of gram-positive bacteria to low pH. Microbiology and Molecular Biology Reviews 67(3):429-453.

Desniar, Rusmana I., Suwanto A., Mubarik N. R., 2013 Characterization of lactic acid bacteria isolated from an Indonesian fermented fish (bekasam) and their antimicrobial activity against pathogenic bacteria. Emirates Journal of Food and Agriculture 25(6):489-494.

Dos Santos Pozza M. S., da Silva Miglioranza L. H., Garcia J. E., Garcia S., Pozza P. C., 2011 Human gastrointestinal tract resistance of *Lactobacillus* strains isolated from infant faeces. Semina: Ciencias Agrarias 32(3):1021-1032.

Ibrahim S. A., Ayivi R. D., Zimmerman T., Siddiqui S. A., Altemimi A. B., Fidan H., Esatbeyoglu T., Bakhshayesh R. V., 2021 Lactic acid bacteria as antimicrobial agents: Food safety and microbial food spoilage prevention. Foods 10(12):3131.

Kimoto-Nira H. K., Mizumachi K., Nomura M., Kobayashi M., Fujita Y., Okamoto T., Suzuki I., Tsuji N. M., Kurosaki J., Ohmomo S., 2007 *Lactococcus* sp. as potential probiotic lactic acid bacteria. Japan Agriculture Research Quarterly 41(3):181-189.

Melia S., Purwati E., Kurnia Y. F., Pratama D. R., 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.

Morales G., Sierra P., Mancilla A., Paredes A., Loyola L. A., Gallardo O., Borquez J., 2003 Secondary metabolites from four medicinal plants from northern Chile: Antimicrobial activity and biotoxicity against *Artemia salina*. Journal of the Chilean Chemical Society 48(2):13-18.

Orlowski A., Bielecka M., 2006 Preliminary characteristics of *Lactobacillus* and *Bifidobacterium* strains as probiotic candidates. Polish Journal of Food and Nutrition Sciences 56(3):269-275.

Pan X., Chen F., Wu T., Tang H., Zhao Z., 2009 The acid, bile tolerance and antimicrobial property of *Lactobacillus acidophilus* NIT. Food Control 20(6):598-602.

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

Purwati E., Gratitude S., Hidayat Z., 2005 [*Lactobacillus* sp. isolation of Bivicophitomega as probiotic]. Proceedings of the Institute of Sciences, Jakarta, Indonesia 5:24. [In Indonesian].

Rashid S., Hassanshahian M., 2014 Screening, isolation and identification of lactic acid bacteria from a traditional dairy product of Sabzevar, Iran. International Journal of Enteric Pathogens 2(4):e18393.

Reuben R. C., Coy P. C., Sarkar S. L., Alam A. S. M. R. U., Jahid I. K., 2019 Characterization and evaluation of lactic acid bacteria from indigenous raw milk for potential probiotic properties. Journal of Dairy Science 103(2):1223-1237.

Roza E., Aritonang S. N., Yellita Y., Susanty H., Rizqan, Pratama Y. E., 2021 Potential of dadiah kapau from Agam District, Indonesia as a source of probiotics for health. Biodiversitas 23:564-571.

Sari M., Suryanto D., Yurnaliza, 2018 Antimicrobial activity of lactic acid bacteria isolated from bekasam against *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC

25922, and *Salmonella* sp. IOP Conference Series: Earth and Environmental Science 130:012011.
Shah N. P., 2000 Probiotic bacteria: Selective enumeration and survival in dairy foods. Journal of Dairy Science 83(4):894-907.
Walker D. K., Gilliland S. E., 1993 Relationships among bile tolerance, bile salt deconjugation, and assimilation of cholesterol by *Lactobacillus acidophilus*. Journal of Dairy Science 76(4):956-961.
Wikandari P. R., Suparno, Marsono, Marsono Y., Rahayu E. S. 2012 [Potential of lactic acid bacteria isolated from bekasam as an angiotensin converting enzyme inhibitor in "bekasam-like" fermentation product]. Argritech 32(3):258-264. [In Indonesian].
Yang Z., Rannala B., 2012 Molecular phylogenetics: principles and practice. Nature Reviews Genetics 13:303-314.
*** http//:www.ncbi.nlm.nih.gov

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Authors:
Dinna Devi, Department of Biomedical, Faculty of Medicine, Andalas University, Jl. Perintis Kemerdekaan No. 94, 25171 Padang, Indonesia, e-mail: devi.dinna@gmail.com
Nuzulia Irawati, Department of Biomedical, Faculty of Medicine, Andalas University, Jl. Perintis Kemerdekaan No. 94, 25171 Padang, Indonesia, e-mail: nuzuliairawati@med.unand.ac.id
Yanwirasti, Department of Biomedical, Faculty of Medicine, Andalas University, Jl. Perintis Kemerdekaan No. 94, 25171 Padang, Indonesia, e-mail: yanwirasti@yahoo.com
Imam Budi Putra, Department of Dermatology and Venerology, Faculty of Medicine, Universitas Sumatera Utara, Jl. Dr. Mansyur No. 5, 20155 Padang Bulan Medan, Indonesia, e-mail: imam@usu.ac.id
Yudha Endra Pratama, Faculty of Animal Science, Andalas University, Jl. Univ. Andalas, Limau Manis, 25171 Padang, Indonesia, e-mail: yudhaendra.pratama@gmail.com

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