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B I O D I V E R S I T A S ISSN: 1412-033X Volume 21, Number 5, May 2020 E-ISSN: 2085-4722 Pages: 2240-2245 DOI: 10.13057/biodiv/d210553 Short Communication: Application of bacteriocin from Lactobacillus plantarum SRCM 1 004 34 strain isolated from okara as a natural preservative in beef sausage SALAM N. ARITONANG♥, ELLY ROZA♥♥, AFRIANI SANDRA♥♥♥ Faculty of Animal Science, Universitas Andalas. Jl. Raya Unand, Kampus Limau Manis, Padang 25163, West Sumatra, Indonesia. Tel.: +62-751-71464 ext 617, Fax.: +62-751-71464, ♥email: snaritonang@ansci.unand.ac.id, ♥♥elroz@ansci.unand.ac.id, ♥♥♥afrianisandra@ansci.unand.ac.id Manuscript received: 15 October 2019. Revision accepted: 27 April 2020. Abstract. Aritonang SN, Roza E, Sandra A. 2020. Short Communication: Application of bacteriocin from Lactobacillus plantarum SRCM 1 004 34 strain isolated from okara as a natural preservative in beef sausage. Biodiversitas 21: 2240-2245. Lactic Acid Bacteria (LAB) can be found in food and most of the LAB have beneficial effects for humans as potential probiotics. LAB isolated from okara (soy milk pulp) produces antimicrobial compounds called bacteriocins which can be used as natural preservatives in sausages. This study aims to determine the effectiveness of this bacteriocin from LAB as a natural preservative in sausages. The bacteriocin was isolated from Lactobacillus plantarum SRCM 1 004 34 strain isolated from Okara.

Completely Randomized Design (CRD) was used in 4 X 4 factorial pattern by three replicates The first factor was the percentage addition of bacteriocin (B): 0% (B0), 0.3% (B1), 0.6% (B2) and 0.9% v/b (B3). The second factor was the length of sausage storage time at 4°C (L): 0 days (L0), 4 days (L1), 8 days (L2) and 12 days (L3). The observed variables were moisture content, protein, fat, pH, total aerobic bacteria, and sausage fatty acid profile. The results showed that the content of protein, fat, and the bacterial count was significantly influenced by the interaction of the percentage addition of bacteriocin and storage time. pH was significantly affected by bacteriocin dose and storage time but was not affected by the interaction of other factors. The moisture content of the sausage was significantly affected by storage time. The results of this study showed that sausages treated with 0.9% bacteriocin (B3) with a maximum storage time of 12 in the refrigerator was still safe to be consumed. Keywords: Bacteriocin, lactic acid bacteria, preservative, storage time, sausage INTRODUCTION Lactic Acid Bacteria (LAB) are bacteria that produce lactic acid from the breakdown of carbohydrates and antibacterial substances including bacteriocins and hydrogen peroxide, which can inhibit the growth of microbes. Therefore it can be used as a preservative or natural antimicrobial (Alakomi et al. 2000). The effectiveness of LAB in inhibiting pathogenic and destroying bacteria is influenced by the density and composition of the media and the strain. Lactic acid bacteria such as Lactobacillus lactis and Streptococcus thermophillus are used to inhibit food spoilage and pathogenic bacteria and preserve the nutritive quality of food (Heller 2001). Lactic Acid Bacteria can be isolated from various natural sources and are produced especially during the fermentation process of some foods. LABs are probiotics that are Generally Recognized as Safe (GRAS) therefore they have no health risks health. Previous studies showed that LAB can reduce cholesterol (Liong and Shah 2005; Jeun et al. 2010), increased the nutritional value of food, control intestinal infections and improve digestion because LABs produce lactase in the digestive tract of humans and animals (LeBlanc et al. 2008). One important characteristic of LAB is its ability to produce antimicrobial compounds bacteriocins that inhibit the growth of pathogenic microorganisms, therefore it can be used as bio-preservatives (Savadogo et al. 2006). There are several classes of bacteriocins, i.e. simple peptides or proteins and others contain lipid molecules (Salminen 2004). They act as bactericidal or bacteriostatic agents against other bacteria. They are easily degraded by proteolytic enzymes and they can inhibit the growth of microorganisms that phylogenetically close to bacteria that produced bacteriocins. (De-Vuyst and Leroy 2007). The bacteriocins produced by LAB can inhibit the growth of pathogenic microbes and those involved in decomposition such as Bacillus cereus, Clostridium botulinum, Clostridium perfringens, Listeria monocytogenes, and Staphylococcus aureus (Diop et al. 2007). The application of bacteriocins in food does not affect the taste and appearance of the product. Bacteriocins produced by LAB can be utilized in the form of supernatant, partially purified, or more completely purified products (Woraprayote et al. 2016). Bacteriocins are commonly used in the food industry especially in fermented foods to inhibit the growth of bacterial contaminants that cause food spoilage and food-borne diseases (Abdelbasset et al. 2008). The antimicrobial compounds may affect bacterial metabolism and toxin production (Rolfe 2000). Sausages are meat products with the addition of fillers and binders such as vegetable flour or starch, seasonings, and other permitted food ingredients to be placed in sausage casings (Hui et al. 2001). In Indonesia, sausages ARITONANG et al. – Bacteriocin from Lactobacillus plantarum as a natural preservative 2241 must contain at least 75% of meat (SNI 1995). According The process of making sausages (Modified Erkkila 2001) to the USDA (2001), fresh sausages stored in the fridge Beef ribs (meat and fat) were washed thoroughly, and (4oC) have a shelf life of 1-2 days. then added 3% salt and 20% ice/ice water followed by The shelf life of fresh sausages can be extended by grinding until smooth. Ground meat was added with 15% adding nitrite, but excessive consumption of nitrites can be tapioca flour, 10% skim milk, and spices and a preservative harmful to health (Stringer and Pin 2005). Nitrites can bind (garlic 0.4%, onion 0.6%, ginger 0.5%, sugar 1.5%, salt to the amino and amides present in meat proteins to form 2%, nitrite 150 ppm, and pepper 0.15%) as a filler and carcinogenic nitrosamines (Zarringhalami et al.2009). The mixed until well blended. Sausage mixture was cured at 4- use of nitrite as a preservative in making sausages can be 7°C for 24 hours. The sausage mixture was divided into 48 reduced by the addition of bacteriocin. The addition of parts and added with supernatant containing bacteriocin 0.3% bacteriocin extracted from Lactobacillus plantarum according to the treatment: 0% (B0), 0.3% (B1), 0.6% (B2) 2C12 was able to inhibit the growth of Escherichia coli, and 0.9% (B3). Stuff mixture into casings and steamed at Staphylococcus aureus, and Salmonella typhimurium in 80°C for 40 minutes, and then cooled down to room meatballs without any changes of the taste (Arief et al. temperature for 2 hours, followed by storing in a 2012). refrigerator according to the treatment of storage time: 0 Lactic Acid Bacteria in this study were isolated from days (L0), 4 days (L1), 8 days (L2) or 12 days (L3). Every Okara. Okara is the residue left from ground soybeans after treatment has three replicates filtration to produce soy milk, however, it still has a high nutritional content. Okara contains 28.36% crude protein, Determination of chemical composition 5.52% fat, 7.6% crude fiber, lysine and methionine amino Chemical properties of sausages: The moisture, protein, acids, and vitamin B (Hsieh and Yang 2003), therefore it and fat content were determined according to AOAC can be used as growth media for bacteria including lactic (2005). acid bacteria. The aim of the research was to study the role Fatty acid composition: was determined by extracting of bacteriocin from L. plantarum SRCM 1 004 34 strain samples using chloroform: methanol (2: 1) solution. The isolated from okara as a natural preservative in beef extract was partitioned with methanol using the Morrison sausage. and Smith method (1964). Fatty acids were separated on a capillary column (30 m × 0.32 mm with 0.25 µm film thickness) that connected to a Gas Chromatography (GC, MATERIALS AND METHODS Model Star 3600, Palo Alto, USA). The GC was conditioned at 250oC for the injection port and 300oC for Materials detectors. The free fatty acids in the samples were Bacteriocin used in this study was isolated from L. identified by comparing their retention time to those of plantarum strain SRCM 1 004 34 from okara (Aritonang et standard fatty acids. The results were expressed as relative al. 2017). percentages based on the total peak area. Total aerobic bacteria count (Fardiaz 1992): Five Research design grams of the sausage was crushed until smooth, then put The study used a Completely Randomized Design into a flask containing 45 ml of NaCl solution and (CRD) in 4x4 factorial pattern by three replicates The first homogenized. Take one ml of the solution and diluted to factor was four levels of bacteriocin addition (B), i.e., 0% 10-7 and 10-8. One hundred µl from each dilution was (B0), 0.3% (B1), 0.6% (B2) and 0.9% (B3). The second pipetted and placed on Plate Count Agar (PCA) media in a factor was the length of sausage storage time (L) at 4°C: 0 petri dish. Petri dishes were incubated at 37°C for 48 hours days (L0), 4 days (L1), 8 days (L2) and 12 days (L3). in an inverted position. The number of bacteria was determined by the plate count method and Standard Plate Data analysis Count (SPC). The data were statistically analyzed using the Statistical Analysis System (SAS). Mean comparisons were carried out by Duncan's Multiple Range Test (DMRT) with RESULTS AND DISCUSSION significance at P <0.05. Moisture content Bacteriocin extraction (Yang et al. 2012) Table 1. showed that the moisture content of beef Ten ml of L. plantarum SRCM 1 004 34 strain culture sausages was not affected by the interaction between was inoculated into 90 ml of MRS-B media, then incubated bacteriocin dose and sausage storage time (P > 0.05) but it in a shaker incubator at a speed of 100 rpm at 37°C for 24 was significantly affected by storage time (P < 0.05). The hours. After incubation, the growth media was centrifuged level of bacteriocin addition did not affect significantly at 4,000 rpm at 4°C for 25 minutes and then filtered using a moisture content (P> 0.05). 0.22 μm membrane filter. The crude bacteriocin Storage time of 12 days (L3) result in the highest level supernatant was used as sausages preservative. of moisture content in sausages but it was not significantly different from moisture content in 8 days (L2) and 4 days (L1) storage time. Naturally, food degraded during storage which can result in increased moisture content (Ray and 2242 B I O D I V E R S I T A S 21 (5): 2240-2243, May 2020 Bhuna 2008). The results of this study showed that 12 days The protein content of sausages containing 0.9% of storage at 4oC did not significantly change the chemical bacteriocin was slightly decreased after being stored for 12 composition of sausages, but the moisture content was days at 4-10oC, but it did not significantly different from slightly increased. The increase of moisture content did not sausages that were not stored (L0B3). The addition of reduce sausage quality, because the moisture content still bacteriocin to 0.9% did not change the nutrient content of meets the Indonesian National Standard (INS 2015) sausages after being stored for 12 days. It might be caused requirements which is less than 67%. Arief et al. (2017) by the ability of bacteriocins to inhibit the growth of showed that the addition of bacteriocin derived from L. microorganisms such as proteolytic bacteria that could plantarum IIA-1A5 produces sausages with the moisture potentially break down protein (Collins et al. 2010). content of 57.57%. The addition of bacteriocin supernatant The protein content of sausages in this study ranged in this study did not significantly affect the moisture from 18-19%, which was above the minimum protein content of beef sausages (P> 0.05 because the primary content of 13%, according to the Indonesian National content of bacteriocin is proteins. Standard (SNI 2015). these results were in line with the results of Melia (2018) that the addition of bacteriocin from Protein Lactobacillus fermentum L23 produced sausages with The protein content of sausages was not significantly protein content range 17-18%. affected by the interaction between the bacteriocin dose and the storage time (P < 0.05) (Table 2). Increasing Fat bacteriocin dose up to 0.9% (B3) result in increasing The fat content of the sausages was significantly protein content due to the addition of protein from affected by the interaction between bacteriocin dose and bacteriocin (Jack et al. 1995). This is because bacteriocins sausage storage time (P < 0.05) (Table 3). The addition of are a precursor protein that carries N-terminals in the main bacteriocin increased the fat content of sausages regardless peptide that remains it is in the cytoplasm to play a role of storage time. Increasing the dose of bacteriocin results in again in the synthesis of bacteriocins (Ray 2004). increasing the fat content of sausages. The increase of fat content in sausages containing bacteriocin due to lipid content in bacteriocins (Ouattara et Table 1. The effect of bacteriocin dose and storage time to the al. 2011). Sausages containing 0.9% bacteriocin (B3) moisture content of sausages stored for 12 days at cold temperatures (4-10oC) have the highest fat content but are not significantly different from Treatment L0 L1 L2 L3 Average sausages that are stored for 4 days and 8 days. B0 52.74 52.12 53.17 54.04 53.02 These results indicated that the addition of bacteriocin B1 52.60 52.32 53.59 54.20 53.18 can maintain the fat content of beef sausages at cold B2 52.85 52.37 53.45 54.10 53.19 storage for 12 days. Bacteriocins contain antimicrobial B3 52.75 52.79 52.87 53.69 53.11 compounds that inhibit lipid decomposition thereby Average 52.74a 52.40b 53.27b 54.01b reducing fat degradation. When bacteriocin peptides attach Note: a, b Means in the same column with a different letter are to target bacterial cell membranes, the positive end of the significantly different (P<0.05). L: Length of storage time. B: peptide binds to the fatty acids present in the membrane Percentage of bacteriocin phospholipid, and then was separated by formation of pores which finally the bacterial death was in own cell (Song and Table 2. The effect of bacteriocin dose and storage time to the Zheng 2015). The fat content of sausages in this study was protein content of sausages (%) around 6% which was below the maximum level (20%) according to the Indonesian National Standard (INS 2015). Treatment L0 L1 L2 L3 This result was in line with the result of Melia (2018) that B0 18.37b 18.22b 18.31b 18.05a the addition of bacteriocin from L. fermentum L23 B1 18.72d 18.48ab 18.46ab 18.21b produced sausage with the fat content of 6.85%. B2 19.07f 18.87e 18.70d 18.51c pH B3 19.53g 19.40g 19.43g 19.39g Table 4. showed that the pH of beef sausages was Note: a-g: Means with a different letter are significantly different (P<0.05) affected significantly by the bacteriocin dose and storage period (P < 0.05) but was not affected by the interaction between these two factors (P > 0.05). Table 3. The effect of bacteriocin dose and storage time to the fat Table 4. The effect of bacteriocin dose and storage time to the pH content of sausages (%) of sausages Treatment L0 L1 L2 L3 Treatment L0 L1 L2 L3 Average B0 6.11c 6.06ab 6.01a 6.07b B0 5.40 5.40 5.50 5.50 5.45a B1 6.40g 6.30f 6.19e 6.15d B1 5.40 5.40 5.40 5.50 5.40a B2 6.47i 6.40g 6.42h 6.22e B2 5.30 5.40 5.40 5.40 5.40a B3 6.52j 6.50ij 6.48ij 6.47i B3 5.20 5.30 5.30 5.30 5.30b Note: a-g: Means with a different letter are significantly different Average 5.32a 5.39b 5.40b 5.43b (P<0.05) Note: a, b Means in the same column /row with a different letter are significantly different (P<0.05) ARITONANG et al. – Bacteriocin from Lactobacillus plantarum as a natural preservative 2243 Table 5. The effect of bacteriocin dose and storage time to the Total Aerobic Bacteria (x 103 Cfu/mL) Fatty acid profile The percentage of bacteriocin addition and storage time affected the lipolysis process in sausages, results in Treatment L0 L1 L2 L3 changing the profile of fatty acids. Fatty acid profile of B0 14.37a 14.86bc 16.57e 19.33f sausages was presented in Table 6. showed there were 13 B1 14.30a 14.60b 15.57c 16.05d types of fatty acids were detected in all samples. B2 14.27a 14.65b 15.35c 16.06d B3 14.26a 14.37a 14.98bc 15.70c Percentage of stearic acid (C18:0), linolenic acid Note: a-f: Means with a different letter are significantly different (C18:3n3), erucic acid (C20:1n9), and arachidonic acid (P<0.05) (C20: 4n6) were significantly different among treatments (P <0.05). The highest level of C18:0 (13.85%) was in sausages treated with 0.9% bacteriocin without storage The highest addition of bacteriocin (0.9%, B3) produced (B3L0), while the lowest was sausages without the addition the lowest sausage pH (5.2). The decrease in pH of beef of bacteriocin and stored 12 days (B0L3). sausages due to the amino acids in bacteriocin which The results suggest that the highest dose of bacteriocin contain hydrogen atoms in the carboxyl group dissociates (0.9%) inhibited fat decomposition by lipolytic bacteria and to produce H+ ions thereby increasing acidity and reducing result in a high level of stearic acid. Bacteriocins are pH. known to inhibit lipolytic bacteria (Abdelbasset et al. The length of storage time from 4 to 12 days (L1, L2, 2008). Levels of highly unsaturated fatty acids such as L3) did not significantly affect the pH of sausages but C18:3n3 and C20:4n6 were significantly higher in sausages slightly higher than control (L0). An increase of sausage pH which were treated with 0.9% bacteriocin and stored for 4 accord along with an increase of storage duration in low days (B3L2) were 0.67% and 0.49% respectively. temperature due to enzyme activity and chemical Unsaturated Fatty Acids (UFA) levels and n6/n3 ratio decomposition of compounds such as proteins which also differed among treatments. UFA levels were higher in results in the production of alkaline compounds such as bacteriocin-treated sausages than controls and being the indole, scatole, and cadaverine (Suradi 2012). highest was in sausages treated with 0.9 % bacteriocin on 0 days of storage (B3L0). The ratio of n6/n3 was lower in Total aerobic bacteria sausages treated with high bacteriocin than control, and Total aerobic bacteria were significantly affected by the being the lowest ratio was sausages with 0.9% bacteriocin interaction between the bacteriocin dose and the storage with a storage period of 12 days. UFA especially PUFAs time of sausage (P <0.05) (Table 5). The addition of such as C18:3n3 have a highly significant influence on bacteriocin significantly inhibits the growth of aerobic human health (Jump 2002), and a high ratio of n6/n3 is bacteria during storage. Bacteriocin addition to 0.9% (B3) good for health (Krauss et al. 1998). Differences in fatty suppressed the increase of total aerobic bacteria. Total acid content between treatments due to differences in the aerobic bacteria in the B3L3 treatment (0.9% bacteriocin, 12 lipase activity of microbes (Chen et al. 2017). Fatty acid days of storage) was 15.70 x 103 CFU/mL which was not levels may be influenced by the antimicrobial activity of significantly different from B2L2 treatment (0.6% bacteriocin that capable of inhibiting the growth of bacteriocin, 8 days of storage) which was 15.35 x microorganisms (Collins et al. 2010). 103Cfu/mL. In conclusion, the protein and fat content, pH, total Total aerobic bacteria in sausages with B3L3 treatment aerobic bacteria, and fatty acid profile of the beef sausages were higher compared to the results of Wibowo et al. were significantly influenced by bacteriocin dose and (2017) showed that the addition of the bacteriocin storage time. The moisture content of sausages was supernatant from L. plantarum IBL-2 to fresh ground beef significantly influenced by storage time. The addition of and stored for 12 days had total aerobic bacteria of 0.96 x 0.9% bacteriocin produces sausages that are still suitable 104 CFU / ml. Because L. plantarum IBL-2 of Wibowo for consumption after 12 days of storage at the refrigerator research added with nisin amide. While the addition of based on the chemical composition and total aerobic nisin influenced decreased bacterial growth. So that why bacteria. total aerobic bacterial was lower than the result of this study. Result of this research is also higher than Arief et al. ACKNOWLEDGEMENTS (2017) study which is Total aerobic bacteria in sausages with the addition of bacteriocin from L. plantarum IIA-1A5 This research was funded by BOPTN Research Funding is 3.66 x 103 CFU/mL. It caused the storage time of from the Andalas University, Padang, Indonesia, Ministry sausage at Arief's et al studied up to 9th days. Total aerobic of Research and Technology of Higher Education of the bacteria are still under the maximum number (1 x 105) Republic of Indonesia through contract No. permitted by SNI (1995). The results suggest that 0.9% 34/UN.16.17/PP.RGB/ LPPM/2018. We are very grateful bacteriocin addition to sausages that sored for 12 in the to the Rector of Andalas University and the Minister of refrigerator are still safe for consumption. SNI (1995). Research and Technology of Higher Education for funding Therefore sausages with the addition of 0. 9% bacteriocin this work. stored for 12 days in the refrigerator are still safe for consumption. B I O D I V E R S I T A S ISSN: 1412-033X Volume 21, Number 5, May 2020 E-ISSN: 2085-4722 Pages: 2240-2245 DOI: 10.13057/biodiv/d210553 Table 6. Percentage of fatty acid in sausage (%) B0.0 B0.4 B0.8 B0.12 B1.0 B1.4 B1.8 B1.12 B2.0 B2.4 B2.8 B2.12 B3.0 B3.4 B3.8 B3.12 C14:0 C16:0 C16:1n7 C18:0 C18:1n9 C18:2n7 C18:2n6 C18:3n6 C18:3n3 C20:1n9 C20:4n6 C20:5n3 C22:4n6 SFA UFA MUFA PUFA n6/n3 1.40 1.41 22.45 22.70 1.25 1.25 12.47b 12.40b 40.90 40.60 0.15 0.14 15.51 15.50 0.06 0.05 0.52a 0.52a 0.62a 0.83bc 0.32ab 0.30a 0.01 0.01 0.13 0.13 38.70 38.70 55.40b 55.25ab 42.42 42.40 17.43 17.37 29.60cd 29.15bc 1.48 1.46 22.85 22.15 1.23 1.15 12.20b 11.25a 40.53 39.95 0.13 0.10 15.48 15.27 0.05 0.04 0.50a 0.49a 0.75b 0.85c 0.26a 0.24a 0.009 0.008 0.12 0.10 38.20 38.00 55.03a 54,92a 42.35 42.05 17.15 17.05 28.87b 28.85b 1.51 1.51 22.87 22.72 1.18 1.38 12.85bc 12.25b 40.85 40.63 0.16 0.14 15.78 15.73 0.06 0.06 0.52a 0.51a 0.87c 0.83b 0.37b 0.33ab 0.013 0.013 0.16 0.15 39.70 39.67 57.90de 57.50d 42.92 42.75 18.00 17.87 29.89d 29.57cd 1.45 22.15 1.25 12.37b 40.67 0.13 15.65 0.05 0.50a 0.81b 0.30 0.011 0.12 38.50 56.47c 42.45 17.63 1.57 22.18 1.23 11.87a 40.45 0.12 15.35 0.04 0.48a 0.76b 0.27a 0.009 0.11 38.10 56.40c 41.95 17.57 29.03bc 28.67b 1.52 23.25 1.57 13.57c 40.95 0.17 16.41 0.07 0.57b

0.95d 0.39c 0.014 0.16 39.87 59.20f 42.81 18.02 29.87e 1.52 23.05 1.40 13.05c 40.70 0.15 16.47 0.07 0.53ab 0.87c 0.37b 0.013 0.16 39.65 58.12e 42.80 18.01 29.76de 1.52 1.55 22.85 22.35 1.48 1.35 13.23c 12.15b 40.25 40.00 0.15 0.14 16.35 16.05 0.07 0.06 0.51a 0.47a 0.85c 0.79a 0.37b 0.31a 0.011 0.009 0.13 0.11 39.10 38.80 57.50d 57.01cd 42.68 42.49 17.85 17.55 29.15bc 28.90b 1.56 1.52 23.60 23.35 1.85 1.60 13.85d 13.50cd 40.73 40.75 0.18 0.15 16.75 16.53 0.05 0.07 0.62bc 0.67c 0.98d 0.95d 0.45cd 0.49d 0.015 0.015 0.18 0.16 39.92 39.85 62.20g 61.81h 42.98 42.87 18.23 18.05 29.30c 28.45a 1.55 1.58 23.20 23.15 1.67 1.45 13.25c 12.87bc 40.78 40.55 0.15 0.10 16.45 16.25 0.06 0.04 0.57b 0.54ab 0.87c 0.84bc 0.39c 0.35ab 0.012 0.009 0.14 0.12 39.50 38.80 61.35h 60.95g 42.62 42.43 17.86 17.51 28.37a 28.23a B I O D I V E R S I T A S ARITONANG et al. – Bacteriocin from Lactobacillus plantarum as a natural preservative ISSN: 14122-023435X Volume 21, Number 5, May 2020 E-ISSN: 2085-4722 Pages: 2240-2245 DOI: 10.13057/biodiv/d210553 REFERENCES LeBlanc JG, Clier FL, Bensaada M, de Giori GS, Guerekobaya T, Sesma F, Juillard V, Rabot S, Piard JC. 2008. Ability of Lactobacillus Abdelbasset M, Djamila K. 2008. Antimicrobial activity of autochthonous fermentum to overcome host α-galactosidase deficiency, as evidenced lactic acid bacteria isolated from Algerian traditional fermented milk by reduction of hydrogen excretion in rats consuming soya α-galacto- "raib". Afr J Biotechnol 7 (16): 2908-2914. oligosaccharides. BMC Microbiol 8: 22. DOI: 10.1186/1471-2180-8- Alakomi AL, Saarela S, Sandholom M, Kala L, Helander. 2000. Lactic 22. acid permeabilized Gram-negative bacteria by disrupting the outer Liong MT, Shah NP. 2005. Acid and bile tolerance and cholesterol membrane. Appl Environ Microbiol 66: 2001-2005. removal ability of lactobacilli strains. J Dairy Sci 88: 55-66. AOAC. 2005. Official Method of Analysis of AOAC International 18th ed. Melia S. 2018. Potensi Isolat Bakteri Asam Laktat Dari Susu Kerbau AOAC International, Gaithersburg, MD. sebagai Probiotik dan Penghasil Bakteriosin Untuk Pangan Arief II, Wulandari Z, Sindya ES. 2017. Application of Purified Fungsional. [Dissertation]. Program Studi Ilmun Peternakan, Fakultas Bacteriocin from Lactobacillus plantarum IIA-IAS as Bio- Peternakan Universitas Andalas, Padang. [Indonesian] preservative of Beef Sausage. Pak J Nutr 16 (6): 444-450. Morrison WR, Smith LM. 1964. Preparation of fatty acid methyl ester a Aritonang SN, Roza E, Rossi E. 2017. Isolation and identification of lactic dimethyl acetals from lipids with boron fluoride methanol. J Lipid acid bacteria from okara and evaluation of their potential as candidate Res 5: 600-608. probiotics. Pak J Nutr 16 (8): 618-628. Ouattara L, Koudou J, Zongo C, Barro N, Savadogo A. 2011. Antioxidant Cahyadi W. 2006. Kedelai Khasiat dan Teknologi. Bumi Aksara. Jakarta. and antibacterial activities of three species of lannea from Burkina [Indonesian] Faso. J Appl Sci 11 (1): 157-162. Chen Q, Kong B, Han Q, Xia X, Xu L. 2017. The role of bacterial Ray B, Bhuna A. 2008. Fundamental Food Microbiology. 4th ed. CRC fermentation in lipolysis and lipid oxidation in Harbin dry sausages Press, Boca Raton, FL. and its flavor development. LWT Food Sci. Technol 77: 389-396. Ray B. 2004. Fundamental Food Microbiology. 3rd ed. CRC Press, New Collins B, Cotter PD, Hill C, Ross R P. 2010. Applications of Lactic York. AcidBacteria - Produced Bacteriocins in Biotechnology of Lactic Rolfe. 2000. The role of probiotic cultures in the control of Acid Bacteria, Novel Application. Willey-Blackwell, New York. gastrointestinal health. J Nutrition 130: 396-402. De-Vuyst L, Leroy F. 2007. Bacteriocins from lactic acid bacteria: Salminen S, Atte VW, Arthur O. 2004. Lactic Acid Bacteria. Marcel production, purification, and food applications. J Mol Microbiol Dekker Inc., New York. Biotechnol 13: 194-199. Savadogo AA, Ouattara TC, Bassole HNI, Traore SA. 2006. Bacteriocins Diop M, Dubois-Dauphin BR, Tine E. 2007. Bacteriocin producers from and lactic acid bacteria-a minireview. Afr J Biotechnol 5 (6): 678- traditional food products. BASE Biotechnol Agron Soc Environ 11 683. (4): 275-281. Song H, Zheng W. 2015. Antimicrobial natural product the battle against Erkkila S. 2001. Bio

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