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Antimicrobial Activity of Lactic Acid Bacteria Thermophilic Isolated from Hot Spring Rimbo Panti of West Sumatera for Food Biopreservatives

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Abstract: Rimbo Panti hot springs located bordering the province of West Sumatra and North Sumatra, precisely in the District of East Pasaman Pasaman regency of West Sumatra Province approximately 200km from the city of Padang. Hot water samples obtained from 5 pools which have a normal temperature of 50- 95°C. The aim of this research was to isolate and identify Lactic Acid Bacteria (LAB) thermophilic activity from hot spring with pathogen bacteria (Escherichia coli O157: H7, Salmonella thypmurium and Listeria monocytogenes) followed identified by PCR. The bacteria isolated were growth on medium thermus cair and then deMan Rogosa and Sharpe (MRS) Agar supplement with CaCO3 1% and then performed purification by plate out on deMan Rogosa and Sharpe (MRS) Agar. It was found 23 isolates of LAB showed with clear zone around the culture and 5 isolates (N2, N4, N6, N9 and N12) has been antimicrobial activity against the growth of pathogenic bacteria. The results showed that isolates N6 had the highest antimicrobial activity against all bacteria test, with a range of inhibition zone 18-30 mm, gram positive, spore former coccus, non motility and catalase negative. LAB isolates that have the widest diameter of the clear zone continued to test the minimum inhibitory concentration (MIC). MIC values of isolates N6 supernatant against pathogenic bacteria Escherichia coli O157: H7 by 60 and 80% of the bacterial pathogen Salmonella thypmurium and 50% of the bacterial pathogen Listeria monocytogenes. Based on morphological examination and PCR analysis, the

isolate N6 was primarily identified as Pediococcus pentosaceus strain A24 bacteria. Key words: Antimicrobial activity, hot spring, isolated, food biopreservatives, west sumatera INTRODUCTION Antimicrobial agent is a general term used to refer to any compound which include antibiotics, food antimicrobial agents, sanitizer, disinfectants and other substances that acts against microorganisms (Katzung, 2004). Lactic acid bacteria as antimicrobial agent are widely distributed in the nature. In this group are included representatives of the genus Lactobacillus, Lactococcus, Pediococcus and Leuconostoc. They could be isolated from soils, waters, plants, silages, waste products and also from the intestinal tract of animals and humans (Tserovska et al., 2002). Hot spring is geothermal habitat diversity of microorganisms and the potential applied to the heating industry involving so intensive research. Khalil et al. (2003), isolation of thermophilic bacteria from hot springs Zerka-Maen and Himma in Jordan to produce plasmid DNA from these isolates. Khalil et al. (2006), further isolation of thermophilic bacteria from Zara hot springs in Jordan Vellay used as an antimicrobial producer. Isolation of thermophilic lactic acid bacteria from hot springs Rimbo Panti West Sumatra due to the location of the overgrown shrubs and trees like the banyan similar Laban (Vitex pubersens), Sicorek (Santina apiculata) and Jawi-Jawi (Ficus sp) as well as ferns, mosses and other lower plants (Thamrin, 2001). Pursuant according Dirnawan et al. (2000), dropping leaves, twigs, stems and dead animals found in hot springs are a source of organic material that can be utilized amylase thermophilic bacteria to grow. Furthermore, according to Rao (1994), the decomposition of organic material (leaves and twigs) were entered into the pool of microorganisms involved in decomposition of both aerobic and anaerobic conditions to produce organic acids such as lactic acid, acetic acid, butyric acid and alcohol. Microorganisms decomposing take water, O2 from the environment and food from the organic material to be converted into products of biological metabolism in the form of CO2, H2O, some humus and energy. Thermophilic microbes are indispensable in the food processing industry involving heating. Thermophilic microbes able to survive at high temperatures, this is caused by several factors: (1) microbial termofil contains enzymes and proteins are heat stable and functioning optimally at high temperatures, (2) protein pensintesis machinery (ribosomes) is heat stable and (3) termofil cell membrane lipids rich in saturated fatty acids form a hydrophobic bond so strong heat resistance (Brock and Madigan, 1991). Furthermore. According to Nosoh and Corresponding Author: Nurjama'yah, Department of Agriculture, Faculty of Agriculture, Al-Azhar University Medan, Medan-20142, Indonesia 465 Sekiguchi (1991), Stability to heat the substance is achieved by a number of amino acid residues that can increase the hydrophobicity of proteins and lead to a more compact structure that is resistant to heat. The growth of spoilage and pathogenic bacteria in food containing LAB is inhibited. This can be due to competition for nutrients and also to pH reduction by the organic acids produced. Furthermore, these bacteria have the ability to produce a variety of antimicrobial substances as a natural competitive means to overcome other microorganisms sharing the same niche, among them, ethanol, formic acid, acetoin, hydrogen peroxide, diacetyl and bacteriocins. The last ones are small, ribosomally synthesized, peptides or proteins which inhibit microorganisms that are

usually closely related to the producer strain (Tserovska et al., 2002). According Jenie et al. (2001), antimicrobial compounds are biological and chemical compounds that can inhibit microbial growth and activity. Lactic acid bacteria are able to act as an antimicrobial either through direct use in food fermentation processes and through the resulting metabolites that serve to extend shelf life, improve product quality and inhibit the growth of pathogenic and spoilage microorganisms. These compounds also serve to prolong the shelf life and improve the safety of food products. Furthermore, according to (Todorov, 2009), Lactic Acid Bacteria hydrolyze sugars (starch, cellulose and hemicellulose) into lactic acid. Fermented lactic acid bacteria are homofermentatif and heterofermentatif. Homofermentatif only produce lactic acid as an end product of glucose metabolism using the EMP pathway. In heterofermentatif be formed lactic acid, CO2 and ethanol or acetate of sugar through phosphoketolase. Lactic acid bacteria are also capable of forming other products such as diacetyl

and aromas eg acetone or bacteriocins Lab or bacteriocins have received increased attention during the last few decades. They have been mainly used in food preservation and safety either separately or in combination with other conventional treatment as part of hurdle technology. Other applications are now being considered including their use as

functional foods (prebiotics, probiotics or nutraceuticals) as well as in human therapy. It also satisfies industrial and consumers demands. Some of the trends of the food industry, such as the need to eliminate the use of artificial ingredients and additives, the demands for minimally-processed and fresher foods, as well as for ready-to-eat food or the request for

functional foods and nutraceuticals could be satisfied (Oliveira et al., 2008). The aims of this research are to isolate

and identify lactic acid bacteria thermophilic from water hot spring and to screen and determine the activity antimicrobial with pathogen bacteria (Escherichia coli O157: H7, Salmonella thypmurium and Listeria monocytogenes) in order apply for food biopreservative. MATERIALS AND METHODS Samples were obtained from an existing 5 in Rimbo hot springs pool parlors sumbar. Suhu and pH were measured with a thermometer and pH paper. The temperature and pH of the five pools sampled consists of: a temperature of 70° C, pH 5, temperature 70°C, pH 6, temperature 52°C, pH 5, temperature 86°C, pH 6 and a temperature of 95°C, pH 6 water samples were taken at depths of as much as 60 mL of 30-50 cm below the surface of the water, then placed in sterile bottles containing 50 mL of medium Thermus. The samples were transferred immediately to the laboratory for microbiological analysis. Pathogenic bacteria strains and culture medium: Escherichia coli O157: H7, Salmonella thyphimurium and Listeria monocytogenes. The culture medium in this research: Thermus liquid, thermus agar, with composition in 1 L consists of: 0.1 g (NH4)2SO4, 0.25 g MgSO4.7H2O, 0,125 g CaCl2.2H2O, 0, 3 g K2HPO4, 1 g NaCl, 2 g yeast extract dan 4 g pepton (Suyono et al., 2008)., MRS agar+CaCO3 1%, MRS broth (Merck), MHA (Merck) and Nutrient Agar (NA) (Merck). Isolation of lactic acid bacteria: modification method Suyono et al. (2008), Sarkono (2005) and Khalil et al. (2006), after the hot water samples incubated at 50-600 C for 2-3 days in Thermus liquid medium is then taken as many as 5 ml plus 45 mL of 0.1% peptone diluent. At 10-3 dilution of 0.5 mL were taken termus grown in medium with a pH that appropriate pH 5 and 6 as well as the habitat was calculated CFU/mL of sample. Samples were also grown in hot water MRSA medium plus 1% CaCO3 at 10-5 dilution of 0.5 mL and then incubated at 37°C for 2-3 days. Colonies that grew alleged BAL if forming a clear zone around the colony and then purified by growing on medium MRS agar. After the pure LAB isolates were then grown back to medium Thermus order. Culture bred alternate between Termus order and MRSA, so obtained are thermophilic LAB isolates. For storage of isolates grown in MRS broth medium for 24 h at 37°C, then centrifuged 10,000 rpm for 20 min. Supernatant was removed, placed in a suspense taken eppendop containing 1 ml. Suspension plus 40% glycerol at -200°C (Savodogo et al., 2044). Characterization of lactic acid bacteria: The identified of the isolates were determined by the standard procedure of gram staining, catalase test, motility and spore former test (Hadioetomo, 1985; Fardiaz, 1989; Lay, 1994). Characterization bacterial strains of LAB determination the using standard methods "Manual for the identification of medical bacteria" (Cowan and Steel, 1975).

Antimicrobial activity test: The modified methods wells that of Cintas et al. (1995), Savodogo et al. (2004) and Girum et al. (2005) were used to determine the antibacterial activities of the isolates. A single isolated colonies were selected from MRS agar plates and transferred to grow in sterile MRS broth. The broth culture was incubated erobically at 37°C for 48 h. After incubation, the culture was centrifuged at 10.000 rpm for 20 min at 4°C to obtain the culture supernatant. The indicator microorganisms (Escherichia coli o157:H7, Salmonella thypimurium and Listeria monocytogenes) were grown in NA for 24 h at 37°C. Prepared media MHA 15 mL of sterile (autoclaved). After a rather cold (lukewarm) then poured into a petri dish. After that freezes, made well by using a blue pipette tip (blue tip) so that the ends are cut large diameter wells 5 mm. A sterile cotton swab was dipped into culture of the indicator microorganisms and rotated several times and the swab was then pressed firmly on the inside wall of the tube above the fluid level to remove excess inoculums. The dried surface of NA was inoculated by streaking the swab over the entire agar surface. This procedure was repeated by streaking two or more times while rotating the plate each time to ensure an even distribution of inoculums. For the bioassay, the sterile filter disc was dipped into the culture supernatant and touched to the side of container to remove excess liquid and it was then placed on a NA plate. After 48-72 h at 37°C of aerobic incubation, each plate was evaluated and the diameters of the inhibition zones, including the diameter of the disc, were measured using a transparent ruler (Tagg and Mc Given, 1995) and Delgado et al. (2001). Minimum inhibitory concentration- broth dilution method: The modified method tube dilution MIC that of Kubo (1993) and Consentino (1999), as many as 10 tubes filled with a solution of Nutrient Broth (NB) each consisting of 9, 8, 7, 6, 5, 4, 3, 2, 1 and 0 mL with a concentration of pathogenic bacteria in it 106 CFU/mL. Then the supernatant plus thermophilic LAB isolates as much as 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 mL of the supernatant in it so that the concentration of 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100%. The tubes were incubated at 370 C for 48 hours. Turbidity levels show the growth of pathogenic bacteria was measured with a spectrophotometer at a wave length of 630 µm. DNA extract, PCR and sequencing: The Genomic DNA was extracted from pure culture of the Pediococcus isolate using genomic DNA extraction kit following instructions of the Marchesi et al. (1998). DNA of CTAB extraction method (Cetyl Trimethil ammonium bromide) by opening the cell membranes in the cell. DNA was precipitated using 100% cold ethanol. The quality of the isolated DNA is checked by 1% aga, further purification using a column aimed at throwing DNA contamination from other organic material rose gel electrophoresis. The concentration of the isolated DNA was measured using a spectrophotometer with 5 uL DNA computation coupled with 495 mL of water. Total DNA samples isolated so there are 50 uL total genomic DNA in 2500 or 2.5 ug/uL. DNA that has been measured is ready for PCR. The 1 kb gene 16S rDNA was amplified by PCR using a pair of universal bacterial 16S rDNA gene primer 63F: 5-CAG GCC TAA CAC ATG CAA GTC-1387 primers 3 and R: 5-GGG CGG WGT GTA CAA GGC-3 s. The total volume of PCR reaction (20 uL) consisted of a mixture of 9 uL kit. MASTER MIX (dNTPs, Taq polymerase) to the reaction mixture kit beyond 1 uL of DNA and water. Purification of PCR products prior to sequencing carried out by PT genetics scince use kit that uses the column. PCR results in sequences edited and aligned using the program (BioEdit Sequence Alignment Editor version 7.0.9.1.), then traced the species or genus in the NCBI (National Center for Biotechnology), which is the software BLASTN. Results of BLASTN with a journal that has been published as a reference. RESULTS AND DISCUSSION Isolation of LAB: The isolation of LAB from hot spring was performed MRS agar supplemented with CaCO3 1% which was obtained at the room temperature 37°C, 48 h of incubation was used as a preliminary screening medium for LAB. It was found that 23 isolates exhibited a clear zone and growth on MRS agar. Isolation of BAL samples of pond water with a temperature of 70°C and pH 5 resulted in 23 colonies that produced a clear zone that was grown on MRS medium plus 1% CaCO3 with a 10- 5 dilution. Retrieved 18 isolates (78%) shaped cocci and 5 isolates (22%) shaped bacillus. A total of 19 isolates were white (82.6%), cream-colored 2 isolates (8.7%) and 2 isolates of white milk (8.7%) and all isolates have flat edges (Table 1). According to Khalil et al. (2006), isolation of antimicrobial-producing thermophilic bacteria from hot springs in Jordan Vellay Zara produces two isolates of Aeromonas hydrophila and Yersinia namely sp. 1, both rod-shaped. Aeromonas hydrophila has a diameter of 16 mm zone of inhibition against Staphylococcus aureus. Detection of antagonistic activity: A total 23 isolated randomly picked from hot spring samples for the morphological identification was selected isolated gave 5 (N2, N4, N6, N9 and N12) isolates has the most extensive zone of inhibition when compared to other Table 1: Observations Morphology 23 isolates that produce a clear zone on MRSA medium +1% CaCO3 at 105 dilution IC Form Color Edges N1 coccus white Entire N2 coccus white Entire N3 coccus white Entire N4 bacil white Entire N5 coccus Krem Entire N6 coccus Milk of white Entire N7 coccus white Entire N8 coccus white Entire N9 bacil white Entire N10 Bulat white Entire N11 coccus White Entire N12 coccus Milk of white Entire N13 coccus white Entire N14 bacil White Entire N15 coccus crem Entire N16 coccus white Entire N17 bacil White Entire N18 coccus white Entire N19 coccus white Entire N20 coccus white Entire N21 coccus white Entire N22 bacil white Entire N23 coccus white Entire IC: Isolate code isolates. Based on the data it appears that the fluid extracellular of eight isolates of the inhibition of the bacterial pathogen/indicator and it appears that the inhibitory activity extracellular N2 and N6 had a higher power resistor. While isolates had the highest inhibitory power is N6 isolates with inhibition of the Escherichia coli 20 mm, against Salmonella thypi 18 mm and the Listeria monocytogenes 30 mm. Isolates that have the widest zone of inhibition against Escherichia coli O157: H7 isolates N2 and N12 that is measuring 15 mm with inhibitory activity mm2/mL 200, against Salmonella thypimurium isolates N2 and N4 that is measuring with inhibition zone diameter of 17 mm with inhibition activity 240 mm2/mL activity. The results showed an average inhibition zone of Escherichia coli ranging from 11 mm (isolate N4) to 20 mm (isolate N6), Salmonella thypimurium ranging from 15 mm (isolate N9) to 18 mm (isolate N6) and Listeria monocytogenes ranging from 10 mm (isolate N2) to 30 mm (isolates N6). Diameter of clear zone formed is shown in Fig. 1. Minimum inhibitory concentration-broth dilution method: MIC test results on the Table 3 shows that the supernatant of N6 isolates had MICs for 60% of the bacteria E. coli O157:H7 and 80% of the bacteria Salmonella thyphimurium after incubation for 48 h, the test results of the MIC values against E. coli O157:H7 and Salmonella thyphimurium different from Triani study (2008) that the MIC for E. coli 80 and 90% for Salmonella thyphimurium after incubated for 24 h. In this reset incubation for 48 h because to the incubation time resulted in a clear zone diameter of the most widespread. Value MiC isolates N6 lower against E. coli O157:H7 bacteria are more sensitive means to BAL. Pursuant according Mckane and Kandel (1985) and Prescott et al. (2002), inhibition of BAL against pathogens is influenced by differences in the cell wall and peptidoglycan layers that make up the cell wall. Gram-negative bacterial peptidoglycan thinner than gram-positive bacteria. Gram-negative bacterial peptidoglycan only 1-2% of the dry weight of the cell whereas gram-positive bacteria reached 20%. While the MIC values against Listeria monocythogenes bacteria which are gram positive by 50%. This is because the isolates N6 is a bacteriocin-producing thermophilic LAB isolates that are heat resistant and has a high ability to inhibit the growth of pathogenic bacteria Listeria monocythogenes. It is appropriate in Barefoot and Nettles, (1993), Nettles and Barefoot (1993) in the Tagg et al. (1995) that the bacteriocin-producing thermophilic bacterium BAL heat resistant (class II bacteriocins) contains a peptide that has a very strong anti Listeria. Bal has antimicrobial activity because the resulting metabolic components can inhibit or kill pathogenic bacteria. Ammor et al. (2006) LAB produce a wide range of products from low molecular mass compounds, such as hydrogen peroxide, carbon dioxide and diacetyl, to high molecular mass compounds, such as bacteriocins. Organic acid produced by LAB leads to a reduction in pH levels and increases the production of hydrogen peroxide (Ponce et al., 2008), enzymes (lactoperoxidase system with hydrogen peroxide and lysozyme), low-molecular metabolites (reuter in, diacetyl and fatty acids) and bacteriocins (nisin and others) (Holzapfel et al., 1995). These products exhibit antibacterial activity against various pathogenic microorganisms, including gram-positive and gram negative bacteria (Maragkoudakis et al., 2009). Khalil et al. (2003), isolation of thermophilic bacteria from hot springs and Zerka-Maen Himma in Jordan resulted in two isolates of Streptococcus thermophilus and Bacillus sp. 1 for to produce plasmid DNA. Furthermore Gilbeth et al. (2005), isolate bacteriocins thermophilin 110 of Steptococcus thermophilus ST 110 is used in the

food industry. Narayan et al. (2008), did isolation and characterization of thermophilic bacteria from hot springs in Savusavu Fiji New Zealand produces 58% of species Anoxybacillus flavithermus, 19% Bacillus licheniformis, Thermus sp. 10% TO 153 and 10% TG 206 Thermus sp. Martirani et al. (2002) isolate bacteriocins of Bacillus licheniformis 490 bacillocin used as an antimicrobial food, especially in milk. Morphological, physiological and biochemical examination of isolate N6 bacteria: Bacteria isolated from the hot Spring (sample N6) was identified as (a) (b) (c) Fig. 1(a-c): Antimicrobial activity of Pediococcus pentosaceus strain A24 against pathogens bacteria: (a) Salmonella thypimurium (b) Escherichia coli O157:H7 and (c) Listeria monocytogenes, by well diffusion agar methods Table 2: Antibacterial activity of 5 isolates of LAB obtained from water hot spring samples against different indicator microorganisms ---------- ---------- Diameter of inhibition zone (mm) ---------- ---- No. of Strains E. coli O157:H7 S. thypimurium L. monocytogenes N2 15 17 20 N4 11 17 14 N6 20 18 30 N9 16 15 13 N12 15 16 10 Pediococcus on physiological and biochemical characteristics (Table 2). The isolate N6 as gram positive, coccus shapeband negative for catalase test having smooth round colonies on the MRS media. The strain was capable of fermenting sugars, namely glucose and sucrose and still can growth at 70°C. These tests based on Manual for the Identification of Medical Bacteria (Cowan and Steel's, 1975) and the bacteria belong to Pediococcus genus Phylogenetically, bacteria belonging to the genus Pediococcus belong to class I of the phylum Firmicutes i.e., the bacilli. Members of the genus Pediococcus are gram-positive, aerobic and non endospore forming bacteria that are characterized by their coccus shaped cell morphology, not catalase production and their ubiquitous distribution. Several species and strains of pediococci which are used as starter cultures in fermentation of meat, sausage products, vegetables and cheddar cheese have also been the

subject of much recent investigation with regard to their bacteriocin- producing ability. Bacteriocins have been characterized from Pediococcus acidilactici and Pediococcus pentosaceus for effective use in foods. Pediocin AcH and pediocin PA-1 produced by different strains of Pediococcus acidilactici were purified and later demonstrated to be identical. Characteristics common to these bacteriocins are that their genetic determinants appear to be plasmid-borne and the bacteriocins are active against a broad spectrum of Gram-positive bacteria, many of which are associated with food spoilage

and food related Table 3: Minimum Inhibition Concentration of supernatant isolates N6 Type patho gens of bacterial Antimicrobial substrate concentration (%) Final of population (Nt) (CFU/mL) % Retardation = 100%-s(Nt/No X 100%) Salmonella thyphimurium Initial population (No) = 6.1 X 106 10 20 30 40 50 60 70 80 90 100 3.1 X 106 2.8 X 106 2.6 X 106 2.3 X 106 1.9 X 106 1.5 X 106 7.4 X 105 5.6 X 105 4.4 X 105 3.2 X 105 49.18 54.10 57.38 62.30 68.85 75.48 87.86 90.81 92.79 94.75 Eshercia coli O157: H7 Initial population (No) = 5.3 X 106 10 20 30 40 50 60 70 80 90 100 2.6 X 106 2.3 X 106 1.8 X 106 8.4 X 105 7.5 X 105 5.0 X 105 4.5 X 105 3.9 X 105 2.6 X 105 1.8 X 105 50.94 56.60 66.04 84.15 85.84 90.60 91.51 92.64 95.09 96.60 Listeria monocythogenes Initial population (No) = 5.0 X 106 10 20 30 40 50 60 70 80 90 100 2.5 X 106 2.1 X 106 1.3 X 106 5.8 X 105 4.9 X 105 4.1 X 105 3.7 X 105 3.2 X 105 2.6 X 105 2.0 X 105 50.00 58.00 74.00 88.40 90.20 91.80 92.60 93.60 94.80 96.00 Table 4: Physiological and biochemical characteristic of isolate N6 Test Isolate N6 Colony morphology white, entrie, tetrad and smooth round colonies Gram staining Gram positive coccus Spore-former Coccus Coloni Surface Convex Coloni size 3 mm Motility Negatif Growth in MRS broth Uniform turbidity Catalase Negatif Production of gas Negatif Growth at: 50°C + 60°C + 70°C + 80°C - health hazards. The ability of these bacteriocins to inhibit many foodborne pathogens, including Listeria monocytogenes, make them attractive as potential food preservation

agents (Osmanagaoglu, 2001) and then do isolation bacteriocin Pediosin P from Bakteria Pediococcus pentosaceus Pep 1 from Vacuum-packed sausages. This Pediosin P effective against foodborne pathogenic bacteria such as

Listeria monocytogenes. There is concern regarding L. monocytogenes in milk and meat products and the use of this bacteriocin may be a way of controlling the growth of this pathogen in milk, meat and other food items. Fermented meat

products are often involved in staphylococcus food poisoning outbreaks and leuconostoc spoilage. Since bacteriocins

from lactic acid producing bacterial starter cultures are present in some naturally fermented food products,

their addition to foods in a purified form should pose no risk to consumers. Starter cultures of Pedioccoccus spp. which possess pediocin P activity may be useful in controlling Listeria, Leuconostoc and Staphylococcus contamination in

fermented meats. Their active proteins may also have potential as biopreservatives in a variety of perishable foods.
Identification of lactic acid bacteria by 16SrRNA: Methods for the detection and identification of Pediococcus pentosaceus are e.g., serotyping, pyrolytic gas chromatography, pyrolytic mass spectrometry, ribotyping, phage typing, plasmid profiles, electrophoresis in pulse electric field and Polymerase Chain Reaction (PCR) using genera specific and species-specific primers. N6 isolates was DNA extraction methods for GES (Marchesi et al., 1998), PCR amplification of 16S rDNA with its gene-specific primers 63F: 5- CAG GCC TAA CAC ATG CAA GTC-1387 primers 3 and R: 5-GGG CGG WGT GTA CAA GGC-3 (Marchesi et al., 1998), PCR product purification by PT genetics scince using a kit that uses the column. Result of purification to analysis against by the program (BioEdit Sequence Alignment Editor version 7.0.9.1.). Then traced the species or genus in the NCBI (National Center for Biotechnology), which is the software AGTTATTTACTCATTTCATTGGGCTTTTTGTACGTGCA
TGTGTACGCCTTGGTACCTCGGCCCAAAGAGATTAAGC TAAACCCTCGCGGGTTTCGCGGACTCGTGTGTACCCAT
CCCATTGTAGCCACGTGTGGTAGCCCCAGGTCAATAAG GGGCATGGATGATTTGACGTCGTCCCCCACCTTCCTCC
GGTTTGGTCACCGGGCAGTCTCCACTAGAGTGCCCAAC TGAATGCTGGCAACTAGTAATAAGGGTTGCGCTCGTT
GCGGGACTTAACCCAACATCTCACGACACGAGCTGACG ACAACCATGCACCACCTGTCATTCTGTCCCCGAAGGGA
ACGCCTAATCTCTTAGGTTGGCAGAAGATGTCAAGACC TGGTAAGGTTCTTCGCGTAGCTTCGAATTAAACCACAT
GCTCCACCGCTTGTGCGGGCCCCCGTCAATTCTTTTGA GTTTCAACCTTGCGGTCGTACTCCCCAGGCGGATTACT
TAATGCGTTAGCTGCAGCACTGAAGGGCGGAAACCCTC CAACACTTAGTAATCATCGTTTACGGCATGGACTACCA
GGGTATCTAATCCTGTTCGCTACCCATGCTTTCGAGCC TCAGCGTCAGTTACAGACCAGACAGCCGCCTTCGCCAC
TGGTGTTCTTCCATATATCTACGCATTTCACCGCTACA CATGGAGTTCCACTGTCCTCTTCTGCACTCAAGTCTCC
CAGTTTCCAATGCACTTCTTCGGTTGAGCCGAAGGCTT TCACATTAGACTTAAAAGACCGCCTGCGCTCGCTTTAC
GCCCAATAAATCCGGATAACGCTTGCCACCTACGTATT ACCGCGGCTGCTGGCACGTAGTTAGCCGTGGCTTTCT
GGTTAAATACCGTCACTGGGTGAACAGTTACTCTCACC CACGTTCTTCTTTAACAACAGAGCTTTACGAGCCGAAA
CCCTTCTTCACTCACGCGGCGTTGCTCCATCAGACTTG CGTCCATTGTGGAAGATTCCCTACTGCTGCCTCCCGTA

GGAGTCTGGGCCGTGTCTCAGTCCCAATGTGGCCGAT TACCCTCTCAGGTCGGCTACGCATCATCGCCTTGGTGA GCCGTTACCTCACCAACTAGCTAATGCGCCGCGGGTCC ATCCAGAAGTGATAGCAGAGCCATCTTTTAAAAGAAAA CCAGGCGGTTTTCTCTGTTATACGGTATTAGCATCTGT TTCCAGGTGTTATCCCCTGCTTCTGGGCAGGTTACCCA CGTGTTACTCACCCGTCCGCCACTCACTTCGTGTTAAA ATCTCATTCAGTGCAAGCACGTCATGATC Fig. 2: Nucleotide sequence of Pediococcus pentosaceus strain A24 (N6) by 16S rDNA sequence BLASTN. Results of BLASTN with a journal that has been published as a reference. Showed that the new isolate was taxonomically very close to Pediococcus pentosaceus strain A24 (Fig. 2). Conclusions: The isolation of LAB from hot spring was found that 23 isolates exhibited a clear zone, 18 isolates (78%) shaped cocci and 5 isolates (22%) shaped bacillus. For the morphological identification was selected isolated gave 5

(N2, N4, N6, N9 and N12) isolates has been antimicrobial activity against the growth of pathogenic bacteria (E. coli O157: H7, S. thypimurium and L. monocytogenes). Isolates N6 had the highest antimicrobial activity against all bacteria

test, with a range of inhibition zone 18-30 mm, gram positive, coccus shape, spore former coccus,

non motility and catalase negative. MIC values of isolates N6 supernatant against pathogenic bacteria Escherichia coli O157: H7 by 60 and 80% of the bacterial pathogen Salmonella thypmurium and 50% of the bacterial pathogen Listeria

monocytogenes. Based on morphological examination and PCR analysis, the isolate N6 was primarily identified

as Pediococcus pentosaceus strain A24 bacteria.

REFERENCES Cowan, S.T. and Steel's, 1975. Manual for the identification medical bacteria. Cambridge University Press, Cambridge, London. Hadioetomo, 1985. Basic microbiology in practice. PT. Scholastic. Jakarta. Fardiaz, 1989. Guidance practical food microbiology. Faculty of Agricultural Technology. Bogor Agriculture University. Ammor, S., G. Tauveron, E. Dufour and I. Chevalier, 2006. Antibacterial activity of lactic acid bacteria against spoilage and pathogenic bacteria isolated from the same meat small-scale, Facility: 1- Screening and Characterization of The Antibacteria Compounds, Food Cont., 17: 454-461. Barefoot, S.F. and C.G. Nettles, 1993. Antibiotics Revisited : bacterious produced by dairy starter cultures. J. Dairy Sci., 76 : 2366-2379. Brock, T.D. and M.T. Madigan, 1991. Biology of microorganisme. Six edition prentice-hall, Inc. Englewood Cliffs, New Jersey. Cintas, L.M., J.M. Rodriguez, M.F. Fernandez, K. Sletten, I.F. Nes, P.E. Hernandez and H. Holo, 1995. Isolation and characterization of pediocin l50, A new bacteriocin from pediococcus acidilactici. Consentino, S., 1999. In vitro antimicrobial activity and chemical composition of Sardinian Thyymus essential oil. Lett. Appl. Microbiol., 29: 130-135. Dirnawan, H.A., Suwanto dan T. Purwadaria, 2000, Exploration of thermophilic bacteria-producing extracellular hydrolytic enzymes and hot springs mountain deconvolution. J. Biol., 7: 52-55. Delgado, A., D. Brito, P. Fereiro, C. Peres and J.F. Margues, 2001. Antimicrobial activity of l. Plantarum isolated from a traditional lactic acid fermentation of table olives, EDP. Sci., 81: 203-215. Gilbeth, S.E. and G.A. Somkati, 2005. Thermophilin 110: A bacteriocin of streptococcus thermophilus ST 110. J. Curr. Microb., 51: 175-182. Girum, T., E. Eden and A. Mogessie, 2005. Assessment of the antimicrobial activity of lactic acid bacteria isolated from borde and shameta, traditional ethiopian fermented beverages, on some foodborne pathogens and effect of growth medium on the inhibitory activity. Int. J. Food Saf., 5: 13-20. Holzapfel, W.H., R. Geisen and U. Schillinger, 1995. Biological preservation of foods with reference to protective cultures, bacteriocins and food-grade enzymes. Int. J. Food Microbiol., 24: 343-362. Jenie, B.S.L., N. Afitah and Suliandari, 2001. Safety and quality improvement pindang mackerel (rastellinger sp.) with sodium acetate combination application of lactic bacteria aam and vacuum packing. J. Food Sci. and Tech., 21-27. Katzung, B.G., 2004. Basic and clinical pharmacology, Lange Medical Books. McGraw-Hill, 9th edition, New York, USA. Khalil, A., 2003. Production of new antimicrobial agents from thermophilic bacterial isolated from hot spring in Jordan andArab Conf. and Exhibition Biotechnology and Genetic Engeneering, Bahrain. Khalil, A.B., A.Z. Musa and A. Mohammad, 2006, Production of antimicrobial agents from thermophilic Yersinia sp and Aeromonas hydrophila isolated from hot spring in Jordan Valley. J. Biotech., 5: 252-256. Kubo, I.H., H. Muroi and M. Himejima, 1993. Antimicrobial activity against mutan of mute tea flavor component. J. Agri. Food Chem., 42: 107-111. Lay, B.W., 1994. Microbial analysis laboratory, Eagle Press, Jakarta. Marchesi, J.R., T. Sato, A.J. Weightman, T.A. Martin, J.C. Fry, S.J. Hiam, D. Dymnock and W.G. Wade, 1998. Design and evaluation of useful bacterium-specific PCR primers that amplify genes coding for bacterial 16S rRNA. Appl. Environ. Microbiol., 64: 795-799. Martirani, L.M. Varcamonto, G. Naclerio and D.E. Felice, 2002. Purification and partial charactaresition of thermophilic strain bacillus licheformis Microba. Cell Facterories. Environ., 4: 350-352. Maragkoudakis, P.A., K.C. Mountzouris, D. Psyrras, S. Cremonese, J. Fischer, M.D. Canter and E. Tsakalidou, 2009. Functional properties of novel protective lactic acid bacteria and application in raw chicken meat against Listeria monocytogenes and Salmonella enteridis. Int. J. Food Microbiol., 130: 219-226. Mckane, L. and J. Kandel, 1985. Microbiology: Essential and application, Mc Graw Hill Book Company, New York. Narayan, V.V., M.A. Hatta, H.W. Morgan and D. Rao, 2008. Isolation and characterization of aerobic thermophilic bacteria from the savusavu hot spring in Fiji, J. Microbes. Nettles, C.G. and S.F. Barefoot, 1993. Biochemical and Genetic Characteristic of Bacteriocins of Food Associated Lactic Acid Bacteria. J. Food Protec., 56: 338-356. Nosoh, Y.T. and Sekiguchi, 1991, Protein Stability and Stabilization Trough Protein Engineering. Ellis Horwood Limited, England. Oliveira, R.B.P., A.D.L. Oliveira and M.B.A. Gloria, 2008, Screening of Lactid Acid Bacteria from Vacum Packaged beef for antimicrobial activity, Braz. J. Microbiol., 39. Prescott, L.M., J.P. Horley and D.A. Klein, 2002. Microbiology 5th ed. Boston: Mc Graw-Hill. Rao, N.S.S., 1994. Soil microorganisms and plant growth, press, University of Indonesia, Jakarta. Savodogo, A., C.A.T. Quattara, I.H.N. Bassole and S.A. Traore, 2004. Antimicrobial activities of lactic acid bacterial strains isolated from Burkina faso fermented milk. Pak. J. Nutr., 3: 174-179. Sarkono, 2005, isolation, selection, characterization and identification of lactic acid bacteria producing bacteriocins from various fruits cook, master thesis, Graduate School, Gadjah Mada University, Yogyakarta. Suyono, Chairunisa, E.K. Sari, I. Sucipto and H. Anwar, 2008. Isolation of lactic acid bacteria-thermophilic from hot springs mountain deconvolution, Bogor Agricultural University. Tagg, J.R. and A.R. McGiven, 1995. Assay system for bacteriocin. J. Appl. Microbiol., 21-943. Thamrin, E, 2001, Cloning genes encoding hydrolases from hot springs Rimbo Panti West Sumatra, Thesis, Graduate Program, Bogor Agricultural University, Bogor. Tserovska, L., S. Stefanova and T. Yordanaya, 2002. Identification lactid acid bacteriaisolated from KATYK Goats milk and cheese. J. Culture Collections, 3: 48-52. Triani, W., 2008. Substrate antimicrobial activity of lactic acid bacteria isolated from beef against bacterial pathogens and the minimum inhibitory concentration Bogor Agricultural University. Ponce, A.G., M.R. Moreira, C.E. Valle and S.I. Roura, 2008. Preliminary characterization of bacteriocin-like substances from lactic acid bacteria from organic leafty vegetables LWT-Food Sci. Technol., 41: 432- 441. Osmanagaoglu, O., 2001. Isolation and characterization of pediocin producing pediococcus pentosaceus Pep 1 from Vacum-Packed Sausages, Turkey J. Biol., 25: 133-143. Todorov, S.D., 2009. Bacteriocins from lactobacillus plantarum production, genetic organization and mode of action, Barz. J. Microbiol., 40: 209-221. Pak. J. Nutr., 13 (8): 465-472, 2014 Pak. J. Nutr., 13 (8): 465-472, 2014 Pak. J. Nutr., 13 (8): 465-472, 2014 Pak. J. Nutr., 13 (8): 465-472, 2014 Pak. J. Nutr., 13 (8): 465-472, 2014 Pak. J. Nutr., 13 (8): 465-472, 2014 Pak. J. Nutr., 13 (8): 465-472, 2014 Pak. J. Nutr., 13 (8): 465-472, 2014 466 467 468 469 470 471 472