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1st Lekantara Annual Conference on Natural Science and Environment (LeNS 2021) IOP Conf. Series: Earth and Environmental Science 1097 (2022) 012025 IOP Publishing doi:10.1088/1755-1315/1097/1/012025 1 Isolate Characterization and Amylolytic Properties of Lactic Acid Bacteria from Traditional Fermented Dadih Mutia Elida*, Agustina Agustina, Ermiati Ermiati, Susi Desminarti Politeknik Pertanian Negeri Payakumbuh, West Sumatera, 26271, Indonesia. *Email: elida_mutia@yahoo.com Abstract. This research aimed to explore, isolate, select, and characterize lactic acid bacteria (LAB) potentially used as amylolytic bacteria from traditional fermented food "dadih".

Dadih was collected from Lareh Sago Halaban and Lintau, West Sumatera Province. The LAB was isolated by the streak method to obtain a uniform color and size colonies. The selected isolates were characterized by the reaction gram and catalase test. The result showed that isolates had the form of coccus, coccobacillus, and basil with gram-positive, catalase-negative, and endospore-negative reactions. The further characterization using the KIT API 50 CHL identified Lactobacillus plantarum (mH1), Lactobacillus paracasei ssp paracasei (mL3), Lactobacillus plantarum (mH4), Lactococcus lactis subsp lactis (mH6), Lactobacillus plantarum (mH7), Lactobacillus plantarum (mH8), Lactobacillus brevis (mH13), and Lactobacillus paracasei subsp paracasei (mL14).

The amylolytic and starch hydrolysis test showed that the isolates with higher amylolytic index ? 2 were Lactobacillus paracasei ssp paracasei mL3, Lactobacillus plantarum mH4, and Lactobacillus paracasei ssp paracasei mL14. The Lactobacillus plantarum mH8 had a lower amylolytic index of 0.7. Therefore, the four isolates (mL3, mH4, mL14, and mH8) are potentially used as fermenting bacteria for high- carbohydrate food. Keywords: Characterization, Amylolitic Bacteria, Dadih, Lactic Acid Bacteria 1. Introduction Dadih is

a traditional fermented product from West Sumatra. It is made of buffalo milk and fermented in a bamboo container for 24-48 hours.

The quality of the produced curd varies because the type of bamboo used as a fermentation vessel is different in each region. Different types of bamboo are suspected to contain different microbes, and each region has certain types of bamboo, such as Gombong, Betung, Reed, and Yellow bamboo. Besi des, the environment and fermentation process greatly affect the culture of lactic acid bacteria (LAB) contained in the product [1]. LAB is a microorganism that does not harm human health.

However, it contributes to world food, such as milk- based products and other derivative products (fun ctional drinks and health food therapies) to improve the function of immunostimulation [2]. Lactic acid bacteria have amylolytic properties to produce amylase enzymes to degrade starch into sugar [3]-[4]. The starch decomposition process can be accelerated using the amylase enzyme biocatalyst produced by amylolytic bacter ia [5]. Amylolytic LAB is widely applied in the food and non- food industries because it is easily produced on a large scale and in a relatively short period [6]. However, the high demand for amylase enzymes is not fulfilled by the high enzyme production.

This problem can be solved by increasing the production of amylase enzymes to find new sources and produce amylase enzymes by utilizing amylolytic bacteria. The amylolytic lactic acid bacteria were isolated and identified by the LAB from growol [7], rice water [8], and sago starch [9]. However, since not many materials have been isolated from curd, this study was conducted to isolate the LAB from the curd and identify its amylolytic ability for application development especially in the food industry.

This study aimed to characterize the isolate and amylolytic properties of LAB from curd to develop fermented starch products. 1st Lekantara Annual Conference on Natural Science and Environment (LeNS 2021) IOP Conf. Series: Earth and Environmental Science 1097 (2022) 012025 IOP Publishing doi:10.1088/1755-1315/1097/1/012025 2 2. Methodology Samples were collected from five curd produc ers in two locations: Lareh Sago Halaban and Lintau. The samples were then brought to the laboratory to test. This research was conducted through five stages as follows. 2.1 Isolation The isolates were isolated or separated from curd by plating them on the selective MRSA media.

The isolation started by suspending 5 g of cu rd into 45 ml of sterile diluent and homogeneous vortex. The next process was diluting to 10 -6 dilutions, and two of the last dilutions were plated into sterile dishes. Then, 15-20 ml of MRSA medium with 0.2% CaCO3 and incubated for 48 at 37°C was added. Colonies forming clear zones were scratched repeatedly until they obtained uniform morphology [10]. Uniform colonies

were inoculated into MRS agar as a culture stock. 2.2

Characterization The characterization was continued by the gram reaction morphology and biochemical tests, including the catalase test, growth test at various temperatures and salt concentrations, glucose fermentative test, and production of diacetyl and acetoin test [11]. 2.3 Identification Using the KIT Api 50 CHL Carbohydrate fermentation patterns were determined using the API 50 CHL test kit (bioMerieux, France 2001). Meanwhile, the strains were identified using the LAB API test kit and software version 3.3.3 from bioMerieux. The Lactobacillus was identified using the API 50 CHL media within 48 hours of incubation.

The test was carried out with five test strips: strip 1 (0-9), strip 2 (10- 19), strip 3 (20-29), strip 4 (30-39), and strip 5 (4 0-49). The 2-day-old bacterial isolates were spotted into the tube containing the API media then incubate at 37 0C for 48 hours. The mineral oil/liquid paraffin was added to create anaerobic conditions. The ability of isolates to use different carbohydrates was indicated by purple to yellow color changes in the medium (positive reaction). The results are processed in the Web API Software to label each isolate with the identity percentage. 2.4

Characterization of LAB Amylolytic Properties The test was carried out qualitatively by scratching the isolates on the MRSA media by adding 1% starch or Starch Agar media and incubating it at 37 oC for 24-48 hours. After the incubation, each petri dish was poured with gram-iodine solution (0.5% iodine crys tal was added to 1.5% KI solution). Strains with amylolytic ability will hydrolyze the starch in the media around the growth site; a clear zone is formed after adding iodine solution for a while [12]. 2.5

LAB Starch Hydrolysis Test The amylolytic characteristics with starch hydrolysis were quantitatively tested to calculate the amylolytic index. The test was carried out by spotting one of the LABs on the Starch medium agar. Bacterial isolates were incubated for 72 hours at 37 ? . Then, two drops of Gram's iodine (KI+I2 reagent) in excess were added, and the clear zone formed was observed. The amylolytic index was calculated by comparing the clear zone formed due to amylolytic enzymatic activities with the colony diameter [13]. 3. Results And Discussion 3.1

Isolation and Characterization of Morphology The initial screening of curd from two collecting areas and five curd producers obtained 27 isolates (see Table 1). Morpholo gically, the colonies were milky white to clear light brown. Microscopically, the bacteria were rod-shaped and round coco-bacilli with purple reactions, namely gram-positive and non-spore groups. Lactobacillus colonies originating from fermented milk are yellow, gray, and brown, shiny with flat colony ed ges, smooth to swollen, and

sometimes irregular. The brown color of the colonies was caused by the acid produced by LAB isolates [14].

1st Lekantara Annual Conference on Natural Science and Environment (LeNS 2021) IOP Conf. Series: Earth and Environmental Science 1097 (2022) 012025 IOP Publishing doi:10.1088/1755-1315/1097/1/012025 3 Table 1. Morphological characteristics of LAB colonies and cells from curd "dadih" Characteristics Collecting Areas Halaban Lintau Amount of 17 10 morphological colonies milky white - light brown Light brown Light brown - dark brown Light brown - white Light brown Light brown Amount of 13 2 2 8 2 - characteristics of cells Bacil Coccus Cocobacil Bacil Coccus Cocobacil Gram + + + + + endosphores - - - - - - The LAB is a gram-positive purple round or rod-shaped, does not form spores, can ferment carbohydrates, is catalase-negative, and is a microaerophilic group [15]. All LABs included the gram- positive bacteria of which the peptidoglycan cell wall was composed of peptides (amino acids) and glycans (carbohydrates) [16]. Table 2.

Physiological and Biochemical Characteristics of LAB from Curd No Isolates Code Origin of Isolates Catalase Test Growth at T (0C) Growth at Salinity (%) Production of CO2 from Glucose Product ion of Diacetil and Acetoin 10 1 5 4 5 4 6.5 1 mH (LB) Halaban (-) + + + + +/- Heterofermentat ive + 2 mH (MW) Halaban (-) + + + + + Heterofermentat ive + 3 mL 7 (LB) Lintau (-) + + + +/- + Heterofermentat ive + 4 mH (LB) Halaban (-) + + + + + Heterofermentat ive + 5 mH1 (LBb) Halaban (-) + + + + - Heterofermentat ive + 6 mH (SW) Halaban (-) + + + +/- + Heterofermentat ive + 7 mH 1 (BW) Halaban (-) + + + + + Heterofermentat ive + 8 mH 2 (LBb) Halaban (-) + + + + - Heterofermentat ive + 9 mH 1(SW) Halaban (-) + + + + +/- Heterofermentat ive + 10 mH3 (LB) Halaban (-) - - + + + Heterofermentat ive - 11 mH4 (LB) Halaban (-) - - + + + Heterofermentat ive - 12 mL3 (SW) Lintau (-) + + + +/- + Heterofermentat ive + 13 mH 10 (SW) Halaban (-) + + + + + Heterofermentat ive + 14 mL 4 Lintau (-) + + + + + Heterofermentat ive + 15 mH 2 (SB) Halaban (-) + + - + + Heterofermentat ive + 1st Lekantara Annual Conference on Natural Science and Environment (LeNS 2021) IOP Conf. Series: Earth and Environmental Science 1097 (2022) 012025 IOP Publishing doi:10.1088/1755-1315/1097/1/012025 4 16 mL (LB) Lintau (-) + + + + + Heterofermentat ive - 17 mH 4 (MW) Halaban (-) - - - - + Heterofermentat ive - Notes: (LB) = Light brown, (MW) = Milky white , (SW) = Small white, (BW) = Big white, (SB) = Small brown 3.2

Characterization of Isolates Seventeen isolates were identified based on th eir similar morphological characteristics, then they were physiologically and biochemically tested (see Table 2). Three isolates had similar physiological and biochemical properties; they were isolates 5 and 8, isolates 6 and 12, as well as isolates 10 and 11. Therefore, isolate 14 was screen ed again to estimate its genus. Glucose fermentation test showed that

isolates 6, 7, and 14 were classified homofermentative, and the others were classified heterofermentative. Homofermentative lactic acid bacteria produced more than 90% of lactic acid from their fermentation.

Therefore, it had to be combined with heterofermentative groups to produce the preferred flavor in fermented products. Besides the lactic acid, the heterofermentative group produced flavor-forming compounds, such as alcohol and other organic acids. The homofermentative LAB produces lactic acid as the main product of sugar metabolism, starting from breaking down glucose with the help of the aldolase enzyme to creating glyceraldehyde- 3-P and dihydroxyacetone-P [17]. Then, the gly ceraldehyde-3-P is converted to pyruvic acid. One molecule of pyruvic acid is further conver ted to two molecules of lactic acid.

Meanwhile, heterofermentative species are a group that produces lactic acid in small quantities, and their resulted products are ethanol, acetic acid, and formic acid [18]. Heterofermentative LAB can produce lactic acid from glucose by 85-90% [19]. The identification of this research obtained three genera: Lactobacillus, Lactococcus, and Leuconostoc, as shown in Table 3. Table 3. Names of Genus and Cell Shapes based on Identification Keys Isolate Number Isolate Code Origin of Isolates Shape of Cells Genus 1 mH Halaban Bacil/coccobacillus Lactobacillus/Lactococcus 2 mH Halaban Streptococcus, Diplococcus, Coccus Streptococcus/Lactococcus 3 mL Lintau Coccus/coccobacillus Lactobacillus/Lactococcus 4 mH Halaban Streptococcus, coccus Streptococcus/ Lactococcus 6 mH Halaban Coccus Streptococcus/ Lactococcus 7 mH Halaban Coccobacillus Lactobacillus 8 mH Halaban Coccobacillus Lactobacillus 9 mH Halaban Coccus Streptococcus/ Lactococcus 10 mH Halaban Coccus Lactococcus 13 mH Halaban Coccobacillus Lactobacillus/ Leuconostoc 14 mL Lintau Coccobacillus Lactobacillus/ Leuconostoc 15 mH Halaban Coccus Streptococcus/ Leuconostoc 16 mL Lintau Coccus Streptococcus/ Leuconostoc 17 mH Halaban Coccus Streptococcus/Leuconostoc 3.3 Identification by API 50 CHL Eight LAB isolates were selected based on their ability to ferment carbohydrates on various API 50 CHL, as presented in Table 4.

 Table 4. Identification of LAB Isolates Using API 50 CHL No Isola tes Isolat e Codes Results Levels of Accura cy * Cell Shapes 1 mH Lactobacillus plantarum 99.6% Bacill/coccobacil 3 mL Lactobacillus paracasei ssp paracasei 99.4% Coccus/ coccobacil 4 mH Lactobacillus plantarum 99.9% Streptococcus, coccus 6 mH Lactococcus lactis ssp lactis 99.6% Coccus 7 mH Lactobacillus plantarum 99.9% Coccobacillus 8 mH Lactobacillus plantarum 99.9% Coccobacillus 13 mH

Lactobacillus brevis 99.4% Coccobacillus 14 mL Lactobacillus paracasei ssp paracasei 99.4% Coccobacillus Notes: * The identification was conducted for 24 and 48 hours of incubation.

The identification results and accuracy were obtained based on the filling color changes in the API 50 CHL media. Isolate 1 was identified as Lactobacillus plantarum 99.6% that could ferment 21 types of glucose. Isolate 3 could ferment 23 types of carbohydrates: ribose, galactose, glucose, fructose, mannose, sorbose, mannitol, sorbitol, N-acetylgluco samine, arbutin, esculine, salycine, cellubiose, maltose, lactose, saccharose, trehalose, inulinone , melezitose, D-turanose, D-ta galose, and potassium gluconate. Moreover, isolate 3 was identified as Lactobacillus paracasei ssp paracasei 99.4%. Isolate 4 could ferment 24 types of glucose and was identified as Lactobacillus plantarum 99.6%.

Isolate 6 was identified as Lactococcus lactis ssp lactis by fermenting 18 types of glucose. Meanwhile, isolates 7 and 8 were identified as Lactobacillus plantarum 99.9% by fermenting 25 types of glucose. Isolate 13 was Lactobacillus brevis 99.4% that could ferment 19 species of sugar. Finally, isolate 14 was identified as Lactobacillus paracasei ssp paracasei 99.4% [20]. 3.4 Characterization of Amylolytic Properties of Lactic Acid Bacteria Five isolates were amylolytic-positive after being dripped with the KI indicator. In this case, no blue color was formed around the colonies, and all colonies were surrounded by clear areas.

The results are shown in Figure 1 and Table 5. Figure 1. Amylotic Activities of LAB Isolates from Curd “Dadih” Table 5. The Results of the Amylolytic Test of LAB Isolates from Curd “Dadih” No Names of Isolates Amylotic activity 1 Lactobacillus plantarum mH1 - 2 L actobacillus paracasei ssp paracasei mL3, + 3 Lactobacillus plantarum mH4, + 4 L actococcus lactis ssp lactis mH6 + 5 Lactobacillus plantarum mH7 - 6 L actobacillus plantarum mH8 + 7 Lactobacillus brevis mH13 - 8 L actobacillus paracasei ssp paracasei mL14.

+ Note: + = Positive reaction to the amylotic test; - = Negative reaction to the amylotic test 1st Lekantara Annual Conference on Natural Science and Environment (LeNS 2021) IOP Conf. Series: Earth and Environmental Science 1097 (2022) 012025 IOP Publishing doi:10.1088/1755-1315/1097/1/012025 6 The LAB could grow by utilizing the starch in the medium and would provide a clear zone around the place where it grew. The clear area around the inoculated bacterial growth was caused by the starch in the media hydrolyzed by the amylase enzyme produced by bacteria into glucose [9].

The clear area was also caused by exoenzymes and organisms that hydrolyzed starch in the agar medium. A clear zone will appear after adding the iodine solution [7]. 3.5 BAL

Starch Hydrolysis Test Starch hydrolysis test of LAB isolates was conducted by looking at their amylolytic index and calculated from the diameter of the clear area. The results of this test are shown in Table 6. Five isolates of Lactococcus lactis ssp lactis mL6 did not have amylolytic activities. The isolate of Lactobacillus plantarum mH8 had a low amylolytic index of 0.7

mm, while the isolates of Lactobacillus paracasei ssp paracasei mL3, Lactobacillus plantarum mH4, and Lactobacillus paracasei ssp paracasei mL14 had high amylolytic indexes ? 2. Table 6. Characterization Results of Starch Hydrolysis Test of LAB Isolates from Curd "Dadih" No Names of Isolates Average Amylolytic Index* 1 Lactobacillus paracasei ssp paracasei mL3, 2.5 2 Lactobacillus plantarum1 mH4, 2.5 3 Lactococcus lactis ssp lactis mH6 - 4 Lactobacillus plantarum mH8 0.7 5 Lactobacillus paracasei ssp paracasei mL14. 2.8 Description: *) value 0-1; low value; 1-2; medium value; >2: high score Several strains of Lactobacillus spp.

produced extracellular amylase and ferment starch directly to lactic acid [21] because the fermentation with amylolytic LAB would combine two processes: enzymatic hydrolysis of carbohydrate substrates (star ch) and fermentation th at utilized the resulted sugar into lactic acid. Fermentation caused changes in the characteristics of starch because the starch granules were attacked by enzymes and acids were released by the involved microorganisms [22]. The LAB culture could hydrolyze starch substrates to produce a crude extract of amylase enzyme; thus, bacteria produced extracellular amylase enzymes and then simultaneously hydrolyzed amylose and amylopectin [7].

This enzyme would break down starch polymer bonds into oligosaccharides or simple sugar molecules. 4. Conclusion The isolation and characterization using the Api 50 LAB Kit from curd obtained eight isolates: Lactobacillus plantarum (mH1), Lactobacillus paracasei ssp paracasei (mL3), Lactobacillus plantarum (mH4), Lactococcus lactis subsp lactis (mH6), Lactobacillus plantarum (mH7), Lactobacillus plantarum (mH8), Lactobacillus brevis (mH13), and Lactobacillus paracasei subsp paracasei (mL14).

The characteristics of amylolytic properties obtained three isolates with high amylolytic indexes: Lactobacillus paracasei ssp paracasei mL3, Lactobacillus plantarum mH4, Lactobacillus paracasei ssp paracasei mL14. Lactobacillus plantarum mH8 with a low amylolytic value of 0.7. References [1] M. Elida, "Profil Bakteri Asam Laktat dari Dadih yang Difermentasi dalam berbagai Jenis Bambu dan Potensinya sebagai Probiotik," Thesis Program Magister, Dept. Food Science, Bogor Agricultural University, Bogor, Indonesia. 2002. [2] M. Elida, "Makanan Fungsional Probiotik Dan Prebiotik Serta Efeknya Terhadap Kesehatan Pencernaan," Jurnal Lumbung, vol 2. No. 1, 2006.

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