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- ID: 1262261195
- Word Count: 3188
- Submitted: 1

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International Journal of ChemTech Research CODEN (USA): IJCRGG, ISSN: 0974-4290, ISSN(Online):2455-9555 Vol.9, No.12, pp 534-540, 2016

Isolation and Screening of Lactic Acid Bacteria from Dadih for Glutamic Acid Production as Precursor of γ-Amino Butyric Acid (GABA) Induced Heat Stress in Broiler

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Abstract : Lactic Acid Bacteria (LAB) is bacteria which has an important role in the process of fermentation of organic matter. Dadih is traditional fermented milk product made from the milk of water buffalo. Dadih is well known as typical tradionall food of West Sumatera (Minang Kabau), Indonesia. This study aims to obtain isolates of Lactic Acid Bacteria (LAB) producer of glutamic aci as precursor of GABA. The study consisted of three stage: stage 1; isolated of LAB from Dadih used of MRS agar contained CaC03, 2%. Stage 2 was the selection of glutamic acid-producing LAB qualitatively and quantitatively with inducers of monosodium glutamate (MSG). Stage 3 was the characterization of selected LAB isolates biochemically. The result found that 10 isolates of LAB producing glutamic acid, namely Y1, Y2, Y3, Y4, Y5, Y6, Y7, Y8, Y9, Y10. After tested the ability to produce a qualitative glutamic acid of 10 isolates of LAB has capability to produce glutamic acid in the extracellular and intracellular which indicator changed the color to purple used 2% ninhidrin solutions, but after the test quantitatively obtained two isolates (Y2 and Y8) which resulted in the production of glutamic acid, the highest yield of glutamic acid were 41.73 mg/L and 40.86 mg/L, respectively. The characterization of two isolates (Y2 and Y8) was bacill, convex surface, white milk, and was a gram positive bacteria and aerobic. Based on catalase test and oxidase test showed that isolate Y2 and Y8 was negative catalase and oxidase, but for the glucose, sucrose and mannitol test the two isolates were positives and negatives to lactose test. Based on the characterization, the two isolates were

Lactobacillus sp. The results of this research, can be concluded that 10 isolates of LAB that isolated from Dadih potentially producer glutamic acid, which the highest production was 41.73 mg/L by isolate Y2 (Lactobacillus sp) can be as precursor of γ-Amino Butyric Acid (GABA) induced heat stress in broiler. Keyword : LAB, glutamic acid, dadih, MRS agar, GABA. Introduction Glutamic acid or glutamate is an important molecule for all living organisms, which plays a role in various metabolic processes. It is a non essential amino acid involved in protein synthesis and other fundamental processes such as glycolysis, gluconeogenesis and the citric acid cycle1. It is also a key metabolite because it serves to link nitrogen and carbon metabolism1. Catabolism of glutamate occurs mainly by the action of either glutamate dehydrogenase or glutamate decarboxylase (GAD) 2. The first enzyme, among other roles, is important for the assimilation of ammonia to amino acids, while the second is important for resistance mainly against acid but also other stresses2. The quality of meat (carcass) of broiler chickens during the development of the poultry industry in Indonesia increased considerably still less concern to the poultry business, because they prioritize production volume rather than quality of production. One attempt to improve the quality of carcass meat is glutamic acid supplementation in feed. Glutamic acid is a non-essential amino acid that plays a role, either on improving the performance and carcass quality to increase the percentage of carcass, abdominal fat loss and gives a taste of umami in broiler. The same research were reported by a group of researchers3-8. Glutamic acid is an amino acid that involved in the perception of multifunction flavors, excitatory neurotransmission and intermediary metabolism2. It plays an important role in this phase of digestion in the stomach with the effects of diversity in the gastrointestinal tract when consumed with nutrition can improve exocrine secretion in the stomach9. Glutamic acid is a specific precursor for other amino acids, namely, arginine and proline as well as for bioactive molecules such as γ-amino butyric acid (GABA) and glutathione. In addition, a number of studies have shown the possibility of the use of glutamic acid to improve nutrition in the elderly and in patients with the condition of malnutrition10-11. At this time, most of the glutamic acid is produced through microbial fermentation for the chemical method produces a racemic mixture of glutamic acid (D- and L-glutamic acid) 12. Numerous studies have reported glutamic acid excretion by various micro-organisms; However, most of the microbes found not food-grade microorganisms. Lactic acid bacteria

(LAB), which is well known to produce a wide range of primary metabolites. In addition, LAB important in the processing of food ingredients and have been widely applied in the food industry13. Exploiting the potential of LAB to produce glutamic acid can facilitate the production of functional food and feed rich in bioactive molecules such as γ-Aminobutyric acid (GABA). The main advantage of glutamic acid production by the LAB is that amino acids are produced in this way is biologically active (L-glutamate) and the production process is considered safe and environmentally friendly. This can be achieved through the selection of appropriate LAB from natural sources that are well adapted to a particular product, more competitive and with high metabolic capacity. A group of researchers have tried to exploit LAB producer of glutamic acid fermented food products like cheese14; Malaysian fermented food15; kimchi from South Korea16; fermented fish from Thailand17; Algerian goats milk18; Indonsian traditional fermented fish sauce bakasang19; Syrian fermented food20; and semi-dried sausages21, while milk buffalo fermented (dadih) that emit a distinctive aroma and flavor that no one has reported. Minang Kabau in the West Sumatera, which is located in the West part of Sumatera island, is one of the major areas in which people produce various fermented milk buffalo products, names is dadih, a traditional fermented milk popular among people of West Sumatra, Indonesia, is made by pouring fresh raw unheated buffalo milk into a bamboo tube capped with a banana leaf, and allowing it to ferment spontaneously at room temperature for two days. The aim of this study was to screen various LAB exhibiting a strong ability to glutamic acid production that can be bioconversion to produce GABA, which are expected to enhance the development of functional feeds to induce heat stress of animals. Material and Methods Samples Five locally available fermented milk buffalo (dadih) were purchased from Bukittinggi, Payakumbuh, Sijunjung, Padang Panjang dan Solok as LAB-strain local sources. Isolation of Lactic Acid Bacteria Isolation of lactic acid bacteria (LAB) from dadih was performed according to the method described by Adnan and Tan22. Each sample (10 g) was separately blended with 90 mL of 0.85% NaCl solution for 2 min (Lab Blender Seward, Stomacher 400). This blended dadih (10 mL) was mixed with MRS broth (90 mL) in a 250 mL Erlenmeyer flask. The broths containing the dadih samples were enriched with glucose (2% w/v). The flasks were incubated at 30 °C, 100 rpm, for 7 days. Aliquots of the culture from each of the flasks were serially diluted from 101 to 1012 times and 0.1 mL of each dilution was spread evenly on MRS agar plates. Colonies of LAB were counted on MRS agar plates after anaerobic incubation for 72 h at 30 °C in GasPaks jars (GasPaks System, BBL) and colonies were reported as log 10 CFU/mL. Colonies with distinct morphological differences such as color, size and shape were randomly picked from countable MRS agar plates and subcultured on fresh MRS agar plates. Pure colonies were maintained in 20% v/v glycerol in MRS broth for

storage at −80 °C. Glutamic Acid Production The medium composition for the production of glutamic acid was as the following: (g/l) Glucose-50.0, Urea-8.0, Biotin-0.002, K2HPO4-1.0, MgSO4.7H2O-2.5, MnSO4. 7H2O-0.1, CaCO3-1.6. The medium pH was adjusted to 7.0 with 1N sodium hydroxide or 1N hydrochloric acid. The fermentation was carried out in 250 ml Erlenmeyer flask. The fermentation medium was inoculated with 1% (v/v) of the overnight culture (LAB strain Y1 –Y10). The production medium was kept in an orbital incubator shaker at 30°C at 120 rpm for 48 hr. Then the cells and debris were removed by centrifugation at 10.000 g at 4 0C for 10 min. Supernatants were used as the crude glutamic acid source for estimation. Glutamic acid estimation Thin layer chromatography was employed for detecting L-glutamic acid in the culture medium and solvent system consisted of nbutanol:acetic acid: water (2:1:1). The visualization of spots was performed by spraying with 0.02% ninhydrin solution and the quantitative estimation of L-glutamic acid in the suspension was done using colorimetric methods Biochemically Identification of LAB The cultures were identified according to their morphological, cultural, physiological and biochemical characteristics23-24. The used tests were: Gram reaction; production of catalase, hydrogen peroxide; gas production from carbohydrates (1 % w/v) - lactose, sucrose, glucose and mannitol in MRS broth devoid of glucose and beef extract with chlorophenol red as indicator; production of acid and gas from 1 % glucose (MRS broth without beef extract) ; methyl red and Voges-Proskauer test in MRVP medium; H&L test in O/F medium; production of ammonia from arginine; nitrate reduction in nitrate broth; indole production in tryptone broth and growth on acetate agar. Result and

discussions Qualitatif Screening of Glutamic Acid Production Isolation of lactic acid bacteria from dadih begins to grow on selective media MRS broth were incubated for 7 days. The research showed that from five locally available dadih found that 45 isolates of lactic acid bacteria that could be seen clear zone around the colony using selected media MRS agar after added 2% CaC03. The research also showed that

10 isolates of 45 that produced glutamic acid were isolated from fermented milk buffalo (dadih). Table 1 showed that the qualitative screening of LAB producing glutamic acid by indicator of change of color of ninhindrin reaction to purple. The glutamic acid production measured by intraseluler and extraselluler. In Table 1, can be seen production of glutamic acid extracelluler higher than intracelluler, whereas isolate Y2, Y7, Y8 and Y9 produced the higher glutamic acid compared the other isolates.

Higher concentrations of glutamic acid produced by LAB strains isolated from dadih showed that these strains were more efficient in biosynthesizing glutamic acid, the same result also have been reported by Zareian et al15 Table 1. Qualitatif glutamic acid production of LAB by extraselluler and Intraselluler No. Name of isolates Qualitative Glutamic Acid Exstraseluler Intraseluler 1 2 3 4 5 6 7 8 9 10 Y1 Y2 Y3 Y4 Y5 Y6 Y7 Y8 Y9 Y10 ++ +++ ++ ++ ++ ++ +++ +++ +++ ++ + + + ++ ++ + + + + + Description: + : faded, ++ : concentrated, +++ : very concentrated Quantative Screening for Glutamic Acid Production For the quantitative screening of glutamic acid from LAB, the results showed that the ten isolates produced glutamic acid can be seen in Figure 1. Figure 1 showed that isolates Y2 and Y8 were as a glutamic acid producer. The glutamic acid produced 41.8 mg/L and 42.7 mg/L for Isolate Y2 and Y8 respectively. The results of glutamic acid from this research lower than has reported by Tarek and Mostafa (2010)25 whereas glutamic acid production were 68.7 mg produced by the LAB species such as Lactobacillus paracasei and Lactobacillus spp. The Gram-positive micro-organisms other than LAB were also shown to produce glutamic acid. for example, Brevibacterium spp. were found to

produce this amino acid between 10 to 46 mmol/ L26 . Figure 1. Quantitative screening of glutamic acid produced by LAB Figure 2. Morphology of isolates Y2 (1000 X) Characterization of Isolates As shown in Table 2, out of 2 colonies, appeared to be positive in lactose utilisation test. These isolates were able to ferment lactose to produce lactic acid that lowers the pH of the MRS media that, in turn, changed the purple indicator dye to yellow indicative of fermentation activities. Gram reaction and morphology studies showed that all of these isolates from dadih as Gram-positive cocci (Figure 2). These are the common features of LAB whereby these organisms constitute a large group of non-sporulating gram positive, catalase and oxidase negative bacill that produce lactic acid as the major metabolite of the carbohydrate fermentation. Tabel 2. Characterication of Isolates Y2 and Y8 biochemically No. Treatments Results Y2 Y8 1 MRSA + + 2 Coloni (Color, shape) white, bacill white, bacill 3 Gram (Morfologi, Spora) + + 4 Aerob + + 5 Catalase - - 6 Oxsidase - - 7 Lactose - - 8 Glucose + + 9 Sucrose + + 10 Mannitol + + 11 Gas production - - Figure 3 . Morphology of isolates Y2 dan Y8 (1000 X) Conclussions The results of this research can be concluded that found of 45 isolates of Lactic Acid Bacteria (LAB) and after screening for glutamic acid production, 10 isolates have capability to produced of glutamid acid, the higher glutamic acid production found that two isolates (Y2 and Y8). The Characterization of two isolates were gram positive, negative catalase and can be as Lactobacillus sp, which glutamic acid production 42.7 mg/L. Acknowledgement Pronounced thanks to the Ministry of Research and Technology and Higher Education of Indonesia for funding the BOPTN Andalas University Grants through Research Cluster Professor of Contract No: 82 / UN.16 / HKRGB / LPPM / 2016. References 1. Kondoh, T.; Mallick, H.N.; Torii, K. 2009. Activation of the gut-brain axis by dietary glutamate and physiologic significance in energy homeostasis. Am. J. Clin. Nutr. 90, 832S–837S. 2. Inoue, K.; Shirai, T.; Ochiai, H.; Kasao, M.; Hayakawa, K.; Kimura, M.; Sansawa, H. 2003. Blood pressure lowering effect of a novel fermented milk containing g amino butyric acid (GABA) in mild hypertensives. Eur. J. Clin. Nutr, 27, 490–495. 3. Aletor VA, Hamid Il., Nieb.E and Pfefler,E. 2000. Low-protein amino acid-supplemented diets in broiler chickens: effects on performance, carcass characteristics, whole-body composition and efficiencies of nutrient utilisation. Journal of the Science of Food and Agriculture , 80 (5), 547–554. 4. Kerr, B.J. and Kidd M. T. .1999. Amino acid supplementation of low protein broiler diet : glutamic acid and indispensable amino acid suplementation J Appl Poult Res 8 (3):298-309 5. Moran, E. T. and Stilborn H. L. 1996. Effect of Glutamic Acid on Broilers Given Submarginal Crude Protein with Adequate Essential Amino Acids Using Feeds High and Low in Potassium1. Poultry Science 75 (1):120-129. 6. Berres.J; Vieira, S. L. Dozier, W. A; Cortês M. E. de Barros M. R., Nogueira E. T. ,‡ and M. Kutschenko. 2010. Broiler responses to reduced-protein diets supplemented with valine, isoleucine, glycine, and glutamic acid. J Appl Poult Res 19 (1):68-79. 7. Wattanachant,S. Benjakul,and D. A. Ledward. 2004. Composition, Color, and Texture of Thai Indigenous and Broiler Chicken Muscles. Poultry Science 83:123–12 8. Dai,S.F;Gao,F;Zhang,W.H; Song,S.X; Xu,X.L and Zhou,G.H. 2011. Effects of dietary glutamine and gamma-aminobutyric acid on performance, carcass characteristics and serum parameters in broilers under circular heat stress. Animal Feed Sciences and Technology. 168 (1-2)51- 60. 9. Zolotarev, V.; Khropycheva, R.; Uneyama, H.; Torii, K. 2009. Effect of free dietary glutamate on gastric secretion in dogs. Ann. N. Y. Acad. Sci. 1170, 87–90. 10. Tomoe, M.; Inoue, Y.; Sanbe, A.; Toyama, K.; Yamamoto, S.; Komatsu, T. Clinical trial of glutamate for the improvement of nutrition and health in the elderly. Ann. N. Y. Acad. Sci. 2009, 1170, 82–86. 11. Yamamoto, S.; Tomoe, M.; Toyama, K.; Kawai, M.; Uneyama, H. 2009. Can dietary supplementation of monosodium glutamate improve the health of the elderly? Am. J. Clin. Nutr. 90, 844S–849S 12. Sano, C. 2009. History of glutamate production. Am. J. Clin. Nutr. 90, 728S–732S. 13. Leroy, F and Devuyst ,E. L. 2004.Lactic acid bacteria as functional starter culture for the food fermentation industry. Trends in Food Science & Technology, v. 15, n. 2, p. 67-78, 2004. http://dx.doi.org/10.1016/j. tifs.2003.09.004 14. Siragusa, S. M. De Angelis R. Di Cagno, C. G. Rizzello, R. Coda, and M. Gobbetti 2007. Synthesis of γ-Aminobutyric Acid by Lactic Acid Bacteria Isolated from a Variety of Italian

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