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Asian Jr. of Microbiol. Biotech. Env. Sc. Vol. 20 (October Suppl.) : 2018 : S118-S121 © Global Science Publications ISSN-0972-3005

EVALUATION ?-AMINOBUTYRIC ACID (GABA) PRODUCED BY LACTIC ACID BACTERIA ISOLATED FROM FERMENTED DURIAN

LILI ANGGRAINI2, HARNENTIS1 AND YETTI MARLIDA1 1 Department of Animal Nutrition and Feed Technology, Faculty of Animal Science, Andalas University, West Sumatera, Indonesia 2 Graduate Students of Master 's Programs Leading to Superior Doctorates in Agricultural Science, Andalas University, West Sumatera, Indonesia (Recieved 30 April, 2018; accepted 28 June, 2018) Key words: Lactic acid bacteria, Lactobacillus sp., GABA, Fermented durian, P. acidilactici Abstract - Fermented durian is known as tempoyak in Indonesia. It is added to sambal chili or pounded into chili paste and then mixed with rice and eaten with fish (that is normally salted) and ulam (salads made of raw cucumbers and other vegetables). This study aimed to evaluation ?-aminobutyric acid (GABA) produce by lactic acid bacteria isolated from fermented durian. The isolated lactic acid bacteria were cultivated on MRS agar containing 2% $CaCO_3$, and those producing ?-aminobutyric acid (GABA) were qualitatively and quantitatively screened with glutamate acid inducers. The selected lactic acid bacteria producing GABA were biochemically characterized, and the selected isolates were identified by 16 S RNA. Fifty-nine isolates of lactic acid bacteria were found, and after qualitative screening by thin-layer chromatography (TLC), 42 isolates were found to be candidates of ã-aminobutyric acid (GABA) producers. The quantitative screening showed that 13 isolates produced higher amounts of GABA, namely, T1, T2, T3, T4, T5, T6, T7, T8, T9, T10, T11, T12 and T13. The most GABA was produced by isolate T8 (22.615 mg/mL), as measured using HPLC. The isolate (T8) was characterized as a Bacillus with a convex surface and white milk, and it was an aerobic, gram-positive bacteria. Isolate T8 tested negative for catalase and oxidase, but the isolates were positive for glucose, sucrose and mannitol test and negatives for lactose. Based on these results, the isolates were characterized as Lactobacillus sp. From the results of this research, it can be concluded that of 13 isolates of lactic acid bacteria from fermented durian producing ?-aminobutyric acid (GABA), only 1, isolate T8, potentially produced the most GABA, 22.615 mg/L. After identification with 16S rRNA, isolate T8 was determined to be Pediococcus acidilactici. INTRODUCTION Minang Kabau, or West Sumatera, is located in the middle of Sumatera Island, Indonesia, and is one of the major areas where various fermented naturally traditional food products are produced, such as fermented fish (ikan budu), fermented buffalo milk (dadih) and fermented durian (tempoyak). These products are known to be lactic acid-fermented foods, and various lactic acid bacteria (LAB) are involved in the production process (Marlida et al., 2016). Until now, many studies have demonstrated that several LAB species produce a ubiquitous four- carbon amino acid, ?-aminobutyric acid (GABA), which is synthesized from glutamic acid via a reaction that is catalyzed by glutamate decarboxylase [EC 4.1.1.15], a pyridoxal 5′- phosphate-dependent enzyme (Shelp et al., 1999). In mammals, GABA has various physiological functions, such as neurotransmission and the induction of hypotensive effects (Hayakawa et al., 2004), and in monogastric animals, such as broiler and layer chickens, GABA can be used as a feed additive to prevent heat stress (Marlida et al.,2016; Chen et al., 2015; Chen and Wang, 2008; Cooper and Washburn, 1998). Although GABA is present in many fruits and vegetables, the amount is very low, ranging from 0. 03–2.00 ?mol/g fresh weight (Fougère et al., 1991), so it is necessary to develop new pharmaceutical and functional foods as well as feed additives that contain a considerable amount of GABA. Many studies have reported the mass production of GABA *Corresponding author's email: yettimarlida@ansci.unand.ac.id using Lactobacillus brevis isolated from alcohol distillery lees (Yokoyama et al., 2008) and kimchi (Cho et al., 2007), Lactobacillus paracasei from fermented fish (Komatsuzaki et al., 2005), and Lactococcus lactis from cheese starters (Ratanaburee et al., 2011). The aim of this study was evaluation of ?-aminobutyric acid (GABA) produced by lactic acid bacteria isolated from fermented durian. MATERIALS AND METHODS Isolation of LAB Lactic acid bacteria (LAB) were isolated by spreading various 101–106 fold-diluted samples of fermented durian (tempoyak) from Minang Kabau onto MRS agar plates containing 2% $CaCO_3$. At least 5 colonies were randomly selected from each fermented durian sample and used for species selection. Isolated colonies were streaked twice and stored in liquid culture at –80°C with 15%–20% (v/ v) glycerol. GABA Production The medium composition for GABA production was as follows (g/L): glucose: 50.0, urea: 8.0, glutamate: 50 mM, K_2HPO_4: 1.0, $MgSO_4.7H_2O$: 2.5, $MnSO_4.7H_2O$: 0.1, and $CaCO_3$: 1.6. The pH of the medium was adjusted to 7.0 with 1N sodium hydroxide or 1N hydrochloric acid, and fermentation was carried out in 250-mL Erlenmeyer flasks. The fermentation medium was inoculated with 1% (v/v) of the overnight culture, and the production medium was kept in an orbital incubator shaking water bath (Thermo Fisher Scientific) at 30°C and 120 rpm for 48 hr. Then, the cells and debris were removed by centrifugation at 10.000 g at 4° CC for 10 min. The supernatants were used as the crude source of GABA for estimation. GABA Estimation Thin-layer chromatography was employed to qualitatively detect GABA in the culture medium; the solvent system consisted of 2:1:1 n-butanol: acetic acid: water. The visualization of spots was performed by spraying with 0.02% ninhydrin solution, and the GABA in the suspension was quantitatively estimated using HPLC. Biochemical Identification of LAB The cultures were identified according to their morphological, cultural, physiological and biochemical characteristics (George and Garrity, 2005; Badis et al., 2004). The tests were as follows: Gram reaction; production of catalase and hydrogen peroxide; production of gas from carbohydrates (1% w/v), i.e., lactose, sucrose, glucose and mannitol, in MRS broth devoid of glucose and beef extract with chlorophenol red as an 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; and indole production in tryptone broth and growth on acetate agar. RESULTS AND DISCUSSION The lactic acid bacteria isolated from fermented durian that began to grow on selective MRS broth media were incubated for 7 days. From four locally available fermented durian, 59 LAB isolates were identified based on the clear zone generated around the colony using selective MRS agar after media with 2% $CaCO_3$. Table 1 shows the number of LAB isolates from the Minang Kabau Region. Based on the results of the study, a total of 59 LAB isolates were found, of which 4 districts were used as sources of the LAB- producing isolates. The highest number of isolates was obtained from fermented durian meat with added salt followed by durian meat alone and durian meat plus chili. It is assumed that salt can rapidly enhance LAB growth by acting as a growth precursor since the salt contains Na and Cl, both of which play a role as metabolic cofactors in LAB cells and thus enhance LAB growth. One of the factors affecting the growth of lactic acid bacteria is competition for nutrients. Lactic acid bacteria naturally live in nutrient-rich environments, such as milk and plant products, and they require nutrients for life and growth that include carbon sources, nitrogen sources, energy sources and growth factors (minerals and vitamins). Salt (NaCl) is one of the most important additives for pickling and preserving food, and Masui et al., (1979) said that NaCl may inhibit the growth of harmful bacteria and enhance desirable fermentation. Shockey and Berger (1991) added that salt inhibits the growth and proteolysis of butyric acid-producing bacteria but not the growth of LAB, and they postulated that the addition of salt and salt-tolerant LAB would improve fermentation quality. Other researchers, Ibourahema et al., (2008) found that bacterial cells S120 LILI ANGGRAINI ET AL cultured with a high concentration of salt could lose turgor pressure, thus affecting their physiology, enzyme activity, water activity and metabolism. Adnan and Tan (2007) concluded that high osmotolerance would be a requirement of LAB strains used commercially because when lactic acid is produced by the strain, alkali must be pumped into the broth to prevent an excessive reduction in pH, so the free acid would be converted to its salt form, increasing the osmotic pressure on the bacterial cells. Figure 1 shows the positive and negative lactic acid bacteria isolated from fermented durian. From the 59 LAB isolates, 42 were found to produce GABA after qualitative screening by TLC (data not shown), of which only 13 isolates can produce GABA (Figure 2). The highest GABA production was observed in isolate T8 followed by isolates T4, T1 and T13. From Figure 2, it can be seen that the ability of LAB to produce GABA depends on the ability of each isolate to change glutamate acid to GABA in the fermentation medium because the same fermentation medium was used but GABA production differed. The results of this research are opposite of that done by Siragusa et al., (2007) who reported that the rate of GABA production by microorganisms is affected by different fermentation factors, among which the most common and essential are pH, temperature, cultivation time and additives in the culture media. There were no differences in the type of LAB isolated from naturally fermented food in Minang Kabau, especially fermented durian from various kabupatens in West Sumatera made with different raw materials (Table 1). After quantitative screening, only 13 isolates were found to have the potential for GABA production due to the different capabilities of the isolates to produce secondary metabolites in the fermentation process. Similar results have been reported by other studies such as Siragusa et al. (2007), who isolated LAB from 22 varieties of Italian cheese and found differences in the capability of the isolates to produce GABA, which varied from 0.26 to 391 mg/kg. In this research, the isolate T8 produced the greatest amount of GABA (22.615 mg/mL) at 40 ºC and a pH of 6.5 after seven days of incubation (Figure 2) followed by isolates T1, T4, T9 and T13. Lactobacillus paracasei isolated from traditional Japanese fermented fish was reported by Komatsuzaki et al., (2005) to produce GABA at a concentration of 302 mM, and Ratanaburee et al., (2011) reported that L. plantarum DW12 produced approximately 4000 mg/L in a fermented red seaweed beverage system after 60 days of incubation. As shown in Table 2, the result of a lactose utilization test of the colony was positive. These isolates were able to ferment lactose to produce lactic acid, which lowered the pH of the MRS media that, in turn, changed the purple indicator dye to yellow, indicating fermentation activities. Gram reaction and morphology studies showed all these isolates from fermented durian to be gram-positive cocci (Figure 3). These are common features of LAB, which constitute a large group of non-sporulating gram-positive and catalase and oxidase-negative bacilli that produce lactic acid as the major metabolite during carbohydrate fermentation. After identification using 16S rRNA, the isolate T8 was identified as Pediococcus acidilactici. The study discovered that P. acidilactici isolated from fermented durian has the ability to produce GABA, which can benefit various physiological functions, such as neurotransmission and the induction of hypotensive effects, or be incorporated into feed additives, such as those used to reduce heat stress in broiler chickens. This study will help broiler breeders in the tropics who maintain animals at numbers that exceed the capacity of the cage, thus causing heat stress that many researchers have not been able to explore. Thus, a new metabolite that produces lactic acid bacteria must be explored in the future. From the results of this research, it can be concluded that, after screening 59 isolates of lactic acid bacteria (LAB) for GABA production, 13 isolates have the capability to produce GABA, and the highest GABA production was found in isolate T8. The isolate was characterized as gram-positive and negative for catalase, and it was determined to be P. acidilactici by 16S rRNA with GABA production of 22.165 mg/mL. ACKNOWLEDGEMENTS This work was supported by the Ministry of Technology Research and Higher Education of the Republic of Indonesia through basic research grants under contract 6/UN.16.17/PP. Fundamental/LPPM/ 2017. REFERENCES Adnan, M. A.F. and Tan, I.K. 2007. Isolation of lactic acid bacteria from Malaysian foods and assessment of the Evaluation ?-aminobutyric acid (GABA) Produce by Lactic Acid Bacteria Isolated from Fermented S121 isolates for industrial potential. Bioresour Technol. 98: 1380–1385. Badis, A., Guetarni, D., Moussa-Boudjema, B., Henni, D.E. and Kihal, M. 2004. 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