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Optimization of Nutrient Medium for Pediococcus acidilactici DS15 to Produce GABA

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Received: 02 Aug. 2019 Accepted: 09 Sept. 2019
ABSTRACT Nutrition is an essential factor for microorganisms to grow and survive. Carbon and nitrogen sources are used in producing primary and secondary metabolites. Gamma-Aminobutyric acid (GABA), a non- coded amino acid, is a secondary metabolite which acts as an inhibitory neurotransmitter of the central nervous system. Pediococcus acidilactici DS15 is a bacterium belonging to the order of Lactic Acid Bacteria. This study aimed to determine the effects of nutrients including glutamate, nitrogen and carbon sources on GABA production by Pediococcus acidilactici DS15. The tests were carried out using a range of 30 mM, 40 mM, 50 mM, 60 mM, and 70 mM glutamate as inducer and carbon sources in the form of peptone, yeast extract, skim milk, NH4NO3, KNO3, whey tofu and soy milk as nitrogen sources, and then glucose, lactose, maltosa, sucrose, palm, and cane sugar as carbon sources. The best sources of both will be tested with levels 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100% for nitrogen and 1, 3, 5, 7, 9, 11, 13 and 15% for carbon. The results of this investigation revealed that the addition of 60 mM glutamate caused the higher amount of GABA production and the best source of nitrogen and carbon for Pediococcus acidilactici DS15 were 100% whey tofu and 15% palm sugar, respectively. Production rate of GABA by Pediococcus acidilactici DS15 could reach up to 311,485 mg / L. Key words: Carbon, GABA, Glutamate, Nitrogen, Pediococcus acidilactici DS15

INTRODUCTION

Microorganisms are widely used in various industrial sectors. In the livestock fields, microorganisms are associated to health advantages and apply as probiotics and Direct-Fed Microbial (DFM) supplementations (Khan et al., 2016). In addition, they play important roles in feed ingredients processing such as fermentation, production of enzymes or other additive compounds. A short process, high production, and easy to use in the production process are some of the supporting factors for the use of microorganisms (Gurung et al, 2013). The growth of microorganisms can be successful if nutritional, environmental and other requirements are properly provided. These available nutrients such as carbon and nitrogen in the growth medium will be used to produce primary and secondary metabolites (Thirumurugan et al., 2018). Primary metabolites are formed intracellularly and have an essential function for the survival of microbes, while secondary metabolites can be used as emergency nutrition to survive or to defend themselves in the final phase of growth or death phase (Thirumurugan et al., 2018). Gamma-Aminobutyric Acid (GABA) is one of the secondary metabolites that can be used as an anti-stress agent for humans or livestock. GABA, a non- proteinogenic amino acid, acts as an inhibitory neurotransmitter of the central nervous system (CNS) (Murray et al, 2003). GABA dilates blood vessels and resulted in lowering blood

pressure and are used as a medication for stroke treatment. Moreover, GABA has diuretics, tranquilizer, anti-oxidant, and pain relief effects and regulates the secretion of growth hormone (Hao and Schmit, 1993; Kono and Himeno, 2000; Leventhal et al., 2003). It is demonstrated that GABA can be also produced by microorganisms such as bacteria, yeast and fungi To cite this paper: Anggraini L, Marlida Y, Wizna W, Jamsari J and Mirzah M (2019). Optimization of Nutrient Medium for Pediococcus acidilactici DS15 to Produce GABA. J. World Poult. Res., 9 (3): 139-146. 139 (Dhakal et al., 2012). lactic acid bacteria (LAB) are considered as useful and safe microorganisms that are capable to produce GABA (Li H et al., 2010). Pediococcus acidilactici is a LAB which has been used as a starter culture in fermented meat, milk, and vegetable which causes distinctive taste changes, improves cleanliness and extends product shelf life (Mora et al., 1997; Porto et al., 2017). Pediococcus acidilactici have also been found in the process of production of traditional food such as dadih (fermented buffalo milk) (Anggraini et al., 2018). Pediococcus acidilactici DS15 requires nitrogen and carbon to meet their daily needs both for living or producing GABA (Donnell et al., 2001; Savijoki et al., 2006). Most LAB strains usually prefer glucose as a carbon source (Kim et al., 2009). Glucose replacement with fructose, lactose, maltose, arabinose, and galactose can reduce GABA production (Cho et al., 2007).

The use of inorganic or organic nitrogen can also affect the growth of LAB. Administration of inorganic nitrogen caused the growth of Lactobacillus buchneri WPZ001 to be severely inhibited, but providing organic nitrogen source leads to better growth of L. buchneri WPZ001 (Zhao et al., 2015). In addition to nitrogen and carbon nutrients, an inducer of glutamate is also needed to increase GABA production. Addition of exogenous glutamic acid can augment GABA synthesis (Kim et al., 2009). There is less data about nutritional requirements of Pediococcus acidilactici DS15 to produce GABA optimally. Therefore, the present study aimed to assess the different media nutrients in term of GABA production by Pediococcus acidilactici DS15. MATERIALS AND METHODS Isolation of Pediococcus acidilactici DS15 Pediococcus acidilactici DS15 was isolated from curds as LAB producing GABA (Anggraini et al., 2018). The bacteria were grown anaerobically at 30oC on MRS broth (Merck, Germany) and stored for further analysis. The experiment was carried out at the Feed Technology Industry Laboratory, Faculty of Animal Science, Andalas University, West Sumatra, Indonesia. GABA production medium The nutritional content of media in one liter consists of di-potassium hydrogen phosphate 2 gr, di-ammonium hydrogen citrate 2 gr, sodium acetate 5 gr, magnesium sulphate 0.2 gr, and manganese sulphate 0. 02 gr. Optimization of source and nitrogen levels As nitrogen sources, GABA-producing LAB used peptone, yeast extract, skim milk, NH_4NO_3, KNO_3, whey tofu, and soy milk. The best results from nitrogen sources are continued with different levels of addition, namely 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100%. The results were determined by calculating Optical Density (OD) using a spectrophotometer with a wavelength of 600 nm. Then, GABA production was measured by centrifuging at a speed of 10,000 rpm, temperature 4oC and analyzed using Shimadzu HPLC (Kyoto, Japan) C18 column (250 mm × 4.6 mm I.D., particle size 5 µm / L, Alltech, IL, USA) Optimization Source and Carbon Levels The treatment was repeated three times. As carbon sources, Pediococcus acidilactici used glucose, lactose, maltose, sucrose, granulated sugar, and palm sugar. The best results from carbon sources are continued with different addition levels, namely 1%, 3%, 5%, 7%, 9%, 11%, 13% and 15%. The results were determined by calculating OD using a spectrophotometer with a wavelength of 600 nm. Then, GABA production was measured by centrifuging at a speed of 10,000 rpm, temperature 4oC and analyzed using Shimadzu HPLC (Kyoto, Japan) C18 column (250 mm × 4.6 mm I.D., particle size 5 µm / L, Alltech, IL, USA). RESULTS AND DISCUSSION Optimization of glutamate as an inducer The effect of giving glutamic acid as an inducer of GABA production and growth of Pediococcus acidilactici DS15 is shown in figure 1. Giving glutamic acid with a range of 0,2-1% did not seem to have much influence on growth, where the obtained OD value was 0.443; 0.459; 0.453; 0.452, and 0.445. Whereas for the production of GABA there is a rise along with an increase in the concentration of glutamate acid given. The highest concentration of GABA was observed in the giving of inducer as much as 0,8%, which was 159,047 mg/L, but at the giving of 1%, there was a slight decrease to 158,344 mg/L. Zhong et al. (2019) indicated that L-sodium glutamate at the concentration of 1% had the greatest capacity to enhance the production of GABA compared with the other concentrations by L. pentosus, However, the highest mycelium biomass was obtained with L-sodium glutamate at 0.5%. In the present study, there is an increase in GABA production due to the increase in the amount of glutamate J. World Poult. Res., 9(3):

139-146, 2019 given that was consistent with the results of a previous study (Komatsuzaki et al., 2005). GABA is synthesized from glutamic acid with the help of Glutamic Acid Decarboxylase (GAD) (Kan et al., 2017) so that with the addition of glutamate, the GAD enzyme will be activated. Bibb (2005) declared that the synthesis of secondary metabolites was triggered by critical conditions of a nutrition or by adding of inducers to a growth medium. GABA synthesis is influenced by the ability of bacteria and also the presence of glutamate in the cell-matrix. The GAD enzyme in LAB is an intracellular enzyme (Huang et al., 2007; Komatsuzaki et al., 2008) that is synthesized as a form of a stress response to an acidic environment (Sanders et al., 1998; Small and Waterman, 1998). L- 180 160 140 GABA yield (mg/L) 120 100 80 60 40 20 0 0.1 0.2 0.4 MSG (%) glutamate concentration can be increased by adding exogenous glutamate acid (Park and Oh, 2005; Seok et al., 2008; Kim et al., 2009; Zhuang et al., 2018), protease to hydrolyze proteins and produce glutamate acid, using LAB to hydrolyze proteins as co-cultures in the fermentation process (Inoue et al., 2003). Figure 1 shows when the concentration of giving MSG exceed 0,8% resulting in decreased GABA production, therefore, there is an optimal limit of MSG as an inducer, which is 0,8%. This finding is compatible with the study that has been reported excess monosodium glutamate can inhibit GABA production (Tung et al., 2011). 0.460 0.458 0.456 0.454 0.452 0.450 0.448 GABA yield 0.446 Cell growth 0.444 0.442 0.440 0.8 1 Cell growth (OD) Figure 1. GABA production and growth curve of Pediococcus acidilactici DS15 based on glutamate dose Utilization of nitrogen sources The uses of various nitrogen sources were investigated to determine optimal GABA production. Figure 2 illustrates that the use of whey as a nitrogen source is breached compared to other nitrogen sources in producing GABA for Pediococcus acidilactici DS15; however, peptone was found as the best source of nitrogen in the growth of bacteria. Figure 2 shows that the use of organic nitrogen sources (peptone, yeast extract, tofu water, and soy milk) increased GABA production and cell growth compared to inorganic nitrogen sources (KNO3, NH4NO3, and urea). This result is in line with the research of Zhao et al. (2015) that reported the use of organic and inorganic nitrogen sources lead to a difference in the yield of GABA produced by LAB. In the mentioned study, when a single inorganic nitrogen source such as urea, ammonium sulfate or citric acid diamine was given, the growth of L. buchneri WPZ001 was severely inhibited and the production of GABA reduced. But when peptone fish meal, meat extract, or stumped peptone were given singly, both cell growth and GABA production were higher. Tofu liquid waste, also called whey tofu, is a by- product in the process of tofu production. Whey tofu contains organic compounds such as organic nitrogen (7.61%), total sugar (0.32%), reducing sugars (0.09%), and minerals (Ghofar et al. 2005). these organic compounds make whey an appropriate growth media for bacteria. The nutrient content of soybeans is what distinguishes whey tofu from commercial nitrogen sources including peptone, yeast extract, NH4NO3, and KNO3. 180 160 140 GABA yield (mg/L) 120 100 80 60 40 20 0 GABA yield Cell growth KNO3 NH4NO3 Urea Pepton Yeast Whey tofu Susu ekstrak Kedelei 0.500 0.450 0.400 0.350 0.300 0.250 0.200 0.150 0.100 0.050 0.000 Cell growth (OD) Nitrogen sources Figure 2. GABA production and growth curve of Pediococcus acidilactici DS15 in various nitrogen sources 300 250 GABA yield (mg/L) 200 150 100 50 0 GABA yield Cell growth 10 20 30 40 50 60 70 80 90 100 0.50 0.45 0.40 0.35 0.30 0.25 0.20 0.15 0.10 0.05 0.00 Cell growth (OD) Whey tofu(%) Figure 3. GABA production and growth curve of Pediococcus acidilactici DS15 based on the level of tofu liquid waste supply Level of use of tofu liquid waste The nitrogen source is used as a constituent material of cell biomass. LAB in the growth phase utilizes protein as a source of nitrogen, which is used by bacteria for protein synthesis, amino acids (Nisa et al., 2001). The highest cell growth was found in 40% of the use of whey tofu, and the use of more than 40% reduced cell growth (Figure 3). This decline is due to the high nitrogen content being the limiting factor for cells to grow. Changes in nutrient availability affect growth and biomass products (Leroy and de Vuyst, 2001). J. World Poult. Res., 9(3): 139-146, 2019 Figure 3 shows that GABA production increased with increasing dosage of whey tofu used. This is because higher the doses of whey tofu are richer in organic compounds such as organic nitrogen and minerals. According to the obtained results, it was found that 100% use from whey tofu could provide a good source of nitrogen for Pediococcus acidilactici DS15 to produce GABA. Utilization of carbon sources In the present study, several types of carbon sources in the form of simple sugars were used to determine the effects of type and amount of carbon sources on GABA production by Pediococcus acidilactici DS15. Figure 4 illustrates that palm sugar is the best source of carbon compared to glucose, maltose, cane sugar, and sucrose which were used for the GABA production. In contrast to GABA production, the graph of cell growth of Pediococcus acidilactici DS15 showed that the highest cell growth was found in glucose as a carbon source, amounting to 0.419, while the lowest was 0.378 in maltose. This difference can indicate that there is no correlation between the level of production of GABA and the number of bacterial cells. The highest GABA production by Pediococcus acidilactici DS15 was 140.6 mg/L in utilizing palm sugar, followed by glucose, sugar cane, lactose, maltose, and sucrose with a production of 115,774; 110.2; 109,554; 94,284 and 79,813 mg/L, respectively. In contrast to the study of Soe et al. (2013) which used Lactobacillus brevis to produce GABA, it was found that sucrose was the best source of carbon compared to fructose and maltose, which was 23.64 mM, while other studies reported that maltose is the best carbon source in GABA production by L.brevis K203 (Binh et al., 2014) and L. brevis HYE1 (Lim et al., 2017).

In addition, xylose was described as the best carbon source L. buchneri WPZ001 to product GABA (Zhao et al., 2014). GABA production is affected by differences in the types and strains of LAB because each strain of

LAB has differences in the use of carbon sources that can have impacts on growth and function of bacteria. The cell growth of the Pediococcus acidilactici DS15 revealed differences in various carbon source. The highest growth is indicated by glucose, followed by sucrose, lactose, palm sugar, cane sugar and maltose. This difference in cell growth is caused by the type of sugar in each source. Palm sugar has a sugar content in the form of sucrose, which is a disaccharide composed by glucose and fructose, and a dextran which is a polysaccharide that has a chain of glucose branches. Glucose is usually a good source of carbon for bacterial growth but interferes with the formation of secondary metabolites (Demain, 1989). Papagianni and Sofia (2009) revealed that Pediococcus acidilactici can use sucrose as a carbon source. The culture medium contained a mixture of simple and complex carbon sources, the simple carbon source is used for cell formation and little or no secondary metabolites formation. Complex carbon sources will be used for idiolites formation after the simple carbon source has been used (Ruiz et al., 2010). 160 140 GABA yield (mg/L) 120 100 80 60 40 20 0 0.450 0.400 0.350 0.300 0.250 0.200 0.150 GABA yield 0.100 Cell growth 0.050 0.000 Glucose Lactose Maltosa Sucrose Palm sugar Cane sugar Carbon sources Cell growth (OD) Figure 4. GABA production and growth curve of Pediococcus acidilactici DS15 in various carbon sources 350 300 GABA yield (mg/L) 250 200 150 100 50 0 1 3 5 7 9 Palm sugar (%) GABA yield Cell growth 11 13 15 0.6 0.5 0.4 0.3 0.2 0.1 0 Cell growth (OD) Figure 5. GABA production and growth curve of Pediococcus acidilactici DS15 based on the level of administration of palm sugar Level of use of palm sugar In the current study, the optimum dose of palm sugar as best carbon source were determined. According to the obtained results, it demonstrated that *Streptococcus thermophilus QYW-LYS1 Isolated from Traditional Fermented Yoghurt", Advanced Materials Research, 2014.">the production of GABA has Streptococcus thermophilus QYW-LYS1 Isolated from Traditional Fermented Yoghurt", Advanced Materials Research, 2014.">increased along Streptococcus thermophilus QYW-LYS1 Isolated from Traditional Fermented Yoghurt", Advanced Materials Research, 2014.">with the increase in the provision Streptococcus thermophilus QYW-LYS1 Isolated from Traditional Fermented Yoghurt", Advanced Materials Research, 2014.">of palm sugar up to 15% by 311,485 mg/L (Figure 5). Previous studies have been reported that in order to GABA production, the best carbon sources to add to MRS media are 4% sucrose for Lactobacillus sakei B2- 16 (Kook et al., 2010), 3% sucrose for L. brevis 340G (Soe et al., 2013), 1% glucose for L.buchneri MS (Cho et al., 2007). Palm sugar has high sucrose content which is used by Pediococcus acidilactici as an energy source. Addition of sucrose can increase the number of carbon sources as an energy source for cell growth so that the production of primary and secondary metabolites will increase. The growth of microorganism including bacteria or fungi was strongly influenced by the presence of sufficient carbon sources, optimal temperatures, suitable pH conditions and other supporting conditions. Carbon sources that act as nutrients are needed for the survival of bacteria in producing primary metabolites as a necessity of life. If the nutrients contained in the media are overgrown in abundant amounts, then cell viability will increase CONCLUSION The best source of nitrogen and carbon in producing GABA by Pediococcus acidilactici DS15 were tofu water and palm sugar, respectively. The best concentration was 100% tofu liquid waste and 15% palm sugar resulted in production rate of GABA up to 311,485 mg / L. DECLARATIONS Acknowledgments We are very grateful to the Minister of Research, Technology and Higher Education of the Republic of Indonesia for funding this research through PMDSU No: 1387/E4./2015 and the support through World Class Professor Program Scheme-B No. 123.57/D2.3/KP/2018. Author's contribution Lili Anggrani and Yetti Marlida conducted the research, prepared data and wrote the article. Wizna, Jamsari and Mirzah cheked and confirmed the final form of article. Competing interests The authors declare that they have no competing interests. J. World Poult. 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