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 capabality 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 cycle[1]. It is also a key metabolite because it serves to link nitrogen and carbon metabolism[1]. 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 stresses[2].

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 researchers[3-8]. Glutamic acid is an amino acid that involved in the perception of multifunction flavors, excitatory neurotransmission and intermediary metabolism[2]. 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 stomach[9]. 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 malnutrition[10-11]. 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 industry[13]. 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 cheese[14]; Malaysian fermented food[15]; kimchi from South Korea[16]; fermented fish from Thailand[17]; Algerian goats milk[18]; Indonsian traditional fermented fish sauce bakasang[19]; Syrian fermented food[20]; and semi-dried sausages[21], 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 Tan[22]. 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 10^1 to 10^{12} 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 ^{0}C 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 characteristics[23-24]. 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 al*[15]

Table 1. Qualitatif glutamic acid production of LAB by extraselluler and Intraselluler

No.	Name of isolates	Qualitative Glutamic Acid	
		Exstraseluler	Intraseluler
1	Y1	++	+
2	Y2	+++	+
3	Y3	++	+
4	Y4	++	++
5	Y5	++	++
6	Y6	++	+
7	Y7	+++	+
8	Y8	+++	+
9	Y9	+++	+
10	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/L[26] .

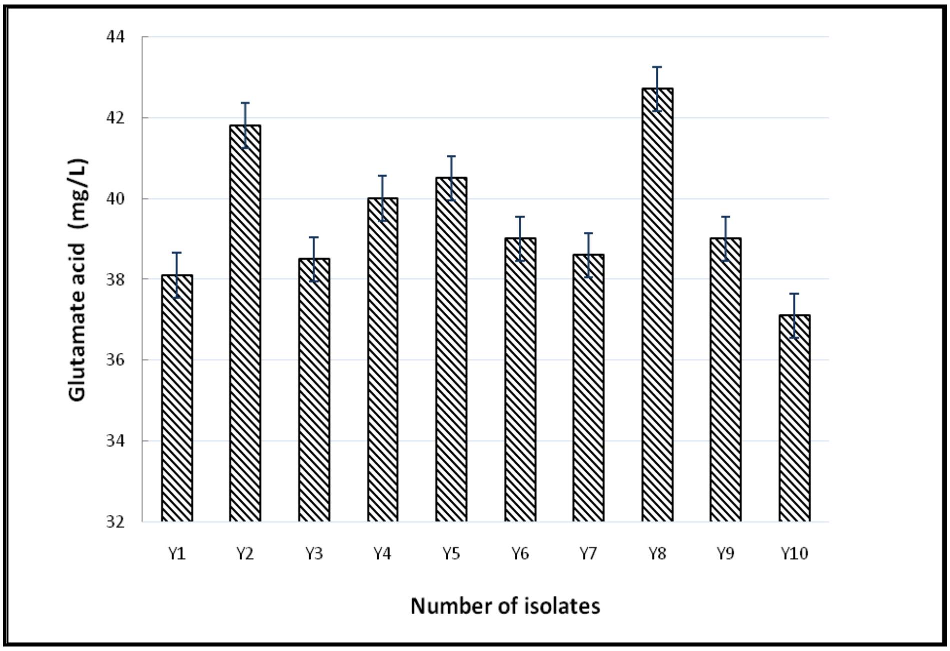

Figure 1. Quantitative screening of glutamic acid produced by LAB

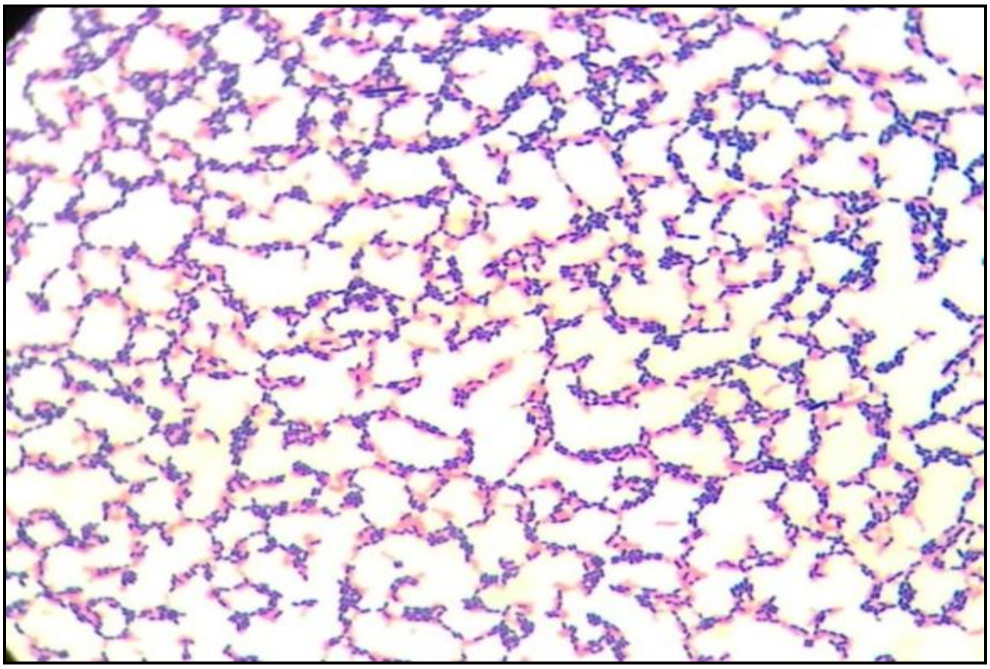

Figure 2. Morphology of isolates Y2 (1000 X)

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	-	-

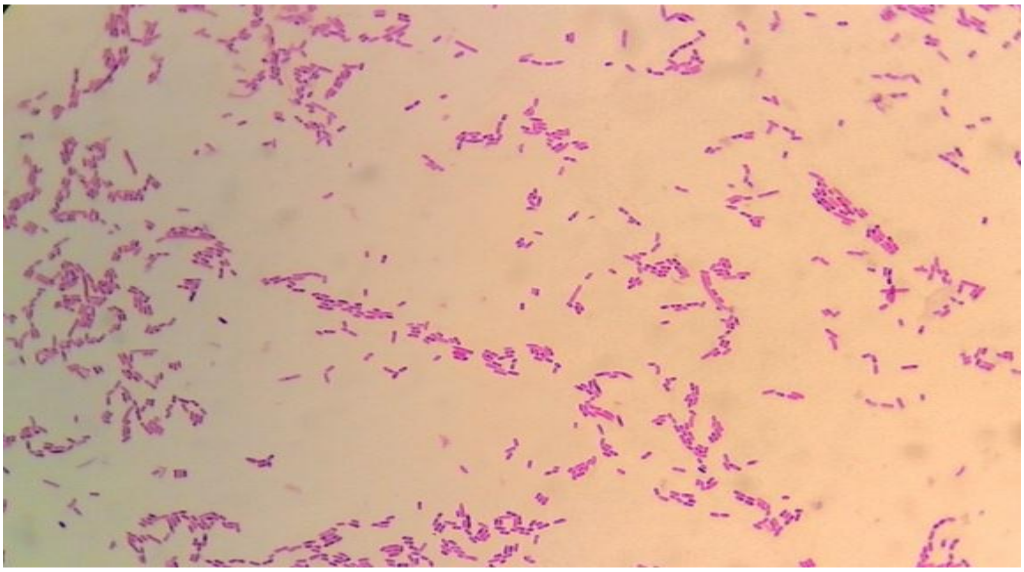

Figure 3 . Morphology of isolates Y2 dan Y8 (1000 X)

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.

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