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The growth and potential of *gamma-aminobutyric acid* (GABA) by lactic acid bacteria isolated from fish fermented food from Maluku, Indonesia

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1. Introduction

Fermented products have traditionally been owned by each region, one of them is processed traditional fermented fish products that is Bekasam. Bekasam is a preservation process using fish, rice as a source of carbohydrates, and the salt that is put into the jar is tightly closed and stored to undergo a fermentation process for several days. The fermentation process in fish bekasam is a fermentation of lactic acid bacteria that can convert 95% glucose into lactic acid [1]. Lactic acid bacteria are closely related to the process of food fermentation and today has grown into the fermented food industry. Lactic acid bacteria can be isolated from fermented foods, one of them being fish fermented. Lactic acid bacteria are a group of bacterium that are most widely used in industry because they have advantages when compared to



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other groups. The main role of lactic acid bacteria in fermentation is generating acid on the fermented food. The acid can inhibit the growth of bacteria that causes disease (pathogens) and food spoilage bacteria [2]. Microbial growth is an increase in the number or volume and size of cells. In its growth, microbes, especially bacteria and yeast, multiply by dividing from one cell into two cells. Bacterial cell growth usually follows a specific growth pattern in the form of a sigmoid growth curve. The growth curve of bacteria can be separated into four main phases, namely: lag phase (slow phase), exponential phase (fast phase), stationary phase (static phase), and population decrease phase (decliners) [3]. Several studies have shown that LAB could reduce the pathological conditions due to oxidative stress indicating that LAB has antioxidant activity. One of the metabolites produced by LAB that can act as an antioxidant is GABA. The function as an antioxidant is shown by *Lactobacillus plantarum* DM5 in ITS ability to produce *Gamma-Aminobutyric Acid* [4].

Gamma-Aminobutyric Acid (GABA) is a non-protein amino acid that is widely distributed in nature and irreversibly generated via α -decarboxylation of L-glutamic acid in a reaction catalyzed by the enzyme of glutamate decarboxylase (GAD). *Glutamic Acid Decarboxylase* (GAD) is an enzyme that catalyzes the decarboxylation of glutamate into GABA and CO₂. GAD and GABA can be found widely in all organisms, ranging from the smallest organisms, such as bacteria, to higher organisms. GABA is known as a neurotransmitter inhibitor in the mammalian central nervous system [5] Abnormal levels of GABA can cause sleep disorders, eating disorders, seizure disorders including epilepsy [6]. The existence of GDH gene which is responsible for the production of glutamic acid becomes the strength of LAB compared to the other microbes [7]. GABA can be produced from lactic acid bacteria such as *Lactobacillus brevis*, *Lactobacillus plantarum*, *Lactobacillus delbrueckii* which are reported to have the activity of *glutamate decarboxylase* enzyme and the ability to produce GABA in the concentration of 10-350 mmol/L depending on the concentration of monosodium glutamate in the fermentation medium [8]. LAB can produce GABA due to its GAD enzyme activity [9].

Thin Layer Chromatography (TLC) is a fairly simple analysis method because it can determine the number of components in a material, it can even identify those components [10]. The working principle of TLC is to separate samples based on the differences in polarity between the sample and the used solvent. This technique usually used stationary phase and in the form of silica plate and its motion is adapted to the type of sample that wants to be separated. The used solution or the mixture of solution is called the eluent. The closer the polarity between the sample and the eluent, the sample will be carried away by the mobile phase.

The identification of GABA can be done by Thin Layer Chromatography (TLC), UV-VIS spectrophotometer, and High Performance Liquid Chromatography (HPLC). Given the growth of LAB and the important function of GABA, this study will examine the growth, the potential of GABA, and know the character of LAB isolated from fish fermented food from Maluku, Indonesia. This study uses Thin Layer Chromatography (TLC).

2. Methods

2.1. Revitalization of LAB isolates

Lactic acid bacteria were derived from the collections of the Laboratory of Biotechnology, University of Diponegoro. The rejuvenation of isolates was conducted by planting isolates preserved in the refrigerator into MRSA medium with quadrant streak method and then incubated at 37°C for 48 hours. Streak quadrant aims to purify isolates that have been kept long, to avoid contamination by unwanted microorganisms. LAB isolates that have been obtained previously went through biochemical characterization tests which include Gram staining, catalase, fermentation, motility and acid formation tests. The tests indicate the characteristics of lactic acid bacteria in general.

2.2. The making of LAB growth curve

2.2.1. Starter making. The starter making for the growth curve is as follows; INS and INS-A2-A4 isolates were inoculated with round-ose, 1 ose was put into the MRSB media in a 25ml volume. After that, it was incubated on a rotary shaker for ± 24 hours, a speed of 150 rpm at 37°C until the number of cells of 10^7 CFU/ml was measured by optical density (OD) to a value of 1 at a wavelength of 600 nm.

2.2.2. LAB isolates growth curve. After the number of cells reached 10^7 CFU/ml and then taken by 5% (v/v), the starter was transferred to 100 ml of MSRB medium with the addition of 2% MSG and without 2% MSG, after that it was incubated on a rotary shaker for 60 hours with a speed of 150 rpm at 37°C. Every 6 hours, sampling was performed to observe the cell growth. The growth curve was made by counting the number of cells with the spectrophotometric method. Bacterial culture on the growth medium was taken 4 ml every 6 hours for 60 hours incubation. Afterward, the absorbance was measured using a spectrophotometer with a wavelength of 600 nm. Furthermore, the measurement of bacterial growth is made into a graph.

2.3. GABA production curve

Bacterial culture on growth medium were respectively taken 4 ml every 6 hours in incubation for 60 hours, the separation between the supernatant and the cells using centrifugation with a speed of 6,000 rpm, 4°C for 20 minutes [3]. The supernatant that has been obtained was then measured with a spectrophotometer at a wavelength of 425 nm. The obtained results of absorbance value would be calculated for its GABA concentration measurement.

2.4. GABA concentration standard curve

The making of standard curve uses pregabalin solution of 150 mg, 225 mg, 300 mg, 375 mg, and 459 mg which were dissolved in 20 ml of distilled water, so a concentration of 7.5 mg/ml, 11.25 mg/ml, 15 mg/ml, 18.75 mg/ml, 22.5 mg/ml were obtained then measured using a spectrophotometer with a wavelength of 425 nm. The obtained absorbance values were used to create a standard curve to obtain the R^2 equation. The result of $Y = ax + b$ was used to obtain the concentration of GABA.

2.5. The identification of GABA production with the TLC method

The identification of GABA production by LAB culture was conducted with the method of Thin Layer Chromatography (TLC) using a solution of 2% MSG as a marker. Bacterial culture in MRSB fermented medium was transferred to each centrifuge tube as much as 2ml. Centrifugation was performed at a speed of 6,000 rpm at 4°C for 20 minutes. The resulting supernatant was transferred into a new tube.

The MSG solution concentration was prepared by diluting as much as 2g of MSG into 100 ml of distilled water to obtain a concentration of 2% MSG. A total of 4 mL concentrations of MSG was spotted or dripped on the TLC plate using a capillary pipette, a pregabalin solution of 75 mg as a positive control, the isolates supernatant of 2% INS-A2 MSG, INS-A2 non-MSG, the supernatant of 2% INS-A4 MSG and non-MSG were sequentially dropped on TLC plates. TLC was run for 30 minutes. TLC was performed using a developer solution (eluent) consisting of a mixture of n-butanol: acetic acid: distilled water with a ratio of 5: 3: 2 [11]. Upon completion, the TLC plate was sprayed with a ninhydrin solution of 0.5% (w/v) and then was dried with the wind. The GABA compound produced by LAB isolates can be seen from the Retention factor (Rf) which is equal to the pregabalin solution as a GABA standard solution that is used [12]. The standard Rf value of GABA is 0.61 [13].

According to [14], the calculation is as follows:

$$R_f = \frac{\text{The distance between the starting point and the center of the resulting spot}}{\text{The distance between the starting point and the distance proceeded by the developer}}$$

3. Results and discussion

3.1. LAB isolates from bekasam

There are two LAB isolates which are found from the bekasam isolation results of a research that has been done before by [15], namely INS-A2 and INS-A4 in which further characterization will be performed. LAB isolates that were isolated using the media of MRSA + CaCO_3 is seen based on the clear zone around its colonies. LAB is a bacterium that is able to produce lactic acid in its growth media. LAB that is obtained from the isolation results, that are 2 isolates, then conducted Gram staining test, catalase, motility, and type of fermentation of INS-A2 and INS-A4 isolates.

Identification is carried out on the obtained isolates by referring to the Bergey's Manual of Determinative Bacteriology, LAB has the characteristics of Gram-positive, non-spore, negative catalase, non-motile [16], [17]. The results of the characterization of INS-A2 and INS-A4 isolates are presented in Table 1.

Table 1. Characteristics of INS-A2 dan INS-A4

Characteristics	Isolate	
	INS-A2	INS-A4
Gram staining	(+)	(+)
Cell shape	Rod	Rod
Motility	(-)	(-)
Catalase	(-)	(-)
Fermentation	Homo	Homo

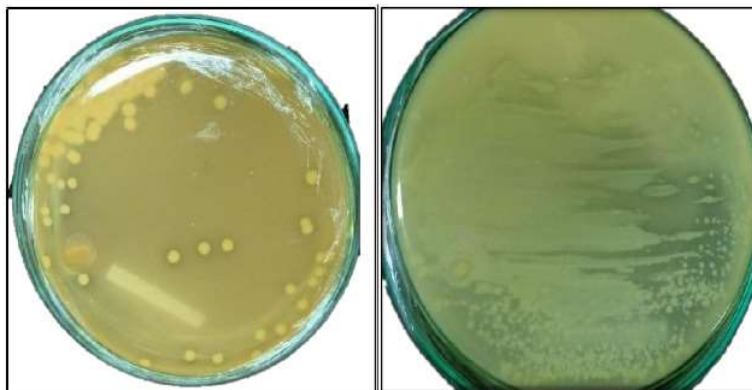


Figure 1. LAB Isolate a) INS-A2 b) INS-A4 in macroscopic

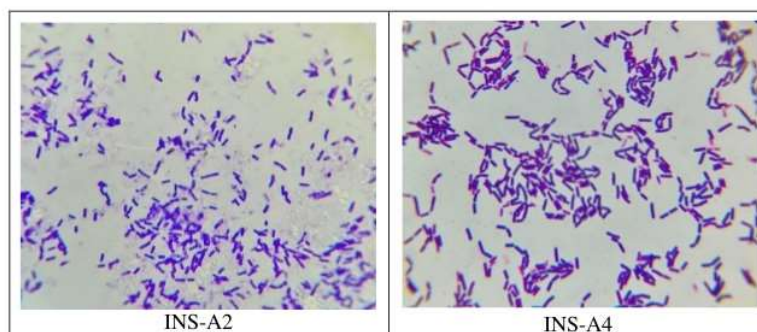


Figure 2. LAB isolate in microscopic

3.2. The Identification of GABA with the TLC Method

LAB culture in the medium of 60 hours fermented result production is transferred into centrifuge tubes. Centrifugation aims to separate bacterial cells in order to obtain the extract in the form of the supernatant. GABA compound can be determined qualitatively with the TLC method using the plate of TLC aluminium (Merck). A total of 4-5 drops of the supernatant was spotted or dripped on the TLC plate using a capillary pipette and then dried with the wind. After spotting, the TLC plate was soaked with eluent solution (developer solution) of *n*-butanol: acetic acid: water at a ratio of 5: 3: 2 and then sprayed with a solution of ninhydrin 0.5% (w/v-ethanol) as a dye reagent, then a purple spot will be formed. The mobile phase used in this study is consistent with the research conducted by [11] with a ratio of *n*-butanol, glacial acetic acid and water (5: 3: 2), and added ninhydrin.

Purple spots indicate the presence of chemical compounds accelerated by the mobile phase of the eluent and stained by the dye solution. Spot number 1 indicates pregabalin at a concentration of 75 mg, spot number 2 shows the MSG solution, spot numbers 3-4 indicate INS-A2 non-MSG and INS-A2 MSG isolates, spot 5-6 show INS-A4 and non-MSG isolates. According to [18], spot of GABA and MSG can be easily confirmed with a standard solution of GABA because GABA and MSG are detected with purple points (spots) when sprayed with ninhydrin. The R_f values of pregabalin which are resulted after being measured are ($R_f = 0.62$), MSG ($R_f = 0.25$), whereas INS-A2 MSG and non-MSG isolates and INS-A4 MSG and non-MSG produced ($R_f = 0.62$), The R_f values of the isolates are equal with the R_f value of pregabalin which proves that all isolates are able to produce GABA.

Based on a research conducted by [13], the identification of GABA from the six isolates of LAB (IFK-10, IFK-11, IFK-12, FN-12, FN-14, FN-15) derived from the fermentation of soybeans and fish showed GABA identification with TLC method with the same standard R_f value of GABA as all of the isolates ($R_f = 0.61$). The used eluent in the identification of GABA with TLC are butanol: acetic acid: distilled water (5: 3: 2). Some strains or species of LAB have been reported as GABA-producing bacteria. Almost all strains or species of GABA-producing bacteria are isolated from traditional fermented foods such as Nham [19], Italian cheese [20], and paocai [21]. Additionally, [20] reported that only four isolates of LAB such as *L. paracasei* PF6, *L. bulgaricus* PR1, *L. lactis* PU1 dan *L. brevis* PM17 are isolated from a variety of cheeses with the highest GABA production and demonstrate the highest level of GABA.

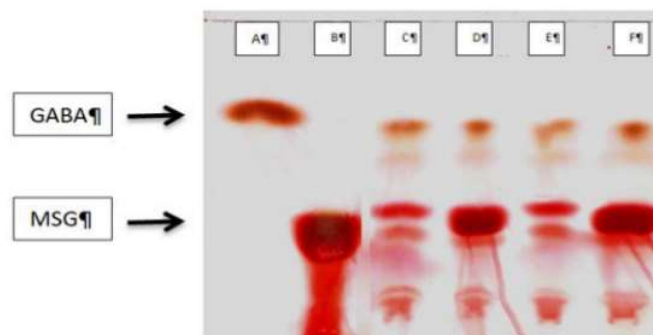


Figure 3. The TLC results of GABA A) GABA standart, b) MSG, c) INS-A4 non MSG, d) INS-A4 MSG, e) INS-A2 non MSG, f) INS-A2 MSG

3.3. Growth of LAB

Several environmental factors that affect the growth of lactic acid are salinity, temperature, pH, and the availability of carbohydrates as a source of food [22]. The ideal growth for lactic acid bacteria needs to create an optimal condition. Isolates are incubated for 60 hours at a temperature of 37°C to determine the growth curve. The measurement of LAB growth of INS-A2 and INS-A4 isolates is treated with the addition of 2% MSG and non-MSG in MRSB media. (Figure 3.4)

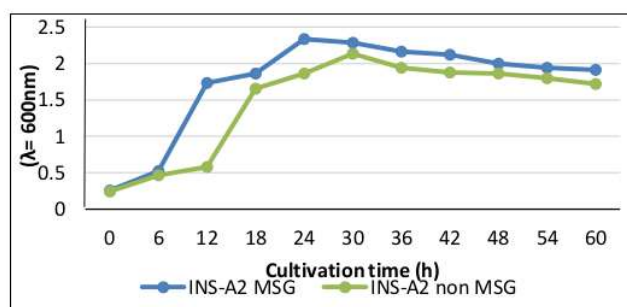


Figure 4. The LAB growth of INS-A2 MSG and non-MSG isolates on the MRSB media

Figure 4 shows the log phase of INS-A2 MSG isolate started at 0 hour to 12 hour, while log phase of INS-A2 non-MSG is indicated at 0 hour to the 24 hour. The LAB growth of INS-A2 MSG isolate reached the stationary phase at the end of 42 hours of storage with the absorbance value of 2.1, while the INS-A2 non-MSG isolate at the 48 hour with the absorbance value of 1.8. The death phase occurred at the 48 hour for INS-A2 MSG isolate and at the 54 hour for INS-A2 non-MSG isolate.

Figure 5 shows the log phase of INS-A4 MSG isolate started at 0 hour to the 24 hour, while the INS-A4 non-MSG isolate started at 0 hour to the 30 hour. The LAB growth of INS-A4 MSG and non-MSG isolates reached stationary phase at the 48 hour. This is in accordance with [23] which states that microbes are transferred into a media will experience an adaptation phase to adjust to the conditions of the surrounding environment. The length of the adaptation phase is determined by the number of cells inoculated, the appropriate physiological and morphological conditions and the necessary cultivation media. According to [1], the size of the stationary phase cells become smaller due to the cell keep dividing although the nutrient is depleted, this growth rate phase will decline because of a lack of growth factors such as vitamins and mineral elements. The stopping of growth can also be due to the reduced of some essential nutrients in the media or by the accumulation of autotoxin in the media or a

combination of both. After the first logarithmic phase, unexpected product accumulation may occur in which its existence can inhibit cell growth. Organic acids produced by LAB such as lactic acid, acetic acid, or pyruvic acid result in the accumulation of acidic final products and a decrease in pH which causes growth retardation. Products that might inhibit the growth of other bacteria, except for lactic acid, can also be in the form of carbon dioxide and other neutral components.

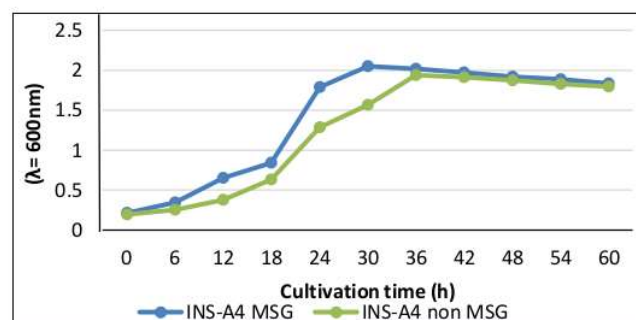


Figure 5. The LAB growth of INS-A4 MSG and non-MSG isolates on the MRSB media

3.4. GABA production

GABA Production is done by using the MRSB medium which is added by Mono Sodium Glutamate (MSG) by 2% and without MSG as a substrate to produce GABA by lactic acid bacteria isolates. The supernatant that has been obtained is measured with a spectrophotometer at a wavelength of 425 nm. The obtained absorbance value results will be calculated for the measurement of its GABA concentration. The measurement results of GABA production can be seen in (Figure 6)

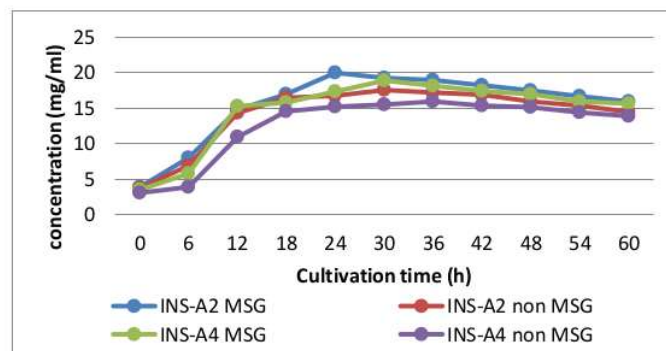


Figure 6. The GABA production of INS-A2 and INS-A4 LAB isolates in the MRSB media

The above results indicate the highest GABA concentration of each isolate with different production time. INS-A2 MSG isolate with a value of 20.005 mg/ml reached its highest at the 24 hour, INS-A2 non-MSG isolate of 17.581 mg/mL is at the 30 hour, INS-A4 MSG of 18.879 mg/mL is at the 30 hour and INS-A4 non-MSG of 15.941 mg/ml is at the 36 hour. It can be concluded that the isolates with the addition of 2% MSG in the production media can accelerate the growth and production of the produced GABA which becomes higher than the isolates that were grown without 2% MSG. Based on the obtained GABA concentrations from the two isolates, it shows that INS-A2 MSG and non-MSG have a higher concentration than INS-A4. MSG is used as a substrate for the production of GABA by LAB. Glutamate

has the principle of increasing the neurotransmitter in the central nervous system [12]. GABA-producing microorganisms, especially LAB receive great attention due to its potential in producing GABA to enrich food.

Research on the high concentration of GABA with microorganisms is still in progress. LAB that is isolated from fermented foods like kimchi and seafood can produce GABA with glutamic acid substrate. PharmaFood company (Japan) has produced GABA with the addition of monosodium glutamate using GABA from kimchi and marketed it as a functional food ingredient. *Lactobacillus brevis* IFO 12005 that was isolated from kimchi for Jugemi soju, that is made from rice, produced 6.3 mM of GABA [24], [25], 302 mM of GABA with the addition of pyridoxal phosphate as a coenzyme of GAD by *Lb. paracasei* NFR17415 from Funasushi, Japanese traditional fermented foods [26]. A research that has been conducted by [27] GABA production from *L. brevis* HYE1 which was cultured for 60 hours at 30°C on 200 ml MRSB media with the addition of 1% MSG with an interval of 12 hours resulted in optimal GABA at the 36th hour with a concentration of 16.94 mM. GABA is one of the secondary metabolites produced by lactic acid bacteria which has antioxidant activity. LAB can produce GABA due to its GAD enzyme activity [9].

4. Conclusion

The growth of INS-A2 and INS-A4 LAB isolates is treated with the addition of 2% MSG and non-MSG. The highest growth of INS-A2 MSG is at the 24 hour, the highest of INS-A2 non-MSG is at the 30 hour. The growth of INS-A4 MSG isolate is the highest at the 30 hour, and the highest of INS-A4 non-MSG is at the 36 hour. The highest concentration of GABA isolates is owned by INS-A2 MSG of 20.005 mg/ml. INS-A2 non-MSG isolate of 17.581 mg/ml, INS-A4 MSG of 18.879 mg/ml and INS-A4 non-MSG of 15.941 mg/ml. The characterizations of INS-A2 and INS-A4 LAB isolates are negative catalase, negative motility, positive Gram staining, a fermentation (homofermentative) type test that is consistent with the characteristics of LAB in general.

References

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