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Test of Antioxidants and Alpha-glucosidase Inhibitors from Lactic Acid Bacteria in Fermentation of Soy Milk (Soygurt)

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Abstract: Soygurt is an innovative product from yogurt which is fermented from soy milk. This research is an experimental study performed by an in vivo approach. The disc diffusion method evaluated the antibacterial activity of lactic acid bacteria isolates from Soygurt. Meanwhile, the antioxidant activity and inhibition of α-glucosidase enzymes were evaluated from cell-free supernatants of lactic acid bacteria isolated by spectroscopic methods. The results of the enumeration of lactic acid bacteria in Soygurt with 5% *Lactobacillus acidophilic* yogurt starter were 8.00 x 104 CFU/ml, and the calculation of lactic acid bacteria in Soygurt with Yakult starter was 5.00 x 107 CFU/ml. Lactic acid bacteria isolates from Soygurt with 5% *Lactobacillus acidophilic* starter showed no antibacterial activities against *Staphylococcus aureus* bacteria by the disc diffusion method. However, this lactic acid bacteria isolate had antioxidant activity and inhibited the α-glucosidase enzyme. It was shown by the percentage of DPPH inhibition, which ranges from 27.06-58.63%. Meanwhile, the moderate inhibitory activity of the α-glucosidase enzyme was 50.55%. Therefore, it can be concluded that lactic acid bacteria isolated from Soygurt with 5% *Lactobacillus acidophilic* yogurt starter have antioxidant and antidiabetic activity.

Keywords: Alpha-glucosidase; Diphenyl-1-Picrylhydrazyl (DPPH); *Lactobacillus acidophilic*; Soygurt

Introduction

Diabetes is a problem faced by various countries in Indonesia. Diabetes can cause various disorders, such as blindness, heart disease, and kidney failure. The International Diabetes Federation (IDF) organization estimates that at least 463 million people aged 20-79 years worldwide have diabetes in 2019, or the equivalent of a prevalence rate of 9.3% of the total population of the same age. Based on gender, IDF estimates that the prevalence of diabetes in 2019 is 8% for women and 9.65% for men. The majority of diabetes is estimated to increase with increasing age of the population to 19.9% (111.2 million people) at the age of 65-79 years. This figure is predicted to increase to 578 million in 2020 and 700 million in 2045 (IDF, 2019).

Countries in the Arab-North Africa and West Pacific regions are ranked first and second with the highest prevalence of diabetes in people aged 20-79 years among seven areas, namely 12.2% and 11.4%. The Southeast Asia region, where Indonesia is located, ranks 3rd with a prevalence of 11.3%. The IDF also projects the number of people with diabetes in residents aged 20-79 in several countries, identifying the ten countries with the highest number of sufferers. China, India, and the United States rank in the top three with 116.4 million, 77 million, and 31 million sufferers. Indonesia is ranked 7th among the ten countries with the highest number of sufferers, 10.7 million. Indonesia is the only country in Southeast Asia on the list so it can be estimated that Indonesia's contribution to diabetes cases in Southeast Asia (IDF, 2019; Kementerian Kesehatan RI., 2020).

Data from RISKESDAS 2013 shows that the number of Diabetes Mellitus (DM) sufferers in Indonesia is huge. With the possibility of an increase in the number of people with DM, it will be a cumbersome burden to be handled by specialist/sub-specialist doctors or even by all existing health workers (Soelistijo et al., 2015).

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Based on the epidemiological data above, diabetes is a problem in various countries worldwide. Therefore, it is important to explore new therapeutic modalities for diabetes mellitus to reduce the increasing prevalence of diabetes mellitus. Currently, functional foods high in antioxidants are being developed to address the problem of metabolic syndrome, which neutralize and accelerate the degradation of free radical compounds to prevent damage to cell macromolecular components. One of the high-antioxidant functional food products developed is fermented foods and beverages such as yogurt containing probiotics from Lactic Acid Bacteria (LAB) (Rustanti et al., 2019).

Lactic Acid Bacteria are gram-positive motile bacteria with coccus or bacilli forms that do not form spores and are facultative anaerobic without catalase activity. Broadly, LAB has been widely used in the food world, where bacteria act as natural preservatives because they produce various metabolites such as *organic acids, bacteriocins, primary metabolites, hydrogen peroxide, diacetyl, carbon dioxide,* and *acetaldehyde* as a result of the fermentation process. The fermentation process found in lactic acid bacteria is a group of homofermentative and heterofermentative lactic acid bacteria. Homofermentative lactic acid bacteria only produce lactic acid, while heterofermentative lactic acid bacteria produce various other compounds, namely acetate, ethanol, carbon dioxide, and lactic acid (Damayanthi et al., 2014; Gawad et al., 2015; Usmiati, 2012).

Many studies have explored the various benefits of lactic acid bacteria. Lactic acid bacteria obtained from tubers of canna (*Canna edulis*) and Kimpul (*Xanthosoma sagittifolium*) have antidiabetic activity through the activity of inhibiting the α-glucosidase enzyme. The inhibitory activity of the α-glucosidase enzyme is caused by the production of exopolysaccharide and inulin compounds by lactic acid bacteria isolated from tubers (Nurhayati et al., 2017).

Another study conducted by Kwun et al. (2020) reported results that were in line with the results of Nurhayati et al. (2017). Kwun et al. (2020) reported that lactic acid bacteria isolated from traditional Korean fermented foods exhibited antidiabetic activity by the same mechanism. The isolated lactic acid bacteria inhibit the α-glucosidase enzyme by 3.91 ± 0.25%. Therefore, lactic acid bacteria from food and drink also have the potential to have α-glucosidase enzyme inhibitory activity. Exploring other fermented foods and beverages with promising antidiabetic effects is important. One of the products, also a fermented product widely researched now, is fermented soy milk, also known as Soyogurt (Kwun et al., 2020; Nurhayati et al., 2017).

Soygurt is an innovative production of yogurt which is made from soy milk. Soygurt has the advantage of higher antioxidant activity than plain yogurt in preventing lipid oxidation. Besides lactic acid bacteria, which act as probiotics, soy milk is rich in beneficial compounds such as essential fats, amino acids, minerals, isoflavones, and saponins. Isoflavones are flavonoid compounds in the form of glycosides such as *genistin, daidzin*, and *glycine*. These isoflavones have high antioxidant activity, are anti-inflammatory, and prevent the formation of atherosclerotic plaques (Putra, 2015; Rustanti et al., 2019).

Several studies have been conducted to explore the pharmacological activity of lactic acid bacteria in Soygurt. One is the research by Nirmagustina et al. (2017), who reported that Soygurt has antibacterial activity against Escherichia coli and Staphylococcus aureus. Likewise, Rustanti et al. (2019) explored other benefits of soy antioxidants and anti-diabetic effects in vivo using pre-metabolic syndrome mice. The results of the study showed that giving Soygurt at a dose of 3.4 g/200 g body weight for four weeks did not significantly reduce fasting blood sugar, serum insulin, and malondialdehyde (MDA) levels.

Based on the background description above, the researchers were interested in examining the antidiabetic activity of Soygurt more deeply with a different approach, namely in vitro, considering previous research by Rustanti et al. (2019) with in vivo approach did not show significant antidiabetic activity. In comparison, other studies regarding lactic acid bacteria from fermented products have been reported to have antidiabetic activity with an in vivo approach through inhibition of the α-glucosidase enzyme. Therefore, these researchers are interested in exploring the antidiabetic effect of Soygurt in vitro through analysis of α-glucosidase enzyme inhibition activity, where several other fermented products have been reported to have α-glucosidase enzyme inhibitory activity.

Method

This study was an experimental study with a Pre and Post-test only control group design to assess the characteristics of the yogurt used in this study and the activity of inhibiting the α-glucosidase enzyme (Notoatmodjo, 2012). The sample in this study was soybean seeds obtained from one of the traditional markets in Brastagi Regency, North Sumatra. Meanwhile, the yogurt starter used in this study was two different types: commercially packaged yogurt starter and mixed yogurt starter.

Research variables are concepts with varying values so researchers can understand that variables can be distinguished into different types or categories (Suwarno & Nugroho, 2023). The variables in this study consist of independent, dependent, and control variables. The independent variable of the research is the type of yogurt starter for Soygurt. The study's dependent variable was the number of lactic acid bacteria colonies, morphological and physiological characteristics, antioxidant activity and α-glucosidase enzyme inhibition. The control variables were the temperature of making yogurt, the duration of yogurt, and the amount of yogurt starter.

Tools and Materials

Autoclave, micropipette, Erlenmeyer, measuring cup, beaker glass, Durham tube, test tube, measuring flask, hotplate magnetic stirrer, filter, grinder, thermometer, object glass, cover glass, dropping pipette, analytical balance, digital balance, microtube, micropipette tip, spreader, petri dish, centrifuge, incubator, laminar airflow, microscope, cuvette, UV-Vis spectrophotometer. Soybean seeds, yogurt starter, gelatin powder, sodium bicarbonate powder, distilled water, hydrogen peroxide, Man-Rogosa-Sharpe (MRS) Broth and MRS Agar media, calcium carbonate powder, ρNPG solution (5 mM), α-glucosidase solution, potassium phosphate buffer, sodium hydroxide, and acarbose.

Tools and objects must be arranged when they are put into the autoclave. So that all parts of the device can be exposed to steam evenly and thoroughly with a pressure of 1.5 kg/cm2 at 12˚C for 15 minutes using an autoclave. Tools and objects must be wrapped to prevent recontamination after leaving the autoclave (Michiko et al., 2020).

Soygurt Making

Making Soygurt begins with making soy milk using the Illinois method. Soy milk in this study was prepared by cleaning the soybeans and then soaking them in a 0.5% sodium bicarbonate ($NaHCO_3$) solution with a ratio of 1:3 for 8 hours. Soybeans were drained and blanched (enzyme inactivation process) for 30 minutes with a balance of 1:3. Then, the soybean skin was removed and washed with water and drained. Furthermore, the soybeans are put into the grinder to be crushed, and the juice is taken. The soybean extract is extracted by adding hot water (80-100cc) with a ratio of 1:6. This refinement process was carried out for seven minutes, after which it was filtered to obtain soybean juice. After that, the soybean extract was heated until it reached a temperature of 50°C, and then added 5% sucrose (w/v) slowly. The soybean extract is pasteurized for 15 minutes at 80-90°C with occasional stirring (Gawad et al., 2015; Rustanti et al., 2019).

After the pasteurization process, the soy milk is put into laminar airflow to prevent contamination. Adding 0.5% powdered gelatin to the soy milk at 70°C to maintain the stability and texture of the Soygurt and occasionally stirring until the soy milk reaches 45°C is done by inoculating the yogurt starter. Inoculation was carried out using 5% starter yogurt from two different types of starters, namely starter on the commercial market (Yakult) and mixed starter *(Lactobacillus bulgaricus: Streptococcus thermopilus*). After being inoculated, the Soygurt was stirred until homogeneous and incubated for five hours. Soygurt should be stored at 4°C for 17 hours (Nirmagustina & Wirawati, 2017; Zuhaira et al., 2018).

Preparation of MRS Broth Media and MRS Agar

MRS Broth media was prepared by dissolving 5.515 grams of MRS Broth powder in 100 ml of distilled water. Then this mixture is heated and homogenized simultaneously using a hotplate magnetic stirrer. Meanwhile, MRS Agar was prepared the same way but with a larger amount of MRS agar powder, 6,715 grams, dissolved in 100 ml of distilled water. Before use, the MRS agar medium will be autoclaved together with the MRS Broth medium.

Bacterial Enrichment of Samples

Enrichment of lactic acid bacteria from Soygurt samples was carried out using MRS broth media. A total of 1 ml of the Soygurt sample was diluted with 9 ml of MRS broth in a test tube, then homogenized using a vortex and incubated at 37°C for 18-24 hours under anaerobic conditions (Fachrial et al., 2018; Fachrial & Harmileni, 2018; Putri et al., 2020).

The number of colonies in this study was counted using the Total Plate Count (TPC) method through multilevel dilution. A 100 µL of enriched Soygurt sample was diluted with 900 µL of sterile MRS Broth in a 1.5 ml microtube to make a 10-1 dilution. Then, 100 µL of the 10-1 Soygurt sample was diluted with 900 µL of sterile MRS Broth to make a 10-2 dilution. Dilution, in the same way, was then continued to make the dilution level up to 10-7. After the multilevel dilution process was carried out, 100 µL of the mixture from each dilution series (10-1 to 10-7) was cultured onto the MRS Agar medium, which had been added CaCO3 (0.75-1%) by spreading it on the surface of the MRS Agar using a spreader (Pour plates). Each Petri of MRS Agar was then labeled according to the concentration series of the Soygurt mixture. Then, all the Petri dishes were incubated for 18 - 24 hours. After incubation, observations were made to count the number of bacterial colonies on MRS Agar

media, which had only 30-300 territories, and the total number of LAB colonies was calculated using the following formula (Putri et al., 2020; Sitohang et al., 2021; Sood et al., 2016; Ulfa et al., 2019).

$$\frac{\text{CFU}}{\text{ml}} = \frac{\text{Number of Colonies}}{\text{The Volume Stretched out in ml}} \text{ x Dilution Factor} \quad (1)$$

Purification of Lactic Acid Bacteria

Lactic acid bacteria were purified by taking colonies from MRS Agar in each dilution series. Lactic acid bacteria will form a clear zone around the territory. A total of 1 settlement from each dilution series was then cultured back into MRS Agar using the quadrant streak plate method to obtain Pure Lactic Acid Bacteria isolates. Then this pure isolate is then inoculated on slant agar media (Irza et al., 2021; Nasution et al., 2020; Putra, 2015).

Characterization of Lactic Acid Bacteria

Lactic acid bacteria were characterized by assessing the morphological and physiological characteristics of Soygurt samples. Assessment of morphological traits was carried out by macroscopic and microscopic observations of colonies on MRS Agar 1% $CaCO_3$ media. In contrast, the physiological aspects of lactic acid bacteria were tested by testing the type of fermentation and catalase from territories on slant agar (Fachrial & Harmileni, 2018; Siburian et al., 2021; Syukur et al., 2014, 2015).

Antibacterial Activity Screening

Screening for Antibacterial Activity in this activity will be carried out by Making Nutrient Agar (NA) Media, Sterilizing Equipment and Materials, Making Suspension of *Staphylococcus aureus* Bacteria, and Testing Antibacterial Activity with the Disc Diffusion Method.

Preparation of Cell-Free Supernatant

Cell-free supernatant was carried out by selecting a sample of Soygurt to be tested for the activity of inhibiting the alpha-glucosidase enzyme. A total of 5 ml of MRS Broth, which lactic acid bacteria had inoculated, was then incubated for 18 - 24 hours at 37°C. Then the MRS Broth was centrifuged at 10,000 RPM for 30 minutes at 4°C. The broth will separate into supernatant and cell biomass where the supernatant part will be used to analyze the inhibitory activity of the alpha-glucosidase enzyme (Nurhayati et al., 2017; Syukur et al., 2014).

Antioxidant Activity Test with DPPH Method

Samples with different concentrations from each treatment group were added to a volume equivalent to 0.05 mM DPPH methanolic solution. The mixture of the two was then left to react at room temperature for 30 minutes in a dark place. Methanol was used as a control. Repetition was carried out two times for each sample group. The absorbance (A) was measured at a wavelength of 518 nm, and the percent inhibition was calculated using the following equation:

$$\% \text{ Inhibisi} = [(Ao - A1)/Ao] \times 100 \quad (2)$$

Ao is the control absorbance, and A1 is the absorbance obtained from the sample (Selvi et al., 2016).

Alpha Glucosidase Enzyme Inhibition Activity Test

Determination of α-glucosidase inhibitory activity was based on the inhibition of ρ-nitrophenol formation from ρ-nitrophenyl- α-D-glucopyranoside (ρNPG) substrate hydrolyzed by α-glucosidase. ρNPG (5 mM) and α-glucosidase solutions from B. stearothermophilus (0.2 U/mL) were diluted in potassium phosphate buffer (0.1 M, pH 6.8). The inhibition test was carried out by mixing cell-free supernatant (50 µL) and α-glucosidase solution (50 µL) mixed and incubated at 37°C. After 5 minutes, 50 µL of ρNPG solution was added, and the reaction was allowed to run at 37°C for 25 minutes. Then, the response was stopped by adding 100 µL of 0.1 M sodium carbonate. After that, the absorbance was measured at a wavelength of 405 nm. α-glucosidase inhibitory activity (%) was determined according to the following equation (acarbose was used as a positive control) (Kwun et al., 2020).

$$\% \text{ α-glucosidase enzyme inhibitors} = (A-B)/B \text{ x } 100\% \quad (3)$$

A is the absorbance with α-glucosidase without the sample, while B is the absorbance with the sample and α-glucosidase.

Molecular Identification of the 16S rRNA Gene

DNA from BAL isolates with the best inhibition and inhibition of α-glucosidase enzymes were isolated using a bacterial DNA isolation kit (Gene Aid). The primers used were universal 27F: 5'-AGAGTTTGATCCTGGCTCAG-3' and 1492R: 5'-CTACGGGCTACCTTGTTACGA-3'. The total volume of the PCR reaction mixture was 25µL. PCR machine conditions were: pre-denaturation at 95°C for 5 minutes, denaturation at 94°C for 1 minute, annealing at 56°C for 1 minute, extension at 72°C for 1.5 minutes, and final extension at 72°C for 5 seconds. DNA templates and PCR products were electrophoresed with 1.5% agarose gel containing EtBr. Electrophoresis results were seen with Gel Doc (Biorad) (Kwun et al., 2020).

The nucleotide sequence of the purified sample was determined by sending the purified sample to

Macrogen, Korea, accompanied by a one-way reading by reverse primer. The data sequences obtained were then analyzed using the BLAST program by comparing database searches found on the NCBI site. Alignment sequences using The ClustalW2 (Siburian et al., 2021).

Data Analysis

All data in this study were analyzed using IBM SPSS 25. The research data including the number of colonies, morphological and physiological characteristics, and the percentage of α-glucosidase enzyme inhibition, were analyzed using narrative, tabular, and graphical descriptive statistics (Ghozali, 2018). After performing descriptive statistical analysis, data analysis was then continued with inferential statistics to compare the average percentage of α-glucosidase inhibition in cell-free supernatant samples from lactic acid bacteria in Soygurt against positive controls in the form of acarbose. The type of inferential statistical analysis is chosen based on the distribution of the data, if the data is normally distributed, then a parametric statistical analysis is performed. However, non-parametric statistical analysis is performed if the data distribution is not normal.

Result and Discussion

This study used a sample of Soygurt made by itself through the fermentation process of soy milk with two different pieces of lactic acid bacteria starter, namely from a commercial yogurt starter in the form of Yakult and a single yogurt starter from 5% *Lactobacillus acidophilic* bacteria and the results of the fermentation of soy milk can be seen in the following this Figure 1.

Figure 1 shows that this study had two different Soygurt samples obtained through Yakult yogurt starter and 5% *Lactobacillus acidophilic*. Then, the samples were labeled SG1 for 5% *Lactobacillus acidophilic* yogurt starter and SG2 for Yakult commercial yogurt starter.

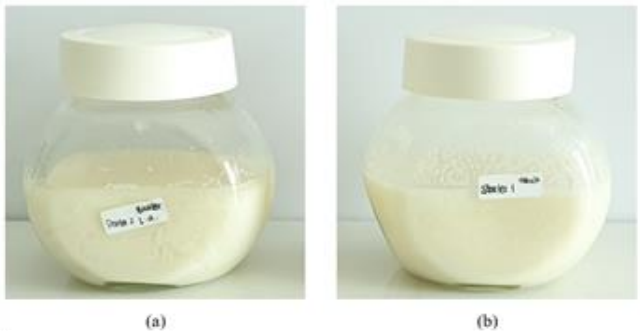


Figure 1. Fermented Soygurt with 5% *Lactobacillus Acidophilic* yogurt starter (a) and Yakult (b)

Isolation and Counting of Lactic Acid Bacteria Colonies

Soygurt samples that have been fermented by 5% *Lactobacillus Acidophilic* yogurt starter and Yakult are first subjected to an enrichment process so that they can be isolated and counted colonies. The results of the Soygurt sample enrichment can be seen in Figure 2.

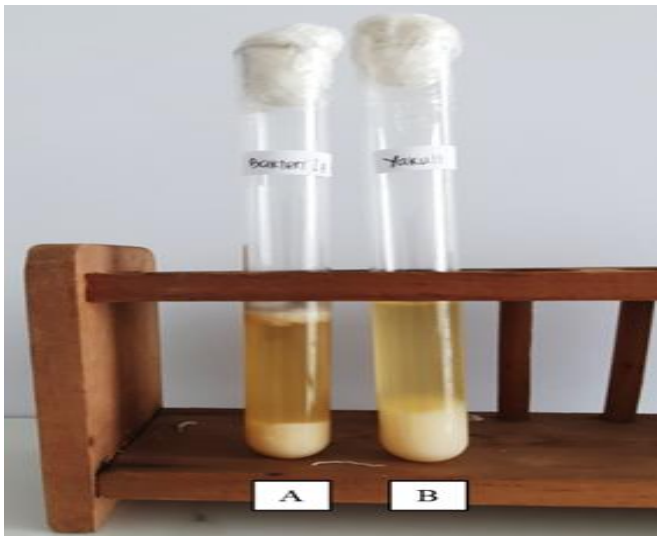


Figure 2. Enrichment of Soygurt samples with 5% *Lactobacillus Acidophilic* yogurt starter (a) and Yakult (b)

The results of the enrichment of each sample of Soygurt with a different starter, then the number of colonies was calculated using the Total Plate Count (TPC) method through Multilevel Dilution, and the results of counting the number of colonies of lactic acid bacteria in each sample can be seen in table 1.

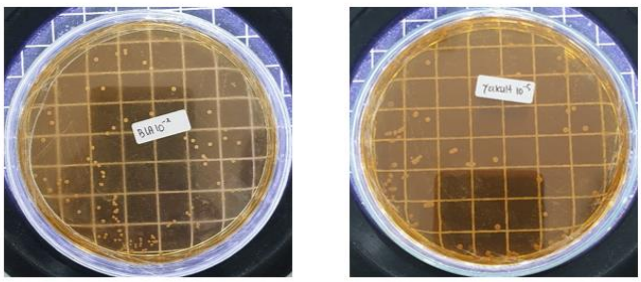
Table 1. Number of Lactic Acid Bacteria Colonies in Soygurt Samples

Sample	Dilution	Number of Colonies	Colony Forming Unit per millilitre (CFU/ ml)
SG1	10^{-2}	80	8.00×10^{4}
SG2	10^{-5}	50	5.00×10^{7}

From the data in Table 1, it can be seen that the number of colonies in the Soygurt sample made with 5% *Lactobacillus acidophilic* (SG1) yogurt starter showed a lower number of colonies compared to the Soygurt sample with Yakult starter where the number of lactic acid bacteria colonies in the Soygurt sample with 5% *Lactobacillus acidophilic* yogurt starter (SG1) was 8.00 x 104 CFU/ml, while in the Soygurt selection with Yakult starter (SG2), it was 5.00 x 107 CFU/ml.

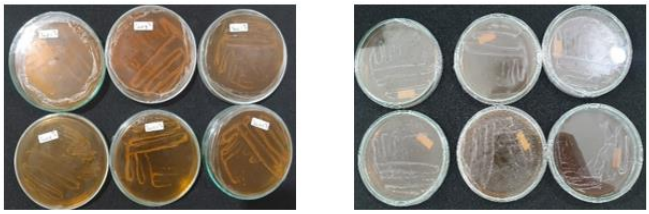
An overview of the colonies formed when calculating the number of colonies in each Soygurt sample using the TPC method can be seen in Figure 3. Then, the lactic acid bacteria colonies growing on MRS Agar media in the TPC method were purified by the four-quadrant method into 6 different Petri dishes, and

the purification results of the lactic acid bacteria colonies from each Soygurt sample can be seen in Figure 4.



(a) (b)

Figure 3. Lactic Acid Bacteria Colonies on MRS Agar Media from Soygurt Starter Yogurt *Lactobacillus Acidophilic* 5% (a) and Yakult (b)



(a) (b)

Figure 4. Purification of Lactic Acid Bacteria in Soygurt with *Lactobacillus Acidophilic* yogurt starter 5% (a) and Yakult (b)

From the purification results of the lactic acid bacteria above, one isolate was taken to examine the characteristics of lactic acid bacteria, namely morphological and physiological characteristics.

Characteristics of Lactic Acid Bacteria

Six isolates of lactic acid bacteria from each type of yogurt were examined for their morphological and physiological characteristics. The morphological characteristics of the lactic acid bacteria include macroscopic and microscopic features of the bacteria, while the physiological characteristics that are assessed are the catalase and fermentation properties of the lactic acid bacteria from each Soygurt sample. The morphological characteristics of the six lactic acid bacteria isolates from SG1 and SG2 showed macroscopically in isolated samples one to six. All of them had a round shape, the color of the colonies was light yellow, the edges of the colonies were flat and the elevations were slightly convex. And the results for microscopy have the form of bacilli, are violet in color, and have a positive weight.

From the results of examining the morphological characteristics of lactic acid bacteria from Soygurt with 5% *Lactobacillus acidophilic* yogurt and Yakult starter, it showed the same description of lactic acid bacteria where the macroscopic appearance of the colonies was round with light yellow color, flat edges, and slightly convex. Then, these colonies were examined microscopically with gram staining and found a violet-colored picture of the bacilli, which indicated a view of gram-positive bacilli, and a microscopic image of lactic acid bacteria with gram staining can be seen in Figure 5.

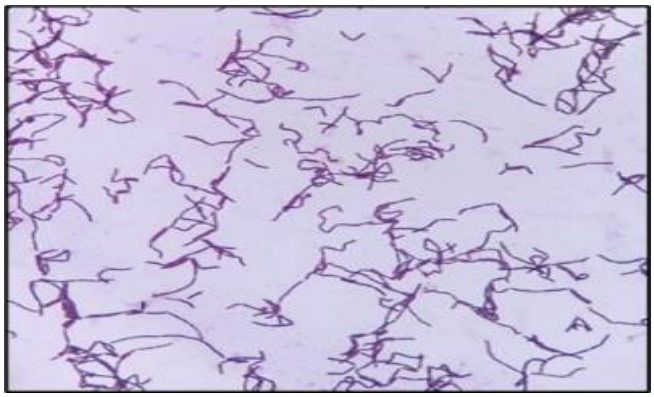


Figure 5. Microscopic Appearance of one isolate from Soygurt with 5% *Lactobacillus Acidophilic* Yogurt starter. Staining: Gram Staining; Magnification: 1000x (immerse oil)

The results of the physiological characteristics of lactic acid bacteria isolates from the two types of Soygurt with different starters were the same, where the lactic acid bacteria isolate in the two Soygurt samples were lactic acid bacteria that did not have catalase activity with a homofermentative type of fermentation. An overview of the types of fermentation of lactic acid bacteria using Durham tubes in all isolates can be seen in Figure 6.

Figure 6. Description of Lactic Acid Bacteria fermentation activities from Soygurt samples

Figure 6 shows that gas bubbles did not form in the Durham tube, which was placed in a test tube containing MRS Broth media. This indicated that all lactic acid bacteria isolate from the two types of yogurts showed homofermentative activity.

Antibacterial Activity Screening

A total of six purified isolates for each type of Soygurt were cultured again in MRS Broth. MRS Broth which has been overgrown with lactic acid bacteria isolates from each Soygurt, was tested for antibacterial activity against Staphylococcus aureus bacteria using the disc diffusion method, and the results of the antibacterial test can be seen in Figure 7. It can be seen that no inhibition zones were formed around the disc paper, which has been diffused by MRS Broth containing lactic acid bacteria isolates from Soygurt with a different starter. This indicates that lactic acid bacteria isolate from each yogurt did not show antibacterial activity, especially against gram-positive bacteria, namely Staphylococcus aureus.

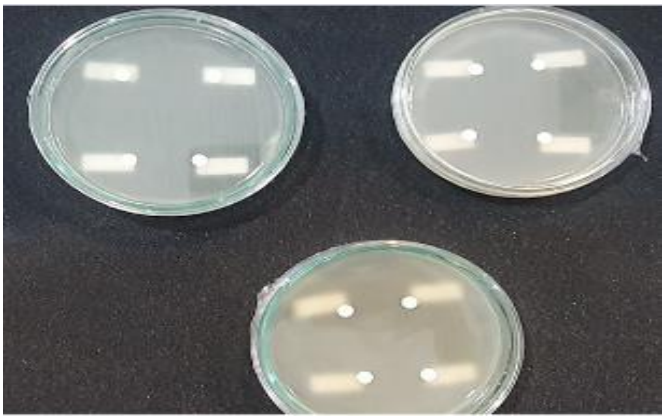


Figure 7. Antibacterial Activity Test of Lactic Acid Bacteria Isolate from Soygurt with different starters against Staphylococcus aureus

Antioxidant Activity

In addition to antibacterial activity, this study evaluated the pharmacological effects of lactic acid bacteria isolates from Soygurt with 5% *Lactobacillus acidophilic* starter, namely antioxidant activity. Antioxidant activity was assessed on the cell-free supernatant obtained from Soygurt with 5% *Lactobacillus acidophilic* starter using the DPPH Scavenging method. An overview of the results of the analysis of antioxidant activity from cell-free supernatant obtained from Soygurt with 5% *Lactobacillus Acidophilic* starter can be seen in Table 2.

The data in Table 2 shows that the highest absorbance value was found in the pro-oxidant control group, which contained MRS Broth, Methanol, and DPPH media, which was 1.020. In contrast, the smallest absorbance was found in the solvent control, which only had methanol. In the antioxidant activity test on Soygurt isolate, the absorbance values varied from 0.422, 0.595, 0.650, 0.672, and 0.710. The highest is 0.744. From the absorbance values obtained, the description of antioxidant activity is described in terms of the inhibition percentage of each sample against the pro-oxidant control group. So that the highest absorption value in this study was found in the solvent control group, namely 99.51%. Meanwhile, the percent inhibition value of this study sample in the form of cell-free supernatant obtained from six Soygurt isolates with 5% *Lactobacillus acidophilic* starter ranged from 27.06-58.63%.

Table 2. Antioxidant Activity of Lactic Acid Bacteria Isolate from Soygurt with 5% *Lactobacillus Acidophilic* Starter

Sample Group	Absorbance	% inhibition DPPH
Pro-Oxidant Control	1.020	Ref
Solvent Control	0.005	99.51
SG1-Isolate 1	0.422	58.63
SG1-Isolate 2	0.719	29.51
SG1-Isolate 3	0.672	34.12
SG1-Isolate 4	0.744	27.06
SG1-Isolate 5	0.595	41.67
SG1-Isolate 6	0.650	36.27

Alpha Glucosidase Inhibitor Activity

Furthermore, this study also evaluated the activity of alpha-glucosidase inhibitors as an antidiabetic effect. Alpha-glucosidase inhibitor activity was assessed from cell-free supernatant obtained from six Soygurt isolates with 5% *Lactobacillus acidophilic* starter. The results of the analysis of alpha-glucosidase inhibitor activity in the samples can be seen in Table 3.

Table 3. Analysis of Alpha Glucosidase Inhibitor Activity

Sample Group	Absorbance		Porsen Inhibitor	
	I	II	I	II
Media Contro (A0)	0	0		
Enzyme Control (A1)	0.091	0.07		
Sample Control (AI0)	0.005	0		
Soygurt strater Lactobacillus acidophilus (AI1)	0.056	0.03	43.96	57.14

From the data in Table 3, it can be seen that the percentage value of alpha-glucosidase enzyme inhibition possessed by Cell-Free Supernatant Isolate Lactic Acid Soygurt Bacteria with 5% *Lactobacillus acidophilic* starter in the first repetition was 43.96%, and in the second repetition was 57.14%. From the value obtained on the repeated test of the activity of the alpha-glucosidase enzyme inhibitor owned by Cell-Free Supernatant Isolate Lactic Acid Bacteria Soygurt with 5% *Lactobacillus acidophilic* Starter, the average magnitude of the percent inhibition of the alpha-glucosidase enzyme owned by Cell-Free Supernatant Isolate Acid Bacteria Soygurt lactate with 5% *Lactobacillus acidophilic* starter is 50.55%.

Molecular Identification of Lactic Acid Bacteria Isolates in Soygurt

In this study, bacterial isolation was performed in Soygurt with 5% *Lactobacillus acidophilic* starter and Yakult. Soygurt with Yakult starter was the control in this study, while the samples in this study that were analyzed starting from antibacterial, antioxidant, and antidiabetic activities were lactic acid bacteria isolates in Soygurt with 5% *Lactobacillus acidophilic* starter. Thus, molecular identification was performed on lactic acid bacteria isolates with 5% *Lactobacillus acidophilic* starter.

Molecular identification of lactic acid bacteria isolates in Soygurt with 5% *Lactobacillus acidophilic* starter was carried out by isolating genetic material in the form of DNA from the bacteria; the isolated results of lactic acid bacteria were then analyzed quantitatively (Nanodrop) and qualitatively (gel electrophoresis), then amplification was carried out. Genetic material using the PCR amplification method with (2x) MyTaq HS Red Mix, and the genetic material amplification results were evaluated for the composition of the genetic material through Bi-Directional Sequencing. As a first step in the molecular identification of lactic acid bacteria genetic material, the quantitative analysis of the genetic material isolated from lactic acid bacteria isolates from this study can be seen in Table 4.

Table 4. Results of Quantitative Analysis of Genetic Material Isolates

Sample	Concentration (ng/µL)	A260/280	A260/230	Volume (µL)
SG1-Isolate 1	43.3	1.93	2.03	35

From the data in Table 4, it can be seen that 35 µL samples of lactic acid bacteria isolated from Soygurt with 5% *Lactobacillus acidophilic* starter isolated 43.3 ng/ µL of genetic material (genomic DNA), with values A260/280 and A260/230 of 1.93 and 2.03. Then, the results of the isolation of genetic material from lactic acid bacteria were also qualitatively analyzed for the content of the genetic material through gel electrophoresis, and the effects of gel electrophoresis can be seen in the gel photo in Figure 8.

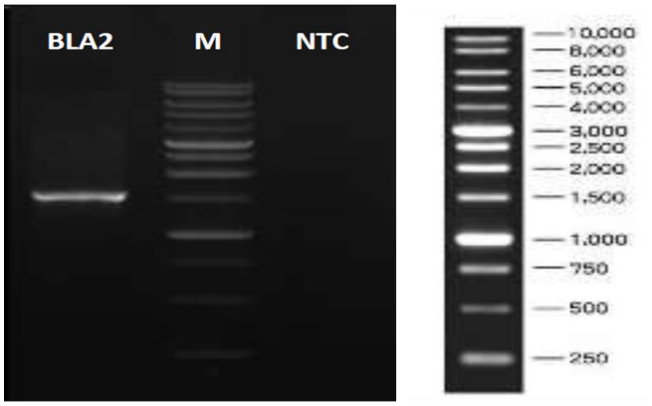


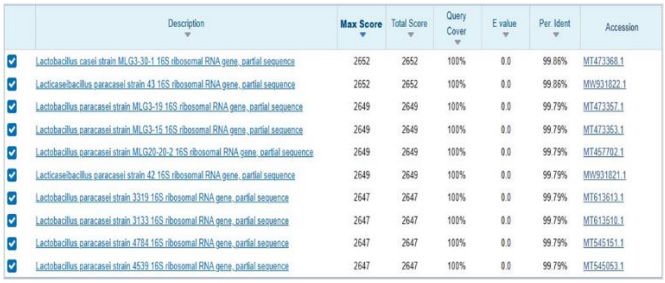
Figure 8. Gel electrophoresis results from genetic material isolation of lactic acid bacteria in Soygurt with Lactobacillus acidophilic starter

From the data above, it can be seen that the genetic material isolated from lactic acid bacteria isolates in Soygurt from 5% *Lactobacillus Acidophilic* starter is a DNA genome with a length of between 1,000-1,500 BP (base pairs), where the isolated DNA genome corresponds to the type of primer used and used to isolate genomic DNA from bacteria, namely 27F – 1492R. The DNA genome isolated from lactic acid bacteria is then analyzed for the composition of its genetic material through the bidirectional sequencing method. The results of this analysis can be seen in Figure 9.

Sequence Assembly 1442bp

```
1     CTATACTGCA GTCGTACGAG TTCTCGTTGA TGATCGGTGC TTGCACCGAG ATTCAACATG
61    GAACGAGTGG CGGACGGGTG AGTAACACGT GGGTAACCTG CCCTTAAGTG GGGGATAACA
121   TTTGGAAACA GATGCTAATA CCGCATAGAT CCAAGAACCG CATGGTTCTT GGCTGAAAGA
181   TGGCGTAAGC TATCGCTTTT GGATGGACCC GCGGCGTATT AGCTAGTTGG TGAGGTAATG
241   GCTCACCAAG GCGATGATAC GTAGCCGAAC TGAGAGGTTG ATCGGCCACA TTGGGACTGA
301   GACACGGCCC AAACTCCTAC GGGAGGCAGC AGTAGGGAAT CTTCCACAAT GGACGCAAGT
361   CTGATGGAGC AACGCCGCGT GAGTGAAGAA GGCTTTCGGG TCGTAAAACT CTGTTGTTGG
421   AGAAGAATGG TCGGCAGAGT AACTGTTGTC GGCGTGACGG TATCCAACCA GAAAGCCACG
481   GCTAACTACG TGCCAGCAGC CGCGGTAATA CGTAGGTGGC AAGCGTTATC CGGATTTATT
541   GGGCGTAAAG CGAGCGCAGG CGGTTTTTTA AGTCTGATGT GAAAGCCCTC GGCTTAACCG
601   AGGAAGCGCA TCGGAAACTG GGAAACTTGA GTGCAGAAGA GGACAGTGGA ACTCCATGTG
661   TAGCGGTGAA ATGCGTAGAT ATATGGAAGA ACACCAGTGG CGAAGGCGGC TGTCTGGTCT
721   GTAACTGACG CTGAGGCTCG AAAGCATGGG TAGCGAACAG GATTAGATAC CCTGGTAGTC
781   CATGCCGTAA ACGATGAATG CTAGGTGTTG GAGGGTTTCC GCTCTTCAGT GCCGCAGCTA
841   ACGCATTAAG CATTCCGCCT GGGGAGTACG ACCGCAAGGT TGAAACTCAA AGGAATTGAC
901   GGGGGCCCGC ACAAGCGGTG GAGCATGTGG TTTAATTCGA AGCAACGCGA AGAACCTTAC
961   CAGGTCTTGA CATCTTTTGA TCACCTGAGA GATCAGGTTT CCCCTTCGGG GGCAAAATGA
1021  CAGGTGGTGC ATGGTTGTCG TCAGCTCGTG TCGTGAGATG TTGGGTTAAG TCCCGCAACG
1081  AGCGCAACCC TTATGACTAG TTGCCAGCAT TTAGTTGGGC ACTCTAGTAA GACTGCCGGT
1141  GACAAACCGG AGGAAGGTGG GGATGACGTC AAATCATCAT GCCCCTTATG ACCTGGGCTA
1201  CACACGTGCT ACAATGGATG GTACAACGAG TTGCGAGACC GCGAGGTCAA GCTAATCTCT
1261  TAAAGCCATT CTCAGTTCGG ACTGTAGGCT GCAACTCGCC TACACGAAGT CGGAATCGCT
1321  AGTAATCGCG GATCAGCACG CCGCGGTGAA TACGTTCCCG GGCCTTGTAC ACACCGCCCG
1381  TCACACCATG AGAGTTTGTA ACACCCGAAG CCGGTGGCGT AACCCTTTAG GGAGCGAGCC
1441  GT
```

Figure 9. Results of Bi-Directional Sequencing of Lactic Acid Bacteria in Soygurt Starter *Lactobacillus Acidophilic* 5%



Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
Lactobacillus casei strain MLG3-38-1 16S ribosomal RNA gene, partial sequence	2652	2652	100%	0.0	99.86%	MT473368.1
Lacticaseibacillus paracasei strain 43 16S ribosomal RNA gene, partial sequence	2652	2652	100%	0.0	99.86%	MW931822.1
Lactobacillus paracasei strain MLG3-19 16S ribosomal RNA gene, partial sequence	2649	2649	100%	0.0	99.79%	MT473367.1
Lactobacillus paracasei strain MLG3-15 16S ribosomal RNA gene, partial sequence	2649	2649	100%	0.0	99.79%	MT473363.1
Lactobacillus paracasei strain MLG20-20-2 16S ribosomal RNA gene, partial sequence	2649	2649	100%	0.0	99.79%	MT457702.1
Lacticaseibacillus paracasei strain 42 16S ribosomal RNA gene, partial sequence	2649	2649	100%	0.0	99.79%	MW931821.1
Lactobacillus paracasei strain 3319 16S ribosomal RNA gene, partial sequence	2647	2647	100%	0.0	99.79%	MT613613.1
Lactobacillus paracasei strain 3133 16S ribosomal RNA gene, partial sequence	2647	2647	100%	0.0	99.79%	MT613510.1
Lactobacillus paracasei strain 4784 16S ribosomal RNA gene, partial sequence	2647	2647	100%	0.0	99.79%	MT545151.1
Lactobacillus paracasei strain 4539 16S ribosomal RNA gene, partial sequence	2647	2647	100%	0.0	99.79%	MT545053.1

Figure 10. Comparison of DNA Genomes of Lactic Acid Bacteria Isolate from Soygurt in the NCBI Database (BLAST Analysis)

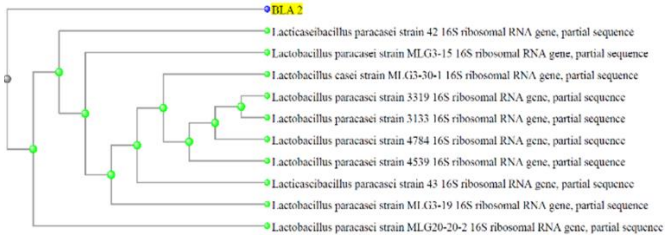


Figure 11. Phylogenetic Tree of Lactic Acid Bacteria Isolate from Soygurt (BLA2)

Figure 9 shows that the genetic material or genomic DNA isolated from lactic acid bacteria in yogurt with 5% *Lactobacillus acidophilic* starter consists of 1443 BP, which can be seen in the picture above. The results of these sequences were then compared with the nucleotide base sequences in the bacterial genome database in the NCBI Database (BLAST Analysis). The results of comparing lactic acid bacteria genomic DNA sequences can be seen in Figure 10.

From Figure 11, it can be seen that the DNA genome isolated from lactic acid bacteria isolates in Soygurt has a similar genomic DNA composition to *Lactobacillus casei* and *Lactobacillus paracasei* bacteria with an identity percentage ranging from 99.79-99.86%. Based on the data obtained, the phylogenetic pedigree of lactic acid bacteria is accepted, as shown in Figure 11.

Discussion

The results of the above research clearly show that the yogurt starter used in this study can affect the number of colonies formed per milliliter of Soygurt, where the effects of counting the number of lactic acid bacteria in Soygurt with 5% *Lactobacillus acidophilic* yogurt starter is 8.00 x 104 CFU/ml and counting the number of lactic acid bacteria in Soygurt with Yakult starter was 5.00 x 107 CFU/ml.

Meanwhile, the identification of lactic acid bacteria in both types of yogurts showed the same morphological and physiological characteristics. In contrast, the identification results showed lactic acid bacteria in the form of gram-positive bacilli did not have catalase activity and were homofermentative. Furthermore, lactic acid bacteria isolated from Soygurt with 5% *Lactobacillus acidophilic* yogurt starter did not show antibacterial activity against gram-positive bacteria in the form of *Staphylococcus aureus*, but lactic acid bacteria isolated from Soygurt with 5% *Lactobacillus acidophilic* yogurt starter showed antioxidant activity and alpha-glucosidase inhibition.

Soygurt is an innovative yogurt product derived from vegetable ingredients, namely soy milk. Soygurt is reported to have several benefits, one of which is antioxidant activity which can be related to the phytochemical content of the soy milk product itself, as well as to the presence of lactic acid bacteria and their metabolite products. Generally, Soygurt is made by fermenting soy milk using lactic acid bacteria such as *Streptococcus thermophiles* and *Lactobacillus bulgaricus*.

However, in this study, Soygurt was fermented with a type of thermophilic lactic acid bacteria, namely *Lactobacillus acidophilic*, where this type of lactic acid bacteria usually produces about 1.5-2.0% lactic acid but does not produce alcohol. Meanwhile, as a comparison, the Soygurt in this study also used a Yakult starter that includes thermophilic lactic acid bacteria but contains a different species of lactic acid, namely *The Lactobacillus Casei Subsp Casei Strain Shirota*. Thermophilic lactic acid bacteria are a type of lactic acid bacteria that will carry out the fermentation process at temperatures > 35°C (Surono & Hosono, 2011).

The difference in the results of the number of colonies in each type of Soygut starter cannot be separated from the kind of starter Soygurt used in this study; the number of colonies formed in Soygurt and Yakult starter shows a higher number of colonies; this is because the number of colonies formed from the start There are quite a lot of them in Yakult, namely 108 CFU/ml which has been standardized from production. Thus, the number of lactic acid bacteria colonies will be higher when compared to the Soygurt with *Lactobacillus acidophilic* starter.

Both Soygurt with 5% *Lactobacillus acidophilic* yogurt starter and Yakult showed lactic acid bacteria with similar characteristics. This cannot be separated from the content of lactic acid bacteria owned by each starter, namely lactic acid bacteria from the genus *Lactobacillus lactic* acid bacteria in the form of bacilli. In general, Lactic acid bacteria are gram-positive, do not form spores, are catalase-negative, are resistant to acidic conditions, and are facultative anaerobes. Therefore, this could explain why the lactic acid bacteria of the two types of yogurts have the appearance of gram-positive bacilli without catalase activity. Meanwhile, based on the fermentation pattern, lactic acid bacteria are divided into two groups, homofermentative and heterofermentative lactic acid bacteria. In this study, lactic acid bacteria isolated from each yogurt were homofermentative.

Homofermentative lactic acid bacteria consist of several genera of lactic acid bacteria, including *Lactobacilli* and most species of *Enterococci, Lactococci, Pediococci, Streptococci, Tetragenococci, and Vagococci*, which ferment hexoses by the Embden-Meyerhof (E-M) pathway and the fermented products contain only lactic acid without the effect such as alcohol, acetate, or carbon dioxide gas. This explains the reason for not forming gas in the Durham tube in all samples because the lactic acid bacteria used in this study as a sourdough starter are lactic acid bacteria from the genus *Lactobacillus*, where

these bacteria do not form other compounds besides lactic acid (homofermentative) (Hugenholtz et al., 2002; Todar, 2012; Widodo et al., 2012).

Analysis of the antibacterial activity of the Soygurt samples used in this study showed that Soygurt did not have antibacterial activity against the gram-positive *Staphylococcus aureus*. The results of this study are in contrast to the effects of previous research conducted by Nirmagustina et al. (2017). Nirmagustina et al. (2017) reported that Soygurt had antibacterial activity against *Escherichia coli* and *Staphylococcus aureus* through the contact test method. The difference in the results of this study was due to differences in the Soygurt starter used in this study and previous studies and differences in the antibacterial activity analysis method used.

In the previous study, Nirmagustina et al. (2017) used commercial yogurt starters on the market in the form of Cimory yogurt and Kin yogurt, which on the packaging stated the content of lactic acid bacteria in the form of *Streptococcus thermophiles* and *Lactobacillus bulgaricus*. Meanwhile, in this study, Soygurt was fermented using lactic acid bacteria in the form of *Lactobacillus Acidophilic* 5% and Yakult containing lactic acid bacteria in the form of *Lactobacillus Casei Subsp Casei Strain Shirota*. In addition, differences in research results can also be caused by differences in the antibacterial activity analysis method used, where in previous studies, the technique used was the contact test, while in this study, the disc diffusion met (Nirmagustina & Wirawati, 2017).

Although the results of the study showed that Soygurt using 5% *Lactobacillus acidophilic* starter did not have antibacterial activity, Soygurt using 5% *Lactobacillus acidophilic* starter had other pharmacological effects, one of which was an antioxidant effect. This study shows that Soygurt with 5% *Lactobacillus acidophilic* starter has antioxidant activity with a percentage range of DPPH inhibition between 27.06-58.63%.

The results of this study are in contrast to the results of a survey conducted by Rustanti et al. (2019) in vivo, who reported that giving Soygurt at a dose of 3.4 g/200 grBW for four weeks did not significantly reduce MDA levels, which an indicator of lipid oxidants in the body. Furthermore, the Soygurt used in their study had a smaller antioxidant activity of 6%.

The antioxidant activity of the bacteria possessed by lactic acid is related to the activity of the lactic acid bacteria that ferment the sample. Natania et al. (2019) reported that the lactic acid bacteria *Lactobacillus plantarum* as heterofermentative bacteria tends to have better antioxidant activity compared to *Lactobacillus acidophilic*, which are homofermentative bacteria. *Lactobacillus plantarum* is said to change the form of phenolic components such as gallic acid and ellagic acid through the activity of tannase enzymes (enzymes that catalyze tannin hydrolysis).

The complex tannin molecule is hydrolyzed to gallic acid and glucose, and then the gallic acid is decarboxylated to pyrogallol. Derived compounds from tannins that have lost glucose groups (aglycones), have more active hydroxyl groups (OH), which show higher antioxidant activity than the initial tannin compounds. Whereas *Lactobacillus acidophilic* tends to use phenolic compounds as substrate growth. Therefore, lactic acid bacteria which are homofermentative tend to use various phytochemical compounds in the fermented material and cause a reduction in the antioxidant activity of these phytochemical compounds.

This could explain the reason for the low DPPH inhibitory activity in soybeans studied by Rustanti et al. (2019) using a yogurt starter which was 10% higher, causing the number of lactic acid bacteria colonies in the yogurt sample to be higher (3.80s x 10^7 CFU/ml) compared to the current study using 5% *Lactobacillus acidophilic* yogurt starter (8.00 x 10^4 CFU/ml). As a result, this causes the antioxidant activity of Soygurt with 5% *Lactobacillus acidophilic* starter in this study to be higher than previous studies using 10% yogurt starter (Natania et al., 2019; Rustanti et al., 2019).

In addition to the antioxidant effect, in this study, lactic acid bacteria in Soygurt also showed alpha-glucosidase enzyme inhibitor activity. Inhibition of the alpha-glucosidase enzyme can interfere with the digestion and absorption of carbohydrates in the small intestine so that it can lower blood sugar levels. In this study, the average percentage of alpha-glucosidase inhibition by Soygurt with 5% *Lactobacillus acidophilic* starter was 57.14%.

The inhibitory activity of the alpha-glucosidase enzyme is related to the content of exopolysaccharide and inulin in lactic acid bacteria, which are inhibitors of the alpha-glucosidase enzyme. The results of this study are not much different from the results of research conducted by Nurhayati et al. (2017) who reported that lactic acid bacteria isolated from canna (*Canna edulis*) and Kimpul (*Xanthosoma sagittifolium*). The alpha-glucosidase enzyme inhibition by canna averaged 32.61-103.18%, while in Kimpul, the average ranged from 7.48-96.19%. Another study conducted by Kwun et al. (2020) also reported that lactic acid bacteria isolated in the form of *Lactobacillus sakei* from kimchi also showed alpha-glucosidase enzyme inhibitory activity of 3.91 ± 0.25%.

From the results of molecular identification of lactic acid bacteria, it was found that the DNA genome as genetic material isolated from lactic acid bacteria in yogurt with 5% *Lactobacillus acidophilic* starter is a bacterial DNA genome similar to the DNA genome of

Lactobacillus casei and *Lactobacillus paracasei* bacteria. Before molecular identification of DNA genomes isolated from lactic acid bacteria, quantitative analysis was carried out on genomic DNA isolated from lactic acid bacteria.

The results of quantitative analysis of lactic acid bacteria genomic DNA isolates through the values of A260/280 and A260/230 were 1.93 and 2.03. The A260/280 value of this research sample is 1.93, where the A260/280 value is used to assess the purity of the isolated DNA. A good A260/280 value is close to 1.80; a lower value indicates contamination from protein, phenol, or other compounds that can increase the A280 value.

Meanwhile, a higher value indicates more isolated genetic material, but in this case, the A260/280 value cannot distinguish between DNA or RNA as isolated genetic material. Therefore, a high A260/280 value can indicate high concentrations of isolated RNA. In addition to the A260/280 value, another parameter used to assess the purity of isolated genomic DNA is the A260/230 value. The value of A260/230 in this study was 2.03, whereas the good deal of A260/230 from genomic DNA isolates was 2.0 - 2.2. A high A260/230 value indicates contamination by protein, guanidine HCL, EDTA, carbohydrates, lipids, salts, and phenols. Based on the importance of A260/280 and A260/230, it can be concluded that genomic DNA isolates obtained from lactic acid bacteria isolate from Soygurt in this study are worthy of identification through molecular identification (Lucena-Aguilar et al., 2016).

The gel electrophoresis results indicated that the length of the DNA genome isolated in this study ranged from 1,000-1,500 BP. The size of the DNA genome used in this study is in line with the primer used in this study, namely 27F-1492R. The DNA primer used in this study is one of the most commonly used in identifying lactic acid bacteria. In another study, they even used primer 27F-1492R as a control to identify various new shorter primers to identify lactic acid bacteria using the single-molecule real-time (SMRT) sequencing analysis method (Hou et al., 2018).

Wang et al. (2022) used primer 27F-1492R to identify *Levilactobacillus brevis* bacteria from sour pulp samples (Sour Porridge). Therefore, this indicates that the primer used in this study is a primer that is commonly used in the molecular identification of lactic acid bacteria (Hou et al., 2018; Wang et al., 2022). From the molecular identification results, lactic acid bacteria isolated from Soygurt in this study were lactic acid bacteria with the highest percentage identity against *Lactobacillus casei*. *Lactobacillus casei* is a group of lactic acid bacteria commonly found in human milk products (human breastmilk) and has potential as a probiotic.

The *Lactobacillus casei* lactic acid bacteria group consists of several bacteria, including *Lactobacillus casei, Lactobacillus paracasei*, and *Lactobacillus rhamnosus*, where all lactic acid bacteria in this group can be exopolysaccharides which can have potential as probiotics. ASI (breast milk) was found to be colonies of lactic acid bacteria which, from the results of molecular identification, are a group of *Lactobacillus casei* bacteria that can produce exopolysaccharides, where these exopolysaccharides molecules were identified through Fourier Transform Infra-Red (FTIR) spectrophotometry (Kusmiyati et al., 2023).

Furthermore, another study conducted by Widodo et al. (2012) reported that feces or feces from babies also contain lactic acid bacteria in the form of *Lactobacillus casei*, where these bacteria come from breast milk consumed by babies. Interestingly, Widodo et al. (2012) also reported that *Lactobacillus casei* bacteria from baby feces could inhibit the growth of *Escherichia coli* and *Bacillus cereus*, so they have the potential as probiotics for babies. Therefore, the bacterium *Lactobacillus casei* has also been studied to develop fermented products such as Soygurt in this study. *Lactobacillus casei* bacteria have been developed of as a probiotic in fruit and leaf extracts of chayote (Kusmiyati et al., 2023; Rahminiwati et al., 2023; Widodo & Taufiq, 2018).

Conclusion

The results of the above study, it can be concluded that the number of lactic acid bacteria colonies in the Soygurt sample with 5% Lactobacillus acidophilic yogurt starter was 8.00 x 104 CFU/ml. In contrast, the Soygurt selection with the Yakult starter was 5.00 x 107 CFU/ml. For each type of Soygurt sample with different starters, lactic acid bacteria were isolated with the characteristics of gram-positive bacilli without catalase activity and homo-fermentative fermentation. Isolate lactic acid bacteria from Soygurt with 5% Lactobacillus acidophilic starter did not show antibacterial activity against Staphylococcus aureus bacteria by disc diffusion. However, lactic acid bacteria isolated from Soygurt with 5% Lactobacillus acidophilic starter did not have antibacterial activity. However, this lactic acid bacteria isolate has antioxidant activity and alpha-glucosidase enzyme inhibition. This is reflected in the percentage of DPPH inhibition of cell-free supernatant isolates of lactic acid bacteria, which ranges from 27.06% - 58.63%. In contrast, the activity of alpha-glucosidase enzyme inhibition from cell-free supernatant isolates of lactic acid bacteria was reflected in the average percentage of alpha-glucosidase enzyme inhibition of 50.55%. The results of molecular identification of 16s rRNA from lactic acid bacteria isolated in Soygurt with

5% Lactobacillus acidophilic starter found that lactic acid bacteria have similar genomic DNA composition to *Lactobacillus casei* and *Lactobacillus paracasei* bacteria with identity percentages ranging from 99.79 - 99.86%. Therefore, it is necessary to carry out further research to assess other characteristics of lactic acid bacteria, such as resistance or resistance of lactic acid bacteria to environmental acidity (high or low pH conditions) and tolerance to bile sacs. In the future, it is also necessary to analyze the antibacterial activity of lactic acid bacteria isolates with other methods and against different pathogens.

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Author Contributions
Cory Oriensia Siahaan is a biomedical master study program student conducting the Antioxidant Test of Lactic Acid Bacteria research, participating in sequence alignment, and compiling manuscripts. I. Nyoman Ehrich Lister is a lecturer at Prima Indonesia University, participating in the alignment of the Alpha-glucosidase Inhibitor test sequence. Ermi Girsang is a lecturer at Prima Indonesia University who participated in Fermenting Soybean Milk (Soygurt), compiling the research, participating in the design and coordination, and helping to compile the manuscript. Edy Fachrial is a lecturer at Prima Indonesia University who participated in making studies and conducting statistical analyses. All authors read and approved the final manuscript.

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Conflicts of Interest
The authors declare that there is no conflict of interest regarding the publication of this paper.

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