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Isolation And Identification of Lactic Acid Bacteria From *Channa* sp. as Potential Probiotic

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Abstract

Lactic acid bacteria (LAB) are a group of bacteria that are naturally present in the digestive tract of vertebrates, including fish such as Channa sp. Lactic acid bacteria are considered the most suitable microbes for use as probiotics. Probiotics can produce antimicrobial metabolites so that can improve the microbial balance in the digestive tract. It is believed that Channa sp. harbors LAB in its digestive tract, which may have probiotic potential and produce antimicrobial metabolites that can inhibit pathogenic bacteria. The aim of this study was to isolate and identify LAB from the intestines of Channa sp. The research involved several steps, including sample preparation, LAB isolation, characterization, and purification of bacterial isolates, biochemical tests, temperature resistance tests, antimicrobial tests, haemolysis tests, lactic acid production tests, and cholesterol-lowering activity tests. Eight LAB isolates with potential probiotic characteristics were isolated and identified as the genus Lactobacillus, which are gram-positive bacteria that produce lactic acid during culture.

Keywords: *Channa, Fish digestive tract, Lactobacillus, probiotics, Lactic acid bacteria*

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INTRODUCTION

Lactic acid bacteria are considered the most suitable microbes for use as probiotics. They are a group of bacteria that can ferment sugars or carbohydrates to produce significant amounts of lactic acid. The 10 genera of lactic acid bacteria include *Aerococcus, Carnobacterium, Enterococcus, Lactobacillus, Lactococcus, Leuconostoc, Pediococcus, Streptococcus, Tetragenococcus, and Vagococcus*. These bacteria have common characteristics

such as being gram-positive and producing products in the form of lactic acid and antimicrobial compounds (Suardana et al., 2017). Testing for lactic acid bacteria can be done using fish to determine the isolates. Lactic acid bacteria are closely related to probiotics, which are preparations of microbial cells that have the ability to produce antimicrobial metabolites that can inhibit pathogenic bacteria. Probiotics are defined as products composed of microbial cultures or microscopic natural feeds that are beneficial to the host (Prameswari et al., 2010)

Probiotics can produce antimicrobial metabolites such as lactic acid, diacetyl, hydrogen peroxide, and bacteriocin compounds. They are also useful for improving the microbial balance in the digestive tract, acting as a barrier against the growth of pathogens by producing compounds that inhibit their growth. Probiotics are intended to help increase digestive activity in the digestive tract of fish. There have been various investigations conducted which indicate that providing probiotic feed to fish can have a beneficial impact by promoting the activity of lactic acid bacteria present in their digestive tracts (Nayak, 2010). This, in turn, can aid the digestion process of fish and help regulate the presence of harmful bacteria and certain enzymes (Mohapatra et al., 2012)

Research in the field of probiotics is crucial in Bangka Belitung Province due to the vast potential for the development of innovative probiotic products. To enhance the quality of such products, it is essential to explore and identify potential local Lactic Acid Bacteria (LAB) isolates. As well as the need for research on the isolation and identification of lactic acid bacteria from the digestive tract of *Channa* sp. to increase productivity in fish aquaculture in Bangka Belitung province.

METHOD

Location and time of the research

The study took place at the Microbiology Laboratory of University of Bangka Belitung from June through November 2022. Sample was taken from several source of aquatic habitat in Bangka Island.

Instruments and Materials

The following tools and equipment were utilized in this study: aluminum foil, autoclave, stirring rod, spreader rod, Bunsen burner, durant bottle, petri dish, funnel, erlenmeyer flask, beaker glass, magnetic stirrer, measuring cup, object glass, scissors, hot plate, incubator, inoculation loop, preparation glass, convex object glass, measuring flask, micropipette, microscope, analytical balance, tweezers, drip pipette, tube rack, Durham tube, test tube, tip, refrigerator, tip box, microwave, laminar air flow, and digital camera.

Additionally, the following materials were utilized in this study: agar, 70% alcohol, crystal violet, decolorizer, H2O2, iodine, cotton, sterile gauze, label paper, Sulfide indole motility (SIM), triple sugar iron agar (TSIA), Vogue proskauer (MR-VP), methyl red, MRSBroth, agar nutrients, nutrient broth, MRS agar, NaCL, CaCO3, blood agar, safranins, tissues, and *Channa* sp.

Procedures

Instruments and Materials Sterilization

The equipment was thoroughly cleaned and dried before proceeding. After that, materials such as MRSB, MRSA, NaCL, and CaCO3 were heated until fully dissolved. Following this, both equipment and materials were sterilized using an autoclave at a temperature of 121℃ and a pressure of 15 psi for a duration of 20 minutes. The entire process, including heating, sterilization, and cooling, took approximately two hours.

Preparation Fish Intestine Collection

Fish specimens were collected from various aquatic habitats prepared with following step: The fish was anesthetized before undergoing surgery, the intestines of the fish were then removed and placed in plastic samples, with the intestine being specifically isolated from *Channa* sp.

Isolation and Selection of Lactic Acid Bacteria

The fish intestines were cut into 1 cm pieces and weighed and then placed in test tubes containing 30 ml of selective media, MRS Broth, and incubated in an incubator at 37℃ for 48 hours. Next, 350 ml of NaCl was prepared, with each test tube containing 9 ml. Additionally, about 350 ml of MRS Agar was prepared and all instruments and materials were sterilized using an autoclave. A sterile dilution was prepared by taking 1 ml of the incubation liquid and diluting it to 10^{-9}. The resulting solution from the 10^{-9} dilution was cultured on MRS Agar using the pour plate method with a continuous streak pattern and incubated for 48 hours at 37℃.

Lactic Acid Bacteria Characterization

Morphological Observation of Lactic Acid Bacteria Colonies

Bacterial isolates were identified based on their morphological characteristics, which included shape, edge, elevation, margin, color, optical character, and surface, with the use of a colony counter and a bacterial identification book.

Gram staining

To prepare the object glass, it was first rinsed with 95% alcohol until it was clean. Drops of distilled water were then placed onto the object glass. Next, the bacterial culture was prepared and the inoculating loop was heated using a bunsen burner. The bacterial culture was then applied to the object glass and fixation was done. The bacterial smear was stained with violet dye for 1 minute, followed by rinsing with distilled water. Iodine was then applied, and the smear was left for 1 minute before rinsing again with distilled water. Decolourizer was then applied and left for 30 seconds before rinsing with distilled water. Lastly, safranin was added and left for 30 seconds before rinsing with distilled water. The smear was then observed under a microscope using an objective lens of 100x. Staining results showed that the bacteria were either gram-positive (purple) or gram-negative (red).

Biochemical Test

Bacterial biochemical testing is a technique utilized to recognize and confirm a bacteria culture's purity by analyzing its physiological traits subsequent to isolation. Simply knowing a bacteria's morphological properties is insufficient for identification; their physiological features must also be considered. The process for assessing biochemical activity follows the procedure developed by previous research (Santoso, 2008).

Probiotic Potency Test

Temperature Resistance Test

The experiment aimed to determine the growth capacity of bacterial isolates under different temperature conditions. This was achieved by cultivating the bacterial isolates on MRSB media and incubating them at three distinct temperatures: room temperature, incubator temperature, and freezing temperature, for a duration of 48 hours. Subsequently, the growth of the bacteria in each tube was carefully monitored and noted.

Antimicrobial Test

To conduct the experiment, 15 mL of Nutrient Agar (NA) was poured into a petri dish and allowed to solidify. Once it had solidified, a sterilized loop was used to scratch the surface of the agar. Next, *Staphylococcus aureus* bacteria was introduced by streaking it onto the nutrient agar (NA) medium. A disc paper pre-soaked with lactic acid bacteria was placed onto the medium that had been inoculated with bacteria. The petri dish was then incubated for 48 hours at a temperature of 30°C.

Cholesterol Lowering Activity Test

The experiment involved four different treatments: bacterial isolates, bacteria-prebiotic media, prebiotic media, and controls without either bacteria or prebiotic media. For each treatment, 2 ml of the solution was added to a test tube and mixed with 5 ml of 140 ppm cholesterol standard. The resulting mixture was then treated with 2 ml of anhydrous acetic acid and 0.1 ml of concentrated sulfuric acid. The solution was left in a dark area for 15 minutes until a green color change was observed. The color results were analyzed using a visible spectrophotometer.

Production of Lactic Acid

In the experiment, qualitative tests were conducted to observe the growth of lactic acid bacteria. The bacteria were grown on MRSA medium containing CaCO3 and incubated for 48 hours. The growth of bacteria was assessed by observing the presence of a clear zone around the colonies of lactic acid bacteria.

RESULTS AND DISCUSSION

Lactic Acid Bacteria Isolates from *Channa* sp.

The study yielded eight bacterial isolates, which exhibit distinct qualities or features. Afterward, all isolates underwent purification to enable further investigation of their properties. Table 1 displays the traits or attributes of the three bacterial isolates.

Table 1. Colony characteristics of Lactic Acid Bacteria

No	Isolate	Size	Shape	Elevation	Margine	Surface	Pigmentation	Optical
1	C1A	Punctiform, small	Circular	Corvex	Entire	Shiny smooth	Cream	Opaque
2	C1B	Punctiform, small	Circular	Corvex	Entire	Shiny smooth	Cream	Opaque
3	C2A	Small	Circular	Corvex	Entire	Shiny smooth	Cream	Opaque
4	C2B	Moderate	Circular	Corvex	Entire	Shiny smooth	Cream	Opaque
5	C3A	Moderate, small	Circular, Punctiform	Raised	Entire	Shiny smooth	Cream	Opaque
6	C3B	Moderate, small	Circular, Punctiform	Raised	Entire	Shiny smooth	Cream	Opaque
7	C4A	Punctiform, small, moderate	Circular	Corvex	Entire	Shiny smooth	Cream	Opaque
8	C4B	Moderate	Circular	Convex	Entire	Shiny smooth	Cream	Opaque

The lactic acid bacteria isolates were characterized through both macroscopic and microscopic observations. Macroscopic observations encompassed the morphology of the colonies, including their shape, edge, elevation, and color. The colonies exhibited a round shape with varying sizes and elevations, appeared creamy white, and produced a clear zone. Meanwhile, microscopic observations focused on the structure and shape of the cells, which were determined using gram staining. The gram staining results indicated that the three samples were bacilli and gram-positive bacteria. Gram-positive bacteria are cells that retain the color of the crystal violet stain, appearing blue-purple, as they cannot release the color. Conversely, gram-negative bacteria can release the crystal violet stain and bind with safranin to appear red. This distinction is related to the composition of the cell wall, as gram-positive bacteria have more peptidoglycan and less fat than gram-negative bacteria (Syulasmi et al., 2005).

Biochemical Properties

The biochemical characteristics of the BAL isolates showed in table 2. These characteristics provide information on the metabolic capabilities of the isolates, such as their ability to utilize certain substrates and produce certain enzymes. The catalase test results were used to identify probiotic bacterial isolates that produce the catalase enzyme, which breaks down hydrogen peroxide produced during aerobic respiration in bacteria. However, the obtained probiotic bacterial isolates yielded negative results for the catalase test, as they did not produce gas bubbles around the bacterial colonies when exposed to a 3% H2O2 solution. A negative result is indicated when no gas bubbles are present in the catalase reaction. These results are consistent with previous studies that also found negative results for the catalase test in lactic acid bacteria (Kusuma, 2009).

Table 2. Biochemical properties of Lactic Acid Bacteria

Characteristic	Lactic acid bacteria isolates							
	C1A	C1B	C2A	C2B	C3A	C3B	C4A	C4B
Catalase	-	-	-	-	-	-	-	-
Motility (SIM)	-	-	-	-	-	-	-	-
MRVP	+	+	+	+	+	+	+	+
TSIA Test (Slant/Butt)	Y/Y	Y/Y	Y/Y	Y/Y	Y/Y	Y/Y	Y/Y	Y/Y
Gas	-	-	-	-	+	-	+	-
H_2S	-	-	-	-	-	-	-	-

Furthermore, all probiotic bacterial isolates showed negative results in the motility test, indicating that they were non-motile. This was evident from the lack of movement and the spread of bacterial growth only on the section of the SIM media where bacteria were inoculated. Since probiotic bacteria have limited biosynthetic abilities, they are non-motile (Surono, 2004). Negative results in the motility test indicate the absence of flagella, which are used for movement in bacteria. Lastly, the methyl red test was performed by adding 3-4 drops of methyl red indicator to the MRVP media containing the bacterial isolates, yielding a positive result when the media turned red. This red color was due to the decrease in the large amount of acid produced from glucose fermentation (Nur et al., 2015). The methyl red test is employed to detect the presence of mixed acid products from glucose fermentation, typically including lactic acid, acetic acid, formic acid, and succinic acid.

The TSIA test was used to observe the fermentation of glucose, lactose, and sucrose in the lactic acid bacteria isolates of the genus Lactobacillus. The higher concentrations of lactose and sucrose allow for further fermentation substrates if glucose is depleted, resulting in the production of yellow-colored acid after 2 days of incubation. The TSIA test results showed that the Lactobacillus isolates had an acidic reaction, which was evident from the change in the color of the media to yellow in the slant and butt portions (Nursyirwani et al., 2018)

Temperature resistance

The growth of lactic acid bacteria can be affected by environmental factors such as temperature (Pelczar et al., 1993). Temperature is controlled to create optimal conditions for the growth of microorganisms in the lactic acid fermentation process. A temperature resistance test was conducted to assess the growth of lactic acid bacteria isolates, and the results are presented in table 3. Table 3 explains that there were three temperature treatments that served as indicators of the growth rate of lactic acid bacteria, namely refrigerator temperature, room temperature, and incubator temperature. The results showed that the growth rate of bacteria was low (-) at refrigerator temperature, medium (+) at room temperature, and fast (++) at incubator temperature.

The optimum temperature for the growth of lactic acid bacteria can be classified into two groups: mesophilic and thermophilic. Mesophilic bacteria grow optimally at temperatures ranging from 25°C to 37°C or 40°C, while thermophilic bacteria grow optimally at temperatures ranging from 37°C to 45°C or 45°C to 52°C. All probiotic bacterial isolates, including mesophilic bacteria, were obtained in this study because they were able to grow at temperatures ranging from 25°C to 40°C. Therefore, it can be concluded that bacterial growth is faster at incubator temperature compared to refrigerator and room temperatures due to the inhibitory effect of low temperature (-6°C) storage for 2x24 hours on bacterial growth. In contrast, 37°C incubator temperature is optimal for bacterial growth

Table 3. Lactic Acid Bacteria Growth on Various Temperature

Isolate	Refrigerator	Room	Incubator
C1A	+	++	+++
C1B	+	++	+++
C2A	+	++	+++
C2B	+	++	+++
C3A	+	++	+++
C3B	+	++	+++
C4A	+	++	+++
C4B	+	++	+++

Lactic Acid Production

Parameters of lactic acid production, the tests carried out still reached the qualitative stage, which was indicated by the presence of a clear zone around the lactic acid bacteria colonies grown on MRSA media supplemented with 1% CaCO3 (Figure 1). The test results showed that lactic acid bacteria isolates were able to produce lactic acid, so that a clear zone was formed. Further testing using a quantitative approach will be carried out to determine the potential of lactic acid bacteria isolate sas probotic agent.

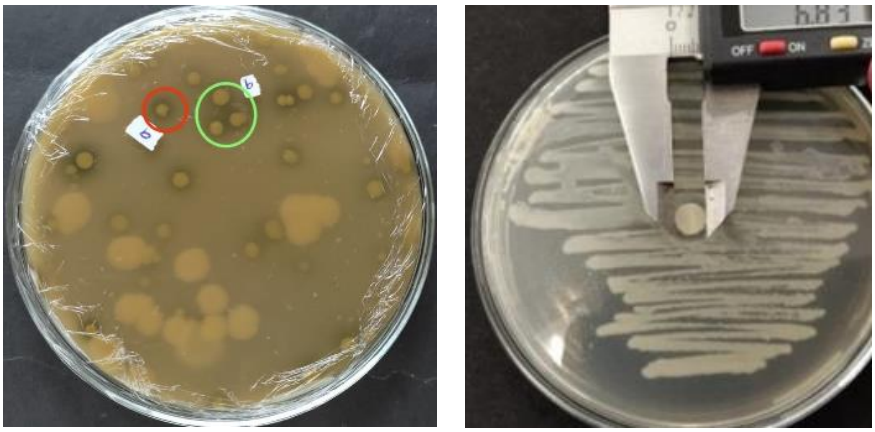

Figure 1. Clear zone formed on MRSA medium supplemented with CaCO3 and antimicrobial acitivity assay.

Antimicrobial Activity

In an antibacterial test, three isolates of lactic acid bacteria (C2B, C4A, C4B) were tested against the pathogenic bacteria, *Staphylococcus aureus*, which causes digestive tract disorders. Results showed that all lactic acid bacteria isolates had the ability to inhibit the pathogenic bacteria, with inhibition zones ranging from 1.90-7.17 mm (Table 4). The probiotic isolates showed moderate antibacterial activity against *Staphylococcus aureus*. The diameter of the inhibition zone was categorized into low, moderate, and high antimicrobial activity, based on the size of the inhibition zone (Prathapan, 2022). The table of antimicrobial test results indicated that S1-3 had the lowest inhibition zone size of 1.90 inches/mm, indicating low antimicrobial activity, while S3-3 had the highest inhibition zone size of 7.17 inches/mm, indicating high antimicrobial activity (Figure 1).

The inhibitory effect could be attributed to the presence of acids or bacteriocins that are capable of inhibiting the growth of pathogenic bacteria (Manalu et al., 2020).

Table 4. Lactic acid bacteria antimicrobial activity

Isolate	Inhibition Zone (mm) *Staphylococcus aureus*
C1A	3.10
C1B	1.90
C2A	3.64
C2B	5.36
C3A	2.44
C3B	3.45
C4A	4.65
C4B	7.17

Cholesterol Lowering Activity

Lactic acid bacteria isolates are believed to be involved in reducing cholesterol levels in animals. One of the lactic acid bacteria isolates, Isolate C2B, was tested and found to have a cholesterol level of 99.32 mg/dL. The graph below shows the absorbance values at different concentrations. This study was conducted as an initial investigation to demonstrate the potential of lactic acid bacteria to reduce cholesterol levels, which has been previously reported by other studies (Ma et al., 2019). Several mechanisms have been suggested for how lactic acid bacteria may potentially lower cholesterol levels i.e the removal of cholesterol from the intestines by the probiotic cells (Lye et al., 2012), inhibition of cholesterol absorption in the small intestine by down-regulating intestinal NPC1L1 protein levels (Huang et al., 2014), and increasing the levels of fecal bile acid excretion through the catalyzation of bile salt hydrolase (BSH) by probiotic cells (Jones et al., 2013).

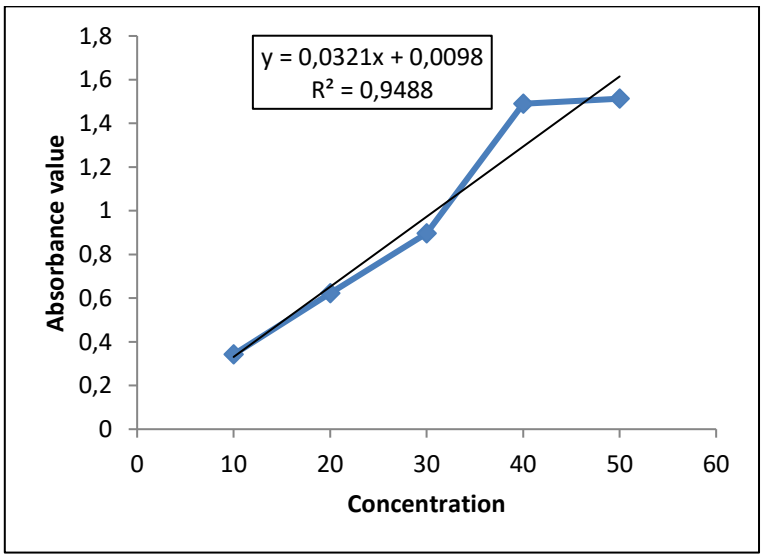

Figure 2. The absorbance value of cholesterol lowering test

CONCLUSION

Based on the research that has been done, it can be concluded that the analysis regarding the identification of lactic acid bacteria from the intestines of *Channa* sp. can be classified that the isolated lactic acid bacteria is *Lactobacillus*. From the results of microscopic and macroscopic observations, as well as the tests carried out, it can be seen that lactic acid bacteria isolate from the intestine of *Channa* sp. has the potential as a probiotic.

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REFERENCES

Huang, Y., Wang, J., Quan, G., Wang, X., Yang, L., & Zhong, L. (2014). Lactobacillus acidophilus ATCC 4356 prevents atherosclerosis via inhibition of intestinal cholesterol absorption in apolipoprotein E-knockout mice. *Applied and Environmental Microbiology*, *80*(24), 7496–7504. https://doi.org/10.1128/AEM.02926-14.

Jones, M.L., Tomaro-Duchesneau, C., Martoni, C.J., & Prakash, S. (2013). Cholesterol lowering with bile salt hydrolase-active probiotic bacteria, mechanism of action, clinical evidence, and future direction for heart health applications. Expert Opin. Biol. Ther. 13:631-642. doi: 10.1517/14712598.2013.758706.

Lye F.S., Alias K.A., Rusul G., Liong M.T. (2012). Ultrasound treatment enhances cholesterol removal ability of lactobacilli. Ultrason. Sonochem. 2012;19:632–641. doi: 10.1016/j.ultsonch.2011.08.004.

Ma, C., Zhang, S., Lu, J., Zhang, C., Pang, X., & Lv, J. (2019). Screening for cholesterol-lowering probiotics from lactic acid bacteria isolated from corn silage based on three hypothesized pathways. *International Journal of Molecular Sciences*, *20*(9). https://doi.org/10.3390/ijms20092073.

Manalu, T.M., Bahri, S., Melisa, & Sarah, S. (2020). Isolasi dan Karakterisasi Bakteri Asam Laktat asal Feses Manusia sebagai Antibakteri *Escherichia coli* dan *Staphylococcus aureus*. Jurnal Ilmu Kefarmasian, 13(10): 55-59.

Mohapatra, S., Chakraborty, T., Prusty, A. K., Das, P., Paniprasad, K., & Mohanta, K. N. (2012). Use of different microbial probiotics in the diet of rohu, Labeo rohita fingerlings: Effects on growth, nutrient digestibility and retention, digestive enzyme activities and intestinal microflora. *Aquaculture Nutrition*, *18*(1), 1–11. https://doi.org/10.1111/j.1365-2095.2011.00866.x

Nayak, S.K. (2010). Probiotics and immunity: A fish perspective. Fish Shelfish Immunol 29: 2-14.

Nursyirwani, V., Dian, B.P., & Effendi, I. (2018). Bakteri Asam Laktat dari Cincaluk dan

Aktivitas Terhadap Vibrio alginolyticus dan Aeromonas hidrophila. Asian. Journal of Aquatic Sciences, 1(1), 35-39.

Nur, F., Wahdiniar, A., Biologi, J., Sains dan Teknologi, F., & Alauddin Makassar Jl Sultan, U. (2015). *Isolasi Bakteri Asam Laktat Berpotensi Probiotik Pada Dangke, Makanan Tradisional dari Susu Kerbau di Curio Kabupaten Enrekang* (Vol. 3, Issue 1).

Pelczar, M.J., E.C.S. Chan Jr, & Krieg, N.R. (1993). Microbiology. 5th ed. New Delhi (India): Tata McGrawHill.

Prathapan, P. (2022). A determination of pan-pathogen antimicrobials? In *Medicine in Drug Discovery* (Vol. 14). Elsevier B.V. https://doi.org/10.1016/j.medidd.2022.100120.

Prameswari, A., Kuntari, S., & Herawati. (2010). Daya Hambat Probiotik terhadap Pertumbuhan Streptococcus mutans. Indonesian Pediatric Dental Journal, 2(2) : 16-19.

Santoso, E. (2008). Bakteri Asam Laktat Pada Cumi-cumi dan aktivitas penghambatannya terhadap bakteri pathogen. Agroteksos, 18(1):1-3.

Sri, A.F.K. (2009). Bakteri Asam Laktat. Jawa Barat: Universitas Padjadjaran.

Suardana, W., Kristal, H., Septiara, A., Suarsana, N., Kesehatan, L., Veteriner, M., & Dokter Hewan Di Denpasar, P. (2017). *Karakteristik Fisikokimia Bakteriosin Asal Bakteri Asam Laktat Enterococcus durans Hasil Isolasi Kolon Sapi Bali (physichochemical chacterization of bacteriocin producing enterococcus durans isolated from colon's bali cattle)*. *9*(2), 2477–2712. https://doi.org/10.21531/bulvet.2017.9.2.209.

Surono, I.S. (2004). Probiotik Susu Fermentasi dan Kesehatan. Jakarta: Tri Cipta Karya.

Syulasmi, A., Hamdiyati, Y., & Kusnadi. (2005). Petunjuk Praktikum Mikrobiologi. Fakultas MIPA. Universitas Pendidikan Indonesia. Bandung.

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