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ISOLATION AND CHARACTERIZATION OF LACTIC ACID BACTERIA FROM BREAST MILK

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A B S T R A C T

Breast milk (breast milk) is one of the sources of Lactic Acid Bacteria (BAL), which functions to maintain the balance of the baby's digestive microflora. BAL derived from breast milk has high viability in the digestive tract and is more adaptive if it comes from the human body. Some BALs are also called probiotics. If Lactic Acid Bacteria Isolate is obtained from breast milk, it can be used as a probiotic candidate in the prevention of stunting and infectious diseases in children. This research applies new biotechnology to produce a food product that is healthy, contains probiotics, and is preferred by the community. The purpose of the study is to isolate and test the probiotic potential of BAL strains isolated from breast milk so that it can have the potential to be applied in the food industry. This research method is an exploratory study to obtain Lactic Acid Bacteria isolate from Breast Milk. The results of the study showed that 14 Lactic Acid Bacteria Isolate was obtained which was shown by the formation of a clear zone area around the colony, 8 isolates of gram-positive lactic acid bacteria, and 6 isolates of gram-negative lactic acid bacteria, and all isolates were catalase negative. Based on the results of biochemical identification, of the 14 BAL isolates that have been successfully identified biochemically, consisting of several genera of BAL, namely Lactobacillus sp. dan Pediococcus sp.

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INTRODUCTION

Microbiota is a group of microorganisms that live in the body of living things, which have an important role in immunity and nutrient absorption. The microbiota of the gastrointestinal tract plays a role in the proliferation and maturation of intestinal epithelial cells, the induction of genes of the human body for nutrient absorption and the development of the mucosal immune system, which is essential for optimal nutrient absorption (Moossavi & Azad, 2020)(Masrul et al., 2020). The unbalanced composition of the gut microbiota contributes to the incidence of stunting. The worse the nutritional status of a child, the more pathogenic microbiota composition in the digestive tract (Suryani, 2020).

Indonesia is among the five countries in the world with the highest number of stunting cases. The stunting rate in Indonesia has decreased from 24.4% to 21.6 in 2022. Based on the results of the Indonesian Nutrition Status Survey (SSGI) of the Ministry of Health, the prevalence of stunting in West Sumatra Province was 25.2% in 2022, an increase from the previous year which was still 23.3%. One group of microbiota that plays a role in the digestive system is Lactic Acid Bacteria (BAL). These bacteria belong to the group of good bacteria that are safe for humans. This is because BAL can naturally inhibit pathogenic microbes. These bacteria can be isolated to produce antimicrobials that can be used as probiotics. Some strains of BAL are also called probiotics.

Probiotics are foods that contain live bacteria, and once ingested, they exert beneficial effects on their hosts. Microorganisms used in probiotic products generally contain Lactobacilli and Bifidobacteria. There are three types of probiotic bacteria: Lactobacillus, Bifidobacterium, and Gram-positive cocci. BAL is the most abundant type of probiotic. Acid production by BAL can prevent the growth of pathogenic bacteria by converting lactose to lactic acid (Jacouton et al., 2017).

Breast milk has been shown to be a good source of probiotics, as it meets some of the key criteria that are generally recommended for humans. These probiotics are derived from the human body, can adapt to products with a milk substrate, and have a history of safe and prolonged intake for babies.

This research is one of the efforts to provide local isolate of BAL indigenus derived from breast milk. Therefore, to obtain local BAL isolate and meet the requirements of GRAS (Generally Recognized as Safe) as a functional food, BAL must be isolated from breast milk. The local potential of BAL from breast milk is expected to be able to adapt to local conditions and ensure its availability.

BAL derived from breast milk has high viability in the digestive tract and is more adaptive if it comes from the human body. Some BALs are also called probiotics. If lactic acid bacteria isolate is obtained from breast milk, it can be used as a probiotic candidate in the prevention of stunting and infectious diseases in children. This research applies new biotechnology to produce a food product that is healthy, contains probiotics, and is preferred by the community. The purpose of the study is to isolate and identify BAL isolated from breast milk so that it can have the potential to be applied in the food industry. Thus, it can support one of the government's programs in the health sector, namely stunting prevention.

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Based on the description above, it is necessary to isolate and identify Lactic Acid Bacteria sourced from Breast Milk which is used as a probiotic agent. Because probiotics that are indigenous to humans such as from breast milk can adapt well to the digestion of babies and toddlers. Isolates from these bacteria will later be used in the production of probiotic food so that they can be consumed by the community, especially toddlers and children. Thus it can help overcome national health problems, especially stunting which is now the center of national attention in the health sector.

METHODS

This study is an exploratory study to obtain Lactic Acid Bacteria isolate from Breast Milk.

Instruments and Materials

The tools needed are50 pieces of reaction apparatus, 100 pieces of Petri (pyrex) dishes, 50 pieces of drip pipettes, 5 pieces of Erlenmeyer (pyrex) 250 ml, 5 pieces of Beker glass (pyrex) 500 ml, 2 pieces of 1000 ml Beker glass (pyrex), 3 pieces of measuring cup (pyrex), 1 piece of measuring pipette, 1 ml micropipette, 1 ml micropipette, 1 piece of blue tip micropipette, 1 piece of incubator, analytical balance 1 piece, ose needle 1 box, microscope 1 piece, glass object 2 boxes, deck glass 2 boxes, test tube rack 5 pieces, spatula 2 pieces, spritus lamp 1 piece, autoclave 1 piece, stirring rod 2 pieces, sample pot 5 pieces, coloni counter 1 piece, hot plate, sterile swab cotton, cotton 1 bale, spray bottle, aluminum foil, gauze, and ATK.

The research materials needed are Media de Mann Rogosa and Sharpe/ MRS agar, CaCO3 1%, gram coloring reagents, methylene blue, sterile aquades, HCl 1 N, methanol, pure agar, alcohol 96% (technical), hydrogen peroxide (H2O2), and Mc farland solution (technical).

Procedure

The stages of the implementation of the research consist of several main stages, namely:

Preparation Stage

The Instruments and media used in this study were previously sterilized using an autoclaf at a temperature of 121^{o}C, pressure of 15 lbs for 20 minutes. Meanwhile, the collection of breast milk samples is collected in sterile sample bottles and taken to the Laboratory with an ice box.

Implementation Stage

Isolation of Lactic acid bacteria from breast milk

Lactic acid bacteria were cultured with MRSA (Man Rogosa Sharpe Agar) medium and the addition of 1% $CaCO_3$. Furthermore, it was incubated at 37^{o}C for 48 hours under anaerobic conditions. Lactic Acid Bacterial Candidates are characterized by colonies that produce clear zones(Suryani et al., 2022).

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Identification of lactic acid bacteria

Pure cultures were carried out on different and single colonies of MRSA media. The growing bacterial colony was made two observations. Macroscopic observations of colonies include color, shape, edges and elevation. Microscopic observations of cells include cell shape, cell arrangement, gram staining and biochemical tests.

Data Analysis

The data obtained were analyzed descriptively. By comparing the data obtained with the literature related to the results of this study.

RESULTS AND DISCUSSION

Isolasi bacteria asam lactate.

Isolation of Lactic Acid Bacteria from breast milk samples was carried out using MRS agar medium of +1% $CaCO_3$. From the isolation work of Lactic Acid Bacteria, 14 isolates were obtained from bacterial colonies that showed a clear zone around them (Figure 1).

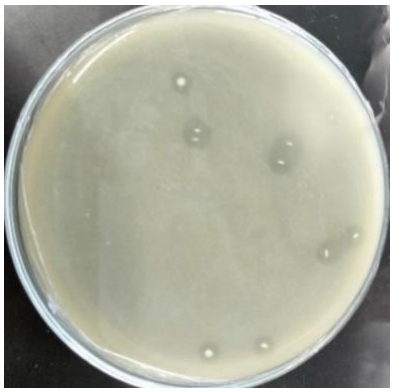

Figure 1. Lactic Acid Bacterial Colonies Isolated from Breast Milk Samples

As seen in Figure 1, a "halo" or clear area will form indicating that the colony is a Lactic Acid Bacteria. The "halo" region occurs because BAL produces large amounts of lactic acid and other organic acids. This acid reacts with the $CaCO_3$ base which is added through a neutralization reaction so as to neutralize the area around the acid-containing colony and give rise to a clean area or zone (Suryani et al., 2017). Next (Anindita, 2022) adding alkalinity $CaCO_3$ neutralizes and localizes acid production by lactic acid bacteria. Kang et al., (2020) have also isolated Lactic Acid Bacteria and obtained 25 isolates, and for further evaluation based on their performance on MRSA media containing $CaCO_3$. In the test, lactic acid bacteria showed a clear zone around the colony, isolation was carried out based on the appearance of BAL and clear zone around the colony on the MRS agar dish given 1% $CaCO_3$ supplement.

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The addition of $CaCO_3$ compound to MRSA media is used to select lactic acid-producing bacteria. Therefore, the formation of a clear zone in MRSA media is caused by a reaction between the organic acids produced by BAL and the compounds contained in the media which will generally be isolated by $CaCO_3$ compounds (Falakh & Astri, 2022).

The LAB that has been successfully isolated is continued by making pure culture by the streak plate method using MRSA media without the addition of $CaCO_3$ to the inclined jaw. Pure cultures are cultures that consist of only one type of microorganism (Leboffe & Pierce, 2011).

The principle of pure culture is the separation or transfer of one type (species) of microbes that comes from a mixture of various microbes. The purpose of this is to characterize, identify, and study previously isolated LAB.

Figure 2. Pure culture of lactic acid bacteria on inclined MRSA media

In MRSA medium without $CaCO_3$, lactic acid bacterial colonies do not appear to form clear regions. This is in accordance with Suryani, et.al. (2020) In MRSA media without the addition of $CaCO_3$, lactic acid bacterial colonies grow and develop well but do not form a "halo" region.

Characteristic Morphology Colony Bacteria Asam Lactate

The bacterial colonies growing on $CaCO_3$ media were then observed macroscopic characteristics of the colony of Lactic acid bacteria in each isolate (Table 1).

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Table 1. Morphology of Lactic Acid Bacterial Colonies from Breast Milk in MRSA Media

No	Isolation Code	Color	Shape	Banks	Surface	Diameter (mm)
1	N1	White	circle	Flat	Convex	2
2	N2	White	circle	Flat	Convex	2
3	N3	White	circle	Flat	Convex	1,5
4	N4	White	circle	Flat	Convex	1,5
5	N5	White	circle	Flat	Convex	1,5
6	N6	White	circle	Flat	Convex	1,5
7	N7	White	circle	Flat	Convex	1,5
8	N8	White	circle	Flat	Convex	1,5
9	N9	White	circle	Flat	Convex	1,5
10	N10	White	circle	Flat	Convex	1,5
11	N11	White	circle	Flat	Convex	1
12	N12	White	circle	Flat	Convex	1
13	N 13	White	circle	Flat	Convex	0,5
14	N 14	White	circle	Flat	Convex	0,5

In accordance with Table 1. The results obtained from 14 lactic acid bacterial isolates showed that some bacteria had different morphological characteristics. The appearance of colonies formed by lactic acid bacteria is in the form of round and elliptical colonies in white. In addition, it can also be seen that the edges of this bacterium are flattened and the surface is convex.

The growing lactic acid bacterial colony has a white color, rounded shape, flat edges, and flat and convex elevations. Based on the character of the bacterial colony, the identification results refer to the genera Lactobacillus and Bifidobacterium. Lactobacillus bacterial colonies on the medium to have convex colony elevations and flat colony edges (Holt et al., 1994 ; Sibarani et al., 2023). Bifidobacterium has a white colony color, flat colony edges, convex colony elevation and a rounded colony shape (Whitman, 2012 ; Sibarani et al., 2023).

Characteristics of Lactic Acid Bacterial Cell Morphology

Observation of the morphological characteristics of lactic acid bacterial cells was carried out by staining the bacterial cells with grams. The results of observation of the morphology of BAL cells from 14 isolates that have been successfully isolated are as follows:

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Table 2. Morphology of lactic acid bacteria cells isolated from breast milk samples

No	Kode Isolat	Pewarnaan Gram	Bentuk sel
1	N1	Gram +	Basil
2	N2	Gram +	Coccus
3	N3	Gram +	Coccus
4	N4	Gram +	Basil
5	N5	Gram +	Coccus
6	N6	Gram +	Basil
7	N7	Gram +	Coccus
8	N8	Gram +	Basil
9	N9	Gram +	Basil
10	N10	Gram +	Basil
11	N11	Gram +	Basil
12	N12	Gram +	Coccus
13	N 13	Gram +	Coccus
14	N 14	Gram +	basil

Based on table 2. Above, the results of observation of the morphology of bacterial cells in the form of bacilli or coccus can be seen. Lactic acid bacterial cells whose morphology is bacillus are arranged in pairs. Furthermore, the results of gram staining from lactic acid bacteria isolate from breast milk are Gram-positive bacteria. Kang et al., (2020) stated that the lactic acid bacteria he observed under the microscope, had rod-shaped and gram-positive characteristics.

When observing lactic acid bacteria under a microscope, the shape of the isolate can also be observed, some are round (coccus) and some are in the shape of a rod (bacil). The appearance of colonies formed by lactic acid bacteria is in the form of white round colonies with elliptical shapes and facultative anaerobes with clear zones formed around the coloni(Anindita, 2022). Lactic acid bacteria belong to Gram-positive bacteria and have a thicker peptidoglycan wall than Gram-negative bacteria (Falakh & Asri, 2022).

Characterization of Lactic Acid Bacterial Isolates by Biochemical Assays

The results of the characterization of aam lactate bacteria isolate using biochemical tests can be seen in Table 3.

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Table 3. Biochemical test observation results from Lactic Acid Bacteria isolate

No	Isolation Code	Catalase	VP	MR	Motil	TSIA			Citrate	Indole
						Glucosa	Sucrosa	Lcktosa		
1	N1	-	-	+	-	-	+	+	+	-
2	N2	-	-	+	-	-	-	-	+	-
3	N3	-	-	+	-	-	+	+	+	-
4	N4	-	-	+	-	-	+	+	+	-
5	N5	-	-	+	-	-	+	+	+	-
6	N6	-	-	+	-	-	+	+	+	-
7	N7	-	-	+	-	-	+	+	+	-
8	N8	-	-	+	-	-	-	-	+	-
9	N9	-	-	+	-	-	+	+	+	-
10	N10	-	-	+	-	-	-	-	+	-
11	N11	-	-	+	-	-	+	+	+	-
12	N12	-	-	+	-	-	-	-	-	-
13	N 13	-	-	+	-	-	+	+	+	-
14	N 14	-	-	+	-	-	+	+	-	-

In the catalase test, 14 isolates that showed a negative catalase reaction showed no gas bubbles were produced when the test was performed. This shows that the 14 isolates do not have the catalase enzyme that functions to break down H_2O_2. The isolate with a Gram-positive reaction and a negative catalase is suspected to be lactic acid bacteria. This is in accordance with Kang et al., (2020) Under the microscope, lactic acid bacteria are characterized in the form of rods and gram-positive, for the catalase activity of all negative strains (no bubbles observed). In the catalase test, four BAL isolates showed negative catalase results indicated by the absence of O_2 bubbles after administration of 3% H_2O_2 (Falakh & Astri, 2022). Bakteri asam laktat tidak dapat memecah hidrogen peroksida karena termasuk bakteri katalase negatif (Sibarani et al., 2023).

According to Rahayu et al., (2000) that the group of BALs in the form of rods, Gram-positive, and catalase negative are BALS of the genus Lactobacillus. This bacterium has a characteristic milky white growth on MRS agar media. BAL is a Gram-positive, non-motile, non-spore-forming, catalase negative, rod-shaped or coccus bacteria, resistant to acidic conditions, anaerobic facultative and ferments carbohydrates into lactic acid as the main product (Anindita, 2022). The isolate suspected to be Lactobacillus by meeting the criteria has catalase negative properties, Gram-positive staining, nonmotile, and stem cell shape.

Lactic acid bacteria are not motile because they do not have flagella. Lactobacillus and Bifidobacterium bacteria are gram-positive, non-motile, and have no spores (Sibarani et al., 2023). The shape of the cells based on the results obtained is in the form of bacilli, the arrangement of paired cells and the size of the cells is about 0.2-5.2 μm. L. brevis and L. casei have a size of about 2.0-4.0 μm. Bacteria of the genus Lactobacillus and the genus Bifidobacterium have bacillus-shaped cells with a single, paired or chained arrangement of

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cells. Lactobacillus generally has a cell size of about 1.0-10.0 μm. Bifidobacterium generally has a cell size of about 1.58.0 μm. The isolate suspected to be Lactobacillus by meeting the criteria has catalase negative properties, Gram-positive staining, nonmotile, and stem cell shape.

The results of the study, bacterial isolate had negative citrate test results, negative indole test, fermentative OF test, and test on TSIA media obtained that this bacteria had a negative H2S result and had a yellow color on the surface and bottom of the media. Bacteria in the indole test that give a positive reaction are due to the presence of tryptophanase enzyme that forms a red layer on the surface of the media given by the kovacs reagent, while bacteria that give a negative reaction are indicated by the presence of a yellow layer on the surface of the media. Lactic acid bacteria have negative citrate test results showing that the bacteria do not use the citrate as a source of energy and carbon. This study also showed that lactic acid bacteria have a yellow color on the surface and bottom of the tubes on TSIA media, which means lactic acid bacteria are able to ferment carbohydrates.

CONCLUSION

Lactic acid bacteria have been successfully isolated from breast milk and have been identified phenotypically and biochemically. The identification results showed that of the 14 BAL isolates that had been successfully isolated, consisting of several BAL genera, namely *Lactobacillus sp*., and *Pediococcus sp.*

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