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ISOLATION AND TESTING OF ANTIMICROBIAL ACTIVITY OF LACTIC ACID BACTERIA FROM CASSAVA FERMENTATION *(MANIHOT ESCULENTA)* IN INHIBITING *ESCHERICHIA COLI* AND *SALMONELLA TYPHI*

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Abstract

Lactic acid produced by lactic acid bacteria can lower the medium pH, inhibiting the contamination from putrefactive bacteria and killing pathogenic bacteria. Lactic acid bacteria can be isolated from various sources, including cassava which is obtained by fermentation process and called as cassava *tape.* Lactic acid bacteria from cassava *tape* are a probiotic that has potency to improve intestinal physiological function. Its presence can prevent the growth of pathogenic bacteria such as *Salmonella typhi* and *Escherichia coli*. This research aims to measure the ability of lactic acid bacteria isolates from cassava *tape* in inhibiting *Escherichia coli* and *Salmonella typhi*. This study was carried out from April to August 2023 in Bacteriology Laboratory of the Health Polytechnic, Ministry of Health in Medan. The cassava *tape* was originated from Sukaramai Market in Medan. The ability of Lactic Acid Bacteria against the pathogenic bacteria is measured by the inhibition zone's size. This study obtained 4 types of lactic acid bacteria isolates. The lactic acid bacteria isolate had inhibition zone against *Escherichia coli* and *Salmonella typhi*, 1.36 cm and 1.26 cm, respectively. People are recommended to consume cassava *tape* as a healthy food because it can suppress the growth of *Escherichia coli* and *Salmonella typhi* bacteria.

Keywords: Cassava *Tape*, *Escherichia Coli, Salmonella Thypi,* Lactic Acid Bacteria.

INTRODUCTION

Lactic Acid Bacteria (LAB) are a group of bacteria that produce lactic acid by fermenting carbohydrates. The antimicrobial effect of lactic acid bacteria is caused by the formation of lactic acid and other inhibitory compounds such as hydrogen peroxide, diacetyl, carbon dioxide, reuterin and bacteriocin (1). Lactic acid produced by lactic acid bacteria can lower the pH of the medium which can inhibit contamination from putrefactive bacteria and also kill pathogenic bacteria in the body (2). Studies that explored the benefits of lactic acid bacteria have been intensively carried out. Lactic acid bacteria can be isolated from various sources, including fermented foods.

Cassava *tape* is a traditional food in Indonesia that is produce by fermentation process. The fermentation uses yeast and mold, such as *Saccharomyces cerevisiae*, *Rhizopus oryzae*, *Aspergillus*, and *Acetobacter* bacteria (3). In natural fermentation there is the growth of mesophilic aerobic bacteria and lactic acid bacteria. This lactic acid bacteria plays a role in preventing the growth of *Salmonella typhi* (4). Lactic acid bacteria in cassava *tape* contains *Lactobacillus bulgaris* and *Streptococcus thermophilus* which are probiotics that have potency to improve the physiological function of the intestine, intestinal microflora which plays a role in optimizing the body's health conditions (5). The presence of probiotic bacteria can prevent the growth of pathogenic bacteria such as *Salmonella sp.* and *Escherichia coli* (6). Meanwhile,

Salmonella typhi is a pathogenic bacteria in human that causes typhus abdominalis (7). Lactic acid bacteria (LAB) have been extensively studied for their antimicrobial properties, which make them potential candidates for inhibiting foodborne pathogens (8). Cassava fermentation, a common food preservation technique in many parts of the world, is a rich source of diverse lactic acid bacteria. This study aims to isolate and test the antimicrobial activity of lactic acid bacteria from cassava fermentation against Escherichia coli and Salmonella typhi, two major foodborne patho gens. A common characteristic among lactic acid bacteria is their ability to produce antimicrobial peptides known as bacteriocins, which have demonstrated antagonistic effects not only against closely related bacteria but also more distantly related pathogenic microorganisms (9,10,11). Ce hun tiau, a fermented beverage, has been previously shown to harbor lactic acid bacteria that produce bacteriocins effective against E. coli, S. aureus, and S. typhi(12).

Lactic acid bacteria were isolated from cassava fermentation using the streak plate method on de Man, Rogosa, and Sharpe (MRS) agar. The isolates were then screened for antimicrobial activity against E. coli and S. typhi using the disc diffusion method. Isolates exhibiting inhibitory effects were further confirmed through proteolytic enzyme treatments, which deactivate bacteriocins(12).

The antimicrobial compounds produced by the lactic acid bacteria isolates were effective in inhibiting the growth of both E. coli and S. typhi, as demonstrated by the zones of inhibition. This indicates the potential of these lactic acid bacteria to serve as natural preservatives in food applications, reducing the need for chemical additives while meeting consumer demand for minimally processed, natural products. In addition to their antimicrobial properties, the fermentation process itself has been shown to degrade harmful toxins, such as cyanide and mycotoxins, that can sometimes contaminate raw food materials like cassava(13).

The aim of this study was to determine the antibacterial potency of lactic acid bacteria derived from cassava *tape* isolate against *Escherichia coli* and *Salmonella typhi* bacteria. The findings of this research highlight the importance of exploring the antimicrobial potential of lactic acid bacteria from traditional food fermentation. These bacteria can provide a natural and beneficial alternative for preventing gastrointestinal diseases and can also be used as chemical preservatives in the food industry.

MATERIALS AND METHODS

The sample of this study was lactic acid bacteria that were isolated from cassava *tape*. The cassava *tape* was obtained from Sukaramai market located in Medan city, Indonesia. This study was conducted from April to August 2023 in Bacteriology Laboratory, Health Polytechnic, Medan. The lactic acid bacteria isolates were identified visually and microscopically. After the biochemical test was carried out, the lactic acid bacteria isolates were tested its resistancy against low pH, salt levels and its antagonist against pathogenic bacteria.

1. Isolation of Lactic Acid Bacteria from Cassava *Tape*

As much as 10 g of cassava *tape* was mashed and added with 90 mL of sterile distilled water. Serial dilutions up to 10^{-5} were carried out and 0.1 mL of each dilution level was inoculated into deMan Rogosa Sharpe Agar (MRSA) media using spread plate method, and incubated for 48 hours at room temperature. The bacterial isolates that grew with various morphologies based on shape, color, edge and elevation of the

colony were collected. The isolate was then grown again in the same media until a pure isolate was obtained (14).

2. Identification of Lactic Acid Bacteria

Identification of lactic acid bacteria was carried out visually and microscopically by comparing their morphology with the Bergeys Manual of Determinative of Bacteriology identification book. Identification was conducted based on the shape, edges, elevation and color of the colony, and also the gram staining, the shape, and the arragement of the bacteria (15).

3. Biochemical Test

The biochemical test was the citrate test, sugar catabolism test, motility test, gelatin test and catalase test (16).

4. Isolate Resistance Test to Low pH

The test was carried out by growing 1% of 24 hour old culture into MRS broth media which had previously been adjusted to pH: 3.5; 3.0; 2.5 and 2.0. Next, it was incubated for 24 hours at a temperature of 37º C. After incubation, the number of bacteria was calculated using the total plate count method on MRS agar media (17).

5. Isolate Resistance Test to Salt Levels

The test was carried out by inoculating 1% of 24 hour old culture into 5 mL of MRS broth media with the addition of bile salts at a concentration of 0.5%; 1.25%; 2.5%; 3.75% and 5%, then incubated at room temperature for 24 hours. The number of bacteria was counted using the total plate count method on MRS agar at the beginning and end of the incubation (17) .

6. Antagonist Test of Lactic Acid Bacteria Isolates with Pathogenic Bacteria

In the antagonist test for bacterial isolates, Mueller Hinton Agar media was used. A suspension of pathogenic bacteria with a cell density of 10^8 Colony Forming Units was spread on the surface of the test media using a sterile cotton swab. A total of 10 µl of lactic acid bacteria suspension was dropped onto a blank paper disc (oxoid). Isolate antagonist testing was carried out using the Kirby-Bauer disk test method. Test paper discs were placed on both sides of the media that had been smeared with pathogenic bacteria at a distance of 3.5 cm. The test plates were incubated at room temperature for 24 hours. The antagonistic activity of bacteria is indicated by the formation of an inhibition zone. Observations began from the second to the fifth day after the incubation period. The inhibition zone formed was then measured and recorded (18).

7. Statistycal analysis

The data was analyzed using a completely randomized ANOVA design (Analysis of variance).

RESULTS

1. The characteristics of the lactic acid bacteria isolate

There are 4 types of lactic acid bacteria isolates obtained from cassava *tape*. The morphology based on colony color, colony shape, colony edge, colony elevation, and the gram staining, the shape and the arrangement of the bacteria.

Table 1: Characteristics of lactic acid bacteria isolates

Isolate	Colony color	Shape	Edge	Elevation	Gram staining	Shape	Structure
Sp1	light yellow	*Circular*	*Entire*	*Convex*	+	Coccus	Mono, diplo, strepto,
Sp2	yellowish white	*Circular*	*Entire*	*Convex*	+	Bacil	Mono, diplo, strepto
Sp3	beige	*Circular*	*Entire*	*Flat*	+	Bacil	Mono, diplo, strepto
Sp4	beige	*Circular*	*Entire*	*Convex*	+	Bacil	Mono, diplo, strepto

All colonies of lactic acid bacteria isolates are circular, edge entire, and the bacteria was gram positive, and mono, diplo, strepto arrangement. The different among them are the colony color and the bacteria shape (Table 1 and Figure 1).

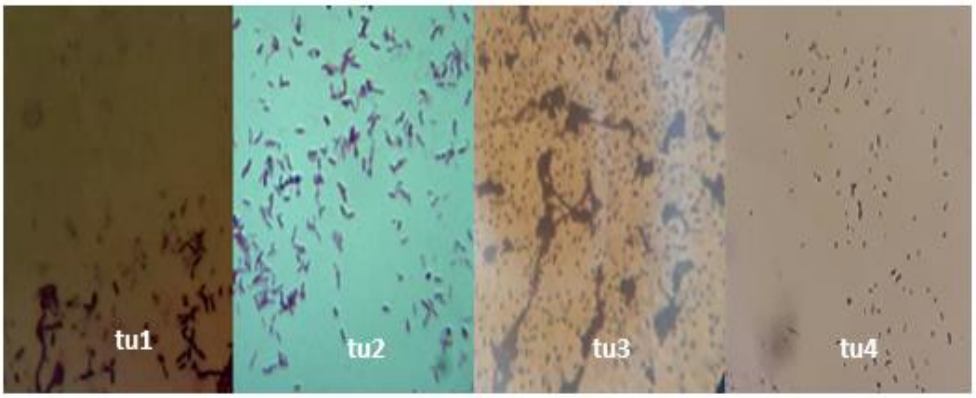

Figure 1: Gram staining of lactic acid bacteria isolates (400 times magnification)

2. The biochemical test and the resistance of lactic acid bacteria isolates to pH and salt levels

Table 2: Biochemical test of lactic acid bacteria isolates and total bacterial growth at varying pH and salt levels

Isolate	Citric fermentation	Sugar fermentation	Motility	Gelatin hydrolysis	Catalase enzyme
Sp1	+	+	+	-	+
Sp2	+	+	+	-	-
Sp3	+	+	+	-	-
Sp4	+	+	+	-	-

Isolate	Total number of bacteria (CFU)							
	pH variation			Salt level variation (%)				
	3.0	2.5	2.0	0.5%	1.25%	2.5%	3.75%	5%
Sp1	32	21	10	90	56	32	0	0
Sp2	46	28	0	78	64	37	0	0
Sp3	18	0	0	56	29	0	0	0
Sp4	27	16	0	78	56	37	0	0

Biochemical tests was carried out to determine the physiological properties of bacteria. All lactic acid bacteria showed positive of citric fermentation, sugar fermentation, and motility but negative of gelatine hydrolisis and only Sp1 that was positive of catalase enzym (Table 2). The positive catalase test was showed by the formation of bubbles in the tested isolate (Figure 2).

Figure 2: Positive test for catalase in Sp3 bacterial isolates

The pH variations that can be tolerated by bacterial isolates range from 3.0 – 2.5. Sp1 bacterial isolate was able to live at pH 2.0 with 10 colonies. Meanwhile, the Sp3 bacterial isolate was able to grow at pH 3.0 with 18 colonies. The variations in pH indicate the ability of lactic acid bacteria isolates to live in acidic conditions (11). Meanwhile, the variations in salt levels showed that lactic acid bacteria isolates were tolerant to the presence of 0.5%, 1.25% and 2.5% salt. At a concentration of 0.5% the highest number of colonies was Sp1 with 90 bacterial colonies. At a concentration of 1.25% the highest total number of colonies was Sp2 with 64 bacterial colonies. At a concentration of 2.5%, the highest total number of colonies was Sp2 and Sp4 with 37 bacterial colonies. The four isolates of lactic acid bacteria were unable to grow at salt concentrations of 3.75% and 5%. The high concentration of salt can cause plasmolization of bacterial cells (19).

3. Antagonistic ability of bacterial isolates against *Escherichia coli*

The results of the antagonist test of lactic acid bacteria isolates against pathogenic bacteria showed that the four isolates had different abilities in inhibiting the growth of *Escherichia coli*. The antagonistic ability of the isolate is characterized by the presence of an inhibitory zone formed in the area where the bacterial colony meets the pathogenic bacteria (20). The inhibition zone is in the form of an elevation thinning overdraft (Figure 3).

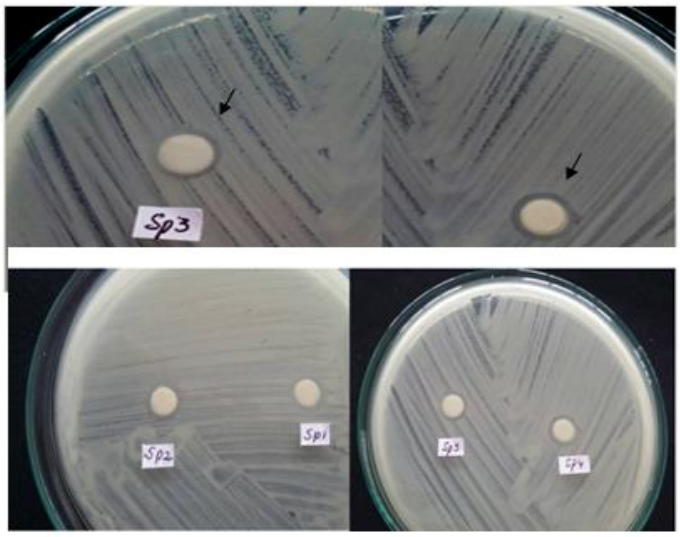

Figure 3: The antagonistic ability of Sp3 and Sp4 bacterial isolates in inhibiting the growth of *Escherichia coli*

Table 3: Inhibition zone (cm) and Duncan Multiple Range Test (DMRT) of lactic acid bacteria isolates against *Escherichia coli*

Isolate	Repetition					Total	Mean	DMRT
	I	II	III	IV	V	Σ X	X	
Sp1	1,1	0.9	1.0	1	0.8	4.8	0.96	c
Sp2	0.8	0.7	0.8	0.9	0.8	4	0.80	c
Sp3	0,9	01,0	1.0	1.2	0.9	5	1,00	b
Sp4	1.3	1.3	1.5	1.5	1.2	6.8	1.36	a

The inhibition zone formed by each isolate of lactic acid bacteria showed different results ranging from 0.7 – 1.5 cm. The results the antagonist test showed that the Sp3 bacterial isolate had the highest of inhibition zone (1.36 cm). Meanwhile, the Sp2 bacterial isolate had the lowest of inhibition zone (0.80 cm) (Table 3).

The ANOVA test analysis showed that the lactic acid bacteria isolates were significantly different in suppressing *Escherichia coli*. The Duncan test of lactic acid bacteria isolates on the diameter of the inhibition zone of *Escherichia coli* bacteria for Sp4 and Sp3 showed significant different.

This means that the lactic acid bacteria isolates Sp4 and Sp3 show different effects in inhibiting the growth of *Escherichia coli* bacteria (21). Meanwhile, the bacterial isolates Sp1 and Sp2 showed no significant differences in the resulting inhibition zones marked with the same letter. This means that the lactic acid bacteria isolates Sp1 and Sp2 showed the same effect in inhibiting the growth of *Escherichia coli* bacteria.

4. Antagonistic ability of bacterial isolates against *Salmonella typhi*

Lactic acid bacteria isolates were also tested against the pathogenic bacteria *Salmonella typhi*. *Salmonella typhi* is also a pathogen that attacks the human digestive tract. The ability of lactic acid bacteria isolates to inhibit the growth of *Salmonella typhi* bacteria was characterized by the presence of an inhibition zone that formed after the incubation period (Figure 4).

The inhibitory ability of lactic acid bacteria isolates is characterized by the size of the inhibition zone formed. The inhibition zone was calculated after the second day after the incubation period (Table 4).

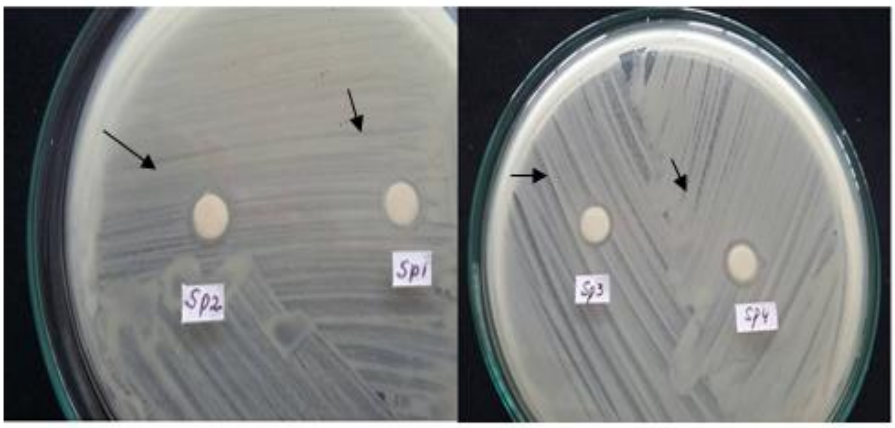

Figure 4: Antagonistic ability of bacterial isolates Sp1, Sp2, Sp3 and Sp4 in inhibiting the growth of *Salmonella thypi*

Table 4: Inhibition zone (cm) and Duncan Multiple Range Test (DMRT) of lactic acid bacteria isolates against *Salmonella typhi*

Isolate	Repetition					Total	Mean	DMRT
	I	II	III	IV	V	Σ X	X	
Sp1	0.8	1.0	0.9	1.3	1.0	5.0	1.00	b
Sp2	0,9	1.1	0.8	1,0	0,9	4.7	0.94	b
Sp3	1.1	1.1	1.2	1.3	1.1	5.8	1.16	a
Sp4	1.0	1.3	1.3	1.4	1.3	6.3	1.26	a

The largest of the inhibitory zone was shown by lactic acid bacteria Sp4 (1.26 cm). Meanwhile, the smallest of inhibition zone was shown by lactic acid bacteria Sp2 (0.94 cm) (Table 4). The differences in inhibitory zone were formed due to differences in isolate types. The ANOVA test analysis showed that the lactic acid bacteria isolates were significantly different in suppressing the growth of *Salmonella typhi*. The Duncan test of lactic acid bacteria isolates on the diameter of the inhibition zone of *Salmonella typhi* bacteria for Sp4 and Sp3 did not show significant differences, marked with the same letter. This means that the lactic acid bacteria isolates Sp4 and Sp3 showed the same effect in inhibiting the growth of *Salmonella typhi* bacteria. If we look at the comparison of the effectiveness of the inhibition zone between bacterial isolates Sp4 and Sp2, it shows a real difference, marked by the appearance of different letter notations. Meanwhile, a comparison between Sp1 and Sp2 bacterial isolates showed that there was no real difference in the resulting inhibition zone marked with the same letter. This means that the lactic acid bacteria isolates Sp1 and Sp2 showed the same effect in inhibiting the growth of *Salmonella typhi* bacteria.

The effectiveness is shown by the size of the inhibitory zone. The larger the inhibitory zone, the more effective of the bacterial isolate in inhibiting the pathogenic bacteria *Escherichia coli* and *Salmonella typhi*. All isolates of lactic acid bacteria were able to inhibit both types of pathogenic bacteria, with different inhibitory zone sizes. The Sp1, Sp2 and Sp3 bacteria are more effective in inhibiting the growth of *Salmonella typhi* bacteria. Meanwhile, Sp4 bacteria are more effective in inhibiting the pathogenic bacteria *Escherichia coli*.

DISCUSSION

The lactic acid bacteria group is a group of positive gram bacteria. Previous study that isolated lactic acid bacteria from the parent abalone and obtained 10 types of isolates which were also gram-positive bacteria in the form of bacilli (22). The other study also found that isolated lactic acid bacteria from Sumbawa horse milk and obtained 35 isolates were positive gram bacteria with variations in the shape of long bacilli and short bacilli (23).

The four isolates did not produce the gelatinase enzyme to hydrolyze gelatine. The Sp1 bacterial isolate was found to be positive in the catalase test, this indicates that the Sp3 isolate was able to break down hydrogen peroxide molecules with the help of the catalase enzyme (24). Lactic acid bacteria are known to have the ability to live in acidic conditions. The citrate fermentation test aims to determine the ability of bacterial isolates to use citrate as the only carbon source. If the bacteria are able to ferment citrate, the acid will be removed from the test medium. Changes in pH will cause the color of the media to change from green to blue (20). The inhibitory zone is formed due to interactions between isolates of lactic acid bacteria which encourage the growth of *Escherichia coli*. Bacteria produce bioactive compounds that can damage the

structural components of the cell walls of pathogenic bacteria. The presence of hydrolytic enzymes produced by isolates of lactic acid bacteria is able to degrade the cell membrane components of pathogenic bacteria. The antagonistic ability of lactic acid bacteria to inhibit the growth of *Escherichia coli* is characterized by inhibiting the growth of these bacteria and the formation of a clear zone around the pathogenic bacterial colonies (21). The differences in the ability of each isolate of lactic acid bacteria to inhibit the growth of *Escherichia coli* may be due to differences in the types of active compounds produced. Several things that influence the size of the inhibitory zone formed by isolates of lactic acid bacteria against *Escherichia coli* include: the interaction between the ability of lactic acid bacteria to produce active compounds or hydrolytic enzymes, the age of the bacterial culture, the number of active compounds are produced, the composition of the medium and the incubation time. A decrease in the inhibition zone can also occur because bacterial isolates have entered the death phase due to limited nutrient sources in the media (25).

CONCLUSIONS

This study concluded that the lactic acid bacteria isolate from the cassava *tape* were able to inhibit the growth of *Escherichia coli* and *Salmonella typhi*. Further study is needed regarding the exploration of lactic acid bacteria from other isolate sources and their ability to inhibit other pathogenic bacteria.

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Ethics statement: This research has obtained ethical clearance from the Ethics Commission of Politeknik Kesehatan Kemenkes Medan.

Author's contribution: SMF, S, and SD contributed in study conception and design, data acquisition, analysis and interpretation, and drafting article. ERM contributed in drafting article. All authors: manuscript critically revised and final approval.

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