

Antibacterial Activities of Lactic Acid Bacteria from Langsat Fruit (*Lansiumdomesticum*) against Pathogenic Bacteria and Spoilage Bacteria

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Abstract— Langsat fruit (*Lansiumdomesticum*) sample collected from 4 district in Minahasa, North Celebes. Lactic Acid Bacteria (LAB) isolates were found at that samples. The study aim to examined the antibacterial activity of Lactic acid bacteria (LAB) from Langsat fruit sample against pathogenic bacteria *Escherichia coli* (Gram Negative Bacteria) and spoilage bacteria *Staphylococcus aureus* (Gram Positive Bacteria) using agar well diffusion method. The isolation result obtained 42 isolates of LAB namely 10 isolate were obtained from Southeast Minahasa district, 8 isolates were obtained from the southern district of Minahasa, 12 isolates were obtained from from the Minahasa district, and 12 isolates were obtained from North Minahasa district. Fourty two isolates LAB identified as *Lactobacillus* genera based on profile matching method were effective against bacteria indicator (pathogenic and spoilage bacteria). The antibacterial activity showed through the formation of a clear zone around well that containing LAB isolates. The diameter inhibition of LAB isolates ranged from 3,0 – 17,0 mm. Overall, the isolated LAB showed the remarkable inhibitory effect against both Gram positive (spoilage bacteria) and Gram negative (pathogenic bacteria). However, the spectrum of inhibition was different for the isolates tested. These results suggest that this potent isolates could be used as a natural biopreservatives agent.

Keyword— Langsat fruit, LAB, Antibacterial, *Lactobacillus*, well diffusion.

I. INTRODUCTION

Langsat Fruit (*Lansiumdomesticum*) is a tropical fruit that will only grow well in tropical regions such as Indonesia and is an endemic fruit in Minahasa, North Sulawesi Province. Langsat fruit has benefits and uses for the human body because it has a high nutritional content. Fresh fruits have a limited shelf life and are easily damaged. This is caused by spoilage microorganisms found in these fruits [16]. Likewise with Langsat fruit, although it has benefits for the human body, this fruit decomposes more quickly when picked from the tree.

Fruits are a substrate for bacterial growth because they have high carbohydrate content and high organic acids. LAB are found in environments that are rich in nutrients such as meat, vegetables, fish, fruits, fermented products and so on. Some studies have succeeded in obtaining LAB in fruits and vegetables including Markissa Fruit Fermentation [9], Dried Fruit (Raisins and Fig) from Morocco [1], Ackee fruit from Nigeria [6], Cabbage Juice [15], Mango fruit [4];[5], and Cayene pepper fermentation [11].

Lactic acid bacteria also produce metabolites and some of them have antibacterial activity including organic acids (lactic acid, acetic acid, propionic acid, sorbic acid, benzoic acid), hydrogen peroxide, bacteriocin and diacetyl [3], but some strains capable of synthesizing bacteriocin antimicrobial compounds [2]. Antimicrobial compounds produced by lactic acid bacteria have the potential as natural preservatives to improve the safety of food products.

This study aims to isolate LAB which has antibacterial activity from Langsat Fruits from Minahasa, North Sulawesi.

II. MATERIALS AND METHODS

Sampling : Langsat fruit samples were collected from 4 districts in Minahasa, North Sulawesi Province, namely Southeast Minahasa districts (Mitra), South Minahasa districts (Minsele), Minahasa districts (MI), and North Minahasa districts (Minut).

Isolation and Purification Lactic Acid Bacteria

The initial stage of LAB isolation from Langsat fruit was enrichment. 10 g of Langsat fruit sample was mashed

and put in a 100 ml erlenmeyer containing MRS broth medium pH (5.5), then grown at room temperature for 2 days.

LAB isolation was carried out using the pour plate method. Take 1 ml of fermented Langsat fruit then put into a test tube containing 9 ml of sterile Aquades (10^{-1} dilution). Dilution is continued until the 10^{-7} dilution series, then from each series of dilutions 10^{-5} , 10^{-6} and 10^{-7} , 1 ml of sample is taken and poured into a petri dish and added with MRSA + 1% CaCO_3 . Incubation is carried out at 37°C for 2-3 days until a clear zone is formed around the bacterial colony. Each colony which has different morphological appearance and clear zone formed is isolated and then purified [7]. LAB colonies were purified on the MRS medium so that by the quadrant streak method until separate colonies were obtained [8].

Cell morphology, Gram staining and catalase test, motility, non-spore forming were performed as a preliminary screening for LAB. The selected LAB were maintained as stock cultures at -80°C in 10% skim milk and 20% glycerol.

Antibacterial Activity of Lactic Acid Bacteria

The antibacterial activity test uses the well diffusion assay against test bacteria (*E.coli* ATCC35218 and *S.aureus* ATCC 25923). Test bacteria were taken as much as 15 ml and then inoculated on Nutrien soft agar (0,75% agar) for each bacterium of 50 μl in petridish inoculated by pour plate. The solid medium was perforated using a 6 mm diameter perforator. MRS hard agar poured on petri dish and allow to solidify, then overlaid with nutrient broth were prepared previously and then in place at a temperature of 4°C for 1 hour. Culture of LAB was taken as much as 50 μl and inserted into the wellbore. Petridish that have been inoculated with test bacterial were incubated at 37°C for 24 hours. After that, the inhibitory zone produced and measured based on the diameter of the antimicrobial area [8].

III. RESULT AND DISCUSSION

Isolation of Lactic Acid Bacteria

Samples of Langsat Fruit were collect from 4 district of Minahasa were used for isolation of LAB. 50 isolates of bacteria-acid producing that produce clear zones around the colony. The formation of a clear zone occurs because CaCO_3 in the MRS medium is to be dissolved by acids produced of bacteria acid production. After being confirmed as LAB, 42 isolates of LAB were obtained (10 isolates were obtained from Southeast Minahasa district, 8 isolates were obtained from the southern district of Minahasa, 12 isolates

were obtained from the Minahasa district, and 12 isolates were obtained from North Minahasa district). All these isolates were gram positive, rods or cocci, appeared singly, in pair, chain, tetrad. Cell were non motile and nonsporing, they gave negative reaction for catalase. These strains were then classified into genus level using profile matching method. Based on the profile matching method that 42 isolates were putatively identified as genus *Lactobacillus*.

Table 1. Screening of lactic acid bacteria isolates into genera level by profile matching method.

Character	Isolate	<i>Lactobacillus</i> *
Amount of isolates	42	
Gram stain	+	+
Shape	Rod	Rod
Cell arrangement	Single, pair and chain,	Single, pair and chain
Production gas from glucose	-	-
Catalase	-	-
Spore formation	-	-
Motility	-	-
	Homofermentati	Homofermentati
Fermentation type	ve	ve

*) Bergey's Manual of Systematic Bacteriology

Antibacterial Activity of Lactic Acid Bacteria

The antibacterial activity of LAB isolates (culture) were tested against pathogenic bacteria and spoilage bacteria are summarized in (Table 2) by using agar well diffusion assay, and figure (1) illustrate the zones of inhibition against pathogenic bacteria and spoilage bacteria under study. The diameters of the inhibition zones were varied it ranged between 2,0 to 17,0 mm. All of LAB isolate have inhibitory activity against pathogenic bacteria and spoilage bacteria. Isolates LMT2, LMS8, LMI7, and LMU2 have the highest diameter of inhibition zones 16,5-17,0 mm (*S.aureus*). Isolates LMT2, LMS8, LMI7, and LMU7 have the highest diameter of inhibition zones 7,0-8,0 mm (*E.coli*) this revealed that the LAB inhibited all the pathogenic bacteria and spoilage bacteria tested.

Previous studies have shown that most of the selected strains of lactic acid bacteria had good antagonistic activity against pathogens including *Escherichia coli* [12]. According to [13] whose mentioned that inhibition was scored positive if the width of the clear zone around the colonies of the producer strain was 0.5 mm or larger.

Generally the antibacterial components produced by LAB negative and the same was stated by [14].
 can inhibit the growth of gram-positive bacteria and gram-

Tabel 5. Selection of LAB Isolates based on antagonistic activity (diameter of the clear zone produced against indicator bacteria)

No	Isolate Code	<i>E.coli</i> ATCC 35218 (mm)	<i>St. aureus</i> ATCC 25923 (mm)
Southeast Minahasa			
1	LMT1	3,0	13,2
2	LMT2	7,0*	16,5*
3	LMT3	6,0	6,0
4	LMT4	3,0	16,0
5	LMT5	2,0	12,5
6	LMT6	3,0	11,2
7	LMT7	5,0	6,0
8	LMT8	5,0	14,5
South Minahasa			
9	LMS1	3,0	15,7
10	LMS2	2,0	16,3
11	LMS3	5,0	13,5
12	LMS4	2,0	7,5
13	LMS5	3,0	11,2
14	LMS6	3,0	11,2
15	LMS7	6,0	13,7
16	LMS8	7,0*	17,0*
17	LMS9	5,0	15,7
18	LMS10	3,0	11,5
Minahasa			
19	LMI1	2,0	10,0
20	LMI2	3,0	3,0
21	LMI3	5,0	10,0
22	LMI4	4,0	11,0
23	LMI5	4,0	13,7
24	LMI6	3,0	13,7
25	LMI7	7,0*	15,0*
26	LMI8	6,0	11,0
27	LMI9	4,0	12,0
28	LMI10	5,0	14,0
29	LMI11	3,0	6,0
30	LMI12	3,0	6,0
North Minahasa			
31	LMU1	6,0	16,0
32	LMU2	5,0	17,0*
33	LMU3	3,0	10,0
34	LMU4	3,0	11,0
35	LMU5	2,0	11,7
36	LMU6	6,0	11,7
37	LMU7	8,0*	12,0

38	LMU8	6,0	15,0
39	LMU9	4,0	12,0
40	LMU10	3,0	12,0
41	LMU11	4,0	10,0
42	LMU12	3,0	10,0

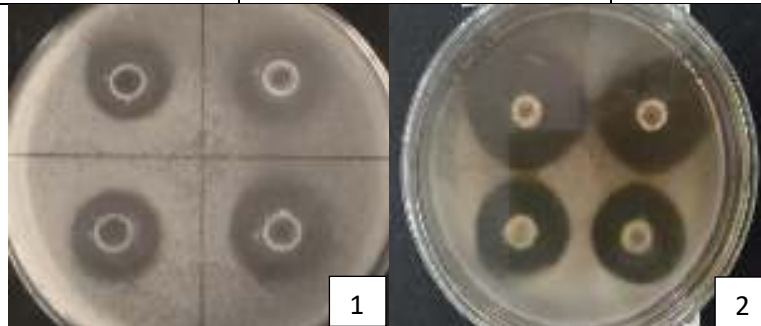


Fig.1: Antibacterial activity of LAB against pathogenic bacteria and spoilage bacteria.
 (1). *Escherichia coli*, (2). *Staphylococcus aureus*

The activity on inhibition of variety of bacteria by LAB due to a combination of many factors produced by LAB e.g. production of lactic acid which reduce pH of Langsat fruit and also other inhibitory substances such as bacteriocins, hydrogen peroxide, diacetyl which are responsible for the most antimicrobial activity [10].

IV. CONCLUSION

The results were obtained 42 isolates of lactic acid bacteria which can be grouped into the genera *Lactobacillus*. All of isolates LAB have antibacterial activity against the growth of pathogenic bacteria and spoilage bacteria. LMT2, LMS8, LMI7, LMU2, and LMU7 have the highest antibacterial activity.

REFERENCES

- [1] Askari, Al, Galal., Kahouadji, A., Khedid, K., Charof, R, and Mennane, Z. 2012. Screening of Lactic Acid Bacteria Isolated from Dried Fruit and Study of Antibacterial Activity. Middle-East Journal of Scientific Research, 11(2) : 209-212.
- [2] Cizeikiene, D., Juodeikiene, G., Paskevicius, A., Bartkiene, E. 2013. Antimicrobial Activity of Lactic Acid Bacteria Against Pathogenic and Spoilage Microorganism Isolated from Food and their Control in Wheat Bread. Journal Food Control, 31 : 539-545.
- [3] Dalie, D.K.D., Deschamps, A.M., and Richard-Forget. 2010. Lactic Acid Bacteria-Potential for Control of Mould Growth and Mycotoxin: review, Food Control, 21: 370-380.
- [4] Fitriyani, I. 2010. Isolasi, Karakterisasi dan Identifikasi Bakteri Asam Laktat (BAL) dari Buah Matang yang berpotensi Menghasilkan Antimikrobia. Skripsi. UIN, Yogyakarta.
- [5] Ibrahim, A., Fridayantian Delvia, F. 2015. Isolasi dan Identifikasi Bakteri Asam Laktat (BAL) dari buah Mangga (*Mangifera indica* L.) Jurnal Ilmiah Manuntung, 1(2):159-163.
- [6] Lawal, R.T., Oyeleke, G.O., Ishola, A.D., and Akinsurojo, M.O. 2018. Determination of Physicochemical Properties and Lactic Acid Bacteria Presence in Ackee (*Blighia sapida*) Fruit. International Journal of Environment, Agriculture and Biotechnology (IJEAB), 3(3): 1079-1082.
- [7] Lawalata V.N. 2012. Rekayasa proses ekstraksi kulit buah langsat (*Lansium domesticum* var *Lansat*) sebagai bahan antibakteri dan antioksidan [disertasi]. Bogor (ID): Institut Pertanian Bogor .
- [8] Lawalata H.J. 2012. Keanekaragaman bakteri asam laktat penghasil antimikrobia selama proses fermentasi bakasang [disertasi]. Universitas Gadjah Mada. Yogyakarta.
- [9] Nurisva Y, M.S. 2013. Isolasi, Karakterisasi, Dan Identifikasi DNA bakteri Asam Laktat (BAL) Yang Berpotensi Sebagai Antimikrobia Dari Fermentasi Markisa Kuning (*Passiflora edulis* var. *flavicarva*). J. Kimia Universitas Andalas, 2(2) : 81-91.
- [10] Ogunbanwo ST. Functional properties of lactic acid bacterial isolated from ogi and fufu, Two Nigerian fermented foods. J food Sci. 2005;27:14-21
- [11] Rustan, I. Reskia. 2013. Studi Isolasi dan Identifikasi Bakteri Asam Laktat dari Fermentasi Cabai Rawit (*Capsicum frutescens* L.). Skripsi, Jurusan Teknologi Pertanian, Universitas Hasanuddin, Makassar.

- [12] Savino,F., Cordisco, L., Tarasco V, Locatelli E, Di Gioia D, Roberto Oggero R and Matteuzzi D. 2011. Antagonistic Effect of Lactobacillus strains against Gas-producing Coliform Isolated from Colicky Infants. *BMC Microbial* 11: 157.
- [13] Schillinger U, and Lucke F. Antimicrobial activity of *Lactobacillus* isolated from meat. *J Appl Environ Microbiol.* 1989;55 :1901-1906.
- [14] Theron MM, and Lues JFR (2011) Organic Acids and Food Preservation. United State: CRC Press. Hlm: 273.
- [15] Utama, C.S., Zuprizal., Chusnul Hanim., Wihandoyo. 2018. Isolasi dan Identifikasi Bakteri Asam Laktat Selulolitik yang berasal dari Jus Kubis Terfermentasi. *Jurnal Aplikasi Teknologi Pangan*, 7(1) :1-6.
- [16] Wahyu, N. 2012. Peranan Bakteri Asam Laktat Terhadap Buah dan Sayuran. <http://apriliana.wahyu.blogspot.com> [25 Agustus 2019].