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Lactic acid bacteria as an exopolysaccharides (EPS) producing starter from pakoba fruit (*Syzygium sp.*), endemic species at Minahasa, North SulawesiHelen J Lawalata ^{1*}¹Dept. of Biology, Faculty of Mathematics, Natural Science, and Earthly, Manado State University, Indonesia

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ABSTRACT

Background: Pakoba fruit (*Syzygium sp.*) is one of the medicinal plants of the Minahasa people and is an endemic species in North Sulawesi. Pakoba can only be found in the Minahasa area and very popular among Minahasa people. The taste is sour, making this fruit as the main ingredient to be processed into rojak, sweets, dodol, jam or syrup. Pakoba fruit is widely used for treatment in the simplest way because this fruit contains many bioactive compounds.

Objective: The study aim to determine the presence of lactic acid bacteria (LAB) in Pakoba fruit and their potential in producing exopolysaccharides.

Results: From the total of 35 producing-acid bacteria, 17 isolates were confirmed as Lactic Acid Bacteria (LAB) isolates with the characteristics of bacilli cells, gram positive, catalase negative, non-motile, non-spore forming, gas production, mesophilic, aciduric, can ferment carbohydrates.

Conclusion: Based on Bergey's Manual of Systematic Bacteriology, the seventeen isolates were identified as member of *Lactobacillus* genus. The seventeen isolates also showed the ability to produce exopolysaccharides in the range of 102-1570 mg /L.

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1. Introduction

Lactic Acid Bacteria (LAB) are beneficial microorganisms and still need to be explored¹ and some lactic acid bacteria are probiotic candidates with certain conditions. Probiotic bacteria have several requirements, namely resistant to low pH conditions (acidic), resistant to bile acids and salts, have the ability to produce antimicrobials so that they are able to develop in the human digestive tract and are able to balance the microflora in the digestive tract and are safe for use^{2,3} Lactic acid bacteria can also produce and secrete Exopolysaccharides (EPS).

LAB have the ability to secrete EPS is important information that has been widely reported regarding its benefits for health.⁴ Some health functions include

immunostimulation,⁵ antioxidant activity,⁶ antitumor and lowering blood cholesterol.⁷ In addition, EPS plays a role in protecting LAB cells from harmful environments and supports in adhesion and biofilms. EPS is also reported to play a role in food technology such as texture formation and rheology,⁸ producing important polymers and food grade so that they are safe to use in food products for example to increase the viscosity of processed food products.^{9,10} Therefore, EPS production becomes an interesting property to consider in the selection of probiotic strains.

The production of EPS of probiotic bacteria is related to genes encoding enzymes that play a role in the reaction of EPS formation, including the fructosyltransferase (*ftf*) gene which encodes the fructosyltransferase enzyme and the glucosyltransferase (*gtf*) gene which encodes the glucosyltransferase enzyme. The polymorphism of the *ftf* gene and *gtf* gene is influenced by the origin and species of

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the LAB strain which has not been studied, especially from local food sources. The identification and screening of *FTF* genes and *GTF genes* in an isolate will have an impact on enzyme variations that play a role in EPS biosynthesis so that the impact will enrich the variations of EPS polymers with unique structures and new functions that have the potential to be developed in use in the pharmaceutical, health and food fields.^{11–13}

LAB exploration from various sources is currently increasing. LAB can be isolated from various foodstuffs for use as probiotics and functional foods such as fruit,^{14,15} vegetables^{16,17} and fermented foods.¹⁸ Pakoba fruit (*Syzygium sp*) as an endemic species of North Sulawesi is known to have the potential to increase endurance because it contains palmitic acid, oleic acid and lenoleic acid and also produces antioxidants¹ and is thought to be the habitat of LAB because the taste of Pakoba fruit is sour.¹⁹

Currently, research on the ability of LAB to produce exopolysaccharides has been widely conducted, especially milk fermentation-based products. However, not much is known about the potential of exopolysaccharides produced by LAB in fruit-based products such as Pakoba fruit. Therefore, this study is important to determine the presence of LAB and how the phenotypic character of LAB isolated from Pakoba fruit (*Syzygium sp.*) and know its potential in producing exopolysaccharides.

2. Materials and Methods

2.1. Isolation lactic acid bacteria

Pakoba Fruit (*Syzygium sp.*) was taken from the North Minahasa Regency, North Sulawesi, Indonesia. The initial growth of LAB from Pakoba fruit is carried out through the enrichment stage. A total of 10 g of fruit flesh samples were cut into small pieces and mashed, and put into a 100 ml erlenmeyer containing MRS broth (Man Ragosa Sharpe) (Merk) pH medium (5.5), then shaker at room temperature for 2 days.

LAB isolation is carried out by pour plate method. In the pipette fermented fruit as much as 1 ml and then put into a test tube containing 9 ml PBS (dilution 10^{-1}). Dilution is continued until the dilution series 10^{-7} , then from each dilution series 10^{-5} , 10^{-6} and 10^{-7} , 1 ml of sample is taken and poured into a petri dish and added MRSA medium + CaCO_3 1% + Sodium acid 1 ppm. Incubation is carried out at 37°C for 2-3 days until a clear zone is formed around the bacterial colony. Each colony with different morphological features and clear zones formed was isolated and further purified.²⁰ LAB colonies are purified on MRS medium in order by quadrant streak method until separate colonies are obtained.

2.2. Phenotypic characterization of lactic acid bacteria (LAB)

Macroscopic observations (seen directly) in the form of visible colony morphology. The parameters observed are color, shape, elevation, edge of the colony. While microscopic observations are carried out using a microscope to see the shape, arrangement, color, presence or absence of endospores from bacterial cells. Cell morphology observations were carried out gram stain to distinguish gram-positive bacteria (purple) from gram-negative bacteria (red). Staining was carried out on a 24-hour old bacterial culture grown on the MRS Agar oblique medium. Staining consists of gram A (Crystal violet) paint. gram B (Lugol solution), gram C (Alcohol), and gram D (Safranin). The staining results were observed under a 1000x magnification microscope. Staining such grams can see the shape, arrangement, color.

In endospore staining using the main stain, namely Malachite green 5% and safranin. Bacterial isolates are etched on the surface of glass objects using ose needles, after which 1-2 drops of Malachite green are then fixated. Next drip 1-2 drops of safranin and dried aerated. The results obtained were given immersion oil and observed under a 1000x magnification microscope. A positive result is the presence of green spores. Lactic acid bacteria are bacteria that do not produce endospores.

Catalase test by dripping H_2O_2 solution 3% on a glass object, then taking a smear of bacteria using ose and applied to the solution on a glass object, seen the reaction formed if no bubbles form indicates a positive reaction.²¹

Motility test is carried out by inserting 1 straight ose bacterial culture into SIM media (Sulfite Indol Motility) by separating 1/4 part of the butt then incubated 48 j, 37°C and observed the presence or absence of propagation around the puncture.²²

2.3. Crude exopolysaccharide test

In crude exopolysaccharide testing is carried out is by gravimetric method. 1 ose isolate was inoculated into 10 mL MRSB which had 5% sucrose added and incubated for 24 hours at 35°C . Separation of bacterial cells is carried out through a centrifugation process with a temperature of 4°C at a speed of 4500 rpm for 45 minutes, the supernatant was taken 5 mL and added 96% cold ethanol as much as 10 mL (2x the sample volume) and allowed to stand for overnight at cold temperatures. Furthermore, centrifuged at a temperature of 4°C at a speed of 4500 rpm for 45 minutes, then the pellets were dried in an oven with a temperature of 100°C for 15 minutes and weighed the dry weight of EPS.¹⁷

3. Result and Discussion

Pakoba fruit samples used in this study were obtained from North Minahasa Regency. Isolation of lactic acid

bacteria (LAB) performed on Pakoba Fruit (*Syzygium sp.*) has succeeded in obtaining 35 colonies of acid-producing bacteria indicated by the presence of clear zones around the colonies grown on MRS Agar selective media containing calcium carbonate (CaCO_3) 1% and sodium azide 1 ppm (Table 1). MRS is a growth medium for acid-producing bacteria because in MRS media there is glucose which is a source of carbon and energy for the growth of these bacteria. Calcium carbonate (CaCO_3) suspension that appears cloudy in the medium so that it will be dissolved by acids produced by acid-producing bacteria including LAB to form a clear zone around the colony. This shows that calcium carbonate (CaCO_3) is an indicator that bacteria growing on MRS media are acid-producing bacteria which include lactic acid bacteria (LAB). Before the isolation process, enrichment is carried out to obtain isolates of acid-producing bacteria for 48 hours at room temperature.^{20,23} Furthermore, purification was carried out on 35 isolates obtained as much as 2x.

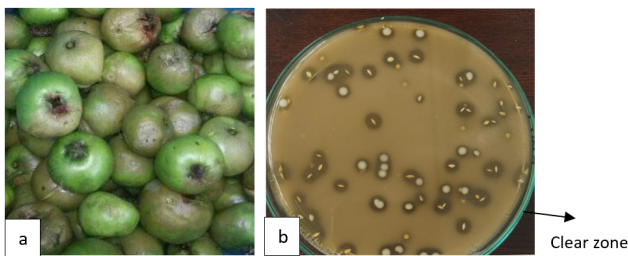


Figure 1: Appearance of Pakoba fruit (*Syzygium sp.*) and Colonies acid-producing bacteria: (a): Pakoba fruit; (b): Bacterial colonies that produce clear zone on MRSA + CaCO_3

Early detection of LAB colonies is based on the presence of clear zones formed around the colony on MRSA- CaCO_3 isolation media. Furthermore, screening of LAB was carried out by conducting a confirmatory test on isolates suspected of being LAB by conducting a Gram staining test, catalase test, endospore staining test, and motility test (Table 2).

LAB screening of Seventeen colonies of acid-producing bacteria that have a circular shape with white and yellowish-white colony colors, showed that Seventeen isolates of these bacteria belong to the lactic acid bacteria (LAB) group. This indicates that LAB isolates have been successfully obtained from samples of Pakoba Fruit (*Syzygium sp.*).

Furthermore, seventeen isolates of LAB obtained from the screening results were identified using the profile matching method. The results of characterization and genus identification (generic assignment) based on profile matching (Table 4) showed that seventeen LAB isolates obtained from samples of Pakoba fruit (*Syzygium sp.*) belongs to a member of the genus *Lactobacillus*. The key characteristics used to distinguish LAB isolates into genus level are cell shape, cell structure, and gas production from glucose.

Table 1: Colonies of acid-producing bacteria (clear zones) on MRSA- CaCO_3 medium isolated from Pakoba fruit

S.No.	Source of Isolates	Colony Color	Isolate Code
1	Pakoba Fruit	Yellowish-white	PM5.1
2		Yellowish-white	PM5.2
3		Yellowish-white	PM5.3
4		Yellowish-white	PM5.4
5		Yellowish-white	PM5.5
6		Milk white	PM5.6
7		Milk white	PM5.7
8		Yellowish-white	PM5.8
9		Yellowish-white	PM5.9
10		Yellowish-white	PM5.10
11		Milk white	PM5.11
12		Milk white	PM5.12
13		Yellowish-white	PM6.1
14		Yellowish-white	PM6.2
15		Yellowish-white	PM6.3
16		Yellowish-white	PM6.4
17		Yellowish-white	PM6.5
18		Yellowish-white	PM6.6
19		Milk white	PM6.7
20		Yellowish-white	PM6.8
21		Milk white	PM6.9
22		Yellowish-white	PM6.10
23		Yellowish-white	PM6.11
24		Yellowish-white	PM7.1
25		Yellowish-white	PM7.2
26		Yellowish-white	PM7.3
27		Yellowish-white	PM7.4
28		Yellowish-white	PM7.5
29		Yellowish-white	PM7.6
30		Yellowish-white	PM7.7
31		Yellowish-white	PM7.8
32		Yellowish-white	PM7.9
33		Yellowish-white	PM7.10
34		Yellowish-white	PM7.11
35		Yellowish-white	PM7.12

The results of bacterial isolation in Pakoba fruit show that not all colonies that produce clear zones on MRS- CaCO_3 media are members of LAB because among the colonies that produce these clear zones, there are also that show Gram negative bacteria, positive catalase, and form spores (Table 2). These bacteria are thought to be *E. coli*, *Bacillus*, *Micrococcus*, and *Pseudomonas*.

In addition to LAB, bacteria members of the genus *Micrococcus*, *Bacillus*, *Pseudomonas*, *Staphylococcus*, *Salmonella*, *Shigella*, *Vibrio*, *Klebsiella* are usually found in fruits and fruit products such as fresh fruit juice (Mango fruit and Avocado fruit), fresh guava fruit juice, banana fruit, papaya fruit, Jujube fruit, fruit, Plum fruit, Apple fruit, Cantaloupe fruit, Watermelon fruit, Dragon fruit.^{24–28}

Selection or screening was carried out, obtained as many as seventeen isolates that are strongly suspected as LAB

Table 2: Screening of LAB and Non-LAB Isolates Growing on MRSA-CaCO₃ Medium Obtained from Buah Pakoba (*Syzygium sp.*)

No	Source of Isolates	Isolate Code	Acid Producer (Clear Zone)	Gram	Catalase	Formation spore	Motility	LAB / NON LAB
1	Pakoba fruit	PM5.1	+	+	-	-	-	LAB
2		PM5.2	+	+	-	-	-	LAB
3		PM5.3	+	+	-	-	-	LAB
4		PM5.4	+	+	-	-	-	LAB
5		PM5.5	+	-	+	-	-	Non LAB
6		PM5.6	+	+	+	-	-	Non LAB
7		PM5.7	+	-	+	-	nd	Non LAB
8		PM5.8	+	+	+	+	nd	Non LAB
9		PM5.9	+	+	+	-	-	Non LAB
10		PM5.10	+	+	+	+	nd	Non LAB
11		PM5.11	+	-	+	-	nd	Non LAB
12		PM5.12	+	+	-	+	nd	Non LAB
13		PM6.1	+	+	+	-	-	Non LAB
14		PM6.2	+	-	+	-	nd	Non LAB
15		PM6.3	+	+	-	-	-	LAB
16		PM6.4	+	+	-	-	-	LAB
17		PM6.5	+	+	-	-	-	LAB
18		PM6.6	+	+	-	-	-	LAB
19		PM6.7	+	+	+	+	nd	Non LAB
20		PM6.8	+	+	+	-	-	Non LAB
21		PM6.9	+	+	+	-	-	Non LAB
22		PM6.10	+	+	-	-	-	LAB
23		PM6.11	+	+	-	-	-	LAB
24		PM7.1	+	+	-	-	-	LAB
25		PM7.2	+	+	-	-	-	LAB
26		PM7.3	+	+	-	-	-	LAB
27		PM7.4	+	+	-	-	-	LAB
28		PM7.5	+	-	+	-	nd	Non LAB
29		PM7.6	+	+	+	-	-	Non LAB
30		PM7.7	+	+	+	+	nd	Non LAB
31		PM7.8	+	+	+	-	-	Non LAB
32		PM7.9	+	+	-	-	-	LAB
33		PM7.10	+	+	-	-	-	LAB
35		PM7.11	+	+	•	-	-	LAB

based on criteria (i) gram-positive, (ii) cocci or rod-shaped cells, (iii) negative catalase, (iv) not spore-forming, and (v) non-motile. Data on the results of screening of LAB were successfully obtained from Pakoba Fruit (*Syzygium sp.*) were presented in Table 3.

LAB screening of Seventeen colonies of acid-producing bacteria that have a circular shape with white and yellowish-white colony colors, showed that seventeen isolates of these bacteria belong to the lactic acid bacteria (LAB) group. This indicates that LAB isolates have been successfully obtained from samples of Pakoba Fruit (*Syzygium sp.*).

Furthermore, Seventeen LAB isolates obtained from the screening results were identified using the profile matching method. The results of characterization and genus identification (generic assignment) based on profile

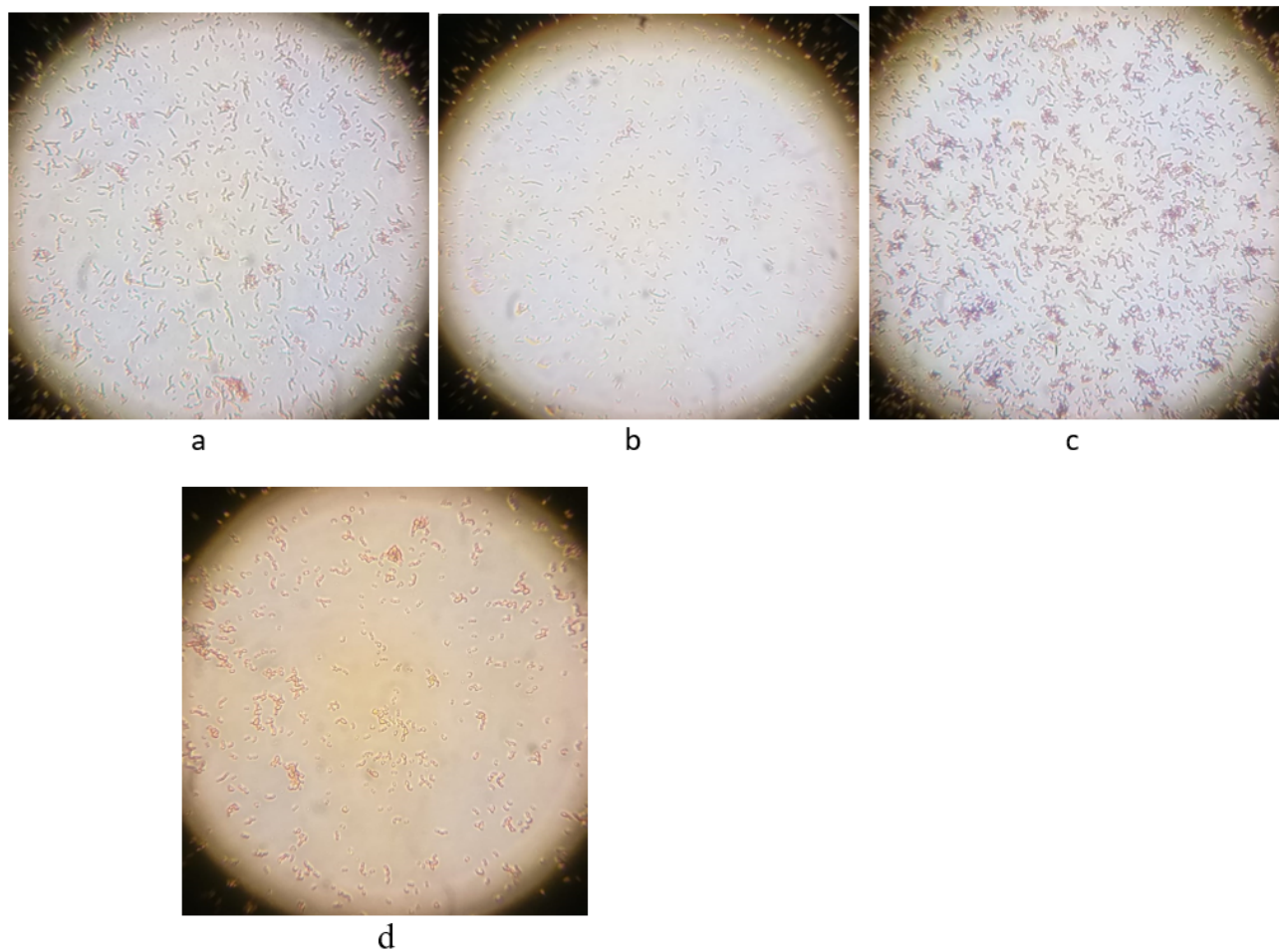
matching (Table 4) showed that Seventeen LAB isolates obtained from samples of Pakoba fruit (*Syzygium sp.*) belonged to members of the genus *Lactobacillus*. The key characteristics used to distinguish LAB isolates into genus level are cell shape, cell structure, and gas production from glucose.²⁹

Thus, characterization based on profile matching can show that as many as Seventeen LAB isolates that have the character of round colony shape, yellowish-white color, stem cell shape (bacilli), single or pair cell arrangement (two-two), gram positive, negative catalase, non motile, do not produce gas, and non form spores so that they belong to members of the genus *Lactobacillus*.²⁹

Fruit is one of the natural habitats for bacteria, including LAB. This is because the fruit contains various chemical

Table 3: Initial characterization (Confirmation Test) of LAB isolates obtained from Pakoba Fruit (*Syzygium* sp.)

No.	Isolate Code	Cell Shape	Cell arrangement	Gram	Catalase	Spore formation	Motility
1	PM5.1	Rod	Single/pair/chain	+	-	-	-
2	PM5.2	Rod	Single/pair/chain	+	-	-	-
3	PM5.3	Rod	Single/pair/chain	+	-	-	-
4	PM5.4	Rod	Single/pair/chain	+	-	-	-
5	PM6.3	Rod	Single/pair/chain	+	-	-	-
6.	PM6.4	Rod	Single/pair/chain	+	-	-	-
7.	PM6.5	Rod	Single/pair/chain	+	-	-	-
8.	PM6.6	Rod	Single/pair/chain	+	-	-	-
9.	PM6.10	Rod	Single/pair/chain	+	-	-	-
10.	PM6.11	Rod	Single/pair/chain	+	-	-	-
11.	PM7.1	Rod	Single/pair/chain	+	-	-	-
12.	PM7.2	Rod	Single/pair/chain	+	-	-	-
13.	PM7.3	Rod	Single/pair/chain	+	-	-	-
14.	PM7.4	Rod	Single/pair/chain	+	-	-	-
15.	PM7.9	Rod	Single/pair/chain	+	-	-	-
16.	PM7.10	Rod	Single/pair/chain	+	-	-	-
17.	PM7.11	Rod	Single/pair/chain	+	-	-	-

**Figure 2:** Cell shape of isolate LAB that obtained from Pakoba fruit (*Syzygium* sp.) **a):** Cell shape of isolate LAB PM6.10; **b):** Cell shape of isolate LAB PM6.4; **c):** Cell shape of isolate LAB PM7.2; **d):** Cell shape of isolate LAB PM5.1

compounds such as water, carbohydrates, fats, vitamins, organic acids, and various minerals needed as nutrients for LAB. Some types of fruit that are thought to be the habitat of LAB include Langsat fruit (*Lactobacillus sp.*),¹⁴ Salak fruit, banana stuff, soursop fruit, mangosteen fruit, guava rice-rice fruit (*Lactobacillus*, *Pediococcus*, *Lactococcus* and *Leuconostoc*),²² Kersen fruit (*Lactobacillus*),³⁰ and kweni mango fruit (*Streptococcus*).²¹

The phenotypic characteristics of LAB isolates determined in the form of, cell morphological characters, biochemical characters and physiological characters have been performed (Table 4). The Seventeen LAB isolates (PM5.1, PM5.2, PM5.3, PM5.4, PM6.3, PM6.4, PM6.5, PM6.6, PM6.10, PM6.11, PM7. 1, PM7.2, PM7.3, PM7.4, PM7.9, PM7.10 and PM7.11) have almost the same colony morphological characteristics, namely round colony shape and yellowish-white colony color, slippery colony edges, *convex* elevation.

3.1. Crude production of exopolysaccharides (EPS) of lactic acid bacteria isolated from pakoba fruit (*Syzygium sp.*)

In this study, crude production of exopolysaccharides (EPS) produced by each LAB isolate obtained from Pakoba fruit was carried out. The Seventeen isolates of LAB from Pakoba fruit (*Syzygium sp.*) It has the ability to produce various amounts of exopolysaccharides, ranging from 102 mg / L - 1570 mg / L.

EPS production by all seventeen LAB isolates from Pakoba fruit showed varying amounts although all seventeen isolates were identified in the same genus *Lactobacillus*. This is due to the influence of its genetic diversity. Species differences refer to differences in genes carried so that they are closely related to the metabolic processes carried out and the number of metabolites produced. Each gene is responsible for overseeing the formation, function and ability of an enzyme to work, so that when there are different genes even though they are still classified as the same species but with different strains, the final results of metabolism can be different.³¹

The difference in the amount of EPS obtained in this study with other studies is thought to be caused by several factors, one of which is the incubation time used. In this study EPS production by LAB was harvested at the 48th hour after being grown on 10 mL of liquid media [nurhasanah]. The maximum production of EPS produced by a culture will be influenced by the growth factors of the culture, where the optimum EPS production occurs during maximum cell production, namely in the stationary phase or the final phase of LAB.³² The stationary phase of LAB isolates begins in the range of 20-24 hours and in the next stage of growth there will be degradation of EPS. This happens because EPS produced by microbes in the stationary phase can be reused as a source of carbon in the

near-death phase because microbes have enzymes that can degrade the EPS. As a result, the extension of incubation time will actually decrease EPS production.^{31,33}

Other factors that can affect the amount of EPS produced by LAB in addition to incubation time are excess carbohydrates in the media, the type of media used, fermentation conditions such as temperature, pH and oxygen levels and the physiology of each bacterium,³⁴ the optimum temperature of LAB is known to be 37°C, so in this study, to obtain maximum polysaccharides, the incubation temperature was lowered to 35°C.

Lactic acid bacteria produce secondary metabolite products in the form of exopolysaccharides that are secreted outside the cell when under unfavorable conditions. Differences in the ability to produce EPS due to differences in species or strains. This difference in strains indicates that the genes contained in these Seventeen isolates of LAB are also different, each gene has different functions and abilities resulting in differences in metabolic processes and differences in the results of metabolites produced.^{13,35}

EPS also tends to be influenced by the bacterial environment such as pH, growth phase, availability of nutrients such as carbon and nitrogen sources, temperature, and fermentation conditions.³⁶ In this test using MRS Broth medium which added 5% Sucrose as a growing medium from lactic acid bacteria because this media contains many nutrients favored by LAB. The added sucrose is an excellent substrate for EPS synthesis with abundant yields. Exopolysaccharide production from LAB isolate is presented in Figure 3.

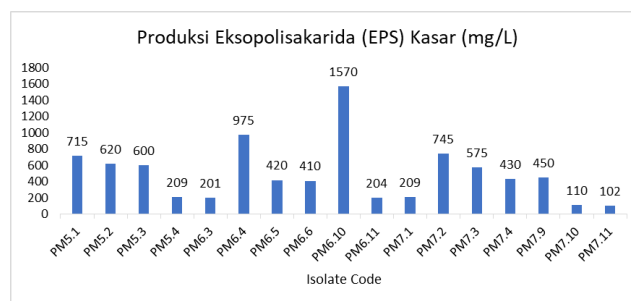


Figure 3: Production of exopolysaccharides (EPS) Crude LAB Isolates from pakoba fruit (*Syzygium sp.*)

EPS production increases with increasing sucrose concentrations and involves extracellular sucrase-type enzymes used by LAB isolates to polymerize EPS, namely fructansucrase and glucansucrase.³⁷ In this test using LAB isolates aged 24 hours. The use of 24-hour age isolates because the logarithmic phase ends at that time and will enter the stationary phase where in this phase bacteria produce EPS optimally. Bacteria begin to experience extreme conditions such as reduced growth nutrients resulting in secondary metabolite products in the form of exopolysaccharides that are released outside the

Table 4: Generic assignment of LAB isolates obtain from Pakoba fruit (*Syzygium* sp.) based on Profile Matching method

Karakter Kunci*	Leuconostoc	Pediococcus	Lactobacillus	I ^a
Number of isolates				17
Shape	Cocci	Cocci	Rods	Rods
Cell arrangement	Pair/chain	Tetrad	Single/pair/chain	Single/pair/chain
Spore formation	-	-	-	-
Catalase	-	-	-	-
Production gas from glucose	+/-	-	-	+/-
Motility	-	-	-	-
Fermentation Type	Hetero	Homo	Hetero/ homo	Hetero/homo
Growth at 10°C	+/-	+/-	+/-	+
Growth at 40°C	+/-	+/-	+/-	+
Growth at 50°C	-	+/-	+/-	-
Growth at pH 3.5	-	+/-	+/-	+
Growth at pH 7.5	+	+	+	-
Growth at pH 9.6	-	-	-	-
Growth at NaCl 6.5%	+/-	+/-	+/-	+
Growth at NaCl 18%	-	-	-	-

*Karakter kunci deskripsi genus *Lactobacillus*, *Leuconostoc* dan *Pediococcus* berdasarkan Bergey's manual Systematics of Bacteriology.²⁹
I^a *Lactobacillus*

cell when conditions are unfavorable. The principle of this test is to separate exopolysaccharides from bacterial cells using centrifugation at 4°C with the aim of preventing protein denaturation. The pellets obtained from the results of the process are dried and weighed until they reach a constant weight. The results obtained are then analyzed quantitatively.

The filtrate obtained was added with 96% cold ethanol by twice the volume of the sample used to precipitate EPS. The use of ethanol with double the sample aims to facilitate the rate of diffusion so that the distribution of particles will be greater with the greater the surface area. 96% ethanol is used as a precipitator for polysaccharides and has a relatively small ability to dissolve polysaccharides, although the ability to dissolve other substances is relatively large.³⁸ Ethanol has a much lower dielectric constant than water so it has lower polarity than water, whereas polysaccharides contain many hydroxyl groups that give their polar characteristics, as a result of which ethanol concentrations increase in solution and cause a decrease in polysaccharide solubility or cause precipitation.³⁹

Exopolysaccharides produced by microbes have great potential for the future, the use of microbes to produce polysaccharides is very profitable in economic terms compared to plant polysaccharides. In producing microbial polysaccharides can be done continuously on a large scale and does not require large areas of land such as plants.³⁹

4. Conclusion

Lactic acid bacteria were successfully isolated from Pakoba Fruit and obtained 35 isolates of acid-producing bacteria and based on LAB screening, Seventeen isolates were obtained (PM5.1, PM5. 2, PM5.3, PM5. 4, PM6. 3, PM6.

4, PM6. 5, PM6. 6, PM6. 10, PM6. 11, PM7.1, PM7. 2, PM7. 3, PM7.4, PM7. 9, PM7. 10 and PM7. 11). Based on phenotypic identification using the profile matching method, the Seventeen LAB isolates belong to members of the genus *Lactobacillus*.

Seventeen isolates of *Lactobacillus* are capable of producing exopolysaccharides (EPS) of 102 mg/L - 1570 mg / L.

5. Conflict of Interest

None.

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References

1. Detha A, Datta FU, Beribe E, Foeh N, Ndaong N. Karakteristik Bakteri Asam Laktat yang Diisolasi dari Susu Kuda Sumba. *Jurnal Kajian Veteriner*. 2019;7(1):85–92.
2. Melia S, Arintonang SN, Juliyarsi I, Kurnia YF, Rusdimansyah, Hernita VO. The Screening of Probiotic Lactic Acid Bacteria from Honey of Stingless bee from Wesr Sumatra, Indonesia and Using as Starter Culture. *Biodiversitas J Biol Divers*. 2022;23(12):6379–85.
3. Seveline S. Kajian Pustaka Teknik Pengeringan Semprot (Spray Drying) untuk Pengawetan dan Produksi Probiotik. *Jurnal Agroindustri Halal*. 2018;3(1):80–6.
4. Caggianiello G, Kleerebezem, Spano G. Exopolysaccharides produced by lactic acid bacteria: from health-promoting benefits to stress tolerance mechanisms. *Appl Microbiol Biotechnol*. 2016;100(9):3877–86.
5. Hidalgo-Cantabrana C, Lopez P, Gueimonde M, Reyes-Gavilán CGL, Suárez A, Margolles A, et al. Immune Modulation Capability

- of Exopolysaccharides Synthesised by Lactic Acid Bacteria and Bifidobacteria. *Probiotics Antimicrob Proteins*. 2012;4(4):227–37.
6. Polak-Berecka M, Choma A, Wasko A, Gorska S, Gamian A, Cybulska J. Physicochemical characterization of exopolysaccharides produced by *Lactobacillus rhamnosus* on various carbon sources. *Carbohydr Polym*. 2015;117:501–9.
 7. Ryan PM, Ross RP, Fitzgerald GF, Caplice N, Stanton C. Sugar-coated: Exopolysaccharide producing lactic acid bacteria for food and human health applications. *Food Funct*. 2015;6(3):679–93.
 8. Torino MI, Valdez GF, Mozzi F. Biopolymers from lactic acid bacteria. Novel applications in foods and beverages. *Front Microbiol*. 2015;6:834.
 9. Malik A, Radji M, Kralj S, Dijkhuizen L. Screening of lactic acid bacteria from Indonesia reveals glucanase and fructanase genes in two different *Weissella confusa* strains from soya. *FEMS Microbiol Lett*. 2009;300(1):131–8.
 10. Ma'unatin A, Harijono H, Zubaidah E, Rifa' M. The isolation of exopolysaccharide-producing lactic acid bacteria from lontar (*Borassus flabellifer* L.) sap. *Iran J Microbiol*. 2020;12(5):437–44.
 11. Alfuruqi HQD, Anindita NS, Bimantara A. Kajian Molekuler Pada Probiotik Asal Air Susu Ibu Dalam Sintesis Ekspolisakarida (EPS). *J Bioteknologi Bios Indonesia*. 2021;8(1):114–22.
 12. Malik A, Ariestanti DM, Nurfactiyani A, Yanuar A. Skrining Gen Glukosiltransferase (GTF) dari Bakteri Asam Laktat Penghasil Ekspolisakarida. *Makara Sains*. 2008;12(1):1–6.
 13. Nurhasanah N, Fu'adah IT, Satria H, Yuwono SD. Analisis Ekspolisakarida Dari Bakteri Asam Laktat Hasil Fermentasi Kefir Kolostrum. *Anal Environ Chem*. 2020;5(1):65–73.
 14. Lawalata HJ, Rengkuan M, Satiman U. Antibacterial Activities of LAB from Langsung Fruit (*Lansium domesticum*) against Pathogenic Bacteria and Spoilage Bacteria. *Int J Environ, Agriculture Biotechnol*. 2019;4(6):1806–10.
 15. Panthavee W, Noda M, Danshiitsoodol N, Kumagai T, Sugiyama M. Characterization of Exopolysaccharides Produced by Thermophilic Lactic Acid Bacteria Isolated from Tropical Fruits of Thailand. *Biol Pharm Bull*. 2017;40(5):621–9.
 16. Bawole KV, Umboh SD, Tallei TE. Uji Ketahanan Bakteri Asam Laktat Hasil Fermentasi Kubis Merah (*Brassica oleracea* L.) Pada pH 3. *J Mipa Unsrat Online*. 2018;7(2):20–3.
 17. Halim CN, Elok ZD. Studi Kemampuan Probiotik Isolat Bakteri Asam Laktat Penghasil Ekspolisakarida Tinggi Asal Sawi Asin (*Brassica juncea*). *J Pangan dan Agroindustri*. 2013;1(2):129–37.
 18. Lawalata H, Suriani W. Antagonistic activity of *Pediococcus* isolated from bakasang against *Pseudomonas fluorescens* (producing-histamine bacteria). *Int J Adv Res Biol Sci*. 2017;4(10):221–5.
 19. Walean M, Rumondor R, Maliangkay HP, Melpin RD. Pengaruh Pemberian Ekstrak Etanol Kulit Batang Pakoba (*Syzygium* sp.) Terhadap Gambaran Histopatologi Ginjal Tikus Putih Yang Diinduksi Etilen Glikol. *Chem Prog*. 2018;11(1):29–34.
 20. Lawalata HJ, Sembiring L, Rahayu ES. Molecular Identification of Lactic Acid Bacteria Producing Antimicrobial Agents From Bakasang, An Indonesian Traditional Fermented Fish Product. *Indonesian J Biotechnol*. 2011;16(2):93–9.
 21. Ibrahim A, Fridayanti A, Delvia F. Isolasi dan identifikasi bakteri asam laktat (BAL) dari buah mangga (*Mangifera indica* L.). *J Ilmiah Manuntung*. 2015;1(2):159–63.
 22. Sari NP, Leni FB, Roza RM. Isolasi dan Karakterisasi Bakteri Asam Laktat (BAL) dari Buah-buahan di Riau; 2013. Available from: <https://repository.unri.ac.id/xmlui/handle/123456789/5914>.
 23. Hwanhlem N, Buradaleng S, Wattanachant S, Benjakul S, Tani A, Maneerat S. Isolation and Screening of Lactic Acid Bacteria From Thai Traditional Fermented Fish (Plasom) and Production of Plasom from Selected Strain. *J Food Cont*. 2011;22:401–7.
 24. Babiye B. Isolation and Identification of Bacteria From Fresh Fruit Juice Prepared in Cafeterias and Restaurants. *Biosci Biotechnol Res Asia*. 2017;14(1):307–13.
 25. Biswas B, Azad AK, Absar N, Islam S, Amin S. Isolation and Identification of Pathogenic Bacteria from Fresh Fruits and Vegetables in Chittagong, Bangladesh. *J Microbiol Res*. 2018;10(2):55–8.
 26. Hasan NA, Zulkahar IM. Isolation and Identification of Bacteria from Spoiled Fruits. *AIP Conf Pro*. 2018;2020. doi:10.1063/1.5062699.
 27. Herwin H, Fitriana F, Nurung AH. Isolasi Bakteri Penghasil Selulosa Dari Buah-Buahan Dipasar Tradisional Makassar. *As-Syifaa J Farmasi*. 2020;12(1):47–50.
 28. Sarker AR, Haque M, Rifa RA, Ema FA, Islam A. Isolation and identification of bacteria from fresh guava (*Psidium guajava*) sold at local markets in Mymensingh and their antibiogram profile. *Vet World*. 2018;11(8):1145–9.
 29. Whitman WB. *Bergey's Manual of Systematic Bacteriology*. New York: Springer-Verlag; 2009.
 30. Giyatno DC, Retnaningrum E. Isolasi dan karakterisasi bakteri asam laktat penghasil ekspolisakarida dari buah kersen (*Muntingia calabura* L.). *J Sains Dasar*. 2020;9(2):42–9.
 31. Nasution AY, Rasyidah R, Mayasari U. Potensi Bakteri Asam Laktat Sebagai Penghasil Ekspolisakarida Dari Dekke Na Niura. 2022;7(3):214–20.
 32. Ihsan B. *Dasar-Dasar Mikrobiologi*. Indonesia: Insan Cendekia Mandiri; 2021.
 33. Petry S, Furlan S, Crepeau MJ, Cerning J, Desmazeaud M. Factors affecting exocellular polysaccharide production by *Lactobacillus delbrueckii* subsp. *bulgaricus* grown in a chemically defined medium. *Appl Environ Microbiol*. 2000;66(8):3427–31.
 34. Asmara KT. Potensi Bakteri Asam Laktat Dari Jerok, Hasil Fermentasi Durian (*Durio zibethinus* L) Khas Karo Sebagai Kandidat Probiotik dan dalam Menghasilkan Ekspolisakarida. Indonesia: University of Sumatera Utara; 2017. Available from: <http://repository.usu.ac.id/handle/123456789/21847>.
 35. Zahro F. Isolasi dan identifikasi bakteri asam laktat asal fermentasi Markisa Ungu (*Pasiflora edulis* var. *Sims*) sebagai penghasil ekspolisakarida; 2014.
 36. Fatih MT. Produksi ekspolisakarida oleh bakteri asam laktat asal susu kacang tanah terfermentasi. Malang; 2020.
 37. Malik A, Sheilla S, Firdaus W, Handayani T, Saepudin E. Sucrase activity and exopolysaccharide partial characterization from three *Weissella confusa* strain. *Hayati J Biosci*. 2015;22(3):130–5.
 38. Kusmawati A, Pratiwi IB. Pengambilan Polisakarida Acemannan dari Aloe Vera Menggunakan Etanol sebagai Pengendap. Jurusan Teknik Kimia, Universitas Diponegoro; 2009.
 39. Klinchongkon K, Bunyakiat T, Khuwijitjaru P, Adachi S. Ethanol Precipitation of Mannooligosaccharides from Subcritical Water-Treated Coconut Meal Hydrolysate. *Food Bioprocess Technol*. 2019;12:1197–1204.

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