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The Influence of Sucrose Addition and Bacterial Strains on the Production of Banana Peel-Based Exopolysaccharides

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Abstract:- Exopolysaccharides, formed from sugars by microorganisms, are mostly produced in dairy-based fermentation products, but can also be produced in fruit or vegetable-based fermentation, such as banana peels. This study aims to produce exopolysaccharides from banana peel waste using *L. acidophilus* and *L. plantarum* bacterial fermentation, with tests to observe the effects of sucrose addition concentration, bacterial type, and functional groups on the resulting exopolysaccharides. The observed parameters were texture, color, weight and sugar content, and identification of functional groups in the produced polysaccharides. The research results showed that the addition of sucrose to both L. acidophilus and L. plantarum isolates resulted in significant differences in texture for each variable. However, the use of different isolates (*L. acidophilus* and *L. plantarum*) did not show significant differences in texture. There was a significant difference in color between the two isolates, with *L. plantarum* being darker. *L. plantarum* isolate was able to produce a higher amount of glucose in EPS than L. acidophilus. The exopolysaccharide produced showed the presence of polysaccharides, which are generally composed of O-H (hydroxyl), C=O (carbonyl), -C-H/C-H_2, -C_4-O_4H, and α(1,6) glycosidic bonds.

Keywords:- *Exopolysaccharides, L. acidophilus, L. plantarum.*

I. INTRODUCTION

Food is one of the primary basic needs of humans. The contents of food are beneficial for energy sources and growth factors of the body. Recently, the demand for food has increased, so starchy or sugary substances have become the main focus compared to other needs. Research and development related to new sources are continuously conducted to produce energy sources from alternative materials that contain carbohydrates, such as sugar or starch and biomass [1].

Exopolysaccharides are residues of monosaccharides formed from sugars and sugar derivatives [2]. Exopolysaccharides are widely used in various industries, including pharmaceuticals, cosmetics, pharmacy, cosmetics, and food [3]. Exopolysaccharides have many benefits, particularly as stabilizers, emulsifiers, gelling agents, and having good fluid binding abilities. Exopolysaccharides (EPS) is one of the polysaccharides produced by microorganisms that are released extracellularly around cells. Lactic acid bacteria can produce long-chain extracellular polysaccharides or exopolysaccharides (EPS), including those that can produce exopolysaccharides, namely *Lactobacillus acidophilus, Lactobacillus rhamnosus, Lactobacillus casei, Lactobacillus plantarum, Lactobacillus reuteri,* and *Bifidobacterium* [4][5]. Exopolysaccharides produced by lactic acid bacteria continue to be used and developed as technology advances, primarily because of their health benefits[6]. In fermented food products, they play a role as taste and texture enhancers, can be used as additives in food, and are useful as anticarcinogenic, antitumor, cholesterol-lowering, and immunomodulatory agents [7][8][9].

Bananas are the most widely produced fruit in Indonesia. According to data from the Indonesian Central Statistics Agency, banana production continues to increase each year, with production reaching 8,818,756 tons in 2020 [10]. The abundant production of bananas results in a large amount of banana peel waste, which is often discarded or used as animal feed. However, if the waste is processed, it can have a high economic value. Therefore, it is necessary to explore ways to utilize banana peel waste and increase its nutritional and economic value [11].

Research shows that banana peels contain high levels of nutrients, such as starch, protein, and other compounds, with a particularly high concentration of carbohydrates. Nutritional content found in banana peels includes 0.32 g of protein, 2.11 g of fat, 715 mg of calcium, 117 mg of phosphorus, 1.6 mg of iron, 0.12 mg of vitamin B, 17.5 mg of vitamin C, and high levels of carbohydrates, specifically 18.5 g [12]. Banana peels can be fermented by lactic acid bacteria, which can produce a product called exopolysaccharide (EPS). Based on this information, it is important to research the ability of lactic acid bacteria to ferment banana peels and produce EPS [13].

Therefore, this research WILL produce exopolysaccharide (EPS) from banana peel waste using lactic acid bacteria (LAB) fermentation, namely *L. acidophilus* and *L.plantarum.* In addition, it aims to specifically examine the effect of sucrose concentration on the produced EPS, examine the effect of the type of LAB

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used on the produced EPS, and examine the effect of functional groups present in the produced EPS using FTIR analysis.

II. EXPERIMENTAL

A. Materials

The materials used are banana peels, 70% alcohol, 95% ethanol, aquadest, *L. acidophilus* bacteria culture, and *L.plantarum* bacteria culture.

B. Equipments

The equipment used in this study are autoclave, electric stove, petri dish, centrifuge, balance, incubator, UV-Vis spectrophotometer, glass beaker, filter paper, HPLC, and FTIR.

C. Methodes

- *Sterilization*

The glassware that will be used in this research is washed thoroughly and dried. Then it is wrapped in paper and placed in heat-resistant plastic. After that, it is sterilized in an autoclave at a temperature of 121 ^{o}C with a pressure of 1 atm for 30 minutes.

- *Banana Peel Extraction*

The banana peels are dried and crushed into powder using a blender. Next, the banana peel powder is extracted with methanol. 100 grams of dried banana peel powder is soaked in ethanol and all the powder is immersed in the solvent for 24 hours. Then, filter the filtrate and soak the precipitate again in ethanol until the compounds contained in the precipitate are completely extracted. The obtained filtrate is collected and evaporated at low pressure and 40°C temperature using a rotary vacuum evaporator to obtain a concentrated ethanol extract (crude extract).

- *Media Preparation*

Weigh of 6.82 grams of MRS agar media and add 2 grams, 4 grams, 6 grams, 8 grams, 10 grams, and 12 grams of sucrose to each variable, respectively. Put each variable into a glass beaker and add 100 ml of distilled water. Heat on a hot plate until boiling and stir evenly using a stirrer. Then, pour each solution into separate Erlenmeyer flasks and cover with cotton plugs, sterilize with an autoclave at a temperature of 121°C and a pressure of 1 atm for 30 minutes. Incubate for 48 hours at room temperature [14].

- *Bacteria Regeneration*

Lactic Acid Bacteria (LAB), such as *L. acidophilus* and *L.plantarum*, to be used, must be regenerated aseptically. Take one colony of bacteria and grow it on the prepared MRS agar, then incubate for 48 hours at room temperature. The regenerated *L. acidophilus* and *L.plantarum* bacteria are then used to make the inoculum [15].

- *Production of Exopolysaccharides*

One hundred grams of banana peel extract was dissolved in 100 ml of distilled water and adjusted to pH 7. Sucrose was added with varying concentrations of 2%, 4%, 6%, 8%, 10%, and 12% (w/v). Yeast extract was added at a concentration of 0.25 g (w/v) for each variable. Then, *L. acidophilus* and *L.plantarum* inoculum were added at a concentration of 10% (v/v) and incubated at room temperature for 48 hours.

- *Extraction of Exopolysaccharides*

Take 20 mL of inoculum and add 20 mL of chloroform. Shake at 100 rpm for 30 minutes at room temperature. Then, centrifuge using a cold centrifuge at 4°C and 5000 rpm for 15 minutes to obtain a supernatant. Take the supernatant containing exopolysaccharides and add 96% cold ethanol at 2x volume, which is 40 mL. Let it sit for 24 hours in the refrigerator. Then, a sediment will be produced which can be separated and dried at 50°C for 6 hours until a constant weight is obtained. Calculate the Exopolysaccharide content using the equation 1:

$$EPS \text{ content} = \frac{\text{weight of EPS (mg)}}{\text{volume (L)}} \quad (1)$$

D. Data Analyses

The glucose HPLC test and FTIR test used in this study were conducted at the Laboratory of Diponegoro University, Semarang.

III. RESULT AND DISCUSSION

A. Exopolysaccharide texture and color

The growth of exopolysaccharides (EPS) in vitro culture can be observed by the visual appearance of texture and color. The texture of EPS is one of the markers used to evaluate the quality of EPS. The observation of EPS texture was carried out at 4 weeks after culture, which produced varying EPS textures as shown in Figure 1.

The research results indicate that the addition of 6 grams of sucrose to both isolates, *L. acidophilus* and *L.plantarum*, had a significant difference in texture compared to other treatments. However, the use of different isolates (*L. acidophilus* and *L.plantarum*) did not show a significant difference in texture. Only a color difference was observed between the two isolates, with *L.plantarum* having a darker color than *L. acidophilus*.

The texture of EPS is a marker used to determine the quality of EPS, which can indicate whether cells are still actively dividing or experiencing cell division stagnation. Good quality EPS is assumed to have a brittle texture, which facilitates separation into individual cells and increases oxygen aeration between cells [16]. Compact EPS is more difficult to separate due to its strong texture. However, compact EPS usually produces high levels of secondary metabolites that are commonly used in the health field. EPS color is one indicator of explant growth in vitro culture. EPS produced from an explant typically emit different colors.

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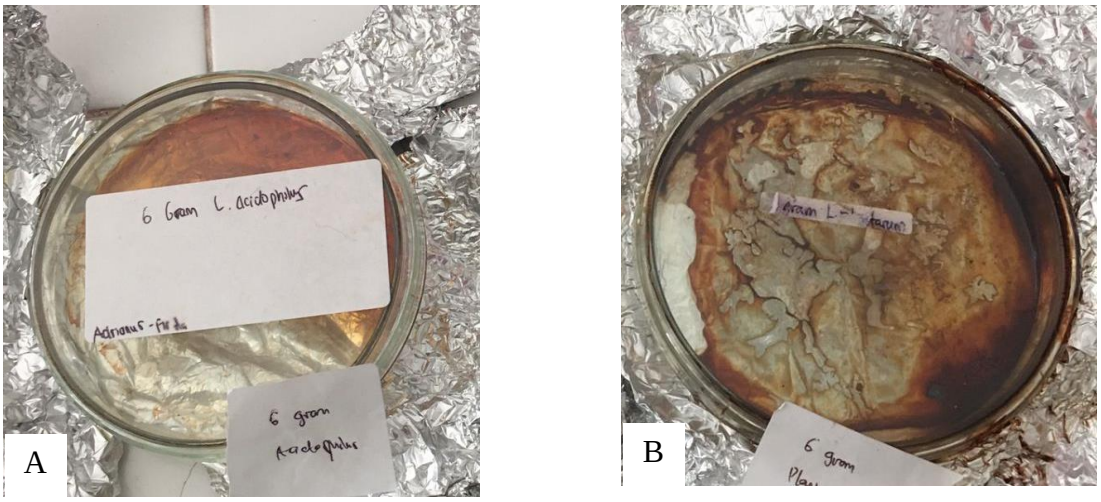

Fig 1. Exopolysaccharide texture and color A. EPS *L.acidophilus.* B. EPS *L.plantaurm*

B. Exopolysaccharide weight and total sugar content

Based on the research results, it is shown that the growth of isolates without the addition of sucrose in liquid MRS broth media does not produce exopolysaccharides. On the other hand, MRS broth media supplemented with sucrose indicates that the isolate is capable of producing exopolysaccharides. In the production of exopolysaccharides, increasing concentrations of sucrose will increase the production of exopolysaccharides. The function of sucrose is as a food source because bacteria can produce invertase enzymes to hydrolyze sucrose into monosaccharides [17].

In this study, the fermentation process was carried out with variations of bacterial species (*L. acidophilus* and *L. plantarum*) with variations in sucrose concentrations of 2%, 4%, 6%, 8%, 10%, and 12%. Fermentation was carried out by mixing sucrose and inoculum and adding 0.1 grams of banana peel extract. The function of adding yeast extract is as a source of nitrogen nutrition. Based on theory, microbial nutrition functions as energy for growth, cell formation, and biosynthesis of metabolites [18]. Microbes require nutrition such as water, carbon sources, nitrogen sources, vitamins, and minerals [19]. The lactic acid bacteria fermentation process takes place anaerobically, but oxygen is required for maintenance before fermentation is carried out for the maintenance itself [20].

Testing of crude exopolysaccharides was carried out by separating exopolysaccharides from bacterial cell membranes using cold centrifugation at 4000 rpm for 30 minutes to obtain the supernatant. The supernatant of bacterial cells containing exopolysaccharides was then precipitated overnight with cold alcohol. The supernatant was then centrifuged again at cold temperatures at 4000 rpm for 30 minutes to remove proteins contained in the supernatant. The resulting pellet was obtained and dried in an oven at 110°C to obtain a constant weight of exopolysaccharides. The constant weight result was calculated by dividing the volume used to produce exopolysaccharides in mg/L units. The yield obtained as shown in Table 1.

Table 1. The yield of exopolysaccharides

Sucrose	***L. plantarum* (mg/L)**	***L. acidophillus* (mg/L)**
2%	1778,5	1654,3
4%	1987,2	1723,6
6%	2111,7	1967,667
8%	2399,9	2109,7
10%	2487,9	2287,5
12%	2856,11	2445,8

Based on the table above, it can be seen that variations in bacterial species and sucrose content affect the level of exopolysaccharides. The highest exopolysaccharide content was obtained from the *L. plantarum* bacterial species and 12% sucrose content. The lowest exopolysaccharide content was obtained from the *L. acidophilus* bacterial species and 2% sucrose content. It can be concluded that the higher the sucrose content, the higher the amount of exopolysaccharides produced, and the best bacterial species for producing exopolysaccharides is *Lactobacillus plantarum*. This is because the reaction rate increases with increasing sucrose concentration [21]. The increase in reaction rate is slower until a limit point is reached where the increase in substrate concentration only slightly increases the reaction rate. *Lactobacillus plantarum* is considered the best bacterial species for producing exopolysaccharides, although the differences in results obtained are not significant [22]. This is due to the difference in the ability to produce exopolysaccharides in lactic acid bacteria isolates, which is caused by differences in species or strain. These differences are influenced by different metabolic processes, resulting in different amounts

of metabolites produced [23][24]. Suryo (2012) stated that gene formation affects the formation of certain enzyme functions and abilities. Therefore, differences in genes in the same species but different strains can result in different outcomes of the metabolic process [25].

Polysaccharides or carbohydrates are included in the group of sugars. The total sugar content was measured using the HPLC method. The average total sugar content of exopolysaccharides is shown in Table 2.

Table 2. The average total sugar content of exopolysaccharides

Sucrose	***L. plantarum* (%)**	***L. acidophilus* (%)**
2%	17	10
4%	22	12
6%	29	15
8%	34	21
10%	46	30
12%	58	39

The glucose content produced in the EPS sample with *L. plantarum* isolate was higher, with a level of 0.058%, while the glucose content with *L. acidophilus* isolate was 0.039%. When compared, there is a significant difference between the two samples, even though both samples were given the same amount of sucrose. The difference in isolation between the two samples can affect the glucose produced. Based on the results of this study, the *L. plantarum* isolate is capable of producing a higher amount of glucose in the EPS compared to *L. acidophilus*. The high glucose content can be attributed to the amount of sugar as a substrate in production and the activity of the invertase enzyme to convert the substrate into its constituent monomers. According to Azizah (2019), the glucose content in EPS is influenced by the amount of sugar present in the production medium, which is MRS. In addition, the low glucose content is also caused by impurities present in the EPS, such as protein.

C. Functional groups of Exopolysaccharides

Exopolysaccharides (EPS) are polymeric compounds consisting of sugar monomers produced by microorganisms such as bacteria and fungi. EPS possesses multiple distinct functional groups, depending on the type of sugar monomer forming it [26]. Some common functional groups in EPS include hydroxyl (-OH), carboxylate (-COOH), amide (-NH_2), and sulfate (-SO_3H) groups [27]. Identification of functional groups in EPS can be performed using various analytical techniques, including Infrared spectroscopy (IR), nuclear magnetic resonance spectroscopy (NMR), and ion chromatography [28].

Identification using FTIR is useful for determining the functional groups present in Exopolysaccharides (EPS) produced by the highest-yielding isolate types, namely *L. acidophilus* and *L.plantarum* bacteria with the addition of 6 grams of sucrose. These two samples were selected to compare the functional groups present in the two different isolate types.

The analysis of EPS functional groups can be concluded based on the peaks that appear in the typical absorption of polysaccharide functional groups. The functional groups of EPS obtained are presented in Table 1.

Table 3. The functional groups of exopolysaccharides

***L.plantarum* (cm^{-1})**	***L. acidophilus* (cm^{-1})**	**literature (cm^{-1})**	**Functional grups**	**literatures**
3273,83	3274,10	3406,35	-OH S	Winahyu et al., 2020
2953,46	2123,75	2925	-C-H (metil)	Bramhachari et al., 2006
1643,32	1639,98	1641	-C=O	Nuwan et al., 2016
1450,78	1451,50	1370-1455	-C-H, C-H_2	Petrovici et al., 1994
1111,98	1110,51	1127	-C_4-O_4H	Petrovici et al., 1994
1014,44	1015,56	1018	α(1,6)	Nuwan et al., 2016

Based on previous research, it has been reported that the polysaccharide compound exhibits O-H absorption at around 3406.35 cm^{-1} with a broad peak stretching [29], C-H absorption at around 2925 cm^{-1} with a stretching vibration [30], C=O absorption at around 1641 cm^{-1} with a broad peak stretching [31], and -C-H, C-H_2 absorption at around 1641 cm^{-1} with a bending vibration [32]. According to Shingel's study in Paulo (2002), the main absorption that characterizes α (1→6) dextran exopolysaccharides can be found at around 1150 cm^{-1}, indicating the presence of glycosidic linkages in the alpha (α) conformation [33]. However, in Nuwan, P., et al (2016) research, the α(1,6) glycosidic bond is absorbed in 1018 cm^{-1} [31]. Therefore, the EPS produced indicates the presence of polysaccharides, which are generally O-H (hydroxyl), C=O (carbonyl), -C-H/ C-H_2, -C_4-O_4H, and α(1,6) glycosidic bonds.

IV. CONCLUSIONS

In this study, the produced EPS contained functional groups such as O-H (hydroxyl), C=O (carbonyl), -C-H/C-H2, -C4-O4H, and α(1,6) glycosidic. The addition of sucrose to both isolates (*L. acidophilus* and *L. plantarum*) affected the EPS texture, but using different isolates did not show a significant difference in texture. EPS produced using *L. acidophilus* and *L. plantarum* with the addition of 6

grams of sucrose was found to have a brittle texture, which facilitated separation into individual cells and improved oxygen aeration between cells. It can be concluded that the higher the sucrose content, the higher the amount of exopolysaccharides produced, and *Lactobacillus plantarum* is the best bacterial species for producing exopolysaccharides. This is because the reaction rate increases with increasing sucrose concentration.

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