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Characterization of Exopolysaccharide Produced by Lactobacillus casei AL15 Isolated from Sap of Arenga pinnata

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ABSTRACT
Exopolysaccharide (EPS) is a polymer of a reducing sugar which has a high molecular weight. This polysaccharide is usually produced by lactic acid bacteria, and has a very large usability for food products and pharmaceutical products. *Lactobacillus casei* AL15 is a type of lactic acid bacteria isolated from sap of *Arenga pinnata* and has a great potential to produce EPS. Productivity in producing EPS of *L. casei* AL15 in MRS broth medium is about 14.1 mg/L. Furthermore, component of carbon was a highest composition of the EPS tested by SEM. The image of SEM showed that the shape of EPS were round to ellipse shape with smooth surface texture and white yellowish color. The spectrum of FTIR produced the wavenumber in the range of 3338 cm^{-1} – 1056 cm^{-1}. The spectrum indicated the presence of O-H in 3338.78 cm^{-1}, C-H in 2962.66 cm^{-1}, C=O in 1649,14 cm^{-1} and C-O-C in 1056.99 cm-1. Those bonds indicated that EPS produced by *L. casei* AL15 was heteropolysaccharide (HePS), since the FTIR spectrum had a same spectrum with sucrose and glucose. This result was also supported by HPLC analysis, which showed that the hydrolyzed EPS was composed by sucrose and glucose. Overall results showed that *L. casei* EPS AL15 was HePS.
Keywords: characterization, exopolysaccharide, *Lactobacillus casei* AL15, *Arenga pinnata*.

1. Introduction

Exopolysaccharide (EPS) was one of polysaccharide produced from microorganisms. EPS is very useful for food as a stabilizer, emulsifier, gelling agent and have a good capability to bind liquid (water). So that EPS can maintain the texture to remain soft during storage (Malik *et al.* 2008). Lactic acid bacteria (LAB) is one of bacteria group that is capable producing EPS. There are a few lactic acid bacteria that can produce EPS such as *Lactobacillus acidophilus, L. rhamnosus, L. casei, L. plantarum, L. reuteri* and *Bifidobacterium* (Tallon *et al.* 2006). Lactic acid bacteria have been isolated from various kinds of food and one of them is fresh of *Arenga pinnata* sap. Previously one species of LAB isolated from *Arenga pinnata* sap, which has already been identified as *Lactobacillus casei* AL15, has a great potential to produce EPS.

Chemically EPS may be classified into two types of EPS which are homopolysaccharide (HoPS) and heteropolysaccharides (HePS). The HoPS is composed of one kind of monosaccharide and HePS is composed of several units of different monosaccharide (deVuyst *et al.* 2001). According Badel *et al.* (2010) HoPS, such as dextran, levan, inulin, reuteran and mutants can be produced by LAB. While the group of HePS, which consists of 3 or 4 or even more combinations of monosaccharide units such as α-glucose, α-galactose, L-rhamnose and in some cases consisting of N-acetylglukosamine, fucose, glucoronic acid, can be produced by LAB (Low *et al.* 1998, deVuyst *et al.* 2001; Harrah *et al.* 2006). There are many LAB produced HoPS such as *Leuconostoc mesenteroides, Streptococcus mutans, Pediococcus* and *Streptococcus salvatus*. The species of LAB that can produce HePS are *L. casei, L. lactis, L. sake, L. rhamnosus* and *S. thermophilus* (deVuyst and Degeest 1999).

Previous studies applying several analytical techniques to determine the characteristics of expected EPS, but until this moment there is no result for which the most appropriate technique to analyze the characteristics of each EPS (Ruas-Madiedo *et al.* 2005). This study used three methods of EPS analysis, such as SEM, FTIR and HPLC to determine the characteristics of EPS produced by *L. casei* AL15.

2. Materials and Methods

2.1 Lactobacillus casei AL15

The culture of *L. casei* AL15 was collected from Integrated Laboratory of Bioscience and Biotechnology Udayana University. The culture was isolated from palm sap and has been screened as a bacteria producing EPS. The culture was refreshed by adding 1 ml of overnight culture to 9 ml of *deMann Rogosa Sharpe Broth* (MRSB) and then the culture was incubated at 37^{o}C for 24 hours.

2.2 Extraction and Measurement of exopolysaccharide from L. casei AL15

Next step for the production of EPS, 10 ml culture that prepared previously was inoculated into 750 ml *MRSB* and incubated at 37°C for 24 hour. Subsequently, the supernatant was collected by centrifugation (Heraus Labofuge 200) at 5000 rpm for 20 minutes. And then the supernatant was transferred to a new container and added by cold 96% ethanol and allowed to stand overnight at 4°C. Furthermore, the supernatant was precipitated by centrifugation at 5000 rpm for 20 minutes, and then ethanol was removed. The precipitation of EPS was washed 2 times per day with sterilize distilled water. The crude EPS was freeze-dried (Hanil Vac Clean Freeze Dryer) for 24 hours at a temperature of -78°C (Liu 2012). Crude EPS was then weighed to find total production of EPS.

2.3 Scanning Electron Microscopy (SEM) Analysis of Crude EPS

The SEM was used to analyze the EPS surface and morphology. Total of 0.2 grams dried sample was added into SEM stubs. The sample was analyzed by scanning SEM (Hitachi TM 3000) at 70°C (Suntherland 1990).

2.4 Fourier Transform Infra-Red Spectroscopy (FTIR) Analysis of Crude EPS

The EPS of *L. casei* AL15 characteristic was tested by FTIR (IRTracer-100 Shimadzu) using existing database at a frequency in the range of 400-4000 wave numbers cm^{-1}. Two mg of crude EPS was mixed with 200 mg potassium bromide (KBr), then the mixture was pressed into 16 mm diameter mold and used for IR spectroscopy for detection band O-H, C-H, C-O, and C-O-C (Nichols *et al.* 2004).

2.5 Analysis of Hydrolyzed-EPS

The hydrolyzed EPS were analyzed with a *High Performance Liquid Chromatography* (HPLC) system (Varian Prostart) equipped with column fermentation Cation- exchange resin (ICI) using Varian Prostar pump, and injector (ICI Instrument AS 2000) and using (Shimadzu Chromatopac) processor. The mobile phase used 1% acetonitrile with a flow rate 0.6 ml/min at 65°C. The separated components were monitored by a *Refractive Index* (RI) detector (Shodex RISE61). The EPS after being hydrolyzed with 2 N H_2SO_4 at 100°C for 2 hours was analyzed its sugar composition by HPLC. The column was calibrated with different molecular mass standard and a standard curve was then established.

3. Results

From the experiment the crude EPS production was of 14.1 mg /L. Apperence of crude EPS after freeze drying for 24 hr produce the rough structure like fiber showed in Figure 1. The crude EPS obtained at this stage was prepared to be used to determine the EPS characteristics in SEM-EDX, FTIR, and HPLC. Quantitative elemental analysis was done by SEM-EDX, which revealed the weight and atomic percentage of different elements present (C, O, Na, Si, P, Cl, and Ca) in the EPS produced by *L. casei* AL15. SEM revealing the surface structure of crude EPS fiber produced by *L. casei* AL15 viewed at 4000 magnification showed in Figure 2. The EPS appeared have a rough structur like grape. Result of SEM-EDX microanalysis showed that the elements of EPS produced by *L. casei* AL15 were composed by 59% of carbon, 38% oxygen and the rest were small amount of impurities element. Propotions of elements compose EPS produced of *L. casei* AL15 as a result of SEM-EDX microanalysis showed in Figure 3.

Figure 1. Appearance of crude EPS fiber produced by *L. casei* AL15 after freeze drying for 24 hr

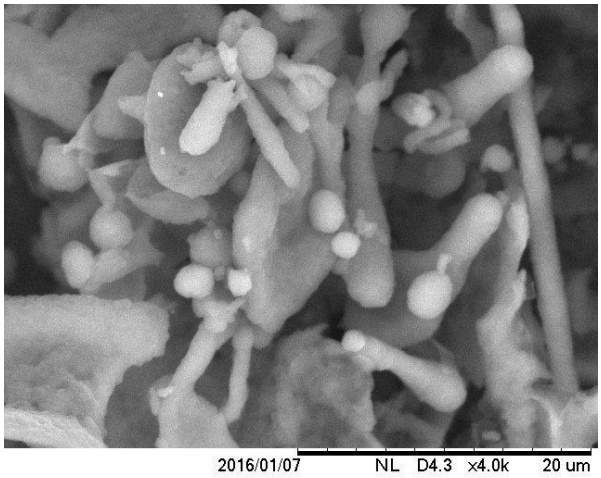

Figure 2. SEM revealing the surface structure EPS fiber produced by *L. casei* AL15

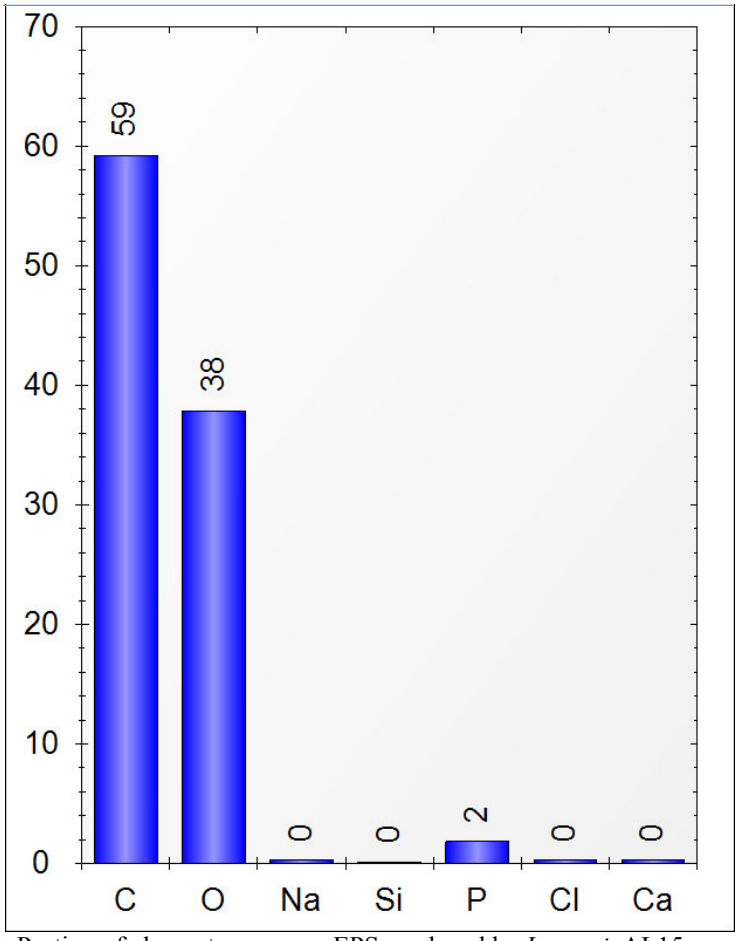

Figure 3. Portion of elements compose EPS produced by *L. casei* AL15 as a result of SEM-EDX microanalysis

The results of IR spectra range of the EPS were divided into four regions, which were the hydroxyl group (O-H) in the wave number of 3338.78 cm^{-1}, the second bond was the group of C-H in the range of 2962.26 cm^{-1}- 2927.94 cm^{-1}, the third group was the bond of C=O in the range of 1649.14 cm^{-1}-1629.85 cm^{-1}, and the fourth group was C-O-C group which in the spectrum of 1056.99 cm^{-1}. IR spectra of EPS produced by *L. casei* AL15 showed in Figure 4. The analysis using HPLC showed that the composition of hydrolyzed EPS were dominated by sucrose, glucose and fructose. This result mean that EPS produced by *L. casei* AL15 was composed by two monosaccharide of glucose and fructose. The chromatogram of hydrolyzed EPS of *L. casei* AL15 showed in Figure 5.

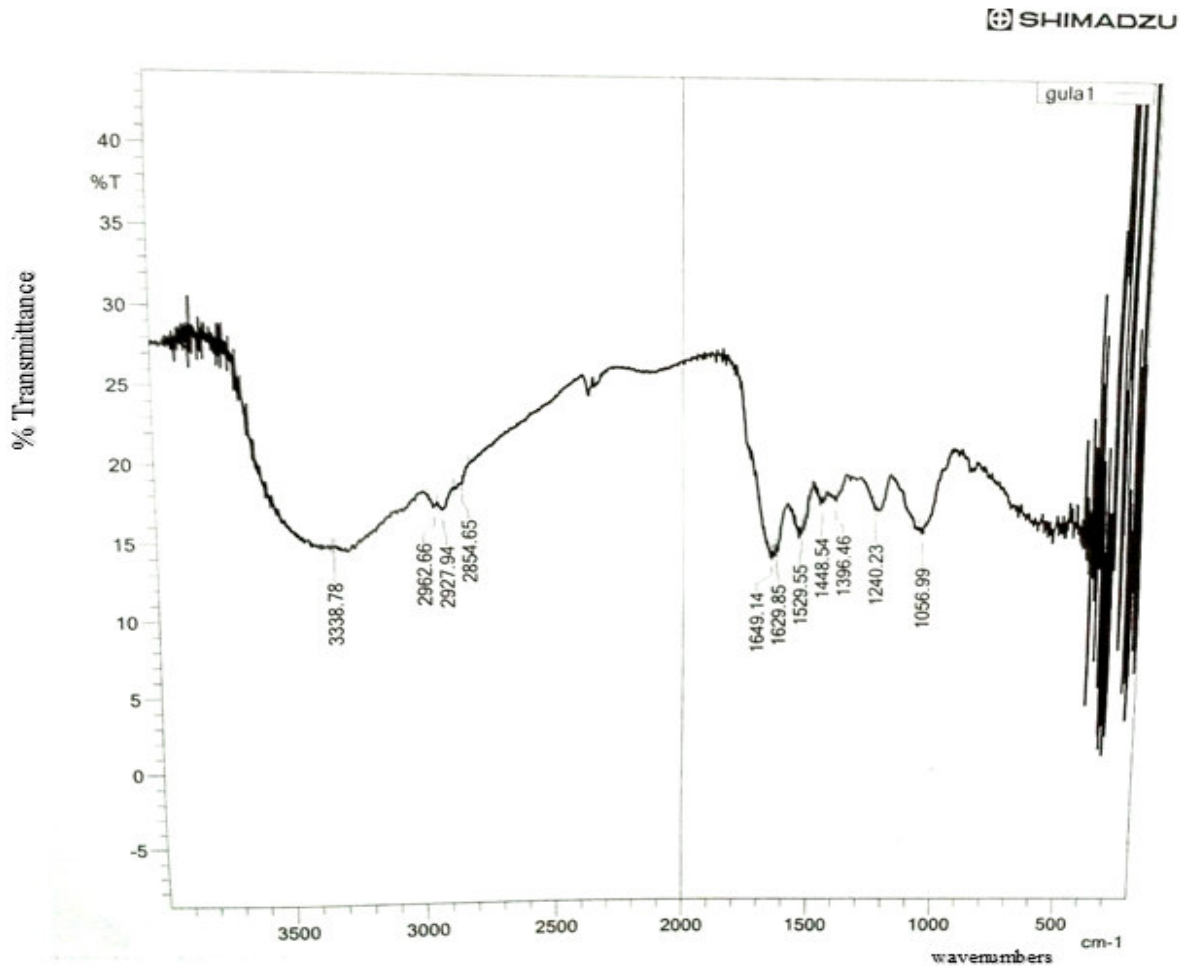

Figure 4. IR Spectra of EPS produced by *L. casei* AL15

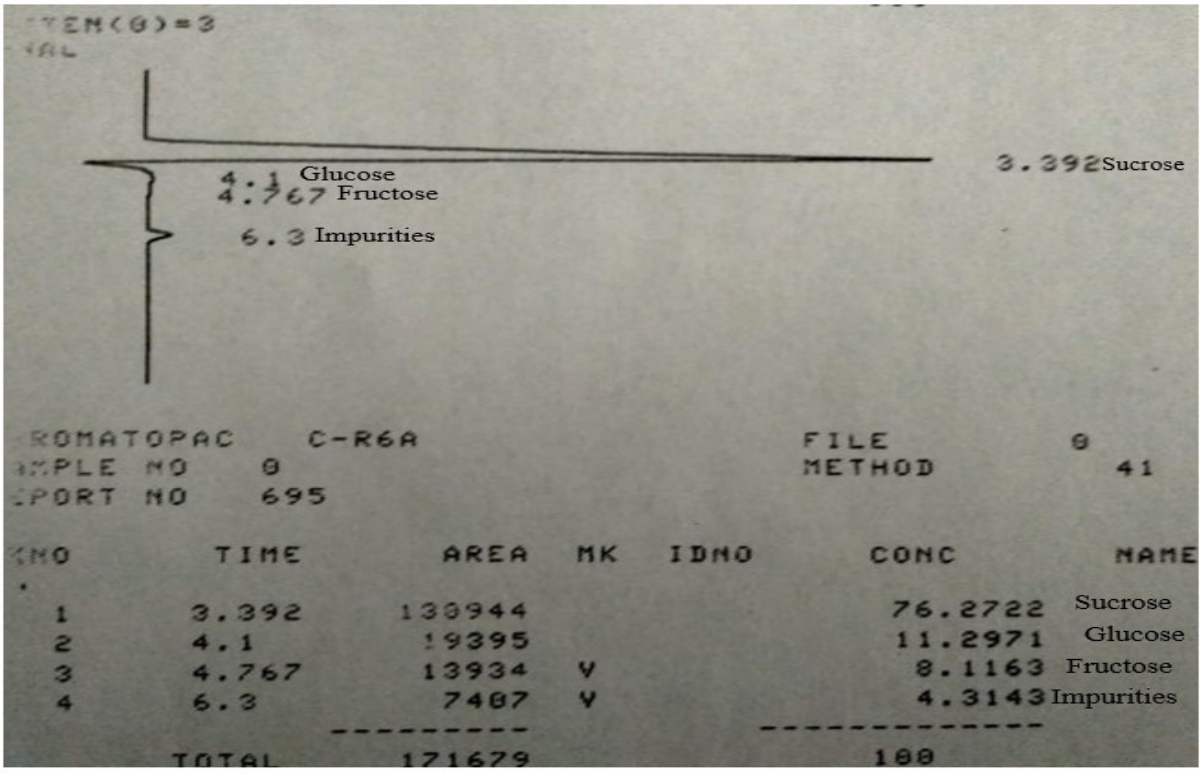

Figure 5. Chromatogram of hydrolyzed EPS of *L. casei* AL15

4. Discussion

The production of crude EPS produced in this study used MRSB medium. The use of standard growth media *Lactic Acid Bacteria* (LAB) is one factor that affects the crude EPS amount produced by the bacteria. In previous study by Nudyanto and Zubaidah (2015) showed that using the MRSB media *L. plantarum* isolates were able to produced 99-427 mg/L EPS, while *L. casei* strain was capable generating 106.33 mg /L of EPS crude.

The FTIR spectrum of *L. casei* AL15 EPS was analyzed and absorption bands were assigned to reveal typical polymeric structure of the carbohydrate. A broad stretching in the region 3338.78 cm^{-1} was observed which represented the stretching vibration of the O-H hydroxyl group of carbohydrate. The absorbtion bands at 2962.66 – 2927.94 cm^{-1} represented the C-H stretching of methyl and methylene groups (Wang *et al.* 2010). The absorption band found in the region 1649.14 cm^{-1} usually represents the C=O stretching vibrations of carboxyl groups. A sharp absorption band at 1059.99 cm^{-1} represents the C-O-C stretching vibration of glycoside bond. Santi, *et al.,* 2013 reported absorption bands of sucrose have a similarity with bands of *L. casei* AL15 EPS. Ortega-Morales *et al.* (2007) which revealed the presence of COOH groups (1600 -1725 cm^{-1}) and –OH (2800-3600 cm^{-1}) groups showing that the samples were EPS. *L. casei* AL15 showed the presence of –OH band at 3338.78 cm^{-1} position and band of COOH groups at 1649.14 cm^{-1}. Vijayabaskar *et al.* (2011) showed the band at 1000 -1500 cm^{-1} which is characteristic to glucan. Similar results have also been reported by Sajna *et al.* (2013), the absorptions band in the region 983-1200 cm^{-1} suggested the presence of sugar monomers such as galactose, manose and glucose.

According to the FTIR spectrum analysis result, proved that EPS *L. casei* AL15 was contained with the group band of hydroxyl, aldehid, keton and glycoside bond. The result prove that the EPS was composed by two monosaccharide such as glucose and fructose. The result was also confirmed with HPLC test which showed two types of monosaccharide such as glucose and fructose. The overall EPS *L. casei* AL15 characteristics test showed the type of two monosaccharide that is glucose and fructose.

5. Conclusion

The results obtained in this study mentions that the EPS of *L. casei* AL15 was composed by two kinds of monosaccharide, which were glucose and fructose. The type of EPS produced by *L. casei* AL15 is supposed to be heteropolysaccharide. Thus EPS *L. casei* has enormous promising used for food and health.

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