Comparative Study Production of Exopolysaccharide (EPS) by Lactic Acid Bacteria (*L. casei* and *L. plantarum*) in Different Media (Dates and Mulberry juice)

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Abstract

Exopolysaccharides (EPS) are polysaccharides that are secreted by some strains of bacteria. E PS contribute in health improvement where it has prebiotic properties, immunostimulatory, anti-tumoral, and hypocholesterolemic effects. A number of Lactic Acid Bacteria (LAB) has ability t o synthesise Exopolysaccharides (EPS). This study aimed to determine the ability LAB (L. casei and L. plantarum B2) to produce EPS in different media (dates and mulberry juice). L. casei sh owed higher EPS production (3413.33 mg/L) than L. plantarum (3316.67 mg / L) were grown in medium dates juices. Based on the type of media, EPS production in dates juice medium higher t han mulberry juice as medium, and there are differences production of EPS by both types of isol ates (L. casei and L. plantarum) in both media. The growth rate of LAB does not always show a positive correlation with EPS formation.

Keywords: *Dates juice, exopolysaccharide, LAB, mulberry juice*

1. INTRODUCTION

Exopolysaccharide (EPS) is a sugar or a polysaccharide polymer that is secreted by the microbes out of the cell. EPS produced by lactic acid bacteria (LAB) have generated increasing attention among researchers for the last few years (Pham *et al*., 2000). LAB is food-grade organisms, and the EPS that they produce contribute to the specific rheology and texture of fermented milk products. EPS have health effects as immunostimulant activity, antitumor, activation of macrophages and lymphocytes to increase their endurance, and as prebiotics (Tallon *et al*., 2006). The amount of EPS produced by microbes influenced by several factors such as the composition of the medium (source of carbon and nitrogen) and incubation conditions (temperature, time and pH) (Tallon et al., 2006). EPS production produced depends on the species of the microorganism used. Many strains of *Lactobacillus*, including *L. casei*, has the ability to produce EPS, that are secreted into the environment (Lam and Lee, 2006). According to Tallon *et al.*, (2006), *L. plantarum* is capable of producing EPS ranged from 126-800 mg / L in milk medium. Mulberry juice as medium for *L. plantarum B2* produce EPS higher than skim milk medium (Zubaidah and Maulida, 2007).

Dates fruit contain compounds of high nutritional value, such as glucose and fructose 20-70% (dry weight), sucrose in the range 0-40%, as well as several other compounds such as niacin (2 mg), thiamin (Vitamin B1) (93 mg), folic acid (5.4 mg), riboflavin (Vitamin B2) (144 mg). This study aimed to determine the ability of *LAB (L. casei and L. plantarum B2)* to produce EPS in different media (dates and mulberry juice).

2. MATERIALS AND METHODS

2.1 Materials

Semi-dry kind of dates (Egypt) obtained

from the fruit shop on Jl. Capt. Piere Tendean, Malang, mulberry fruit obtained from Batu City, Malang, *Lactobacillus plantarum* B2 cultures obtained from Microbiology Laboratory, University of Brawijaya, Malang, *Lactobacillus casei* cultures obtained from Lab. PAU. UGM, Yogyakarta.

2.2 Experimental Procedure

2.2.1 Preparation Starter Culture of *L. plantarum* and *L. casei*

A volume of 10 ml sterile MRS broth in 2 test tubes was inoculated with agar slant culture of L. plantarum and L. casei, and incubated at 37°C for 24 hours.

2.2.2 Preparation of Fermentation Medium

Dates juice fermentation medium: dates are separated from the seeds, crushed, added with water 1: 3, then filtered, and sterilized. Mulberry fermentation medium: Crushed mulberry fruit, added with water 1: 9, filtered, then sterilized.

2.2.3 Fermentation Process

A volume of 100 ml of each medium dates and mulberry, sterilization at 100 ° C for 3 minutes, then cooled to 37°C. A starter *L. plantarum* and *L. casei* inoculation as much as 2% (v / v) and then incubated at 37 ° C for 48 hours.

2.2.4 Analysis

Analysis of total LAB (*L. casei* and *L. plantarum*) and EPS production carried out for 0, 24, 36, 48 hours of fermentation. EPS were separated by centrifugation for 6000 x g at 15 min. to eliminate bacteria from media according the method of Tallon with few modifications. Three volumes of cold ethanol were added to supernatant and stored overnight at 4 °C. Precipitated materials were collected by centrifugation (20 min at 5000 x g) and re-suspended in demineralized water and mixed with three volumes of cold ethanol and centrifuged at 5000 x g for 20 min. The pellets were dried at 100 °C (Tallon, 2006).

3. RESULTS AND DISCUSSION

3.1 Total Lactic Acid Bacteria (LAB)

Figure 1 shows the increase total LAB during fermentation, and then decreased after 24 hour. Both types of isolates (*L. casei* and *L. plantarum*) show a similar growth curve. But on the type of fermentation medium (dates fruit and mulberry juice) there are significant differences, the date palm juice medium produce total LAB higher than the mulberry juice medium and isolates o f *L. casei* grow higher than *L. plantarum*.

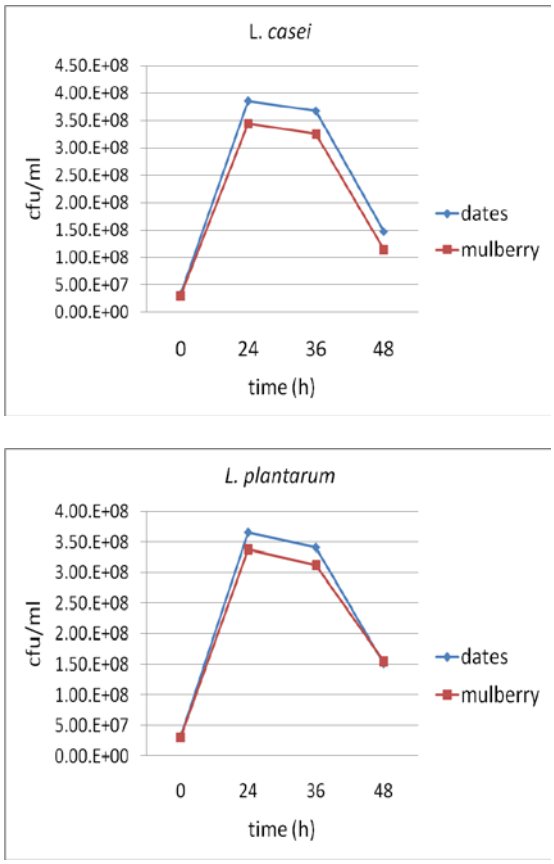

Figure 1. Effect of substrate types (dates and mulberry juice) to ward total LAB (*L. casei* and *L. plantarum*) during fermentation.

Figures 1 shows total LAB for both types of isolates of *L. casei* and *L. plantarum* grown on dates juices fermentation medium are higher (3.18 - 3.76 $x10^8$ cfu/ml) than the mulberry juice fermentation (2.98 - 3.41 $x10^8$ cfu/ml). This is presumably due to the composition of medium, the dates juices has higher nutrient content than that of mulberry juice, such as Niacin (2 mg), Thiamin (Vitamin B1) (93 m g), folic acid (5.4 mg), riboflavin (Vitamin B2)

(144 mg) which can support microbial growth.

Isolates of *L. casei* has a higher growth rate than *L. plantarum*. This is presumably due to the differences of the two types of isolates in their ability to metabolize the substrate. Based on the presence of aldolase and phosphoketolase, *L. casei* classified as facultative heterofermentatif bacteria, that metabolize hexoses to produce lactic acid, and metabolize pentose and gluconate to produce lactic and acetic acid (Fox *et al*., 2001). *L. plantarum* are classified into homofermentatif, able metabolize the sugars from hexoses. *L. plantarum* have limitations in ferment ketoses (fructose) that found in many fruits, so *L. casei* grow better than *L. plantarum* on both medium (dates and mulberry juice).

3.2 Exopolysaccharides (EPS)

The results of extracellular polysaccharides by *L. casei* and *L. plantarum* are shown in figure 2 which shows difference in ability of to produce EPS in the presence of different media (dates and mulberry juice).

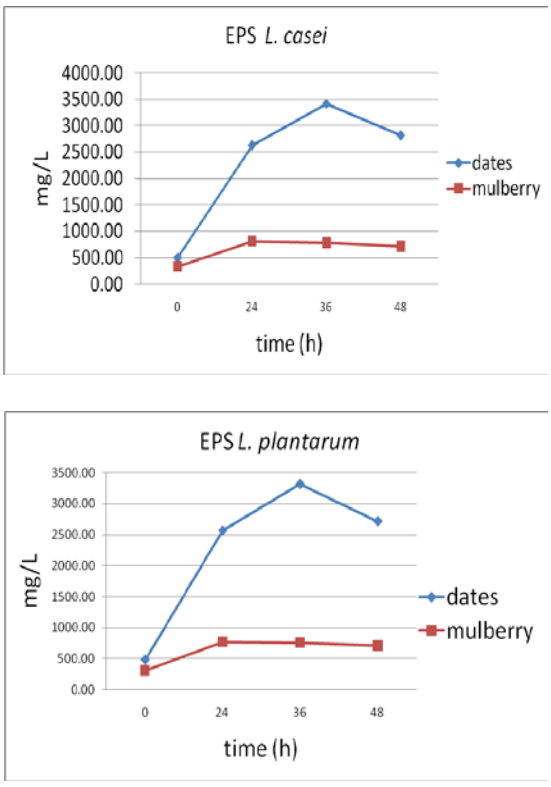

Figure 2. Effect of substrate types (dates and mulberry juice) toward EPS production *L. casei* and *L. plantarum* during fermentation.

The amount of EPS production is increased by the time. This is presumable because the cells are threatened by metabolite product during fermentation, such as lactic acid. it will be encouraged to form EPS as a self-protection mechanism of cells. EPS produced by bacteria function as a self-protection from extreme environmental conditions. EPS serve as a protective cell from desiccation, toxic substances, bacteriophages, and the osmotic pressure for biofilm formation (Harrah et al., 2006). Polysaccharide secretion depend on several environmental responses, such as stress and growth condition, and vary widely among different species.

The amount of EPS produced declined upon prolonged fermentation (more than 32 h for dates medium and 24 h for mulberry medium). The decrease in viscosities and molecular weights of EPS withdrawn at different cultivation times permitted to suspect the presence of a depolymerizing enzyme in the fermentation medium (Pham et al., (2000) The amount of EPS depends on the carbon, nitrogen sources and physico-chemical conditions for bacterial growth as temperature, pH, oxygen rate, etc. Specific carbon substrate differs from one species to another. During prolonged incubation the quantity of produced polysaccharides decreases, suggesting but not concluding about their function of carbon reserve, (Hallemeersch et al., 2002). Nonetheless, only few species have all the necessary enzymes to degrade their polysaccharide by release of glycohydrolases in their culture medium (Cerning et al., 1992; De Vuyst and Degeest, 1999).

The result of EPS production showed that the amount of EPS produced by *L. casei* and *L. plantarum* in the dates medium was higher than in the mulberry medium. Isolates of *L. casei* showed higher EPS production (3413.33 mg / L) than *L. plantarum* (3316.67 mg / L) were grown in medium dates juices. While in mulberry juice EPS was the minimum quantity of EPS produced (Table 1).

Table 1. EPS production (mg/L) by *L. casei* and *L. plantarum* in dates and mulberry juice during fermentation

No	Isolates	Media	EPS production (mg/L)			
			Fermentation time (h)			
			0	24	36	48
1.	*L. casei*	Dates	497.67	2630.00	3413.33	2815.00
		Mullberry	334.00	813.33	783.00	715.00
2.	*L. plantarum*	Dates	479.00	2566.67	3316.67	2716.67
		Mullberry	306.67	770.00	758.33	710.00

EPS produced by each isolate (*L. casei* and *L. plantarum*) in different medium fermentation show a very significant difference. Differences in the ability of EPS production by both types of isolates could be due to differences in genotype of each isolate. Based on carbohydrate fermentation patterns, *L. casei* is facultative heterofermentatif, whereas *L. plantarum* is ho mofermentatif. Facultative heterofermentatif will produce more ATP than homofermentatif pattern. The more ATP generated the more component cells will be formed. The influences of different medium on EPS production are shown on Table 2.

Table 2. Effect types of medium toward EPS production (mg / L)

No.	Type of Media	EPS production (mg/L)			
		Fermentation time (h)			
		0	24	36	48
1.	Dates	488,33	2598,33	3365,00	2765,83
2.	Mullberry	320,33	791,67	770,67	712,50

Table 2 shows that EPS production in the medium of dates juice higher than mulberry juice. This is due to the difference in the nutritional content of each media. Numerous studies show that EPS production is affected by medium composition. Carbon sources in addition to use as primary energy, also used as a raw material EPS polymer formation. According to Velasco et al., (2006), a higher sugar concentration rescued the sustainability of EPS formation in stationary phase. As prop osed by Lam and Lee (2011) high glucose concentrations can stimulate EPS synt hesis. Based on analysis sugar component, date juice contains total sugar 28.146%, while mulberry juice 6.266%.

3.3 The relationship the growth rate and EPS production

Figure 3 show the relationship between total LAB and EPS production during fermentation time. Dates juice as medium, the number of LAB in the range of 3.76 x10^8 - 3.54 x10^8 cfu / ml, with EPS production could reach 3365.00 mg /L. Its contrary with mulberry juice as medium, although the number of cells range between 3.41 - 3.19 x10^8 cfu/ml, but the EPS produced only reached 791.67 mg / L. This should indicate that the growth of total LAB cells with EPS production does not always show a positive correlation.

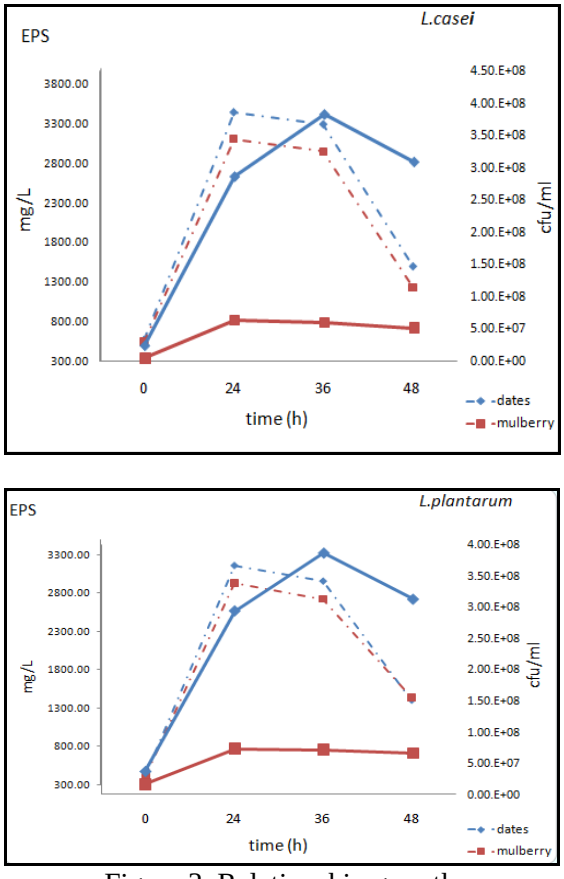

Figure 3. Relationship growth rate and EPS production isolates of *L. casei* and *L. plantarum*

EPS production usually correlated with bacterial cell growth. The higher number of cells, the higher EPS

production. However EPS production is not only dependent on the quantity of cells, but also the cell's response to environmental and component medium. Some researchers have suggested that EPS production not correlated positively to the growth of EPS-producing bacteria. According to Velasco (2006), Expression of enzymes by bacteria EPS producers due to the response to the environment correlated with several environmental responses, such as stress and growth conditions, vary widely a mong different species, and also to the strains of the same species. The high EPS on dates juices presumably caused by the influence of the presence of compounds of iron ions such as Mn, Mg, and Fe that assist the formation of EPS. EPS formation is catalyzed by enzymes glucosyltransferase that proved by EPS formation requires iron compounds as enzyme cofactors, particularly magnesium (M g) and manganese (Mn) (Velasco, 2006).

CONCLUSION

L. casei showed higher EPS production (3413.33 mg/L) than *L. plantarum* (3316.67 mg / L) were grown in medium dates juices. Based on the type of media, EPS production in dates juice medium higher than mulberry juice as medium, and there are differences production of EPS by both types of isolates (*L. casei* and *L. plantarum*) in both media. The growth rate of LAB does not always show a positive correlation with EPS formation.

REFERENCES

Cerning J, Bouillanne C, Landon M, Desmazeaud M. 1992. Isolation and characterization of exopolysaccharides from slime-forming mesophilic lactic acid bacteria. *J Dairy Sci*, 75:692–9.

De Vuyst L, De Vin F, Kamerling JP. (2007). Exopolysaccharides from lactic acid bacteria. In: *Comprehensive Glycoscience*, Vol. 2. (pp. 477–518). (Editor: Kamerling JP). Oxford: Elsevier.

Fox, P.F., T.P. Guinee, T. M. Cogan, P. L. H. McSweeny. 2000. Fundamental of Cheese Science. *An aspen Publicat*. Maryland

Hallemeersch I, De Baet S, Vandamme EJ. In: Steinbüchel A, editor. Exopolysaccharides of lactic acid bacteria. *Biopolymers*. Wiley VCH: Weinheim; 2002. p. 407–29.

Harrah, T., B. Panilaitis and D. Kaplan. 2006. Microbial Exopolysaccharides. (http://www.jds.fass.org/cgi/content/full.html)

Lam J.H. and Lee Y.K. 2006. The Influence of Glucose Concentration on the Production of Exopolysaccharides (EPS) by *Lactobacillus casei* Shirota and the Effect of EPS on the Adherence of *Escherichia coli* O157:H7 to Human Intestinal Cells. Department of Microbiology, Yong Loo Lin School of Medicine, National University of Singapore

Pham P. L, Dupont I., Roy D., Lapointe G, and Cerning J. 2000. Production of Exopolisaccaride by Lactobacillus rhamnosus R and Analysis of its Enzymatic Degradation during Prolonged fermentation. *Appl Environ Microbiol*. American Society for Microbiology. Kanada

Tallon, R., P. Bressollier *and* M.C. Urdaci. 2006. Isolation and Characterization of Two Exopolysaccharides Produced by *Lactobacillus plantarum* EP56. (http://pasteur.fontismedia.com/infiles.doc.)

Velasco et al., 2006. Environmental factors influencing growth of and exopolysaccharide formation by Pediococcus parvulus 2.6. International *Journal of Food Microbiology* 111 (2006) 252–258