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Research Article

Exploration and characterization of exopolysaccharide-producing bacteria from soil in West Kalimantan for improving sandy soil aggregation

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Abstract : Exopolysaccharide (EPS) is a complex mix of macro-molecular electrolyte contained in the outer cells of bacteria excreted as mucus and has a role in soil aggregation. This study aims to obtain bacteria that have a high potential for exopolysaccharide-producing bacteria. A total of 112 isolates of exopolysaccharide-producing bacteria were isolated from rubber soil rhizosphere, secondary forest, and shrubs in PT. Hutan Ketapang Industri is the result of isolation on ATCC no.14 medium. Based on the observations of a morphological colony of these isolates, most of them had similarities of color and shape but only 25 colonies are different isolates were obtained based on colony morphology. However, only 10 isolates formed a thick mucus or slimy when cultured on MacConcey agar. The results show that the obtained three isolates of exopolysaccharide-producing bacteria have a higher value of the dry weight i.e. isolates RB292 (7.53 mg/mL) followed by RB51 (7.55 mg/mL), and RB241 (1.75 mg/mL) with 2% sucrose. Isolates RB51 and RB292 increased significantly soil aggregate stability at 2% dosage of organic matter with soil aggregate stability index from 30.61% to 47.87% and 45.79%. Homology of the isolates with known bacteria i.e. isolate RB51 was 98.86% homolog with *Klebsiella sp*. LW-13, isolate RB241 was 98.65% homolog with *Klebsiella pneumonia* strain DSM 30104 and isolate RB292 was 98.83% homolog with *Burkholderia anthina* strain MYSP113.

Keywords*: bacteria, exopolysaccharide, rhizosphere, soil aggregate stability*

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Introduction

The type of soil in West Kalimantan consists of Andisol (162.446 ha), Entisol (3.882.986 ha), Inceptisol (8.175.970 ha), and Spodosol (1.944.534 ha) (Fairhurst and McLaughlin, 2009). In West Kalimantan today, the development of plantations, especially for rubber and oil palm plantations, has been conducted extensively. The available potential land for plantation development generally varies widely such as high, medium, and low potential land. Spodosol, one of soil type has low potency, formed from quartz sand, has a layer of gray sand (albic horizon) and an accumulated layer of iron, aluminum and organic material (spodic layer) (Hardjowigeno, 1993). Spodosol are found in West Kalimantan (Suharta and Suratman, 2004), and South Kalimantan (Suharta et al., 1999). Spodosol has two limiting factors such as the depth of the spodic layer and the sandy texture. The depth of the spodic layer is related to the ease of roots in penetrating the soil, whereas the texture of sandy soil will result in lower soil capability in water retention and the greater chance washing of nutrients (Syarovy et al., 2015). Sandy soil has very low aggregate stability. According to Kusuma et al. (2016), soil aggregation is an important factor for the development of agricultural land and plantation functions. The use of indigenous exopolysaccharide-producing

bacteria is a solution that can be used to improve soil aggregation. Exopolysaccharide are high molecular weight polymers consisting of monosaccharide and some non-carbohydrate ingredients such as acetate, pyruvate, succinate and phosphate (Mu'minah et al., 2016). Santi et al. (2008) isolated exopolysaccharide-producing bacteria from rhizosphere in palm oil (*Elaeis guineensis* Jacq.) and obtained 71 bacterial isolates from soil in Central Kalimantan. Some exopolysaccharide-producing bacteria have been reported are *Pseudomonas aeruginosa*, *Erwinia, Ralstonia,* and *Azotobacter vinelandii* (Pawar et al., 2013). The contribution of a microorganism to soil aggregate stability is very important due to it involves the mechanism of interaction between exopolysaccharide-producing bacteria and sandy soil or organic matter. This study was aimed to obtain bacteria that have a high potential for exopolysaccharide-producing bacteria.

Materials and Methods

The research was conducted at Soil Biotechnology Laboratory, Department of Soil Science and Land Resources, Faculty of Agriculture, Bogor Agricultural University (IPB). This research was conducted from July 2017 until February 2018.

Screening and isolation of exopolysaccharide-producing bacteria

A total of one gram of soil material aseptically suspended in physiological saline solution (0.85%) and serial dilutions were made to 10^{-6}, with Duplo and incubated in medium ATCC no. 14 (per liter of medium): 0.2 g KH_2PO_4; 0.8 g K_2HPO_4; 0.2 g$MgSO_4.7H_2O$; 0.1 g $CaSO_4.2H_2O$; 2.0 mg$FeCl_3$; $Na_2MoO_4.2H_2O$ (trace); 0.5 g yeast extract; 20 g sucrose; and 15 g agar bacto with pH 7.2 and NB medium for seven days at a temperature of 28^{o}C (Remel, 2005; Santi et al. 2008). Selected bacteria which produceEPS characterized by colonies of bacteria that form thick slime (mucoid) (Tallgren et al. 1999) were purified by streaking the four quadrants to obtain single colonies. Selection of potential exopolysaccharide-producing bacteria that form thick slime in MacConkey medium was conducted in two stages:

Single stage selection: single colony was grown on MacConkey medium using different carbon sources of sucrose and glucose with concentrations: 1, 2, and 3% (b/v) (Moreno et al. 1999; Serrato et al., 2006).

Second stage selection: potential exopolysaccharide-producing bacteria in single stage selection were inoculated in medium ATCC no. 14, added glucose and sucrose as source carbon. According to Bertin et al. (2003), root exudates contain some low molecular weight organic compounds such as simple sugars and polysaccharides (arabinose, lactose, glucose, maltose, mannose). These compounds can promote the growth and development of soil microorganisms. From those, some glucose concentrations (1%, 1.5%, 2%) and sucrose (2%, 2.5%, and 3%) were added and incubated at 28^{0}C for three days and at 200 rpm of rotary shaker. At the end of incubation, cells were harvested with 1 mM EDTA by adding 500 mL, then shaken until homogeneous and then centrifuged at 9000 g for 10 minutes. The supernatant separated from the bacterial cell deposition was taken, coupled with cold acetone solution with a ratio of 1: 2. It was then performed again with the speed centrifugation 15000 g for 2 times on 30 minutes. Deposition of biomass in the form of exopolysaccharide was then washed with distilled water and dried at 60^{o}C for 24 hours or until dry weights obtained were fixed.

Total population of exopolysaccharide-producing bacteria

Isolates of exopolysaccharide-producing bacteria were grown on Nutrient Broth medium with pH 3, 4, 5, 7, 8, 9, 10 and 11, incubated at room temperature for ± 48-72 hours. After that, it wascalculated by the Total Plate Count (TPC) according to the method proposed by Enriquez et al. (1995).

Characterization and identification of exopolysaccharide-producing bacteria

The selected EPS isolates were characterized and identifiedmorphologically, physiologically, and molecularly. Some of the characteristics observed include morphological (shape, color, surface and gram staining) and biochemical (catalase, oxidase, fermentation test of some carbohydrates).The 16S rRNA sequence analysis of pure DNA, the nucleotide sequence data of selected isolates was searched for by its nearest homology with another strain in the 16S rRNA gene database using BLAST (http://www.nebi.nlm.nih.gov) and FASTA 3

Data analysis

The data of this study were analyzed by analysis of variance, and results of the study were analyzed by F test, while the difference between treatments was tested by Duncan's Multiple Range Test at 5 % level using SAS software version 9.4 (Steel and Torrie, 1980).

Results and Discussion

Screening and isolation of exopolysaccharide-producing bacteria

A total of 112 isolates of exopolysaccharide-producing bacteria isolated from soil in the rhizosphere of rubber, secondary forest, and shrubs in PT. Hutan Ketapang Industri were the result of isolation on ATCC no.14 medium. Based on the observations morphological colony of these isolates, most of them had similarities of color and shape but only 25 colonies had different characteristic.

Selection of exopolysaccharide-producing bacteria on McConkey medium

A total of 25 bacterial isolates obtained by morphological screening results on ATCC no. 14 were re-grown on MacConkey agar. The bacterial isolates that were able to grow well on the MacConkey agar were characterized by thick slime (Figure 1) after which the colony diameter was measured.

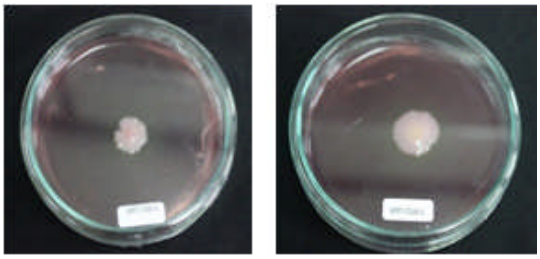

Figure 1. Formation of slime in exopolysaccharide-producing bacteria in MacConkey medium

A total of 10 isolates grown on MacConkey agar could produce thick slime. The isolates having high colony diameter were RB51, RB241, RB10, RB 221, RB292, RB81, RB291, RB102, RB 161 and RB293 reached 1.75-2.25 cm and isolates having low colony diameter i.e. HS221 (0.55 cm). Isolates capable of growing on MacConkey agar are grouped in Gram-negative bacteria (Mu'minah et al., 2015).

Population exopolysaccharide-producing bacteria at various pH

All potential EPS bacteria could grow well in the pH range of 4 to 11. The resistance of bacteria with the isolate code RB51, 241, 101, 292, 293, 291 and RB 161 on NB medium with pH 4 (106 CFU/mL) grew better compared to three isolate bacteria with isolate code RB221, RB102, and RB81 (105CFU/mL). Based on testing of bacterial resistance to acidity, all isolates of exopolysaccharide-producing bacteria were able to grow and adapted well to pH 4 until pH 11. According to research of Torres et al. (2012), the maximum production of exopolysaccharides is >7 g/L at 25-35^{0}C and pH 6.0-8.0. Other studies suggest that optimum pH and temperature are 7.0 and 30^{0}C, for growth synthesis and EPS by *E. cloacae* WD7 (Prasertsan et al., 2008) and *E. agglomerans* WD50 (Prasertsan et al., 2006). According to Imran et al. (2016), in neutral pH, EPS production improved both in *L. plantarum* NTMI05 (0.35 ± 0.03 g/L) and NTMI20 (0.32 ± 0.02 g/L).

Selection of exopolysaccharide-producing bacteria on ATCC no.14 medium with glucose and sucrose as sources of carbon

Application of carbon sources and concentration differences interacted significantly with the dry weight of exopolysaccharides. Table 1 shows that increasing the concentration of sucrose (2, 2.5 and 3%) lead to a significantly different reduction in dry weight of exopolysaccharides. Similarly, increased glucose concentrations (1, 1.5, and 2%) also decreased the dry weight of exopolysaccharides. The highest dry weight of exopolysaccharide and significantly different from the other treatment were obtained with 2% sucrose carbon source. At the same concentration of sucrose and glucose (2%), the bacteria produced 5 times higher exopolysaccharide dry weight if given sucrose compared to glucose. It is estimated that these isolates readily metabolize the sucrose than glucose.

Table 1. Average dry weight of exopolysaccharide in ATCC no.14 medium with two carbon sources incubated for 72 hours

Source of carbon	Dosage (% b/v)	Dry weight of exopolysaccharide (mg/mL)	
Sucrose	2	6.45	a
	2.5	5.05	b
	3	1.21	de
Glucose	1	1.81	c
	1.5	0.94	de
	2	1.39	d
Coefficient of variation (%)		19.60	

Remarks: Mean values within a column followed by the same letters are not significantly different at p<0.05 according to Duncan's Multiple Range Test.

The ease of using sucrose as a source of this energy allows for the growth and formation of optimum exopolysaccharide bacterial cell biomass.

Table 2. Average dry weight of exopolysaccharide in ATCC no.14 medium with two carbon sources incubated for 72 hours

Isolate	source of carbon	Dosage (% b/v)	Dry weight EPS	(mg/mL)	Isolate	Source of Carbon	Dosage (% b/v)	Dry weight	EPS (mg/mL)
RB101	Glucose	1	3.82	jkl	RB293	Glucose	1	2.10	nopqr
		1.5	0.99	rstu			1.5	0.15	u
		2	0.25	u			2	0.69	stu
	Sucrose	2	4.87	fghi		Sucrose	2	6.89	abc
		2.5	4.17	ijkl			2.5	3.77	kl
		3	0.75	stu			3	0.35	tu
RB102	Glucose	1	1.57	rsqop	RB51	Glucose	1	5.22	efgh
		1.5	1.40	rsqtp			1.5	0.34	tu
		2	0.75	stu			2	4.27	hijkl
	Sucrose	2	6.05	cde		Sucrose	2	**7.53**	**a**
		2.5	5.50	defg			2.5	6.07	cde
		3	0.62	stu			3	2.71	n
RB161	Glucose	1	0.34	tu	RB81	Glucose	1	2.82	mn
		1.5	0.18	u			1.5	1.57	opqrs
		2	2.58	no			2	0.38	tu
	Sucrose	2	5.88	def		Sucrose	2	6.07	cde
		2.5	4.69	ghijk			2.5	5.57	defg
		3	1.54	opqrs			3	0.37	tu
RB221	Glucose	1	0.63	stu	RB241	Glucose	1	0.29	u
		1.5	2.11	nopq			1.5	1.23	qrstu
		2	1.24	qrstu			2	0.28	u
	Sucrose	2	5.69	defg		Sucrose	2	**7.59**	**a**
		2.5	5.05	efghi			2.5	7.29	ab
		3	0.59	stu			3	0.86	stu
RB292	Glucose	1	0.73	stu	RB291	Glucose	1	0.70	stu
		1.5	0.75	stu			1.5	0,69	stu
		2	1.41	pqrst			2	2,11	nopq
	Sucrose	2	**7.55**	**a**		Sucrose	2	6,41	bcd
		2.5	4.79	ghij			2.5	3,67	lm
		3	1.97	opqr			3	2,33	nop
	Coefficient of variation (%)							19.60	

Remarks: Mean values within a column followed by the same letters are not significantly different at $p<0.05$ according to Duncan's Multiple Range Test.

This statement is supported by Pawar et al. (2013) that the maximum production of exopolysaccharide is in Nutrient Broth containing 2% sucrose. Other supporting studies (Santi et al., 2008), the best carbon source for *P. fluorescens* PG7II.1 and *P. diminuta* PG7II.9 is sucrose, each with a concentration of 2 and 3% (w/v), with the weight of the exopolysaccharides produced from the two isolates were 8.04 and 1.82 mg/mL respectively with an incubation period of 72 hours at room temperature. Testing of ten isolates with sucrose and glucose as carbon sources as presented in Table 2 shows that at 2% sucrose concentration, three isolates (RB51, RB292 and RB241) yielded exopolysaccharide dry weight (7.53, 5.55 and 7.59 mg/mL, respectively) were not significantly different from each other, and higher than 6 other isolates. However, if glucose of the same concentration of 2% was used, the three isolates produced only the dry weight of the exopolysaccharide 4.27, 0.28 and 1.41 mg/mL. The results of this study corresponded to that has been done by Emtiazi et al. (2004) where sucrose is the best source of carbon for the production of exopolysaccharides from *Azotobacter* strains AC2 and *Pseudomonas diminuta*.

Characterization and identification of exopolysaccharide-producing bacteria

Morphological characterization of three potential EPS isolates (RB51, RB241 and RB292) had characteristic of milky white and yellowish colonies, and irregular colonies and convex and umbonate elevation forms. Microscopic features were as follows: round cell and stem cells, Gram-negative, motile and non-motile, glucose fermentation, sucrose, and mannitol are positive. Homology of the isolates with known bacteria i.e. isolate RB51 was 98.86% homolog with *Klebsiella sp*. LW-13 (Table 3), isolate RB241 was 98.65% homolog with *Klebsiella pneumonia* strain DSM 30104 (Table 4) and isolate RB292 was 98.83% homolog with *Burkholderia anthina* strain MYSP113(Table 5).

Table 3. Comparison of 16S rRNA sequences and RB51 isolate homology (1292 bp) with another strain on Gene Bank NCBI.

Strain	**Length of sequences (bp)**	**Gap**	**Homology (%)**	**Accession No.**
Klebsiella sp. LW-13	1432	6/1143	98.86	KR258763
Klebsiella quasipneumoniae strain HKUOPL4	5087945	6/1132	98.84	CP014156
Klebsiella sp. strain G280	1165	3/1152	98.52	KY655220
Klebsiella pneumoniae strain PBCUK21	1319	7/1191	98.07	LC216325
Klebsiella pneumoniae strain D101	1275	3/1178	97.70	KY888166

Table 4. Comparison of 16S rRNA sequences and RB241 isolate homology (1386 bp) with another strain on Gene Bank NCBI

Strain	**Length of sequences (bp)**	**Gap**	**Homology (%)**	**Accession No.**
Klebsiella pneumoniae strain DSM 30104	1381	2/1336	98.65	KX274129
Klebsiella pneumoniae strain BCH1	1437	4/1378	98.11	GU327663
Klebsiella pneumoniae strain CAV1042	5424949	4/1373	98.03	CP018671
Klebsiella pneumoniae strain VM18	1449	4/1382	97.90	MF953265
Klebsiella pneumoniae strain CCFM8369	1427	4/1382	97.97	KJ803926

Table 5. Comparison of 16S rRNA sequences and RB292 isolate homology (1460 bp) with another strain on Gene Bank NCBI

Strain	Length of sequences (bp)	Gap	Homology (%)	Accession No.
Burkholderiaanthina strain MYSP113	1400	2/1372	98.83	KR827429
Burkholderia sp. T3	1416	3/1406	98.29	KC462881
Burkholderiacenocepacia strain ZYB002	1483	3/1413	98.22	EU684748
Burkholderiacepacia strain TTN1	1435	4/1418	98.10	JX901049
Burkholderia territorii strain RF8	3421021	3/1408	98.08	CP013366

Conclusion

This study obtained three isolates of exopolysaccharide-producing bacteria having a higher value of the dry weight i.e. isolates RB292 (7.53 mg/mL) followed by RB51 (7.55 mg/mL), and RB241 (1.75 mg/mL) with 2% sucrose. Isolates RB51 and RB292 increased significantly soil aggregate stability at 2% dosage of organic matter with soil aggregate stability index from 30.61% to 47.87% and 45.79%. Homology of the isolates with known bacteria i.e. isolate RB51 was 98.86% homolog with *Klebsiella sp*. LW-13, isolate RB241 was 98.65% homolog with *Klebsiella pneumonia* strain DSM 30104 and isolate RB292 was 98.83% homolog with *Burkholderia anthina* strain MYSP113

References

Bertin, C., Yang, X. and Weston, L.A. 2003. The role of root exudates and allelochemicals in the rhizosphere. *Plant and Soil* 2(6):67–83.

De Leenheer, L. and De Boodt, M. 1959. Determination of aggregate stability by the change in mean weight diameter. In: *Proceeding of International Symposium on Soil Structure*, Ghent. Belgium.

Emtiazi, G., Ethemadifar, Z. and Habibi, M.H. 2004. Production of extracellular polymer in *Azotobacter* and biosorption of metal by exopolymer. *African Journal of Biotechnology* 3(6): 330-333.

Eneje, R.C., Oguike, P.C. and Osuaku, S. 2007. Temporal variations inorganic carbon, soil reactivity and aggregate stability in soils of contrasting cropping history. *African Journal of Biotechnology* 6(4): 369-374.

Enriquez, G.L., Saniel, L.S., Matias, R.R. and Garibay, G.1995. General Microbiology Laboratory Manual. Diliman: University of the Philippines Press.

Fairhurst, T. and McLaughlin, D. 2009. Sustainable Oil Palm Development on Degraded Land in Kalimantan. WWF, United States of America. 22p.

Hardjowigeno, S. 1993. Soil Classification and Pedogenesis First Edition. Publisher Akademika Pressindo. Jakarta.

Imran, M.M., Reehana, K.A., Jayaraj, A., Ahamed, P.D., Dhanasekaran, N., Naif, T.S. and Muralitharan, A.G. 2016. Statistical optimization of exopolysaccharide production by *Lactobacillus plantarum* NTMI05 and NTMI20. *Journal of Biological Macromolecules* 66:213-225.

Kusuma, C.A., Wicaksono, K.S. and Prasetya, B. 2016. Improvement of physical properties and chemistry of sandy soils through application of *Lactobacillus fermentum* bacteria. *Jurnal Tanah dan Sumberdaya Lahan* 3(2): 401-410 (*in Indonesian*).

Moreno, J., Garcia, C.V., Lopez, M.J. and Serrano, G.S. 1999. Growth and exopolysaccharide production by *Azotobacter vinelandii* in media containing phenolic acids. *Journal Applied Microbiology* 86(3): 439-445.

Mu'minah, Baharuddin, Subair, H. and Fahruddin. 2015. Isolation and screening Bacterial Exopolysaccharide (EPS) from potato rhizosphere in highland and the potential as a producer Indole Acetic Acid (IAA). *Procedia Food Science* (3): 74 –81.

Mu'minah, Baharuddin, Subair, H., Fahruddin and Darwisah, B. 2016. The potential of producing bacteria exopolysaccharide from rhizosphere potato for produce exopolysaccharide. *Journal Sains and Technology* 16 (2):101–106.

Pawar, S.T., Bhosale, A.A., Gawade, T.B. and Nale, T.R.2013. Isolation, Screening, and optimization of exopolysaccharide producing-bacterium from the saline soil. *Journal of Microbiology and Biotechnology* 3(3): 24-31.

Prasertsan, P., Dermlim, W., Doelle, H. and Kennedy, J.F. 2006. Screening, characterization and flocculating property of carbohydrate polymer from newly isolated *Enterobacter cloacae* WD7. *Carbohydrate Polymers* 66: 289–297.

Prasertsan, P., Wichienchot, S., Doelle, H. and Kennedy, J.F. 2008. Optimization for biopolymer production by *Enterobacter cloacae* WD7. *Carbohydrate Polymers* 71:468-475.

Remel. 2005. Microbiology Products: Instructions for use of MacConkey Agar. http://www.remelinc.com.[28 Jun 2017]

Santi, L.P., Dariah, Ai, and Goenadi, D.H. 2008. Increased stability of mineral soil aggregates by Exopolysaccharide-producing bacteria. *Menara Perkebunan* 76(2): 92-102.

Serrato, R.V., Sassaki, G.L., Cruz, L.M., Pedrosa, F.O.,

Gorin, P.A.J. and Iacomini, M. 2006. Culture conditions for the production of an acidic exopolysaccharide by the nitrogen-fixing bacterium *Burkholderia tropica*. *Journal of Microbiology* 52(5):489-493.

Soil Survey Staff. 1993. Soil Survey Manual. USDA Handbook No. 18.Washington. The USA.

Steel, R.G.D. and Torrie, J.H. 1980. Principles and Procedures of Statistics. A Biometrical Approach. 2nd ed. McGraw-Hill. New York.

Suharta, N. and Suratman. 2004. Characterization and Analysis of Land Resources for Regional Development in Eastern Indonesia, scale 1: 250,000. The Land Resource Research Project and the Participatory Agricultural Technology Assessment (PAATP). Pusat Penelitian Tanah dan Agroklimat. Balitan.

Suharta, N., Anda, M. and Suwandi, V. 1999. Survey and Mapping of Land Review South Kalimantan Province (First Part), scale 1: 250,000. Land Resource and Agroforestry Research and Development Project. Pusat Penelitian Tanah dan Agroklimat. Badan Litbang Pertanian.

Syarovy, M., Ginting, E.N., Wiratmoko, D. and Santoso, H. 2015. Optimization of oil palm plant growth in Spodosol land. *Jurnal Pertanian Tropik* 2(3): 340- 347.

Tallgren, A.H., Airaksinen, U., von Weissenberg, R., Ojamo, H., Kuusisto, J. and Leisola, M. 1999. Exopolysaccharide-producing bacteria from sugar beets. *Journal Applied Environmental Microbiology* 65(2):862-864.

Torres, C.A.V., Marques, R., Antunes, S., Alves, V.D., Sousa, I., Ramos, A.M., Oliveira, R., Freitas, F. and Reis, M.A.M. 2012. Study of the interactive effect of temperature and pH on exopolysaccharide production by Enterobacter A47 using multivariate statistical analysis. *Journal of Biotechnology* 119: 148–156.

Yatno, E. 2011. Role of organic materials in improving soil physical quality and plants production. *Jurnal Sumberdaya Lahan* 5 (1):175-182.