



Original Research Article

Isolation and Screening of Exopolysaccharide Producing Bacterial (EPS) from Potato Rhizosphere for 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 exopolysaccharide-producing isolates of potential origin of potatoes that can risosfer soil aggregates. Soil samples were collected from the rhizosphere of potato in three different land slope respectively 15%, 25% and 35% at altitudes □ 1500 m above sea level in Malino, South Sulawesi. Soil samples were diluted to 10⁻⁹ ATCC and cultured in media with the aim of selecting the bacteria that produce EPS-. There are 74 isolates of exopolysaccharide-producing bacteria were isolated and grouped into gram-negative bacteria. However, only 34 isolates formed a thick slime or mucus when cultured on media MacConkey. The results showed that 34 isolates of potential produces exopolysaccharide and 15 isolates produce exopolysaccharide best dry weight in the range of 0.10 to 2.24 mg / ml. Of the 15 isolates of EPS-producing bacteria that have a higher value of the dry weight is Isolates code P3.69 (2.24 mg / ml) followed by P2.60 (1.96 mg / ml), P2.37 (1.79 mg / ml) and P2.57 (1.75 mg / ml).

Keywords

Potato,
Rhizosphere,
Bacteria,
Exopolysaccharide,
Soil aggregation

Introduction

Planting potatoes in upland slopes are generally more diupayakan to increase production, so that the land conservation issues are often ignored. Conditions of dry land on the plateau sloping generally classified as unstable, prone to erosion and

landslides. Farmers cultivate potato plants on land with slopes of 15% and 35% without regard to principles of conservation of soil and water in the form of planting on ridges in the direction of the slope (Kesaulya *et al.*, 2014). In this connection, according to

Arifin and Soleh (2002), planting potatoes in the ridges in the direction of slope erosion potato growing season of 14 to 16.5 tons ha⁻¹. Research results of Arifin *et al.* (2003) suggested that planting potatoes done in the same direction on a slope 15%–30% with a variety Granola, the rate of run-off and erosion can reach 568.72 m³ ha⁻¹ and 20.83 ton ha⁻¹. This condition leads to deterioration of land productivity, which would lower the potato production and farmers' income. Erosion / degradation directly influence the rate of decline in land productivity, infiltration capacity, soil moisture, and the erosion of topsoil and depletion of nutrients, all of which will determine the availability of water for plants for optimal growth (Kesaulya *et al.*, 2014). Therefore, efforts to maintain the productivity of the land by means of microbiological conservation, the use of microorganisms in the rhizosphere for the plant roots can improve soil structure by means of aggregating soil by microorganism's exopolysaccharide-producing bacteria that indigenous (Baharuddin *et al.*, 2015).

The basic concept of aggregation is the formation of secondary particles through the incorporation of mineral particles with organic and inorganic materials. Aggregation dynamics are complex and influenced by the interaction of several factors such as environment, management of soil, plant, mineral composition, texture, soil organic carbon concentration, pedogenesis processes, the activity of soil microorganisms, ions can be exchanged, reserves of nutrients in the soil, and moisture (Bronick and Lal, 2005).

Some exopolysaccharide-producing bacteria have been reported include *Pseudomonas aeruginosa*, *Erwinia*, *Ralstonia* and *Azotobacter vinelandii*. Exopolysaccharide protects the bacteria from a variety of

environmental stresses (Iqbal *et al.*, 2002), protects cells from antimicrobial compounds, antibodies, and bacteriophages, or for sticking to other bacteria, animal and plant tissues (Wingender *et al.*, 1999). Information on the mechanism of interaction with soil microorganisms associated with soil aggregate stability and providing nutrients for agricultural crops and plantations in Indonesia is still very limited. Therefore, the utilization of exopolysaccharide-producing bacteria for stabilizing soil aggregates in planting vegetables, especially potato plateau need to be developed. The purpose of this study was (i) isolate exopolysaccharide-producing bacteria to aggregate the rhizosphere soil of potato plants (ii) obtain bacterial isolates shaped exopolysaccharide slime

Materials and Methods

The research was conducted in Malino Gowa in South Sulawesi to the rhizosphere soil sampling for isolation of exopolysaccharide-producing bacteria. The series of activities isolation, selection and identification of exopolysaccharide-producing bacteria was conducted in the laboratory of Agricultural Biotechnology, Center for Research activities (PKP) Hasanuddin University, Makassar

Isolation and purification of bacteria producing exopolysaccharide (EPS)

Isolation of bacteria producing exopolysaccharide (EPS) was performed on several samples of soil were taken based on the slope is 15%, 25% and 35% in rhizosphere potato (*Solanum tuberosum* L). Soil material was taken from a depth of 0–20 cm. 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 $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$; 0.1 g $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$; 2.0 mg FeCl_3 ; $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$ (trace); 0.5 g Yeast Ekstrac; 20 g sucrose; and 15 g agar bacto with pH 7.2 and NB medium for seven days at a temperature of 28°C (Remel, 2005; Santi *et al.*, 2008) Bacteria that produce EPS characterized by colonies of bacteria that form thick slime (mucoid) subsequently selected (Tallgren *et al.*, 1999) and purified by streaking the four quadrants to obtain single colonies. Selection of bacterial exopolysaccharide-producing potential by setting the dry weight of bacterial exopolysaccharide produced according to the method proposed by Emtiazi *et al.* (2004).

Screening bacteria producing exopolysaccharide

Selection and screening bacterial exopolysaccharide-producing potential by setting the dry weight exopolysaccharide produced by bacteria in liquid medium ATCC no. 14 (per liter of medium): 0.2 g KH_2PO_4 ; 0.8 g K_2HPO_4 ; 0.2 g $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$; 0.1 g $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$; 2.0 mg FeCl_3 ; $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$ (trace); 0.5 g Yeast Extrac, 20 g sucrose; with pH 7.2 using sucrose as a carbon source method proposed by Emtiazi *et al.* (2004) and Santi *et al.* (2008). Colonies of bacteria that form thick slime (mucoid) on solid medium no.14 ATCC were grown in 50 ml liquid medium ATCC no. 14 and incubated at a temperature of 28°C for three days at the top of the machine shaker with 200 rpm rotation. At the end of incubation, cells were harvested with 1 mM EDTA by adding 500 µl, then shaken until homogeneous and then centrifuged at 9000 rpm for 10 min. The supernatant was separated from the bacterial cell deposition was taken, coupled with cold

acetone solution with a ratio of 1: 3. Then performed again with the speed centrifugation 15000 rpm for 2 times 30 minutes. Deposition of biomass in the form of exopolysaccharide then washed with distilled water and dried at 60°C for 24 hours or until dry weights obtained were fixed.

Gram reaction test

Pure bacterial colonies tested gram reaction, whether including bacteria positive or gram negative salt. Colonies of bacteria taken from a pure culture on medium ATCC No. 14 and the NGA by using a loopful then placed on glass slides were already drops 3% KOH solution. Regularly colonies of bacteria and the solution was stirred with a loopful until completely mixed as he pulled up as high as 0.5 to 1 cm. Koloni that bernampak slimy and attached showed positive reaction indicates that the bacteria are also classified as Gram negative (-), and vice versa are not berlendiran apart is the negative reaction is a Gram positive (+).

Results and Discussion

A total of 24 isolates were obtained from the rhizosphere soil material origin potato crops on slopes of 15%, with an altitude of 1500 m above sea level (Table 1). The bacterial growth in the medium NGA (NB and that wallet as compactor) shows the diversity of morphology of bacteria that grow both shape and color of a single colony, so that the selected 24 isolates suspected to produce exopolysaccharide to characterize the bacterial colony forming slimy slime. Of the number of these isolates, based on test results produce gram-negative gram sebanyak 11 isolates and 13 isolates of gram-positive bacteria. Gram-negative bacteria produced marked with slimy bacteria after reacted with KOH and vice versa gram-positive bacteria are not slimy.

Similarly, the slope of 25%, with an altitude of 1500 m asl A total of 12 isolates were obtained from the rhizosphere soil material origin of potato plants (Table 2). The bacterial growth in the medium NGA (NB and that wallet as compactor) shows the diversity of bacteria that grow, so that selected 12 isolates were allegedly produces exopolysaccharide to characterize the bacterial colony forming slimy slime. Of the number of these isolates, based on test results produce gram-negative gram sebayak 4 and 8 isolates of gram-positive bacterial isolates. Gram-negative bacteria produced marked with slimy bacteria after reacted with KOH and vice versa gram-positive bacteria are not slimy.

Isolation of bacterial exopolysaccharide-producing activities conducted on soil material with clayey loam texture origin potato plant rhizosphere in Malino on slopes of 15%, 25%, and 35% at an altitude of 1500 m above sea level showed that the abundance of bacteria in the rhizosphere of potato crops on slopes 15% higher if compared with the 25% slope. It is assumed that the development of plant roots in the rhizosphere greatly influenced by the structure and size of the soil particles, the water content in the soil and buffer capacity. Plant roots are a source of carbon for energy and food soil microorganisms.

Good root development on slopes of 15% will increase the growth of soil microorganisms and their interaction with plant roots. Research of Hassink *et al.* (1993) showed that bacterial cells more prevalent in clayey soil and clay dominated by smaller pore space when compared to the texture of sand. In mikroagregat (2–20 m) were taken from the depth of the clay layer massive and less porous structure containing bacterial biomass is lower when compared with the same clay layer but granular

structure. Sessitsch *et al.* (2001) stated that in the surface of clay particles with a size smaller than 2 μm is a niche for aerobic and anaerobic bacteria, while the larger particle size is dominated by aerobic bacteria. Abundance of Gram-negative bacteria in the mikroagregat been reported by Hattori (1988) which states that the majority of bacteria stabilizing aggregate mainly Gram-negative bacteria are found scattered in the capillary pores having a diameter of less than 250 μm .

In table 3 above shows that the difference in the growth of bacteria isolated in semi-selective medium is medium ATCC (American Type Culture Collection) by the general media are NGA (Glucose Nutrient Agar). Where the media NGA diverse bacterial growth that must be screened to select a slimy bacteria or gram-negative bacteria to specific media tested exopolysaccharide. While in ATCC medium which is a semi-specific medium so that the bacteria are grown is a bacterial exopolysaccharide. For more selective capture exopolysaccharide gram-negative bacteria do more testing on the MacConkey media to capture as much as possible exopolysaccharide-producing gram-negative bacteria forming slime (Baharuddin *et al.*, 2015).

Four of potential bacterial exopolysaccharide producing each of the isolates code P2 (37), P2 (57), P2 (60) and P3 (69) can produce dry weight exopolysaccharide from 1.75 to 2.24 mg / ml of medium. Dry weight exopolysaccharide weighing results indicate that the bacterial isolates code P3 (69) resulted in a higher dry weight than the other isolates of bacteria with the code. Excreting bacteria growth exopolysaccharide around the neighborhood. The amount and composition of this exopolysaccharide vary

greatly depending on the genus and species of bacteria (Baharuddin *et al.*, 2015) Bacteria in desperate need of energy to produce exopolysaccharide. Therefore, the presence of a carbon source in the growth

medium in addition to functioning as a component forming cells may also serve as a source of energy that is required for the synthesis and excretion of exopolysaccharide (Santi *et al.*, 2008).

Table.1 Isolation of bacteria producing exopolysaccharide (EPS) on a 15% slope at an altitude > 1500 m asl

No	Isolat Code Description	Coloni Color Media NGA	Gram Test		
			Positive	Negative	
1.	1.1	White turbid	+	-	Not Slimy
2.	1.2	White turbid	-	-	<i>Slimy</i>
3.	1.3	White turbid	-	-	<i>Slimy</i>
4.	1.4	Yellowsh white	+	-	Not Slimy
5.	1.5	Yellow	+	-	Not slimy
6.	1.6	Yellowsh white	-	-	<i>Slimy</i>
7.	1.7	White turbid	+	-	Not Slimy
8.	1.8	White	+	-	Not Slimy
9.	1.9	White turbid	+	-	Not Slimy
10.	1.10	Yellowsh white	+	-	Not Slimy
11.	1.11	Yellows white	+	-	Not Slimy
12.	1.12	Yellows white	+	-	Not Slimy
13.	1.13	White turbid	-	-	<i>Slimy</i>
14.	1.14	Yellowsh	+	-	Not Slimy
15.	1.15	White turbid	+	-	Not Slimy
16.	1.16	Translucent white	-	-	<i>Slimy</i>
17.	1.17	White turbid	+	-	Not Slimy
18.	1.18	White	-	-	<i>Slimy</i>
19.	1.19	Translucent white	-	-	<i>Very Slimy</i>
20.	1.20	White turbid	-	-	<i>Slimy</i>
21.	1.21	Yellowsh white	-	-	<i>Slimy</i>
22.	1.22	White turbid	+	-	Not Slimy
23.	1.23	White turbid	-	-	<i>Very Slimy</i>
24.	1.24	White turbid	-	-	<i>Slimy</i>

* Very Slimy = - - - - Slimy = - - - Slimy enough = - - Somewhat slimy = -

Table.2 Isolation of bacteria producing exopolysaccharide (EPS) on a 25% slope at an altitude > 1500 m asl in Malino

No	Isolat Code	Coloni Color Media NGA	Gram Test		Description
			Positive	Negative	
1.	2.1	Creme	+	-	Not slimy
2.	2.2	Yellow	+	-	Not Slimy
3.	2.3	Creme	+	-	Not Slimy
4.	2.4	White	+	-	Not Slimy
5.	2.5	Crème	+	-	Not Slimy
6.	2.6	Creme	+	-	Not Slimy
7.	2.7	White	+	-	Not Slimy

8.	2.8	White turbid	-	<i>Very Slimy</i>
9.	2.9	Turbid	-	<i>Very Slimy</i>
10.	2.10	Yellowsh white	-	<i>Very Slimy</i>
11.	2.11	White turbid	+	Not Slimy
12.	2.12	Yellowish	-	<i>Somewhat slimy</i>

* Very slimy = - - - - Slimy = - - - - Enough slimy = - - Somewhat slimy = -

Table.3 Isolation of bacteria producing exopolysaccharide (EPS) on a 35% slope at an altitude > 1500 m asl in Malino

No	Isolat Code	Coloni Color Media ATCC	Gram Test		Description
			Positive	Negative	
1.	1.1	White turbid		-	<i>Slimy</i>
2.	1.2	White turbid		-	<i>Very Slimy</i>
3.	1.3	White turbid		-	<i>Somewhat Slimy</i>
4.	1.4	Yellowish white	+		Not Slimy
5.	1.5	Yellow		-	<i>Slimy</i>
6.	1.6	Yellowish white		-	<i>Somewhat Slimy</i>
7.	1.7	White turbid	+		Not Slimy
8.	1.8	White		-	<i>Somewhat Slimy</i>
9.	1.9	White turbid	+		Not Slimy
10.	1.10	Yellowish white	+		Not Slimy
11.	1.11	Yellowish white	+		Not Slimy
12.	1.12	Yellowish white	+		Not Slimy
13.	1.13	White turbid	+		Not Slimy
14.	1.14	Yellowish	+		Not Slimy
15.	1.15	White turbid	+		Not Slimy
16.	1.16	White turbid		-	<i>Somewhat Slimy</i>
17.	1.17	White turbid		-	<i>Slimyr</i>
18.	1.18	White	+		Not Slimy
19.	1.19	Translucent white	+		Not Slimy
20.	1.20	White turbid	+		Not Slimy
21.	1.21	Yellowish white	+		Not Slimy
22.	1.22	White turbid		-	<i>Somewhat</i>
<i>Slimy</i>					
23.	1.23	White turbid	+		Not Slimy
24.	1.24	White turbid	+		Not Slimy
25.	1.25	Turbid	+		Not Slimy
26.	1.26	White	+		Not Slimy
27.	1.27	White turbid	+		Not Slimy

* Very Slimy = - - - - Slimy = - - - - Enough Slimy = - - Somewhat Slimy = -

Table.4 Dry matter exopolysaccharide in exopolysaccharide production medium for 72 h of incubation

No	Isolates Code	Dry matter EPS (mg/ml)	No	Isolates Code	Dry matter EPS (mg/ml)
1	P2 (67)	0,87	18	P2 (16)	0,40
2	P1 (7)	0,45	19	P3 (51)	0,30
3	P3 (70)	1,67	20	P2 (66)	0,22
4	P3 (53)	0,30	21	P2 (34)	1,30
5	P3 (42)	0,10	22	P3 (50)	1,67
6	P3 (68)	0,45	23	P2 (37)	1,79
7	P2 ((20)	0,45	24	P3 (69)	2,24
8	P2 (65)	0,45	25	P1 (6)	1,04

9	P2 (56)	0,50	26	P2 (58)	0,54
10	P2 (32)	0,43	27	P3 (41)	1,52
11	P2 (27)	0,48	28	P3 (46)	1,17
12	P2 (21)	0,28	29	P3 (38)	1,70
13	P2 (33)	0,49	30	P3 (39)	0,8
14	P3 (49)	0,91	31	P2 (57)	1,75
15	P3 (72)	0,32	32	P2 (60)	1,96
16	P3 (48)	0,33	33	P1 (4)	0,76
17	P3 (73)	0,57	34	P2 (15)	0,64

Figure.1 Bacterial isolates producing EPS with MacConkey media



Figure.2 Formation of slime (arrow) bacteria EPS on ATCC 14 media



In the soil, bacteria are always associated with clay and other polysaccharides or excretions of plant decomposition. This association generally occurs in mikroagregat contained in the root zone. Lupwayi *et al.* (2001) found the ratio of bacteria: fungi in makroagregat lower when compared with in mikroagregat. This is due to the activity of bacteria occurred on mikroagregat while the activity of fungi is more prevalent in makroagregat. Isolation of exopolysaccharide-producing bacteria in the

rhizosphere made potato plants contained in the soil matrix. Soil matrix is a plant root development, production of root exudates internal metabolic outcomes plants which generally contain a lot of carbon compounds, and the growth of macro and micro soil biota. Therefore, by taking the material in the soil around the roots, is expected to be obtained with a diversity of soil bacteria are quite high. As noted by Bertin *et al.* (2003) that the root exudates contain some organic compounds with low

molecular weight such as simple sugars and polysaccharides (arabinose, fructose, glucose, maltose, mannose), oligosaccharides, amino acids (arginine, asparagine, aspartate, cysteine, cystine, glutamine), acid Organic (acetic, ascorbic, benzoic acid, and malic) and phenolic compounds. Some of these compounds can enhance the growth and development of soil microorganisms.

Four isolates that have a value of potential in producing exopolysaccharide are P3 (69), P2 (37), P2 (57) and P3 (70), isolates code P3 (69) exopolysaccharide producing high amounts of 2.24 mg / ml compared with other isolates. Bacterial isolates that produce exopolysaccharide seen from the origin of the samples derived from the slope of the bacteria both P2 (25%) and P3 (35%).

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