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PRODUCTION OF EXOPOLYSACCHARIDE (EPS) ISOLATED FROM BACTERIAL POTATO RHIZOSFER ON SEVERAL SOURCES OF CARBON

Mu'minah[1*], Baharuddin[2], Hazarin Subair[3], Fahruddin[4]

[1]Graduate School, Hasanuddin University, Makassar 90245 Indonesia
[1]Department of Plantation Crop, Agriculture Polytechnic Pangkep, Pangkep 90655 Indonesia
**email:mutmainah2009@gmail.com*
[2]Center for Biotechnology, University of Hasanuddin Makassar 90245 Indonesia
[3]Department of Soil Science, Faculty of Agriculture, Hasanuddin University Makassar 90245 Indonesia
[4]Department of Biology, Faculty of MIPA, Hasanuddin University Makassar 90245, Indonesia

Abstract

This study aimed to establish the optimum type of carbon source for the production of exopolysaccharide from potato rhizosphere bacteria. Soil samples were collected from the rhizosphere of potatoes in three different land slope respectively 15%, 25% and 35% at an altitude more than 1500 m above sea level in Malino, South Sulawesi. There were 74 isolates of exopolysaccharide-producing bacteria were isolated and grouped into gram-negative bacteria. However, only 34 isolates produce a thick slime or slimy when cultured on media MacConcey. The results showed that 34 of potential bacterial isolates produced exopolysaccharide and 15 isolates of bacteria produce dry matter betwen 0.10 mg/ml to 2.24 mg/ml as the highest exopolysaccharide produced. The best carbon source for the production of exopolysaccharide is sucrose with concentration 2 %. There are 4 isolates that had higher dry matter are P3.69 isolates (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) respectively.

Keywords: *Potato, rhizosphere bacteria, Exopolysaccharide, Soil aggregation*

1. INTRODUCTION

Intensive land use on horticultural crops in the highlands is the trigger of high erosion. Planting potatoes in sloping lands are generally more usable to increase production, so the issue of land conservation is often overlooked [10]. This condition leads to deterioration of land productivity, which will lower the potato production and farmers' income [10]. Therefore, efforts to maintain the productivity of the land by means of microbiological conservation, the use of microorganisms in the rhizosphere roots of potato plants can improve soil structure by way of aggregating soil by microorganisms are bacteria producing exopolysaccharide (EPS).

The role of soil microorganisms on the formation, stability, and degradation of soil aggregates has been investigated [1,4,5]. Accumulation of cells and the formation of colonies of bacteria that coats the grains of primary and secondary particles (aggregates) have an important influence on the structure of the soil [16]. The mechanism is under natural conditions, soil bacteria produce organic compounds such as exopolysaccharide (EPS). Exopolysaccharide (EPS) is

a complex mixture of macromolecular electrolytes found on the outside of the bacterial cell is excreted as mucus that contributes as an adhesive soil aggregation.

Exopolysaccharide-producing bacteria can interact with soil particles through the formation of polymer bridges that have a role in the formation of mikroagregat and more important is the ability of exopolysaccharide in stabilizing soil aggregates. [9] argues that the number of eroded soil particles depends on the type and population of microorganisms that are added. The opinion concluded from experiments the addition of a number of bacteria (*Azotobacter chroococcum* and *Pseudomonas sp.*) And yeast (*Lypomyces starkeyi*) which turned out to increase the stability of the aggregate of the power of water.

Potential exopolysaccharide-producing bacteria (BPE) to help reduce environmental stress in plants growing in high salinity environments reported by [2,7,8]. The mechanism is the EPS can bind cations including Na^+, which is in the rhizosphere. Increasing the density of the bacterial population in the root zone will reduce the content of Na^+ available to crops. The results of the study [17] showed that the exopolysaccharide from *Enterobacter sp, Arthrobacter sp* and *Azotobacter sp* can assist solubility of tricalcium phosphate in the growth medium. The ability of exopolysaccharide in holding phosphorus may be an important factor in helping the solubility of tricalcium phosphate in addition to organic acids. *Azotobacter beijerinckii* WDN-01 produces water-soluble exopolysaccharide. *Rhizobium tropici* accumulate poly-3-hydroxybutyrate [P (3HB)], exopolysaccharide and glycogen as a source of energy and carbon. Catabolism of intracellular carbon storage is a strategy adopted by some species of bacteria to survive in conditions of sub-optimal nutrition [11].

Soil structure with stable aggregates will increase the porosity, soil fertility and crop productivity, and reduce erodibility. Exploration of indigenous bacteria that can potentially produce exopolysaccharide an early stage to develop knowledge about the role of exopolysaccharide-producing bacteria in soil aggregation.

2. MATERIALS AND METHODS

2.1. Isolation and Purification of Bacteria Producing exopolysaccharide (EPS)

Isolation of bacteria producing exopolysaccharide (BPE) on some soil samples taken by a slope of 15%, 25% and 35% in rhisosfer potato (*Solanum tuberosum* L). Soil material 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 then made serial dilutions 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 of $FeCl_3$; $Na_2MoO_4.2H_2O$ (trace); 0.5 g of Yast Ekstrac; 20 g sucrose; and 15 g bakto that with pH 7.2 and NB medium for seven days at a temperature of 28°C [12,13] Bacteria that produce EPS characterized by colonies of bacteria that form a thick slime (mucoid) was then selected [14] and purified by streaking in four quadrants to obtain single colonies. Selection of bacterial exopolysaccharide-producing potential by setting dry weight exopolysaccharide produced by bacteria according to the method proposed by [6].

2.2. Scerening exopolysaccharide Producing Bacteria

Scerening bacterial exopolysaccharide-producing potential by setting 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 $MgSO_4.7H_2O$; 0.1 g $CaSO_4.2H_2O$; 2.0 mg of $FeCl_3$; $Na_2MoO_4.2H_2O$ (trace); 0.5 g of Yeast Ekstrac; 20 g sucrose; pH 7.2 using sucrose as a carbon source method proposed by [6,12]. Colonies of bacteria that form a 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 0C for three days at the top of the machine shaker with 200 rpm rotation. At the end of incubation, cells were harvested by adding 1 mM EDTA 500 mL, then shaken until homogeneous and then centrifuged at 9000 g 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. Further back with speed centrifugation 15000 g 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 a fixed dry weight.

2.3. Optimization of exopolysaccharide production

In order to study the optimization of the production of exopolysaccharide used parameters of the incubation period (1-3 days), carbon source (sucrose, glucose and mannitol) with concentration (1, 2 and 3%)). A total of 15 samples of bacteria were inoculated in 100 ml of production medium teriri of (g / l): peptone 10 g, Yeast Ekstrac 3 grams, 5 grams of NaCl and 20 g sucrose. Medium sterilized at 121 0 C for 20 min, the pH was adjusted to 6.5 - 7. Dinkubasi on shaker at room temperature for 72 hours [14]

3. RESULTS AND DISCUSSION

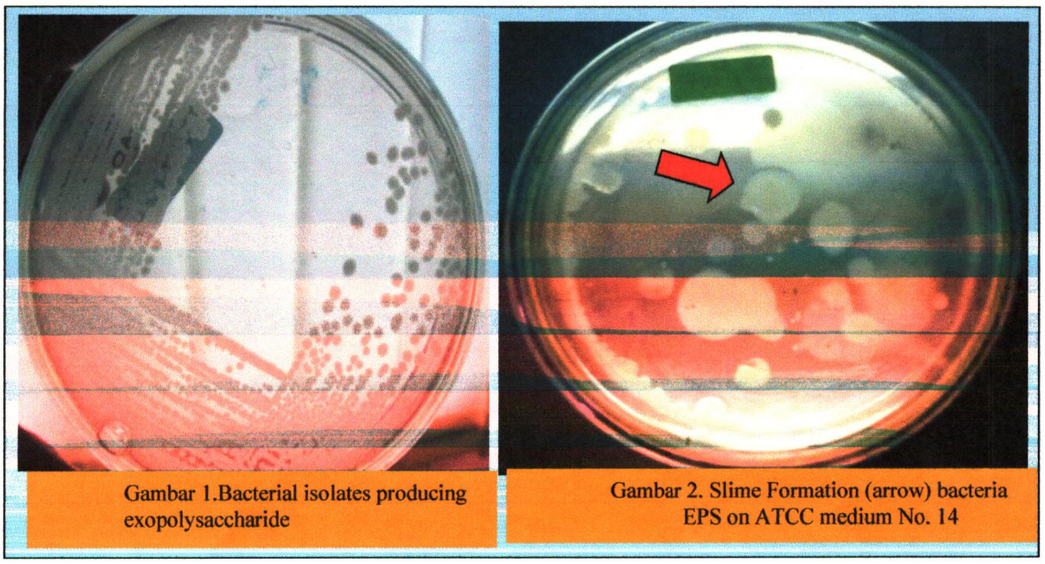
There were 74 bacterial isolates was obtained from the rhizosphere of potato plants derived from the District of Malino High Muzzle, Gowa, South Sulawesi Province (Table 1). After further testing with 34 isolates of bacteria that have the potential to produce exopolysaccharide were grown on agar Mac Concey (selective medium for gram-negative bacteria) with the category level less (+) to very good (++++) that form a thick slime (mucoid).

Isolation of exopolysaccharide-producing bacteria in the rhizosphere of potato plants made widely available within the soil matrix. Where the soil matrix was a root development, production of root exudates of plant metabolic results that contain lots of carbon compounds and the growth of the macro and micro soil biota. As proposed by [3] that the root exudates contain some organic compounds with low molecular weight such as simple sugars and polysaccharides (arabinose, lactose, glucose, maltose, mannose), oligosaccharides, amino acids (arginine, parangin, aspartate, Cysteine, cystine, glutamine), organic acids (acetic, ascorbic, benzoic acid and malic) and phenolic compounds. Some of these compounds can enhance the growth and development of soil microorganisms.

Table1. Isolation of bacteria producing exopolysaccharide origin potato rhizosphere

No.	Isolates Code	The color of colonies in agar Mac Concey	Growth of colonies on medium Mac concey Colonies 7 days	The diameter of colonies for 7 days (cm)	Gram test
1	P1 (4)	Crème pink	+	0,1	Negative
2	P1 (6)	slightly pink	+++	0,3	Negative
3	P1 (7)	Crème pink	++	0,2	Negative
4	P2 (15)	Red	+	0,1	Negative
5	P2 (16)	Pink	+	0,1	Negative
6	P2 ((20)	Crème/Salem	++++	0,5	Negative
7	P2 (21)	slightly pink	++	0,2	Negative
8	P2 (27)	Transparent crème	++	0,2	Negative
9	P2 (32)	Transparent pink	+++	0,4	Negative
10	P2 (33)	Transparent crème	+++	0,3	Negative
11	P2 (34)	Transparent crème	+++	0,2	Negative
12	P2 (37)	Crème/salem	+++	0,3	Negative
13	P3 (38)	slightly pink	+	0,2	Negative
14	P3 (39)	Transparent white	++	0,2	Negative
15	P3 (41)	Transparent	+	0,2	Negative
16	P3 (42)	Transparent slightly pink	++	0,3	Negative
17	P3 (46)	slightly pink	+	0,2	Negative
18	P3 (48)	Transparent crème	++	0,2	Negative
19	P3 (49)	Transparent red	++	0,2	Negative
20	P3 (50)	slightly pink	++	0,2	Negative
21	P3 (51)	old pink	+++	0,4	Negative
22	P3 (53)	Transparent pink	+++	0,3	Negative
23	P2 (56)	Pink	++++	0,4	Negative
24	P2 (57)	slightly pink	++	0,2	Negative
25	P2 (58)	slightly pink	+	0,1	Negative
26	P2 (60)	Red	++	0,1	Negative
27	P2 (65)	Transparent pink	+++	0,2	Negative
28	P2 (6.6)	Old pink	+++	0,6	Negative
29	P2 (67)	Pink	++++	0,5	Negative
30	P3 (68)	Transparent pink	+++	0,3	Negative
31	P3 (69)	Transparent	++	0,1	Negative
32	P3 (70)	Red	+++	0,3	Negative
33	P3 (72)	Transparent	+++	0,2	Negative
34	P3 (73)	Transparent pink	+++	0,3	Negative

Based on the results of measurements of dry weight exopolysaccharide (mg / ml) as shown in Table 2, which shows that the bacterial isolates coded P2 (37), P2 (57), P2 (60) and P3 (69) has a better potential when compared with other types of isolates. The amount of the dry weight of the four bacterial exopolysaccharide of potential are respectively 1.75, 1.79, 1, 96 and 2.24 mg / ml of the medium.



Gambar 1.Bacterial isolates producing exopolysaccharide

Gambar 2. Slime Formation (arrow) bacteria EPS on ATCC medium No. 14

Figure 3. Production of exopolysaccharide by bacteria

Four of potential bacteria producing exopolysaccharide each isolate code P2 (37), P2 (57), P2 (60) and P3 (69) can produce dry weight exopolysaccharide from 1.75 to 2.24 mg / ml of the medium. Dry weight exopolysaccharide weighing results indicate that the bacterial isolates code P3 (69) resulted in a higher dry weight of the bacterial isolates with other codes. Bacteria excrete exopolysaccharide around the neighborhood growth. The amount and composition of the exopolysaccharide is highly variable depending on the genus and species of bacteria. Bacteria in desperate need of energy to produce exopolysaccharide. Therefore, the presence of a carbon source in the medium to grow addition to functioning as a component of cell formation may also serve as a source of energy that is required for exopolysaccharide synthesis and excretion of [11]

Table 2. 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

The results showed that the best carbon source for bacterial exopolysaccharide production risosfer tananaman potato sucrose concentration is 2% dry weight exopolysaccharide produced an average of 2.24 mg / ml. whereas carbon sumbaer glucose and mannitol dry weight exopolysaccharide yield an average 0:55 and 0.78 mg / ml.

In general, exopolysaccharide-producing bacteria will grow well in the medium with the carbon source is oxidized. By using sucrose yield dry weight exopolysaccharide higher when compared to the use of glucose and mannitol in the medium exopolysaccharide production. It is estimated that the potato plant rhizosphere bacterial isolates is easier to metabolize sucrose compared with the use of other carbon sources. Ease in utilizing sucrose as an energy source allows for the growth and formation of cell biomass.

The ability of exopolysaccharide-producing bacteria may be due to soil aggregates due to gum or mucus that serves to glue the land

Table 3. Optimization of exopolysaccharide production on various carbon sources

No	Isolates Code	Exopolysaccharide production in a wide range of Carbon Sources		
		Sucrose (mg/ml)	Glukosa(mg/ml)	Manitol (mg/ml)
1	P2 (67)	0.87	0.67	0.52
2	P3 (70)	1.67	0.57	0.67
3	P3(49)	0.91	0.73	0.77
4	P2 (34)	1.34	0.73	0.55
5	P3 (50)	1.67	0.87	0.77
6	P2 (37)	1.79	1.24	0.73
7	P3 (69)	2.24	0.55	0.78
8	P1 (6)	1.04	0.8	0.67
9	P3 (41)	1.52	0.63	0.76
10	P3 (46)	1.17	0.73	0.5
11	P3 (38)	1.70	0.3	0.83
12	P2 (57)	1.75	1.3	0.7
13	P2 (60)	1.96	0.5	0.57
14	P2 (29)	1.10	0.64	1.16
15	P3 (63)	0.93	0.73	0.5

4. CONCLUSION

Four isolates that have a value of potential in producing exopolysaccharide each 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. Of the three carbon sources tested carbon sources sucrose produces the best exopolysaccharide production. 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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