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The Potential of Exopolysaccharide Bacterial Isolate from the Rhizosphere of Potato as Nitrogen Fixation

Mu'minah[1], Junyah Leli Isnaini[2], Baso Darwisah[3]

[1,2,3] Department of Plantation Crop, Agriculture Polytechnic Pangkep, Pangkep 90655, Indonesia

Abstract: ***This study aims to examine the potential of exopolysaccharide (EPS) bacteria isolated from potato rizosphere to nitrogen fixation. The soil samples were taken from three different altitudes of 1000 m, 1300 m and 1500 m above sea level in Malino Tinggi Moncong districts, Gowa regency, South Sulawesi. Soil samples were cultured on ATCC No. 14 medium to 10^{-8} further EPS bacteria cultured on Mac Concay medium for grouping of gram-negative bacteria. There are 15 isolates capable of fixing the free nitrogen cultured on Burk medium categorized based on percentage of growth ability obtained by 4 isolates (26.67%) have strong growth ability (+++), 6 (40%) isolates have medium growth ability (++) and 5 isolates (33.33%) had weak growth ability (+). Isolate producer of eksopolisakarida bacteria that has the ability to grow strong with the highest percentage of total nitrogen found in P3T63 isolates of 1.42% followed by P3A49 of 1.15% and P3A50 of 0.92%.***

Keywords: exopolysaccharide, nitrogen, rhizosphere, potato

1. Introduction

Land productivity is maintained with various conservation efforts. One effort that can be done is through microbiological conservation efforts, namely by utilization of soil microorganisms especially rizosbakteria. namely the use of microorganisms in the plant rhizosphere to improve the structure of the soil by way of aggregating the soil by microorganisms, which are produced indegenius exopolysaccharide bacteria [1].

The function of soil aggregating rhizosphere bacteria is commonly known as exopolisakarida bacteria. Exopolysaccharide bacteria interact with soil particles through the formation of polymer bridges that play a role in the formation of microaggregates, in particular the ability of exopolysaccharides to stabilize the soil aggregates. The interaction between clay and exopolysaccharide is necessary to solidify the aggregate of the soil. [2]. [3] contend that the number of eroded soil particles depends on the type and population of the microorganisms being added. This opinion was concluded from the experiment of adding a number of bacteria (*Azotobacter chroococcum and Pseudomonas sp*.) And yeast (*Lypomyces starkeyi*) which proved to increase the aggregate stability of water strength.

Rhizobacteria non-symbiotic N2 blockers of the genus Azospirillum are commonly found in the area around rooting and have a beneficial effect on the growth and productivity of some important crops and other plantations [4,5]. Non-symbiotic N2 blocking bacteria such as *Azospirillum, Klebsiella and Azotobacter* are known to have the ability to increase aggregation and soil flocculation. Such capability is strongly influenced by the dissemination and survival of these microorganisms in the soil.
EPS products can protect against adverse conditions such as osmotic stress, dryness, phagocytosis, toxid compounds and bacteriophage infections [6]. However by [7] it is stated that the protection afforded by EPS may be very limited and other benefits of EPS for the growth or survival of bacterial cells remain unclear. For now, the function of EPS bacteria is not fully known [8].

2. Material and Method

2.1 Isolation and Purification of Exopolysaccharide Producing Bacteria (EPS)

Isolation of exopolysaccharide-producing bacteria was taken in several soil samples in potato rhizosphere based on the altitude of the site from the sea level of 1000 m, 1300 m and 1500 m above sea level. As many as one gram of soil material was aseptically suspended in a physiological saline solution (0.85%) and then serial dilutions were made to 10-8 with Duplo and incubated in ATCC no. 14 (per liter 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 Yast Ekstrac; 20 g of sucrose; and 15 g of bacto that with pH 7.2 and NB media for seven days at a temperature of 28^oC [1,4] The bacteria that produced EPS were characterized by bacterial colonies, which produced a thick mucoid (mucoid) then elected [9] and purified by bolted within four quadrants to obtain a single colony. Selection of potential exopolysaccharide bacteria by determining the exopolysaccharide dry weight produced by bacteria according to the method proposed by [9].

2.2 Scerening Bacteria Manufacturer exopolysaccharide (EPS)

Potential to produce exopolysaccharide potential by establishing exopolysaccharide dry weight produced by bacteria in ATCC no. 14 (per liter medium): 0.2 g KH_2PO4; 0.8 g K_2HPO4; 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 of Yeast Extract; 20 g of sucrose; pH 7.2 uses sucrose as the carbon source method proposed by [10,4]. Colonies of bacteria that form thick mucoid (mukoid) in a solid medium of 14 ATCC are grown in 50 ml ATCC no. 14 and incubated at 28 ° C for three days at the top of the shaker machine with a rotation of 200 rpm. At the end of incubation, the 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 is separated from the deposition of the captured

bacterial cell, coupled with a cold acetone solution with a ratio of 1: 3. Then again with a centrifugation rate of 15000 g for 2 times 30 minutes. The biomass deposition in the form of exopolysaccharide is then washed with distilled water and dried at 60 ° C. for 24 hours or until dry weight.

2.3 Optimization of exopolisakarida production

To study the optimization of exopolysaccharide production there are several parameters taken such as incubation period (1-3 days), carbon source (sucrose, glucose and mannitol) with concentration (1, 2 and 3%). A total of 15 bacterial samples were inoculated in 100 ml of production medium (g / l): 10 g pepton, 3 gram Ekstrac, 5 grams NaCl and 20 g sucrose. Medium sterilized at 121 0 C for 20 min, pH adjusted to 6.5-7. And incubated on a shaker at room temperature for 72 hours [11].

2.4 Testing Bacteria in Nitrogen Fixing

Testing the ability of bacterial isolates binding to free nitrogen was tested using Burk N-free media. Burk N-free media composition: 20 g of sucrose, 0.64 g K_2HPO4, 0.16 g KH_2PO4, 0.20 g $MgSO_4.7H_2O$, 0.20 g NaCl, 0.05 g $CaSO_4.2H_2O$, $Na_2MoO_42H_2O$ (0.05 g %) 5.0 ml; $FeSO_4.7H_2O$ (0.3%) 5.0 ml and 15 g bacto agar. The pH of the media is adjusted to pH 7.3 before the autoclave $Na_2MoO_4.2H_2O$ and $FeSO_4.7H_2O$ are filtered before the medium is sterilized [12]. Bacteria grown on Burk N-free media are indicated as bacteria that are able to fix nitrogen. The total nitrogen content measurement was performed using K-jedall method.

3. Results and Discussion

There are 15 bacterial isolates that have the potential to produce exopolysaccharide when grown on a medium for Mac Concey, which is a selective medium for capturing bacteria from the gram-negative group, characterized by thick mucus and colony changes. [1]. However, after further testing there were 6 potential isolates which were tested as plant growth promoters.

Based on the measurement of the dry weight of exopolysaccharide (mg / ml) as shown in Fig1, it showed that four potential exopolysaccharide bacteria yield each code of P2B isolate 29, P3B (46), P3T (63) and P3A (50) exopolysaccharide dry weight of 0,3 – 0,74 mg / ml medium. Exopolysaccharide dry weight test results showed that P3A code bacteria (50) produced higher dry weight than other bacterial isolates (Fig1). The amount and composition of this exopolysaccharide vary greatly depending on the genus and bacterial species [9]. Bacteria desperately need energy to produce exopolysaccharide. Therefore, the presence of carbon sources in the growth medium other than functioning as a cell-building component can also serve as the energy source necessary for the synthesis and excretion of exopolysaccharides [4]. The results showed that the best carbon source for the production of exopolysaccharides was sucrose at a concentration of 2 wt. Of exopolysaccharide dry weight yielding an average of 0,74 mg / ml.

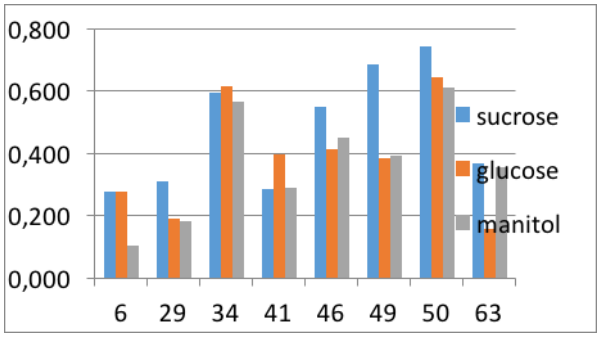

Figure 1: Exopolysaccharide production on sucrose, glucose and mannitol carbon sources at 2% concentration with 72 hours incubation.

Table1: Characterization of isolates of exopolisakarida-producing bacteria from potato rhizosphere.

Isolate Kode	Nitrogen Fixation		characterization bacteria				
	Nitrogen Production	N Total (%)	Growth Colony	Diameter Colony (cm)	Color Colony	Growth medium YDC	pH
P1B6	+	0.61	++	0.65	Cloudy white	+++	5.6
P2B29	+	0.67	++	0.65	Cloudy white	+++	5.5
P2B34	+	0.50	++	0.60	Cloudy white	+	5.7
P2B37	+	0.70	+	0.65	Yellowish white	++	6.2
P3B38	++	0.81	+	0.70	Yellowish white	+++	6.2
P3B41	++	0.64	+	0.65	white	++	5.7
P3B46	+	0.61	++	0.95	white	+	5.7
P3A49	+++	1.15	+++	0,65	Cloudy white	+++	5.6
P3A50	++	0.98	+++	1,20	white	+++	5.6
P2A57	++	0.92	+++	0.50	Yellowish white	+++	5.7
P2T60	++	0.76	++	0.65	white	++	5.8
P3T63	+++	1.42	+++	0.95	white	+++	5.9
P2T67	++	0.98	+++	0.50	Yellowish white	+++	5.6
P3T69	+++	0.87	+	0.70	white	++	6.2
P3T70	+++	0.90	+++	0.75	White	++	6.2

+: less good ++: Good +++: Very Good

Isolation of exopolysacride-producing bacteria in potato rhizosphere is widely available in soil matrix. Where the soil matrix is a root development, the plant roots of production emit metabolic yields that contain many carbon compounds and place the macro and micro growth of the soil biota. As proposed by [13] that root exudates contain several low molecular weight organic compounds such as simple sugars and polysaccharides (arabinose, lactose, glucose, maltose, mannose), oligosaccharides, amino acids (arginine, parangin, aspartate, cysteine, cystine), glutamine), organic acids (acetate, ascorbate, benzoic acid and malate) and phenolic compounds. Some of these compounds can promote the growth and development of soil microorganisms.

The results obtained in the Fixation Tests of Nitrogen Exopolisakarida-producing bacteria, presented in Fig. 2 show that the P3T isolate code (63) showed the highest results in free nitrogen fixation of 1.42%, followed by P3A (50) of

1.24%, P3A (49) 1,15% N total. Besides, the ability of bacteria isolate producing eksopolisakarida grow on Burk medium showed the best growth percentage that is on isolate P3T 63 (+++), P3A 49 (+++) and P3A 50 (++). EPS bacterial characterization results showed that P3T (63) and P3A (50) bacterial isolates showed the best potential isolates from colony growth testing, colony diameter, ability to grow at 33 ° C and growth in YDC medium.

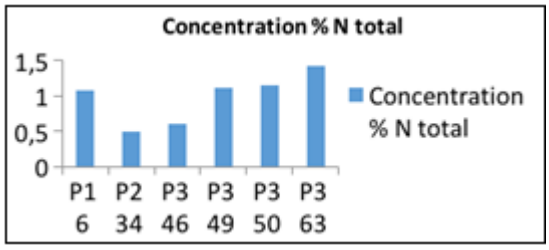

Figure 2: Production nitrogen free fixationon from exopolysaccharide bacteria

4. Conclusion

Four isolates that had potential values to produce P2B exopolysaccharide (29), P3B (46), P3T (63) and P3A (50) resulted in exopolysaccharide of dry weight of 0,74 mg / ml of medium compared with other isolates. Of the three carbon sources tested, the carbon source of sucrose produced the best exopolysaccharide production. The P3T isolate code (63) has a high concentration in Nitrogen fixation of 1, 42% N total and the ability to grow on an excellent Burk medium (+++). The results of characterization test of EPS bacteria showed that the best isolate was bacterial isolate P3T code (63) and P3A (50). The bacterial isolates that produce the best exopolysaccharide and nitrogen fixation, seen from the origin of the bacterial samples are from the 1500 m altitude above sea level.

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