THE POPULATION OF NITROGEN FIXING BACTERIA AND PHOSPHATE SOLUBILIZING BACTERIA IN THE RHIZOSPHERE FROM GUNUNG HALIMUN NATIONAL PARK

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ABSTRACT

The biodiversity of nitrogen fixing bacteria *(Rhizobium, Azotobacter and Azospirillum)* and phosphate solubilizing bacteria in rhizosphere collected from Gunung Halimun National Park were studied. Bacterial population was counted from soil rhizosphere collected from various ecosystem types (determined based on elevation) namely 600 m, 1000 m, 1100 m, 1500 m and 1800 m above sea level. The results showed that the highest population of N-fixing and P solubilizing bacteria at Cikaniki (1100 asl) are founded in the plant rhizosphere of *Schima wallichii* in plot Suzuki A I and *Altingia exelsa* in Suzuki A III, at Gunung Botol area is in 1000 m asl, and Ciptarasa area is in 600 m asl. The population of N-fixing and P solubilizing bacteria at Gunung Halimun was influenced by the vegetation type, soil pH, and the elevation of area. *Rhizobium, Azotobacter, Azospirillum* (N-fixing bacteria); *Pseudomonas* sp, and *Bacillus megaterium* (P solubilizing bacteria), are most dominant at 600 m, 1000 m and 1100 m asl.

Key words : Bakteri pengikat nitrogen/nitrogen fixing bacteria, bakteri pelarut fosfat/phosphate solubilizing bacteria, Taman Nasional Gunung Halimun/Gunung Halimun National Park.

INTRODUCTION

Gunung Halimun National Park (GHNP) is the biggest tropical rain forest conservation area that still exist in Indonesia, and it also the largest tropical forest mountain ecosystem in West Java. This area is located between 106'16' - 10638' EL and 6'37' - 6'53' SL with the elevation between 500 m - 1929 m above sea level. The GHNP area is about 40,000 ha. This area endowed with high diversity of flora, fauna and microorganisms. Though verification of microbial diversity have not yet intensively studied, however it is believed that the higher the diversity of plant will indirectly indicate high diversity of plant microbes.

Soil is a unity of subsistence that includes the varieties of microbe, because microbe's community is one of the important components of soil, therefore the microbial activities and species compositions is generally influenced by the physical characteristic and soil chemical properties, climate and vegetation (Jha *et al.,* 1992).

Soil microbe has an important role to the subsistence on earth, because it has the role on biological and chemical cycling among the flora, fauna, and the life of microbe it self. Nevertheless, not every soil bacteria is suitable and compatible with the habitate and its host, and it is well known that they can perform symbiotic and comensalism.

Soil microbe play key important role on the biotransformation of organic material (as decomposer), and provide organic substances that stimulate plant growth, and finally determine ecosystem stability. On the other hand, organic and an-organic materials released by the plants into their areas (in the form of exudate), will be useful for life continuity of soil microbe (Setiadi, 1989).

Some groups of soil bacteria are useful as bio fertilizer. For example, the bacteria *(Rhizobium, Azotobacter* and *Azospirillum)* are able to fix the nitrogen from the air. There is a symbiosis mutualystic between the bacteria with the host plant (legume and non-legume). The other soil bacteria are *Pseudomonas* and *Bacillus.* Those bacteria are able to solubilize unavailable forms Fe bound P, Ca bound P, Mg bound P and Al bound P. This is happening because the bacteria is producing organic acid which are able to formed stabilize complex with the N fixation cation inside the soil (Rao, 1994).

The soluble P will be available for the plants, and also for the continuity of the symbiosis mutualystic between the nitrogen fixing bacteria and the host plants.

The aim of this research is to know the population of the nitrogen fixing bacteria and phosphate solubilizing bacteria at different elevation of GHNP.

MATERIALS AND METHODS

The soil were collected randomly from 3 study sites namely rizhosphere soil at Cikaniki, Gunung Botol and Ciptarasa of GHNP.

To study microbes plant interaction at Cikaniki, the soil rhizosphere were taken from 0 - 15 cm of the plant of rasamala *(Altingia exelsa),* puspa *(Schima wallichii),* and kianak *(Castanopsis javanica)* at Suzuki plot I, II and III. The elevation of the study sites were 1100 m above sea level (asl). At the forest floor of Gunung Botol, the soil were collected at 1000, 1500 and 1800 m asl. At Ciptarasa, the soils were collected from the rhizosphere of legumes at 600-1500 m asl. Samples soil from Gn. Halimun forest were kept in black plastic bags and in the Laboratory these samples were air-dried before the analysis of pH, total bacteria, nitrogen fixing bacteria and phosphate solubilizing bacteria population.

Total bacteria, nitrogen fixing bacteria and phosphate solubilizing bacteria population were determined by serial dillution plate count technique (Thompson, 1989; Ravina *et al,* 1993). Sample suspension were prepared by shaking 10 gram dry soil and 90 ml sterile distilled water on a wrist-action shaker for one hour to provide mechanical disaggregation of bacterial cell. Subsequent dilutions were prepared by manually shaking the suspension for 10 seconds to resuspend the soil. Then transferring 1 ml an aliquot with a sterile pipettes to 9 ml sterile distilled water in a test tube. This suspension was shaken manually for 10 seconds, and subsequent serial dilution were prepared as noted above to 10^8. From each serial dilutions, 0.2 ml of soil suspension was transferred to sterile petridish and poured with media (50°C). The number of total bacteria were determined by nutrient agar medium (3g beef extract, 5g peptone, 20g agar, diluted in 1 liter H_20, pH = 6.6 - 7.0). The bacteria of *Rhizobium* was determined by plating on congo red with yeast extract manitol agar medium (2.5 ml/1 in the 1 % solvent + lOg manitol, 0.5g K_2HPO_4, 0.2g $MgSO_4.7H_2O$, O.lg NaCl, lg yeast extract, 20g agar, diluted in 1 liter H_2O, pH = 6.8). The phosphates solubilizing bacteria grown on Pikovskaya medium plates (5g $Ca_3(PO_4)_2$, lOg Glucose, 0.2g NaCl, 0.2g KC1, O.lg $MgSO_4.7H_2O$, 0.5g NH_4SO_4, 0.5g Yeast extract, litle $MnSO_4$ and $FeSO_4$, diluted in 1 liter H_2O, pH = 6.8 and the hollozone around the growth spot indicates the phosphatase activity of the bacteria (Gaur, 1981). *Azospirillum* grown on Okon medium plates (6g K_2 HPO_4, 4g KH_2PO_4, 0.2g $MgSO_4$, O.lg NaCl, 0.02g $CaCl_3$, lg NH_4C1, 5g DL malic acid, 3g NaOH, O.lg yeast extract, O.Olg $FeCl_3$, 2.mg Na_2 MoO_4 , 2.1mg $MnSO_4$, 2mg H_3BO_3, 0.04mg $Cu(NO_3)_2$, 0.24mg $ZnSO_4$, 2 ml bromtimol blue/ 0.5% in etanol, 20g agar; diluted in 1 liter H_2O, pH = 6.8 (Okon, 1997) and Ashby manitol agar plates. For *Azotobacter* (20g manitol, 0.2g K_2HPO_4, 0.2g $MgSO_4.7H_2$ O, 0.2g NaCl, O.lg K_2 SO_4, 5g $CaCO_3$, 20g agar, diluted in 1 liter H_2O, pH = 6.8 (Rao, 1994/ The number of bacteria colony was estimated after 1 week of incubation at 28° C. The isolates were identified follows Bergey's manual for bacteriology method systematic (Krieg, 1984).

RESULTS

Table 1 showed that number of N-fixing and P solubilizing bacteria of rhizosphere soil of *A. exelsa* (rasamalaj, *S. wallichii* (puspa/ and *C. javanica* (kianak), were higher than that of the area without plants. The highest population of total bacteria in the rhizosphere of *A. exelsa* (8.5 x 10^7 cell/gram soil) at Suzuki plot A n, N-Fixing bacteria is in the rhizosphere of *S. wallichii* at Suzuki plot A I (4.0 x 10^7 cell/gram soil) and phosphate solubilizing bacteria is in the rhizosphere of *A. exelsa* in Suzuki plot A III (7.5 x 10^6 cell/gram soil).

Table 1. The population of N fixing and P solubilizing bacteria in the rhizosphere of plants at 1100 m asl, Cikaniki, GHNP

Plot	No.	Rhizosphere Plant	The number of bacteria (cell/gram soil)				
			Total bacteria	Nitrogen fixing bacteria			PSB
				Rhizobium	Azotobacter	Azospirillum	
Suzuki A	425	*Altingia excelsa*	7.4x10'	2.0 xlO^{5}	4.8 xlO^{6}	2.4x10'	2.3x10'
I	486	*Schima wallichii*	1.5x10'	1.5x10'	4.0x10'	4.0x10'	3.7x10'
	494	*Castanopsis javanicus*	4.0x10'	4.0x10'	3.0x10'	1.5x10'	5.7x10'
Suzuki A	524	*Altingia excelsa*	8.5 xlO^{7}	2.0x10'	2.6 xlO^{6}	6.0x10'	1.9x10'
II	518	*Schima wallichii*	5.0x10^{7}	1.0 xlO^{7}	2.0x10'	5.0x10'	6.8x10'
	519	*Castanopsis javanicus*	2.8 xlO^{5}	8.1 xlO"	3.7 xlO^{5}	4.2x10"	4.2x10'
Suzuki A	580	*Altingia excelsa*	5.0x10'	1.0 xlO'	5.0x10'	4.6x10'	7.5x10'
III	579	*Schima wallichii*	l.OxlO^{7}	1.5x10'	2.3 xlO^{7}	5.0x10'	1.0 xlO'
	567	*Castanopsis javanicus*	7.5 xlO^{5}	2.5 xlO"	1.3x10'	2.2x10'	3.5x10'
Mix	M	I + II + III	7.5 xlO^{6}	6.2 xlO^{6}	1.0 xlO^{7}	3.1 xlO^{6}	2.5x10'
Control	C	Without plant	1.8x10"	5.5 xlO^{3}	1.0 xlO^{3}	3.5 xlO^{3}	2.4 xlO"
Suzuki	113	*Altingia excelsa*	2.4x10'	8.0x10'	3.5x10'	2.0x10'	1.3x10'
B	120	*Schima wallichii*	1.3x10'	2.3x10'	1.4x10'	3.2x10"	1.5x10'
I	96	*Castanopsis javanicus*	3.2x10'	4.2x10'	4.0x10'	4.0 xlO"	2.0x10'
Suzuki	45	*Altingia excelsa*	1.0 xlO'	1.0 xlO^{5}	1.5x10'	1.0 xlO'	1.5x10'
B	53	*Schima wallichii*	7.5 xlO'	4.0x10'	3.0x10'	2.0x10'	2.0x10'
II	63	*Castanopsis javanicus*	5.0x10'	1.0 xlO'	2.0x10'	4.5 xlO'	3.5 xlO'
Suzuki	6	*Altingia excelsa*	2.5 xlO'	3.2 xlO'	1.0 xlO^{6}	5.0x10'	3.5 xlO'
B	19	*Schima wallichii*	2.9x10"	3.5 xlO^{3}	2.0 xlO^{3}	7.5 xlO^{3}	2.5 xlO^{3}
III	7	*Castanopsis javanicus*	5.0x10'	5.0x10"	1.0 xlO^{5}	6.0x10'	3.5 xlO'
Mix	M	I + II + III	3.7 xlO^{6}	8.2 xlO^{6}	2.2 xlO^{6}	4.0x10'	2.1 xlO^{6}
Control	C	Without plant	3.5x10'	9.1 xlO^{3}	2.3 xlO^{3}	2.0 xlO^{3}	1.1 xlO^{4}

Explanation: PSB = P solubilizing bacteria

Table 2. The population of N fixing and P solubilizing bacteria at Gunung Botol, GHNP.

Sample number	Altitude (asl)	Soil PH	The number of bacteria (cell/gram soil)				
			Total bacteria	Nitrogen fixing bacteria			PSB
				Rhizobium	Azotobacter	Azospirillum	
1	1000 m	5-6	6.0 x 10^{8}	3.5 x 10^{s}	2.8 xlO^{8}	2.1 x 10^{8}	5.8 xlO^{8}
2	1500 m	5-6	1.3 x 10'	4.5 x 10'	7.9 x 10'	2.3 x 10'	4.0 xlO'
3	1800 m	5-6	3.5 x 10'	5.5 x 10^{6}	4.0x10^{6}	4.0 x 10'	4.0 xlO^{7}

Table 3. The population of N fixing and P solubilizing bacteria at Ciptarasa, GHNP

Sample number	Altitude (asl)	Soil PH	The number of bacteria (cell/gram soil)				
			Total bacteria	Nitrogen fixing bacteria			PSB
				Rhizobium	Azotobacter	Azospirillum	
A	600 m	6.0	3. 5x 10°	1.0 x 10°	2.0 x 10°	6.5 x 10'	5.0x10'
B	600 m	6.0	6.0 x 10^{6}	1.0 x 10'	6.5 x 10'	2.0x10'	1.0 x 10'
C	1000 m	3.9	1.2 x 10'	5.0 x 10^{s}	5.5 x 10'	2.5 x 10^{5}	2.0 x 10'
D	1000 m	3.9	2.2 x 10'	1.0 x 10"	5.0 x 10^{s}	5.0 xlO^{4}	5.0x10'
E	1500 m	3.5	2.0 x 10'	5.0 x 10"	2.0 x 10'	5.0x10"	2.0 x 10'
G	1500 m	3.5	1.5 x 10'	5.0 x 104	7.5 x 10'	5.0x10"	1.5 x 10'

Table 2 showed that the number of the total bacteria, N fixing and P solubilizing bacteria at different elevation are around 10^7- 10^s, IO^6- 10^s, 10^7 - 10^8 cell/gram dry soil at Gunung Botol study site.

Table 3, that in A and B with the elevation of 600 m and soil pH 6.0, the population of N fixing and solubilizing P bacteria was higher (10^6 cell/gram soil) than that of elevation 1000 m, 1500 m asl, with pH 4.0, 3.9 and 3.5 respectively.

DISCUSSION

The number of bacterial population

Soil forest composed of large portion of organic material providing carbon and energy sources for plants, fauna and microbe. As shown by Table 1, number of total bacteria, N-fixing and P solubilizing bacteria of rhizosphere soil of *A. exelsa, S. wallichii* and *C. javanica,* were higher than that of the area without plants. This could be affected by altered soil physics-chemical, and soil climate as caused by the lost of vegetation. Organic material and nutrient leaching may occured, and result in less nutrient avalailable for bacterial growth. Setiadi (1989) noted the effect of altered environment on the growth of microbes depend on microbes itself, soil fertility, humidity, light penetration and temperature. The number of total bacteria, nitrogen fixing bacteria and phosphate solubilizing bacteria were varied depending on altitude. The population of bacteria on the lowest altitude show that the highest population of total bacteria, nitrogen fixing bacteria and phosphate solubilizing bacteria (see Table 2 and Tabel 3). Heterogeneous population of N-fixing bacteria and P solubilizing bacteria could be due to variability of organic nutrient composition, microclimate, and diversity of soil microbes. Jha *et al.* (1992) proposed that the composition of population and soil microbe activity are influenced by the different climate and vegetation.

The highest total number of total bacteria, *Azotobacter, Azospirillum, Rhizobium* and P solubilizing bacteria were 10^s cell/gram soil, obtained from the elevation of 1000 m asl, with soil pH 5 - 6. Whereas on the elevation between 1500 m and 1800 m asl, the number of population was lower (total bacteria = 10^7 cell/gram soil, *Azotobacter, Azospirillum,* and *Rhizobium* = 10^6 - 10^7 cell/gram soil , and P solubilizing bacteria = 10^7 cell/gram soil). It's probably caused by the different elevation and soil pH. As shown on Table 3, that in A and B with the elevation of 600 m and soil pH 6.0, the population of N fixing and solubilizing P bacteria was higher (10^6 cell/gram soil) than that of elevation 1000 m, and 1500 m asl, with pH 3.9, and 3.5 respectively. This could be due to several influencing factors such as soil acidity that may affect bacterial growth and symbiosis of *Rhizobium* with legume plant and the failure of root nodulation on legume plants. Gates *et al.* (1974) proposed that nodulation and the N fixation process require a high concentration of phosphate, but if there is an excessive hindrance to the *Rhizobium* growth on rhizosphere, then it will decrease the N fixation, or root nodulation, and also affect other free-living nitrogen fixing bacteria *(Azotobacter)* which life freely and able to use N_2 from organic and an organic substances and from atmosphere. This bacteria is able to survive on the extreme soil condition, but generally failed to fix N_2 under pH 6.0 (Setiadi, 1989). Beside that, it was possible influenced by the different elevation. As shown on Table 2 and Table 3, the population of N fixing and P solubilizing bacteria was varied and fluctuated. Another influencing factors are temperature, humidity, light, oxygen, carbohydrate, and nutrition factors like, phosphor, sulphur, potassium, nitrogen, calcium, boron, hydrogen, cobalt, iron, and copper at each elevation.

At elevation of 1100 m asl the highest N fixing bacteria population (10^7 cell/gram soil) was on rhizosphere of *S. wallichii,* except at Suzuki plot B II, B III (10^3 - 10^7 cell/gram soil). Whereas the P solubilizing bacteria was 10^6 cell/gram soil on

A. exselsa, S. wallichii plant rhizosphere, except *S. wallichii* plant rhizosphere at Suzuki plot B III (10^3 cell/gram soil).

The population of N-fixing bacteria and P solubilizing bacteria in Gn. Botol study site were dominant at 1000 m asl, whereas at Ciptarasa was at 600 m asl. Plant root exudate released into soil rhizosphere create a new environment (niche) for growth of microorganism (Setiadi,1989).

Identification of bacteria

The results of P solubilizing bacteria identification showed that the elevation of 600 m (Ciptarasa), 1000 m (Gn. Botol) and 1100 m asl (Cikaniki) were dominated by *Pseudomonas* sp and *Bacillus megaterium,* and also dominated by *Rhizobium* sp, *Azotobacter* sp, and *Azospirillum* sp. Beside that, another kind of bacteria was found in that area, namely: *Chromobacterium* sp and *Spaerotilus natans.* Rao (1982) proposed that bacteria is a group of microbe inside the soil which most dominant and probably covered half of the biomass, whereas *Pseudomonas, Bacillus, Micrococcus, Flavobacterium, Clostridium,* and *Escherchia* are common bacteria in forest soil for their occurence4s.

CONCLUSION

The highest population of N-fixing and P solubilizing bacteria at Cikaniki (1100 asl.) are in the plant rhizosphere of *Schima wallichii* at Suzuki plot A I and *Altingia exelsa* at Suzuki plot A III, at Gunung Botol was faounded is in 1000 m asl., and at Ciptarasa area is in 600m asl.

The number of N fixing and P solubilizing bacteria at Gunung Halimun was influenced by the different vegetation type, soil pH and the elevation of area.

Nitrogen fixing bacteria *(Rhizobium, Azotobacter,* and *Azospirillum)* and P solubilizing *(Pseudomonas* sp, and *Bacillus megaterium)* are most dominant at 600 m (Ciptarasa), 1000 m (Gn. Botol), and 1100 m asl (Cikaniki).

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