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Isolation and Identification of Phosphate Solubilizing and Nitrogen Fixing Bacteria from Soil in Wamena Biological Garden, Jayawijaya, Papua

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ABSTRACT

A study was undertaken to investigate the occurrence of phosphate solubilizing bacteria (PSB) and nitrogen-fixing bacteria (NFB) from soil samples of Wamena Biological Garden (WbiG). Eleven soil samples were collected randomly to estimate microbial population which used plate count method. The result showed that the microbial population ranged from $5.0x10^3$-$7.5x10^{6\ cells}$ of bacteria/gram of soil and $5.0x10^3$-$1.5x10^7$ cells of bacteria/gram of soil for PSB and NFB respectively. There were 17 isolates which have been identified till genus and species. The isolated microorganism were identified as PSB i.e. *Bacillus* sp*., B. pantothenticus, B. megatherium, Flavobacterium* sp*., F. breve, Klebsiella* sp*., K. aerogenes, Chromobacterium lividum, Enterobacter alvei, E. agglomerans, Pseudomonas* sp*., Proteus* sp. and as NFB i.e*. Azotobacter* sp*., A. chroococcum, A. paspalii, Rhizobium* sp., and *Azospirillum* sp*.*

Key words: phosphate- solubilizing bacteria, Nitrogen-fixing bacteria, Wamena Biological Garden.

INTRODUCTION

Wamena Biological Garden (WBiG) is one of mountain range-biota ex-situ conservation at eastern part of Indonesia. The ex-situ conservation is the first conservation built by Biological Research Center, Indonesian Institute of Sciences. WBiG has unique vegetation and many variations of soil colors. Soil is a unity of subsistence that includes the varieties of microbe, because microbes' community is one of the important components of soil. Activity and species composition of microbes are generally influenced by many factors including physic-chemical properties of the soil, temperature and vegetation (Jha et al*.,* 1992).

The most important role of soil organism in ecosystem is decomposing of organic matters, synthesize and release them into inorganic forms that plant can use (Setiadi, 1989). Most microbes in terrestrial ecosystem are in soil. Bacteria are the most dominant group of soil microbes. In the fertile soil, there are 10^6-10^8 cells of bacteria/ gram of soil. Some groups of soil bacteria (nitrogen-fixing bacteria and phosphate-solubilizing bacteria) are useful as biofertilizer.

Some plants and microbes species have developed symbiosis or mutually beneficial relationships. *Rhizobium* is the root of legumes host nitrogen fixing bacteria which can invade root and get sugars from the plant. In return, they convert large amounts of dinitrogen (N_2) from the atmosphere into forms that the plants can use (Zahran, 1999). Another nitrogen fixing bacteria living in soil are *Azotobacter* sp. and *Azosprillum* sp. Several species of *Azotobacter* and *Azosprillum* are known to fix nitrogen under field condition in association with roots of plant. Nitrogen-fixing *Azosprillum* strains have been isolated from tropical and some temperate grass root surface. Some species of *Azotobacter* from root associations with seasonal grasses are specific hosts, e.g. *A. paspalii* associated with the root of *Paspalum notatum* (Berreiner et al*.,* 1976, Elmerich, 1984, Okon, 1985, Okon and Labandera-Gonzales, 1994). The other biofertilizer bacteria are genus of *Pseudomonas* and *Bacillus*. Those bacteria are able to solubilize available forms of Fe, Ca, Mg, Al bound P. The solubilization effect is generally due to the production of organic acids (Kucey, 1983).

The present investigation was carried out to study the occurrence of PSB and NFB from WBiG. The isolated microbes were identified.

MATERIALS AND METHODS

Soil

Surface (0-15 cm) soil samples were collected randomly from 11 different sites of Gunung Susu which was one of WBiG sites. All samples were kept in plastic bags and transported to the laboratory and stored in 4^o C prior to be analyzed. These samples were air-dried and ground to pass 2 mm sieve before the chemical analyses. The samples were analyzed for pH, soil chemistry and texture, (Table 1 and 2).

Microbial count

Microbial population was estimated by plate count method (Ravina et al, 1992; Thompson, 1989; Vincent, 1982). Ten grams soil was suspended in 90 mL sterile distilled water in Erlenmeyer flask and mixed thoroughly for 30 minutes using a mechanical shaker at 110 rpm. Then 1 mL an aliquot transferred with sterile pipettes to 9 mL sterile

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distilled water in test tube. This suspension was stir for 10 second. A subsequent serial dilution was prepared as above to 10^7. From each serial dilution, 0.2 mL of aliquot was transferred to sterile putridity and over poured and dispersed swirling with agar media (50^{o}C).

PSB was grown on Pikovskaya agar media (Sundara Rao and Sinha, 1963, Gaur, 1981), containing of 5g $Ca_3(PO_4)_2$; ten g glucose; 0.2g NaCl; 2.5mg $MgSO_4.7H_2O$; 2.5mg $MnSO_4.2H_2O$; 2.5mg $FeSO_4.7H_2O$; 5g yeast extract; 20g agar, diluted in 1 l distilled water. The plats were incubated for 7 days at room temperature. Colonies of PSB were detected by clear zones of solubilization around them.

For growing *Rhizobium* was used yeast extract mannitol agar (YEMA) (Subba Rao,1994), containing 10g mannitol; 0.5g K_2HPO_4; 0.1g NaCl; 0.2g $MgSO_4.7H_2O$; 1g yeast extract; 2.5 mL congo red 1%; 20g agar, diluted in 1 l distilled water. The plats were incubated for 7 days at room temperature. Rhizobia colonies have transparent white color, shining.

Mannitol Ashby agar medium was used for isolating *Azotobacter* containing of 20g mannitol; 0.2g K_2HPO_4; 0.2g NaCl; 0.2g $MgSO_4.7H_2O$; 0.1g K_2SO_4; 5.0g $CaCO_3$; 20g agar, diluted in 1 l distilled water. The plats were incubated for 7 days at room temperature (Subba Rao, 1994).

The numbers of *Azospirillum* bacteria counted on a Okon medium containing of 6.0g K_2HPO_4; 4.0g KH_2PO_4; 0.2g $MgSO_4$; 0.1g NaCl; 5g Sucrose; 0.02g $CaCl_2$; 1.0g NH_4 Cal; 5.0g NaOH; 2.1mg $MnSO_4$; 10.0mg $FeCl_3$; 2.0mg $Na_2M_oO_4$.; 0.1g yeast extract; 2.0 mg H_3 BO_3; 0.04mg $Cu(NO_3)_2$; 0.2 mg $ZnSO_4$; 2 mL bromthymol blue 0.5%; 20.0 agar, diluted in 1 L distilled water (Okon et al., 1977).

The number of bacterial colony was estimated after 7 days of incubation at room temperature. The isolates were identified follows Bergey's manual for bacteriology methods systematic (Krieg and Hold, 1984).

RESULTS AND DISCUSSION

Table 1 and 2 showed that the result analyses of soil chemical properties varied depending on the vegetations and soil types. Soils that were dominated by *Imperata cylindrica* indicated to be deficiency of soil nutrient. Setiadi (1989) proposed that the land in area of *Imperata cylindrica* has eroded, because the plants are less effective to avoid the erosion. In the eroded land, organic matter and nutrient leaching may generally occur, and results in fewer nutrients available.

Table 1. Soil physic from 11 sites in Wamena Biological Garden

Soil samples	Soil color	Vegetation	Soil Texture (%)		
			Sand	Clay	Dust
1	Black	*Pittosporum ramiflorum*	5.79	51.37	42.84
2	Dark brown	*Grevillea papuana*	7.38	52.08	40.54
3	Brown	*Castanopsis accuminattisima*	20.17	18.56	61.25
4	Brown	*Vaccinium varingiaefolium*	16.73	28.01	55.26
5	Brown reddish	*Imperata cylindrica*	16.36	43.45	40.19
6	Red	*Imperata cylindrica*	11.51	65.75	22.69
7	Yellow	*Imperata cylindrica*	17.51	41.42	41.06
8	Gray	*Imperata cylindrica*	10.89	38.82	49.28
9	Black	*Imperata* cylindrica	18.03	38.06	43.01
10	Gray	*Imperata cylindrica*	10.89	38.82	49.28
11	Brown	*Imperata cylindrica*	15.65	35.74	48.61

Table 2. Soil chemistry from 11 sites of Wamena Biological Garden.

Soil samples	N (%)	P (ppm)	K (me/100g)	C (%)	C/N	Ca (Me/100g)	PH
1	0.21 (m)	4.8	0.53 (m)	2.54 (m)	12.10 (m)	26.00 (v.h)	5.30 (acid)
2	0.30 (m)	3.9 (l)	0.36 (l)	3.05 (h)	10.17 (l)	19.23 (h)	4.80 (acid)
3	0.22 (m)	1.6 (v.l)	0.15 (l)	3.12 (h)	14.48 (m)	9.34 (m)	4.35 (acid)
4	0.23 (m)	3.3 (l)	0.26 (l)	3.85 (m)	16.74 (h)	8.82 (m)	4.90 (acid)
5	0.06 (v.l)	0.2 (v.l)	0.07 (v.l)	0.69 (v.l)	11.50 (m)	8.88 (m)	5.00 (acid)
6	0.06 (v.l)	0.3 (v.l)	0.07 (v.l)	0.62 (v.l)	10.33 (l)	9.55 (m)	6.05 (acid)
7	0.05 (v.l)	0.4 (v.l)	0.05 (v.l)	0.51 (l)	10.20 (l)	9.34 (m)	5.25 (acid)
8	0.04 (v.l)	1.6 (v.l)	0.13 (l)	0.36 (v.l)	9.00 (l)	9.73 (m)	4.60 (acid)
9	0.25 (m)	2.1 (l)	0.06 (v.l)	2.76 (m)	11.04 (m)	32.90 (v.h)	5.26 (acid)
10	0.23 (m)	2.7 (l)	0.10 (l)	2.47 (m)	10.74 (l)	19.23 (h)	4.25 (acid)
11	0.03 (v.l)	0.1 (v.l)	0.05 (v.l)	0.36 (v.l)	12.00 (m)	9.03 (m)	4.65 (acid)

Note: v.l = very low; l = low; m = moderate; h = high; v.h = very high

The estimates of total bacteria, PSB and NFB at each sites ranging from $2.95x10^5$-$2.5x10^8$cells of bacteria/ gram soil. Soil samples number 2, 3, 4, 9, 10 reveal the number of total bacteria which are higher than samples number 5, 6, 7, 8, 11 (Table 3).

Table 3. The population of microbes isolated from 11 sites of Wamena Biological Garden.

Soil samples	Total bacteria (cell/g soil)	PSB (cell/g soil)	NFB (cell/g soil)		
			Rhizobium sp.	*Azospirillum* sp.	*Azotobacter* sp.
1	$2.5x10^8$	$5.5x10^4$	$2.5x10^6$	$5.8x10^5$	$1.5x10^7$
2	$5.0x10^7$	$1.5x10^6$	$1.5x10^6$	$1.0x10^6$	$1.5x10^6$
3	$2.5x10^7$	$1.1x10^5$	$2.0x10^6$	$5.0x10^6$	$1.5x10^6$
4	$2.0x10^8$	$1.5x10^6$	$2.0x10^6$	$1.5x10^6$	$2.5x10^6$
5	$3.5x10^6$	$2.6x10^6$	$4.4x10^5$	$6.0x10^4$	$2.0x10^4$
6	$2.4x10^6$	$3.5x10^4$	$1.9x10^5$	$1.0x10^5$	$1.0x10^6$
7	$2.4x10^6$	$5.0x10^3$	$2.2x10^5$	-	$1.5x10^5$
8	$3.0x10^5$	$5.5x10^4$	$2.1x10^5$	$2.0x10^4$	$5.0x10^3$
9	$5.0x10^6$	$7.5x10^6$	$1.5x10^6$	$8.5x10^4$	$5.5x10^4$
10	$5.0x10^7$	$2.5x10^6$	$5.0x10^6$	$1.0x10^6$	$2.5x10^6$
11	$5.5x10^6$	$5.4x10^5$	$2.5x10^6$	$1.2x10^5$	$1.5x10^5$

Table 4 showed that the most dominant phosphate solubilizing bacteria found were aerobic spore forming bacteria. Identification of this group showed that *Bacillus* sp. was the most predominant PSB was found in all of soils tested, followed by *B. panthothenticus* and *B. megatherium* were in soil samples numbers 1, 2, 3, 6 and 9 respectively. Other PSB involved were *Flavobacterium* sp*., F. breve, Klebsiella* sp., *K. aerogenes, Chromobacterium lividum, Enterobacter alvei, E. agglomerans, Pseudomonas* sp*. and Proteus* sp. The finding of predominance of spore formers was in harmony with the work of Taha et al. (1969). He also observed that sporeformer was well known to resist adverse conditions such as high temperature and dryness. Thus the important PSB can overcome such unfavorable conditions.

Swaby and Sperber *cit*. Taha et al. (1969) found that the principal genera of PSB were *Arthobacter, Pseudomonas, Xanthomonas, Achromobacter* and *Flavobacterium.* It is well known that the interplay of so many factors such as physico-chemical properties of soil, vegetation crop, rotation and environmental conditions greatly influence soil microbial flora.

Table 4. Identified isolates from 11 sites of Wamena Biological Garden.

Soil samples	Phosphate solubilizing bacteria	Nitrogen-fixing bacteria
1	*Bacillus* sp. *B. panthothenticus,* *Enterobacter agglomerans*	*Rhizobium* sp. *Azospirillum* sp. *Azotobacter* sp.
2	*Bacillus* sp. *B. panthothenticus,* *Chromobacterium lividum,* *Flavobacterium* sp.	*Rhizobium* sp. *Azospirillum* sp. *Azotobacter* sp.
3	*Bacillus* sp. *B. panthothenticus,* *Flavobacterium* sp. *Klebsiella* sp. *K. aerogenes*	*Rhizobium* sp. *Azospirillum* sp. *Azotobacter* sp. *A. paspalii*
4	*Bacillus* sp. *Enterobacter agglomerans*	*Rhizobium* sp. *Azospirillum* sp. *Azotobacter* sp.
5	*Bacillus* sp. *Flavobacterium breve,* *Pseudomonas* sp.	*Rhizobium* sp. *Azospirillum* sp. *Azotobacter* sp. *A. paspalii* *A. chroococcum*
6	*Bacillus* sp. *B. panthothenticus,* *Klebsiella aerogenes,* *Enterobacter alvei*	*Rhizobium* sp. *Azospirillum* sp. *Azotobacter* sp.
7	*Bacillus* sp. *Chromobacterium lividum,* *Enterobacter agglomerans*	*Rhizobium* sp. *Azotobacter* sp.
8	*Bacillus* sp. *Flavobacterium* sp. *Proteus* sp.	*Rhizobium* sp. *Azospirillum* sp. *Azotobacter* sp. *A. paspalii,* *A. chroococcum*
9	*Bacillus* sp. *B. megatherium,* *Enterobacter alvei*	*Rhizobium* sp. *Azospirillum* sp. *Azotobacter* sp. *A. paspalii*
10	*Bacillus* sp.	*Rhizobium* sp. *Azospirillum* sp. *Azotobacter* sp.
11	*Bacillus* sp. *Pseudomonas* sp.	*Rhizobium* sp. *Azospirillum* sp. *Azotobacter* sp. *A. paspalii* *A. chroococcum*

Nitrogen-fixing bacteria such as *Rhizobium* sp. and *Azotobacter* sp were found in all of soil tested. *Azospirillum* was found in almost soil samples except in soil number 7. The isolates of *Rhizobium* and *Azospirillum* have already been identified till genus level. Isolates of *Azotobacter* till genus and species level. Both of *Azotobacter paspali* and *A. chroococum* were obtained in soil samples numbers 5, 8, 11. *Rhizobium*, *Azotobacter* and *Azospirillum* are heterotrophic bacteria which depend on outside carbon sources to fix nitrogen. Soil microbes require energy and essential nutrient to grow and reproduce, while plants derive their energy from carbon acquired from the atmosphere by means of photosynthesis. The carbon in organic matter decomposing provides soil microbes with their energy supply (John et al*.,* 2001).

The result showed that different soil nutrient status and vegetation type in the investigated sites resulted in the different bacterial population and bacterial type. The difference was caused by releasing organic and inorganic root exudates that can be used by surrounding organism. Jha et al*.* (1992) and Setiadi (1989) found that biological activity and composition of soil microbes are generally affected by many factors including physico-chemical properties of soil, temperature and vegetation. C availability in soil may affect the numbers and activities of microbes directly. Setiadi (1989) reported that the roots of higher plants might affect significantly the activity and the development of soil microbes. (Katznelson *cit*. Mukerji and Subba-Rao, 1982) reported that the influence of the root on soil microbes starts immediately after seed germination which increases as the plant grow and reach maximum when plans have reaches the peak of the vegetative growth

Stenton *cit*. Mukerji and Subba-Rao (1982) proposed that the roots of higher plants provide an ecological niche to soil microbes within the soil. Mishara (1969) also reported that different plant species grown in the same type of soil could harbor different microbes in the rhizosphere.

CONCLUSSION

The microbial population range from $5.0x10^3$-$7.5x10^6$ cells of bacteria/g of soil and $5.0x10^3$-$1.5x10^7$ cells of bacteria/g of soil for phosphate-solubilizing bacteria and nitrogen-fixing bacteria respectively. There were 17 isolates which have been identified till genus and species. The isolated microorganism were identified as PSB i.e. *Bacillus* sp*., B. pantothenticus, B. megatherium, Flavobacterium* sp*., F. breve, Klebsiella* sp*., K. aerogenes, Chromobacterium lividum, Enterobacter alvei, E. agglomerans, Pseudomonas* sp*., Proteus* sp. and as NFB i.e*. Azotobacter* sp*., A. chroococum, A. paspali, Rhizobium* sp., and *Azospirillum* sp*.*

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