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Full Length Research Paper

Phosphatase-producing bacteria isolated from Sanggabuana forest and their capability to hydrolyze organic phosphate

Betty Natalie Fitriatin[1]*, Dedeh H. Arief[1], Tualar Simarmata[1], Dwi A. Santosa[2] and Benny Joy[1]

[1]Department of Soil Science Faculty of Agriculture, University Padjadjaran, Indonesia.
[2]Department of Soil and Land Resources Faculty of Agriculture, Bogor Agriculture Institute, Indonesia.

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Some free living microorganisms in soil have the capability to produce extracellular enzymes such as phosphatase. This enzyme is able to mineralize organic phosphates into inorganic phosphates that provides high P for plant. Exploration and laboratory experiments were carried out to obtain the most excellent bacterial isolates for producing phosphatase and solubilizing phosphate and also to study the capabilities of pre-eminent isolates to hydrolyze synthetic and natural organic phosphate. Exploration and selection processes resulted in ten isolates which were then tested to examine their capabilities to hydrolyze synthetic organic phosphate (phytic acid) and natural organic phosphate (extract of cow dung manure). Three pre-eminent isolates [*Bacillus mycoides* (obtained from rhizosphere of *Gleichenia linearis*), *Bacillus laterosporus* (from rhizosphere of Lithocarpus sundaicus) and *Flavobacterium balustinum* (from rhizosphere of *Altingia excelsa*)] were found to have excellent capabilities in mineralizing organic phosphate. Hydrolysis of organic phosphate was affected by the types of organic P substrates. Phytic acid as organic P substrate gave higher phosphatase activity and dissolved P higher than the extract of cow dung manure.

Key words: Phosphatase, hydrolyze, organic phosphate (P), Sanggabuana.

INTRODUCTION

Some part of the phosphorus in soils is bound organically. Organic P is often dominant form in top soils, though largely unavailable for plants. A large propotion of P that is applied to soil as fertilizers rapidly becomes unavailable to plants, accumulating in inorganic P fractions that are fixed by chemical adsorption and precipitation, and organic P fractions that are immobilized in soil organic matter and microbial biomass. The average content of organic phosphorous in cultivated soils ranges from 5 - 50 percent of total P (Sarapatka, 2003). Fertilized soils contain a significant amount total soil P, which some 50 - 80% may exist in inorganic form (McLaughlin et al., 1990). The importance of soil organic P as source of plant available P depends on its rate of solubilization and the rate of inorganic P release.

*Corresponding author. E-mail: fitriatin@yahoo.com.

Some free living microorganisms in soil have the capability to produce extracellular enzymes such as phosphatase (George et al., 2002). This enzyme is able to mineralize organic phosphates into inorganic phosphates that provides high P for plants. Soil phosphatases play a major role in the mineralization processes (dephosphorilation) of organic P substrates. The enzymes in soils originate from animal, plant and microbial sources and the resulting soil biological activity includes the metabolic processes of all organism (Cookson, 2002). The literature shows that under favorable conditions, microorganisms supply most of the soil enzyme activity (Sarapatka et al., 2004).

Activity of phosphatase enzyme is affected by some factors, that is, the amount and kind of substrate (Fitriatin et al., 2008), pH, temperature, material of inhibitor and activator, concentration of enzyme and product, and also the kind of solvent used (Saparatka, 2002). Besides, soil phosphatase activity also affected by properties of chemical and physical of soil , that is, soil type, organic

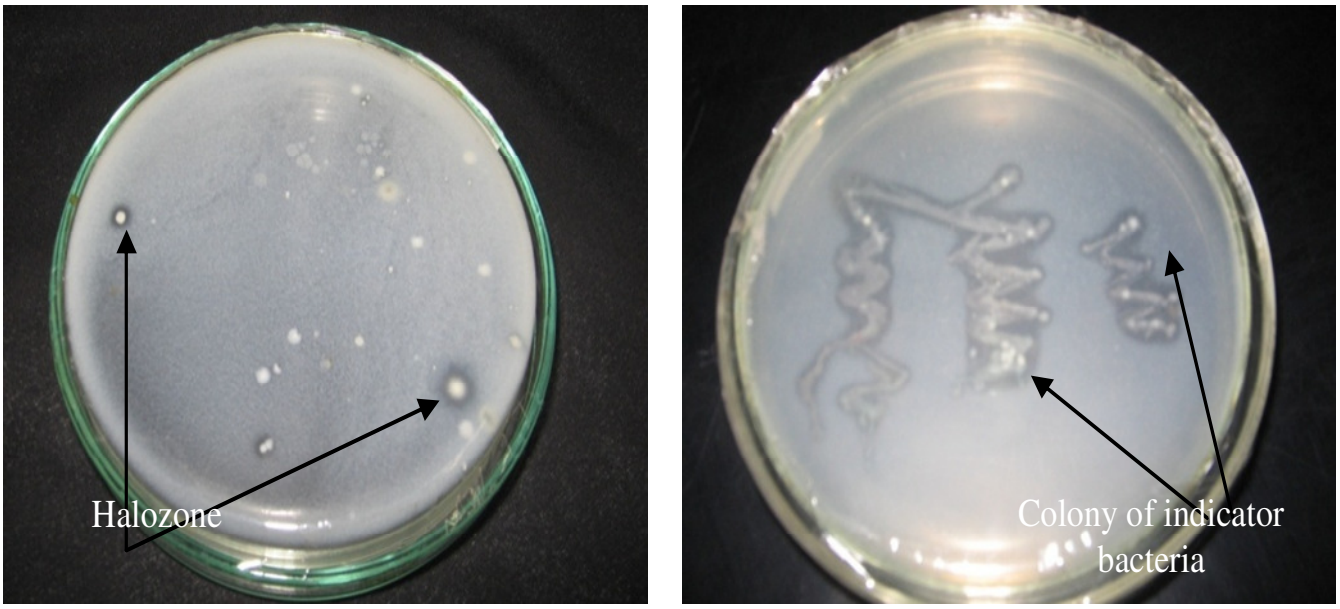

Figure 1. Screening of phosphatase producing bacteria by isolated on selective medium of phosphatase and double layer test method.

matter content, total N content, C/N ratio and Total P content (Dick, 1997; Djordjevic et al., 2003).

In this study, we isolated and screened (explorated) phosphatase producing bacteria from Sanggabuana forest. Laboratory experiments were carried out to obtain the most excellent bacterial isolates for producing phosphatase and solubilizing phosphate, and also to study the capabilities of pre-eminent isolates to hydrolyze synthetic and natural organic phosphate.

MATERIALS AND METHODS

A survey for phosphatases producing bacteria was undertaken from eight areas of Sanggabuana forest (Cibeureum, Gerbang Batu Datar, Gombong, Simpang Pamoyanan, Kiara, Simpang Jatiluhur I and Simpang Jatilihur II) elevated at 1279 m above sea levels. Soil samples was taken form rhizosphere of various vegetations. For screening of phosphastase producing bacteria, pikovskaya medium was used to select the phosphatase medium which show the clear zone (halozone), as capability isolate to solubilize phosphate and producing phosphatase (Figure 1). Double layer method used to determine isolate which producing phosphatase on medium (Chen, 1998).

Acid phosphatase activity was measured spectrophotometrically by measuring the release of para-nitrophenol from para-nitrophenyl phosphate (PNPP) at 400 nm by the method as described by Schinner et al. (1996). Exploration and selection processes resulted in ten isolates which then tested to examine their capabilities to hydrolyze synthetic organic phosphate (phytic acid) and natural organic phosphate (extract of cow dung manure) on murashige and skoog (MS). After 3, 5 and 7 days of incubation, phosphatase activity and dissolve P were measured.

The cluster analyis was used to obtain the most excellent bacterial isolates for producing phosphatase and solubilizing phosphate and also capabilities of pre-eminent isolates to hydrolyze synthetic and natural organic phosphate.

RESULTS AND DISCUSSION

Fifty seven isolates were found in soil samples of rhizosphere of various vegetations from Sanggabuana forest which were capable to solubilizing phosphate and producing phosphatase. The results measurement phosphatase activity and dissolved P generally indicates that isolates with a high phosphatase activity produces high dissolved P as well. This research is supported by Sakurai et al. (2008) who found that there is a positive correlation between phosphatase activity with the content of P soluble.

Ponmurugan and Gopi (2006) stated that there is a positive correlation between the ability of dissolving phosphate with phosphatase activity of bacteria. The results of the cluster analysis of 57 isolates based on phosphatase activity and dissolved P obtained ten isolates of phosphatase producing bacteria have highest phosphatase activity and dissolved P. The phospahatase activity and dissolve P ranged from 44.71 to 74.76 µg *p*-NP ml^{-1} h^{-1} and 16.69 to 32.94 mg L^{-1} respectively. The results of identification of phosphatase-producing bacterial isolates selected are presented in Table 1.

Phosphatases activity

Phosphatase activity of ten selected isolates ranged from 0.35 to 4.96 µg *p*NP ml^{-1} h^{-1} on MS medium with phytic acid as organic P susbtrate. Meanwhile, in medium containing extract of cow dung manure, phosphatase activity ranged from 0.20 to 4.26 µg *p*NP ml^{-1} h^{-1} (Table

Table 1. Identification of selected isolate of phosphatase producing bacteria.

Result of identification	Source of isolate (Rhizosphere)	Location
Bacillus megaterium	*Garcinia mangostana*	Simpang Jatiluhur I
Bacillus laterosporus	*Lithocarpus sundaicus*	Gombong
Peusedomonas pseudoalcaligenes	*Litsea chrysocoma*	Simpang Jatiluhur I
Micrococcus luteus	*Cycas rumphii*	Kiara
Staphylococcus chromogenes	*Litsea javanica*	Simpang Jatiluhur I
Bacillus mycoides	*Gleihenia linearis*	Simpang Jatiluhur I
Bacillus pantothenticus	*Litsea cubeba*	Simpang pamoyanan
Erwinia chrysanthemi	*Lithocarpus sundaicus*	Simpang Jatiluhur II
Bacillus macerans	*Litsea cubeba*	Gombong
Flavobacterium balustinum	*Altingia excelsa*	Cibeureum

Table 2. Phosphatase activity of selected isolates in MS medium.

Treatments	Phytic acid			Extract of cow's manure		
	3 d	5 d	7 d	3 d	5 d	7 d
	Phosphatase (µg pNP ml^{-1} h^{-1})					
Control	0.24	0.19	0.17	0.17	0.53	0.63
B. megaterium	3.91	2.00	2.06	1.56	1.67	1.75
B. laterosporus	3.81	2.15	2.68	2.63	2.09	2.10
P. pseudoalcaligenes	1.48	0.82	0.35	0.58	0.20	1.22
M. luteus	2.32	1.18	1.05	1.48	2.07	2.35
S. chromogenes	1.78	1.54	0.66	1.94	0.73	2.40
B. mycoides	2.45	1.70	1.08	1.95	0.83	0.48
B. pantothenticus	1.94	1.87	1.41	1.11	1.15	1.12
E. chrysanthemi	1.09	0.72	0.61	1.02	3.27	3.84
B. macerans	1.79	2.27	1.53	1.56	1.00	1.20
F. balustinum	4.96	2.75	2.86	4.26	2.31	2.13

2). From Table 2, it can be seen that the higher phosphatase activity on MS medium containing phytic acid than extract of cow dung manure.

Approximately 40% of the ten isolates tested, produced phosphatase activity > 2.0 µg pNP ml^{-1} h^{-1} on MS medium with phytic acid as organic P substrate while for the medium containing the extract of cow dung manure, only 20% of the isolates produced phosphatase activity > 2, 0 µg pNP ml^{-1} h^{-1}. The highest phosphatase activity was (4.96 µg pNP ml^{-1} h^{-1}) achieved by *F. balustinum* on MS medium containing phytic acid (Table 2).

The results of this experiment showed that organic P substrate are affecting phosphatase activity. The results of this experiment consistent with the research of Moura et al. (2001) who explains that the different organic P substrates affects bacterial phosphatase activity. Other researchers reinforce the results of this study, is to Wyss et al. (1999) who examined the influence of different organic P substrates of phosphatase activity of fungi and bacteria. They explained that phosphatase activity of *Aspergillus terreus, A. niger* and *Escherichia coli* in culture with phytic acid as organic P substrate was higher than phenyl phosphate, α- glycerophosphate, fructose 6-phosphate and glucose 6-phosphate. The research of Fitriatin et al. (2008) showed that the phosphatase activity of soil microorganisms (*Pseudomonas mallei, Bacillus subtilis, Aspergillus niger* and *Penicillium* sp.) higher in phytic acid medium compared with α-glycerophosphate, phenyl phosphate and D-glucose-1-phosphate.

Phosphatase activity in medium with phytic acid tended to decrease with the length of incubation. However, in the medium containing extract of cow dung manure showed that phosphatase activity with a high diversity of each isolate at each time of observation. It is expected that the extract of cow dung manure as organic P subatrate was more slowly hydrolyzed by the bacterial isolates compared with phytic acid.

Dissolved P

The ability of phosphatase enzymes to hydrolyze organic P can be determined by measuring the phosphate that is

Table 3. Dissolved P of selected isolates in MS medium.

Treatments	Phytic acid			Extract of cow dung manure		
	3 d	5 d	7 d	3 d	5 d	7 d
	Dissolved P (mgl^{-1})					
Control	10.29	12.65	0.62	8.06	5.83	0.64
B. megaterium	13.66	16.64	0.39	11.99	8.53	0.27
B. laterosporus	27.67	27.56	0.77	11.85	9.56	1.29
P. pseudoalcaligenes	17.36	17.06	0.34	5.68	6.41	0.22
M. luteus	13.14	16.78	0.62	7.91	7.33	0.69
S. chromogenes	12.90	12.64	0.78	16.60	8.20	0.76
B. mycoides	15.53	15.01	1.13	14.02	8.55	0.64
B. pantothenticus	18.52	11.91	0.36	9.90	10.33	0.63
E. chrysanthemi	23.99	14.39	0.53	13.72	8.70	0.56
B. macerans	22.33	9.86	1.02	6.32	10.74	0.58
F. balustinum	18.84	15.11	0.76	13.39	9.33	0.68

formed or dissolved P in the MS medium by hydrolysis of organic P into inorganic P. The results of the experiment at this stage indicates that the dissolved P in medium with phytic acid given, tends to be higher than medium containing extract of cow dung manure. This can be seen in Table 3. This is allegedly related to phosphatase activity of bacteria is higher in medium containing phytic acid compared with extract of cow dung manure as described in Table 2. High phosphatase activity that would cause the hydrolysis of organic P is higher.

Bacillus laterosporus in MS medium containing phytic acid, produced the highest dissolved P (27.67 mg L^{-1}). Each isolate has the ability to hydrolyze different organic P in the medium. The results of this experiment consistent with the research of Yadav and Tarafdar (2003) which indicates that P is released from the hydrolysis by fungi in the medium with glycerophosphate ranging from 2.12 to 4.85 µg P released $minute^{-1}$, whereas for the medium with phytin, it ranges from 0.92 to 2.10 µg P released $minute^{-1}$. Their study also explaned that each isolate has a different ability in hydrolyzed organic P. The result of cluster analysis, we obtained three isolates have excellent capabilities in hydrolyzed organic phosphate, that is, *Bacillus mycoides*, *B. laterosporus* and *Flavobacterium balustinum.*

Conclusion

The phosphate solubilizing bacteria has capability to produce phosphatase enzyme which able to mineralized of organic P to inorganic P. Organic P substrate affects the phosphatase activity. Phytic acid as organic P substrate gave higher phosphatase activity and dissolved P higher than the extract of cow dung manure. We obtained three isolates have excellent capabilities in hydrolyzed organic phosphate that is, *Bacillus mycoides*, *B.laterosporus* and *Flavobacterium balustinum.*

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