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Phosphatase activity of phosphate solubilizing microbes as affected by organic P substrate and acidity

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Abstract

Phosphate solubilizing microbe in soil has capable to produce extracelluler enzyme, i.e. group of phosphatase enzyme which able to mineralized of organic P to inorganic P so that prepare high P for plant. The objective of this experiment was to examine the phosphatase activity from soil bacteria *Pseudomonas mallei*, *Bacillus subtilis* and fungi *Aspergilus niger* and *Penicillium* sp. in media containing different organic phosphorous and acidity. The result of experiment showed that the kind of organic phosphorous of medium affect phosphatase activity of *Pseudomonas mallei, Bacillus subtilis, Aspergilus niger* and *Penicillium* sp. In general, all microbe grown on medium with organic P substrate of phytic acid (myo-inositol hexakisphosphate) showed highest phosphatase activity compare to those grown on organic P substrate from glycerophosphate disodium salt, phenil phosphate or α-D-glucose 1-phosphate dosidium salt. Furthermore, the highest dissolve P was obtained from medium which contain glycerophosphate disodium salt.

Keywords: Mineralization; Organic phosphorous; Phosphatase; Soil microbes

1. Introduction

Generally, the content of organic P in soil is around 20 – 80% of P total of soil [1], [2]. It is the source of P available which is potential for plant. However, P in organic form can not used by plant and should be transformed to inorganic P form pass through mineralization and catalyzed by soil enzyme process [3], [4].

The main problem of phosphorus in soil is only small part of phosphorus is available for plant [5]. The availability of soil phosphorus depend on characteristic and properties of soil and also soil management by human [6]. Application of P in big amount on marginal soil as fertilizer is not ready available for plant and may be accumulated as inorganic P fraction and fixed through adsorption and precipitation process chemically and also organic P fraction which is immobilized as soil organic matter [7].

Many soil bacteria and fungi have the ability to solubilize P and make it available to growing plants [8]. Microbes are central to the soil P cycle and play a significant role in mediating the transfer of P between different inorganic and organic soil P fractions, subsequently releasing available P for plant acquisition. There are two aspects in microbial P solubilization: 1) P released by solubilization processes [9], and 2) P released from accumulated P in biomass of microbes [10]. Inorganic phosphate solubilizing microbes (PSM) constitute various portions of the soil microbial population and vary from soil to soil [11], [12].

The numbers of PSM are more important in rhizosphere than non-rhizosphere soil [13]. PSM occur in both fertile and P-deficient soils and the fastest initial rates of P incorporation were observed in P-deficient soils [14].

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Phosphate solubilizing fungi are superior to their bacterial counterpart for P solubilization both on precipitated agar and in liquid [15]. Fungal hyphae in liquid culture were attached to P mineral particles shown by scanning electron microscopy, whereas were not for bacteria [16]. Furthermore, because of their hyphae, fungi are able to reach greater distances more easily in soil than bacteria [17].

Penicillium and *Aspergillus niger*. were isolated from soil that they are dominant P solubilizing fungi found in rhizosphere. *Pseudomonas mallei* and *Bacillus subtilis* are phosphate solubilizing bacteria that were isolated from rhizosphere [18]. Furthermore, their ability to mineralize organic P need investigate more.

Some free living microbe in soil has capable to produce extracelluler enzyme, i.e. group of phosphatase enzyme which able to mineralized of organic P to inorganic P so that prepare high P for plant. Due to the low availability of inorganic P in soil, the organic P mainly contributes to plant nutrition and to microbial uptake through its mineralization and subsequent release of inorganic P [19]. P mineralization rate depends on microbial activity and on the activity of phosphatases [20]. Consequently, the release of inorganic P through the destruction of the organic matter is usually divided in biological mineralization and biochemical mineralization. Biological mineralization involves the release of inorganic P as a consequence of the carbon oxidation and the microbial growth and turnover, while in biochemical mineralization the release of inorganic P, independent of microbial respiration, is controlled by the supply and need for P and involves the hydrolysis of ester-phosphates by extra-cellular hydrolytic enzymes (phosphatases) both free in solution and stabilised by sorption to the colloidal fraction [21].

There are several soil phosphatases and the most commonly determined are: phosphomonoesterases, phosphodiesterases and phytases. Phosphomonoesterases act on phosphate monoesters and according to their optimum pH are divided in acid and alkaline phosphomonoesterases. Both are adaptive enzymes: acid phosphomonoesterase predominates in acid soils while alkaline phosphomonoesterase predominates in neutral and basic soils [22], [23].

Activity of phosphatase enzyme is affected by some factors, i.e. the amount and kind of substrate, pH, temperature, material of inhibitor and activator, concentration of enzyme and product, and also the kind of solvent used [3]. Besides, soil phosphatase activity also affected by properties of chemical and physical of soil i.e. soil type, organic matter content, total N content, C/N ratio and total P content [24].

The optimum of medium to induct extracellular phosphatase of microbes is necessary in order to reach optimal activity of phosphatase enzyme. The treatments of specific substrate aimed to find out substrate which's able to increase synthesis of extracellular phosphatase of microbe and the effect of optimal pH.

2. Materials and methods

Some kind of organic P substrate were used to find out kind of substrate effect on activity of phosphatase enzyme of phosphate solubilizing microbes which derive from isolation and selection process from staple food rhizosphere and has identified and tested its ability to dissolve inorganic P (in preliminary experiment) i.e. *Pseudomonas mallei., Bacillus subtilis, Aspergillus niger* and *Penicillium* sp.

Test of organic P substrate and the influence of pH on medium were conducted using four organic P substrates with concentration 5 mM consisted of:

- Phytic acid (*myo*-inositol heksakisphosphate),
- Glycerophosphate disodium salt,
- Phenyl phosphate
- α-D-glucose 1-phosphate disodium salt

Those treatments above was tested on some pH values (4.5 ; 5.5 dan 6.5). Phosphatase activity and dissolve P were observed on 3 and 5 days after incubation. Phosphatase activity analyzed with methods as described by Eiviazi and Tabatabai in Margesin [25] and dissolve P determined by Colorimetry.

3. Results and discussion

Both Phosphatase activity (Table 1 and 2) and dissolve P (Table 3 and 4) were observed on 3 and 5 days after incubation.

Table 1 Effect of kind of organic P substrate and pH of medium on phosphatase activity of microbes (1. *Penicillium sp.,* 2. *Aspergillus niger,* 3. *Bacillus subtilis,* 4. *Pseudomonas mallei*) on 3 days After Incubation)

PSM	Organic P substrate											
	Phytic acid			Glycerophosphate			Phenyl phosphate			α-D-glucose 1-phosphate		
	pH 4.5	pH 5.5	pH 6.5	pH 4.5	pH 5.5	pH 6.5	pH 4.5	pH 5.5	pH 6.5	pH 4.5	pH 5.5	pH 6.5
Phosphatase activity (µg pNP/ml/hour)												
1	18.26	18.44	5.92	8.68	1.99	7.26	0.02	0.07	0.34	0.12	0.03	0.13
2	0.73	1.73	2.51	10.07	4.18	4.8	3.82	2.35	5.29	2.65	2.18	4.27
3	0.55	2.83	1.51	4.13	10.25	0.96	0.87	1.94	3.10	0.01	0.58	0.95
4	8.11	5.33	23.22	8.11	6.14	11.72	0.55	2.03	2.02	3.54	0.72	0.63

Table 2 Effect of kind of organic P substrate and pH of medium on phosphatase activity of microbes (1. *Penicillium sp.,* 2. *Aspergillus niger,* 3. *Bacillus subtilis,* 4. *Pseudomonas mallei*) on 5 days after Incubation)

PSM	Organic P substrate											
	Phytic acid			Glycerophosphate			Phenyl phosphate			α-D-glucose 1-phosphate		
	pH 4.5	pH 5.5	pH 6.5	pH 4.5	pH 5.5	pH 6.5	pH 4.5	pH 5.5	pH 6.5	pH 4.5	pH 5.5	pH 6.5
Phosphatase activity (µg pNP/ml/hour)												
1	42.43	46.08	57.40	0.95	12.04	10.63	0.25	4.44	0.78	3.18	4.28	0.23
2	13.07	13.75	17.53	3.19	0.32	3.06	0.26	1.05	1.18	1.72	1.82	1.32
3	4.02	4.10	4.30	0.54	0.86	1.86	1.60	1.83	2.59	0.40	6.37	6.90
4	13.51	16.04	15.65	2.09	3.09	5.86	0.55	0.69	0.62	1.59	1.82	1.18

The ability of phosphatase enzyme to dissolve organic P can found out by measure phosphate which is formed due to hydrolysis of organic P (derive from organic P substrate) to be inorganic P form and measure dissolve P in medium as shown on Tables 3 and 4.

Table 3 Effect of kind of organic P substrate and pH of medium on dissolve P by microbe (1. *Penicillium sp.,* 2. *Aspergillus niger,* 3. *Bacillus subtilis,* 4. *Pseudomonas mallei*) on 3 days after incubation)

PSM	**Organic P**											
	Phytic acid			Glycerophosphate			Phenyl phosphate			α-D-glucose 1-phosphate		
	pH 4.5	pH 5.5	pH 6.5	pH 4.5	pH 5.5	pH 6.5	pH 4.5	pH 5.5	pH 6.5	pH 4.5	pH 5.5	pH 6.5
Dissolve P (mg/L)												
1	112.4	58.1	126.3	193.6	224.4	168.5	32.1	31.2	31.1	30.1	30.1	40.2
2	114.1	192.7	130.1	147.3	158.7	181.4	31.9	3.1	3.1	120.1	84.5	53.2
3	29.7	42.66	7.78	144.1	213.9	115.9	3.13	1.66	1.67	40.2	65.2	73.1
4	40.3	26.4	41.0	230.9	163.6	214.7	1.66	1.69	1.69	60.3	54.2	63.2

Table 4 Effect of kind of organic P substrate and pH of medium on dissolve P by microbe (1. *Penicillium sp.,* 2. *Aspergillus niger,* 3. *Bacillus subtilis,* 4. *Pseudomonas mallei*) on 5 days after Incubation)

PSM	**Organic P**											
	Phytic acid			Glycerophosphate			Phenyl phosphate			α-D-glucose 1-phosphate		
	pH 4.5	pH 5.5	pH 6.5	pH 4.5	pH 5.5	pH 6.5	pH 4.5	pH 5.5	pH 6.5	pH 4.5	pH 5.5	pH 6.5
Dissolve P (mg/L)												
1	50.8	42.7	118.9	160.2	201.4	141.4	10.6	2.9	3.2	23.2	32.93	77.6
2	21.6	67.0	57.5	134.1	140.1	153.5	10.6	8.4	12.4	116.5	76.7	71.1
3	49.9	61.3	28.9	144.2	190.2	91.8	11.8	17.5	1.67	32.1	45.1	67.0
4	21.6	16.2	35.16	215.2	143.1	130.1	4.1	8.21	1.69	58.9	43.5	54.0

Analysis of phosphatase showed that phosphatase activity of fungus was higher than bacteria on all substrate of organic P. Based on result of experiment, Wyss *et al.* [26] reported that fitase (a kind of phosphatase) activity of fungus group is higher than *E. coli.*.

Respons of phosphatase activity of each microbe on organic P substrate on 3 DAI and 5 DAI were various. In the whole, phytic acid substrate in medium has higher phosphatase activity in compare with the other substrates. However, based on data of dissolve P, substrate of glycerolphosphate gave the higher dissolve P than the other organic P substrates. The experiment of Wyss *et al.* [26] using *Aspergillus* and *E. coli* showed that more P release on medium contained phytic acid in compare with organic P substrate derived from phenyl phosphate, sugar phosphate, *p*-nitrophenyl phosphate, α and β-glycerophosphates, and phosphoenolphyruvate.

Result of experiment showed that phosphatase activity of both *Aspergillus niger* and *Penicillium sp* were higher on pH value of 4.5 than pH 6.5. On the contrary, activity phosphatase of *Bacillus subtilis* and *Pseudomonas mallei* were higher on pH 6.5 in compare with pH 4.5.

4. Conclusion

The kind of substrate and pH affect the phosphatase activity of *Pseudomonas mallei, Bacillus subtilis, Aspergillus niger* and *Penicillium sp*. Furthermore, Phytic acid (*myo*-inositol heksakisphosphate) is a substrate with highest phosphatase activity while the highest dissolve P was obtained from medium contained glycerophosphate disodium salt.

Compliance with ethical standards

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Disclosure of conflict of interest

No conflict of interest to be disclosed.

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