Isolation and Effect of Al-Tolerant Phosphate Solubilizing Microorganism for Production and Phosphate Absorption of Grasses and Phosphour Dissolution Mechanism

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Abstract. The objective of this research was to study the isolation and effect of Al-tolerant phosphate solubilizing microorganisms to growth, production of grasses and phosphate dissolution mechanism. The planting materials used were *S. splendida* and *C. gayana* pols. The treatment consisted of four selected isolates, namely P_o = without phosphate solubilizing bacteria(PSB), P_1 = RJM.30.2, P_2 = FT.3.2, P_3 = FT.3.4, P_4 = B8016495, P_5 = B8016498**,** P_6 = the mixture from four isolates P_2-P_5. Observed variables were pH, shoot and root dry weight, and P absorption. The best phosphate solubilizing microorganism on acid soil were FP.3.2, FP.3.3, B8016495 and B8016498. Phosphate solubilizing microorganism could not yet increase shoot and root dry weight production on grasses *S. splendida* and *C. gayana*, but had shown increasing P shoot and root content and P uptake. On grass *S. splendida* the best isolate to increase P shoot and root of shoot and root, organic acid. The best phosphate solubilizing microorganism on acid soil content and P uptake was FT.3.3. On grass C gayana the best isolate to increase P shoot and root content and P uptake were RJM.30.2. and FT.3.3. Organic acid exudated by FT.3.3. were oxalic and acetic acid as phosphate dissolution mechanism.

Keywords: phosphate solubilizing microorganism, acid soil, forage grasses, *Setaria splendida, Chloris gayana*

Abstrak. Tujuan penelitian ini adalah untuk mempelajari isolasi dan pengaruh mikroorganisme pelarut P yang toleran terhadap Al terhadap pertumbuhan, produksi rumput dan mekanisme pelarutan fosfat. Bahan tanam yang digunakan adalah pols dari rumput *Setaria splendida* dan *Chloris gayana.* Perlakuan terdiri dari empat isolat yang terpilih dan satu isolat sebagai pembanding (RJM.30.2). Perlakuan terdiri dari P_o = tanpa bakteri pelarut fosfat (PSB), P_1 = RJM.30.2, P_2 = FP.3.2, P_3 = FP.3.3, P_4 = B8016495, P_5 = B8016498, P_6 = campuran dari empat isolat P_2-P_5. Peubah yang diamati adalah pH, berat kering tajuk dan akar, dan serapan P. Mikroorganisme pelarut Fosfat pada tanah asam yang terbaik dalam pelarutan P adalah FP.3.2, FP.3.3, B8016495, dan B8016498. Mikroorganisme pelarut fosfat belum dapat meningkatkan produksi berat kering tajuk dan akar pada *S. splendida*. Mikroorganisme pelarut fosfat terbaik untuk meningkatkan kadar dan serapan P adalah FP.3.3. Pada *C. gayana* telah menunjukkan peningkatan produksi berat kering tajuk dan akar dan peningkatan kadar dan serapan P. Mikroorganisme pelarut fosfat terbaik untuk meningkatkan serapan dan kadar P adalah RJM.30.2. dan FP.3.3. Eksudat asam organik yang dikeluarkan oleh FP.3.3. adalah asam oksalat dan asetat sebagai mekanisme pelarutan fosfat.

Kata kunci: mikroorganisme pelarut fosfat, tanah asam, rumput pakan, *Setaria splendida, Chloris gayana*

Introduction

Phosphorus is one the most essential elements for plant growth after nitrogen. Phosphorus (P) is a major growth-limiting nutrient, and unlike the case for nitrogen, there is no large atmospheric source that can be made biologically available (Ezawa et al*.,* 2002). However, the availability of this nutrient for plants is limited by different chemical reactions. Phosphorus plays a significant role in several physiological and biochemical plant activities like photosynthesis, transformation of sugar to starch, and transportation of the genetic traits (Mehrvarz, 2008).

Inorganic phosphates in acidic soils are associated with iron (Fe) and aluminium (Al)

compounds where-as calcium (Ca)-phosphatesare predominant form of inorganic phosphates in neutralor calcareous soils (Gyaneshwar et al., 2002). Chemical fertilizers play a significant role in the green revolution, but unbalanced use of them leads to reduction in soil fertility and to environmental degradation (Gyaneshwar et al., 2002). In addition, unfavourable pH and high reactivity of aluminium and iron in soils decrease P availability as well as P-fertilizer efficiency also with high total of P contents (Börling et al., 2001; Hao et al., 2002). Microorganisms are involved in a range of process that affects the transformation of soil phosphorus which was an integral component of the soil P cycle (Deubel and Merbach, 2005). In particular, soil micro-organisms are effective in releasing P from inorganic P through solubilization. Fe and Al bounding by resulting organic acid, phosphates solubilizing microorganism will reduce inorganic phosphate fixation.

Biological fertilizers (phosphate solubilizing bacteria/PSB) are considered among the most effective plant assistants to supply phosphorus at a favorable level Phosphate solubilizing microorganisms refer to agroup of soil microorganisms that as components of phosphorus cycle, could release it from insoluble sources by different mechanisms, phosphate solubilizing fungi and bacteria are known as effective organisms in this process (Salehrastin, 1999). Zhao and Lin (2001) reported that many PSB have been isolated including, for example, those in *Bacillus*, *Pseudomonas*, *Erwinia*, *Agrobacterium, Serratia*, *Flavobacterium*, *Enterobacter*, *Micrococcus*, *Azotobacter*, *Bradyrhizobium*, *Salmonella*, *Alcaligenes*, *Chromobacterium*, *Arthrobacter*, *Streptomyces*, *Thiobacillus,* and *Escherichia.* The objective of this research was to study isolation and effect of Al-tolerant phosphate solubilizing microorganisms to growth and production of Grasses and phosphate dissolution mechanisms.

Materials and Methods

The study was conducted in the Forest and Environment Biotechnology Laboratory, Biological Resources Research Center of Biotechnology, in the Laboratory Field and in Laboratory of Agrostology, Faculty of Animal Science, Bogor Agricultural University and in Laboratory of Biochemistry, Biotechnology Research Institute for Food Crops, Cimanggu, Bogor, West Java. The planting materials used were *S. splendida* (Al-tolerant grass) and *C. gayana* (Al-sensitive grass) pots obtained from Agrostology Laboratory, Faculty of Animal Science, Bogor Agricultural University. The soil used was Red-yellow podzolic from Cigudeg, Jasinga, Bogor. This soil contained 28.15 me Al_{dd}.

Soil sampling. Composite soil from a depth of 0-20 cm was taken, subsequently released from plant residues, stones, gravel, and well stirred. To obtain a uniform grain size, soil was sifted with a 2-mm sieve.

Isolation of bacteria. Ten grams of soil to be isolated were dissolved in 90 ml of physiological solution (NaCl 0.85%), then serially diluted up to 10^5 times the of dilution levels. One ml of this suspension were cultured in aseptically into test tubes containing 9 ml *Pikovskaya* medium. One ml suspension was bred on the cup containing *Pikovskaya* and incubated on room temperature for 2 days. This medium was feculent white chromatic because it contained $Ca_3 (PO_4)_2$ colony, surrounded by a bright, clear zone indicated the dissolution of $Ca_3 (PO_4)_2$. Desirable colonies were then purified. Isolates acquired at collection in the oblique with medium *Pikovskaya* were then kept at 4^{o}C, and rejuvenated every 2 months.

Selection of isolates based on phosphate solubilizing. Isolates, previously collected and selected with a clear zone was formed by the dissolution, then continued to improve the ability of soil P available. 25 g of sterile soil was

placed in a tube, then inoculated with one ml of isolate and incubated at room temperature for 14 days. At the end of the incubation, pH H_2O, water soluble P and total P were measured. Soil was sterilized by heating at 121^{o}C for 30 minutes and repeated 3 times. This test was performed with two replicates chosen based on their ability to increase P available.

Standardization of the population bacteria. Standard curve of selected isolates was determined to facilitate the technique of inoculation for the next experiment. This curve was the relationship between the optical value (optical density) suspension isolates with colony-forming units (CFU), which was determined by pour plate method, so that inoculation in experiments would be able to use a uniform population. The suspension isolates in nutrient broth medium were sequentially diluted 2, 4, 8 and 16 times and then measured using a spectrophotometer at a wave length of 620 nm. Next to each level of dilution, the population of isolates was determined using plate count. Population isolates and optical value were associated with linear regression equation which was used as standard curve of isolate populations within the medium.

Organic Acid Analysis. A number of 1.0 x 10^{10} cells of microorganisms were inoculated in 100 ml Pikovskaya medium, incubated at room temperature for 3 days with 100 rpm shaking. At the end of incubation the culture was centrifuged at 7500 rpm at 25°C for 20 minutes. The filtrate obtained was used to determine the level of organic acids. The determination of organic acids used High Performance Liquid Chromatography (HPLC).

Pot Experiment. Pot experiment was conducted in the greenhouse, using a completely randomized design with 6 treatments and 3 replications in two separate types of grass namely *S. splendida* (Al-tolerant) and *C. gayana* (Al-sensitive). The treatment consisted of four selected isolates, namely P_o = without phosphate solubilizing bacteria (PSB), P_1 = RJM.30.2, P_2 = FT.3.2, P_3 =FT.3.4, P_4 = B8016495, P_5 = B8016498**,** P_6 = the mixture into the four isolates P_2-P_5. This experiment consisted of 42 experimental units measuring 2x7x3. Data obtained were statistically tested with analysis of variance and significant effect would be tested with the Duncan test.

Implementation technique

Sterilization. Soil was cleared from various roots then screened and air-dried on room temperature. Afterwards it was sterilized using wet method or braising up to 12 hours at 100^{0}C, and incubated for 2 weeks.

Fertilization. 4 kg soil was dashed with 0.4 g/pots manure basics as manure of composites until homogeneous then put into plastic pot.

Inoculation and cultivation. Inoculum for phosphate solubilizing bacteria was given 1 ml bacteria suspension containing 1.0 x 10 ^{11} cell (CFU) around grass root. Each pot was cultivated with one pols grasses *S. splendida* and 2 pols *C. gayana* with pols high average 20 cm. Observed variables were 1) pH analysis from soil, 2) titrates p analysis of soil, done by utilizing atomic absorption spectrophotometer (AAS), 3) shoot dry weight, measured at the end of research by air drying the upper part of the plant then heated in oven at 70°C for 24 hours, 4) root dry weight, measured at the end of research by air drying part of root, then heated in oven at 70°C for 24 hours, 5) P titrates analysis on plant tissue (coronet and root), P titrates analysis was done by Watanabe and Olsen's method (1965) utilizing Atomic Absorption Spectrophotometer (AAS), and 6) P absorption. Total Phosphorus absorption was found from total acquired multiple shoot dry weight (SDW) with shoot phosphorus content (SPC) added by root dry weight (RDW) multiplied by root phosphorus content (RPC). Total absorption phosphorus content was counted as shoot phosphorus absorption =

SDW(g)xSPC(%), root Phosphorus absorption = RDW(g)xRPC(%), total Phosphorus absorption = SDW(g)xSPC(%)+RDW(g)xRPC(%).

Results and Discussion

Phosphate solubilizing bacteria isolation and selection of isolate which is tolerant of high Al

Result of microorganisms isolating solubilizing phosphate from Kalimantan East, Papua and Northern Sulawesi presented on Table 1. Phosphate solubilizing microorganisms isolate from Kalimantan East, as much as 26 soil sample resulted in 42 isolates. To get preeminent isolate, selection was firstly made via isolate's ability to dissolve phosphorus, by observing transparent zone at surrounding isolate. The greater transparent zone from an isolate showed greater dissolvent possibility of phosphorus. In Kalimantan's region selection East bases good transparent zone which is diameter vicinity 1 cm and to be gotten 2 isolate.

A total of 20 soil samples from Northern Sulawesi showed 24 isolate microorganism phosphate dissolving. First selection from Northern Sulawesi by observing transparent zone resulted in 3 isolate. From Papua showed 4 isolates where most selection was by observing effect of transparent zone phosphorus dissolution. Result selection to pass through transparent zone was selected 9 isolate, then drawned out by selection phase secondly. The next selection was by observing whether microorganism was able to dissolve phosphorus well deep into neutral soil and also acid shown in Table 2. Neutral pH obtained by dissolution P was significantly different (P<0.01) on B8016495, FP.3.2, FP.3.4, Obissht. 4, if compared to control. On dissolutions in acid soil significantly higher P dissolution was found on FP.3.2, FP.3.3, B8016495, and B8016498. (For successful observation, the best isolate was that with the best ability to dissolve P on acid pH). Succeeding observation chose isolat with

Table 1. Microorganisms isolating result dissolving Phosphate from East Kalimantan, Papua and Northern Sulawesi

Origin	Total soil samples	Total isolates that grew	First selection from transparent zone
East Kalimantan	26	42	2
Northern Sulawesi	20	24	3
Papua	-	-	4

Table 2. Analysis pH, potential P and available P for second selection

Isolate	pH	Available P(ppm)	Potential P (ppm)	pH	Available P (ppm)	Potential P (ppm)
Control	6.35^{b}	3.61^{c}	8.90^{bc}	4.19^{a}	7.21^{b}	12.73^{ab}
B8016498	6.35^{bc}	11.24^{a}	10.23^{b}	4.29^{a}	10.61^{b}	15.69^{ab}
B8016495	6.59^{a}	10.18^{ab}	11.03^{a}	4.26^{a}	11.67^{ab}	16.12^{a}
BD/1/81	6.25^{c}	11.24^{a}	7.63^{c}	4.28^{a}	8.49^{ab}	11.46^{b}
FP.3.1.	6.35^{bc}	9.76^{a}	10.60^{b}	4.25^{a}	11.45^{ab}	15.69^{ab}
FP.3.2.	6.28^{bc}	7.42^{ab}	12.72^{a}	4.25^{a}	12.73^{a}	19.09^{a}
FP.3.3.	6.56^{a}	6.99^{ab}	10.71^{b}	4.28^{a}	12.03^{a}	16.76^{a}
FP.3.4.	6.26^{bc}	5.73^{b}	11.34^{a}	4.27^{a}	10.61^{a}	15.69^{ab}
Obis.ssht4	6.49^{a}	7.21^{ab}	12.29^{a}	4.19^{a}	13.61^{a}	15.71^{ab}
PO2.sob	5.92^{c}	5.30^{c}	8.91^{bc}	3.92^{b}	7.26^{b}	8.24^{c}

Values bearing different superscript at the same column differ significantly (P<0.01)

ability to dissolve P the best one on acid pH which was FP.3.2, FP.3.3, B8016495 and B8016498. Only four isolates were taken due to limitation in observation. For further research it was needed to utilize another isolate with better phosphate dissolution than control.

Populations default curve from isolate which was chosen and contrasting isolate could be seen on Table 3. Populations default curves that was utilized for further research that population amount that is utilized for each isolat uniform. After isolate was selected, pottest was conducted to know the best isolate if grown podzolic soil.

Table 3. Population curves for PSB

Isolate Type	Standard Curve
F.P.3.2.	$Y = -0.433\ 10^{-3} + 1.846\ 10^{-13} X$
F.P.3.3.	$Y = 0.053 + 3.217\ 10^{-13} X$
B.80.1649.5	$Y = 0.005 + 2.421\ 10^{-13} X$
B.80.1649.8	$Y = -3.555\ 10^{-3} + 2.401\ 10^{-13} X$
RJM.30.2.	$Y = 0.015 + 2.701\ 10^{-13} X$

Y = optical density; X: colony formation unit

The plant response of the addition from phosphate solubilizing microorganism

Data on the effect of PSB treatment on shoot and root dry weight on *S. Splendida* and *C. gayana* are presented in Table 4. The result of variance test showed that the addition of phosphate solubilizing bacteria did not produce significant effect of the shoot and root dry weight. Soil utilized in this research was red yellow podzolic soil (PMK) that had constraint namely Al^{3+} that one got to poison plant. Another constraint was with that with low macro element as N, P, K, Ca. On this research it was observed that tolerant plant (*S. splendida*) with added phosphates solubiliing bacteria was not enhanced on shoot and root dry weight. On tolerant plant availibility of element P was accomplished through dissolution by issuing acid organic which is oxalic acid, malic acid, and citrate acid. P one is of service pass through dissolution on tolerant plant without dissolving bacteria increased phosphate which caused no significant difference to shoot and root dry weight production with treatment that was added with phosphates dissolving bacteria.

On susceptible plant (*C. gayana*), constrained growth was observed due to the absence of mechanism for detoxcificated Al^{3+}, although phosphate dissolving bacteria to dissolve bonded phosphate presented. Mark sense Al^{3+} dissolved one talling to cause inhibited growth, could be seen from its root development. Root growth on susceptible plantwas inferior to tolerant plant. Growth comes to fruition that inhibited because of Al^{3+} one that replaced Ca^{2+} on apoplas's region. Besides Al was tied by fosfolipid on double membrane and tied-up DNA and also RNA, that made nutrient element absorption constrained and fission process and amplification of cell stopped, and eventually caused crop plant inhibited (Matsumoto, 1991; Marschner, 1995).

Organic acid exudation was generally believed to play critical roles in ameliorating Al toxicity through forming non-toxic Al chelates, which had been well documented in several species, such as citric acid from roots of soybean (Yang et al., 2001), common bean (Shen et al., 2002). It was known that an abundant quantity of low molecular weight organic acids was found in root exudates (Somers et al. 2004). It was thought that these organic acids acted to chelate metal cations in the rhizosphere. The solubility and toxicity of Al was greater in soils with low pH. To escape the strong toxicity of Al^{3+} ions, many plants developed the ability to secrete citrate or malate from their roots. Sasaki et al. (2004) first identified the ALMT1 gene encoding the Al-activated malate transporter in wheat. ALMT1 was the key factor permitting malate exudation from roots in the Al-tolerant wheat cultivar ET8. Similarly, the MATE (multidrug and toxic compound extrusion)-type citrate transporter was identified in barley (Furukawa et al., 2007), and contributed to Al tolerance by citrate exudation. Organic acid exudation was also

stimulated by nutrient deficiency. Root-secreted organic acids were important in P acquisition. When plants grew under P-deficient conditions, organic acid exudation was stimulated to mobilize P from sparingly soluble forms combined with metal ions in the soil. The citrate channel appears to control the abundant citrate exudation observed from the roots of P-deficient white lupin plants (Zhang et al*.,* 2004). It was also suggested that this citrate exudation was coupled with H^+-ATPase (Tomasi et al., 2009). In contrast to Al-activated organic acid exudation, the genes encoding transporters for malate or citrate exudation under P deficiency had not yet been identified. The kinetics of malate and citrate exudation differed between P deficiency and Al stress (Ma, 2000); organic acid exudation stimulated by P deficiency could be controlled by the transporter independently from Al-activated mechanisms. Beside increased P available, several organic acid could reduce Al_{dd} , Al could be interchanged on cotton plant (Hue, Craddock and Adams, 1986).

The result of analysis of variance showed that the addition of phosphate solubilizing bacteria did not produce any significant effect toward the pH and P level of soil on either *S. splendida* or *C. gayana* grass (Table 5). The effect of the addition of phosphate solubilizing microorganism on P percentage of shoot and root, P absorption at *S splendida* and *C. gayana* were shown at Table 6 and 7.

Analysis of variance showed that the addition of phosphate solubilizing bacteria weresignificantly (P<0.05) increased P level of shoot at *S. splendida* grass (Table 6). The advance test showed that the highest P level of shoot was inoculated with FP.3.3, had a significantly difference (P<0.05) with the control, RJM.30.2., FT.3.2., B8016495, B8016498 and the mixtured. The grass inoculated with B8016498 and the mixtured had a significant (P<0.05) higher P level of the shoot compared with the grass inoculated with RJM.30.2, FT.3.2., and control. The grass inoculated with RJM.30.2 and FT.3.2. had a significantly (P<0.05) highershoot P level compared with the control.

Analysis of variance showed that the addition of phosphate solubilizing bacteria had a significant effect (P<0.05) on P level of *C. gayana* grass (Table 7). The advance test at Table 7 showed that the highest P level of shoot at grass inoculated with RJM. 30.2, did not have any significant difference with grass inoculated with FT.3.3., and the mixtured, but had a significantdifference (P<0.05) with the control and the grass inoculated with FT.3.2, B8016495, B8016498.

Analysis of variance showed that the addition of phosphate solubilizing bacteria had a significant effect (P<0.05) on P level of root at *C. gayana* grass (Table 7). The advance test at Table 7 showed that the highest root P level of the grass inoculated with RJM.30.2. had a significant difference (P<0.05) with the control and the grass inoculated with FT.3.2, FT.3.3, B8016495, B8016498 and the mixtured. The grass inoculated with FT.3.3 and B8016495 had a significant (P<0.05) higher P level compared with the control and the grass inoculated with FT.3.2, B8016498, and the mixtured. The grass inoculated with B8016498, and the mixtured had a significantly (P<0.05) higher root P level compared to the control and the grass inoculated with FT.3.2.

The analysis of variance result was that phosphate solubilizing microorganism was significantly different (P<0.05) increasing phosphate uptake P on *C. gayana* (Table 7). The advance tests on Table 7 showed P roots value on grass RJM.30.2 was not different from FT.3.3, B8016495 and the mixture, but higher (P<0.05) then control. FT.3.2. and B8016498. FT.3.3. on *S. splendida*, B8016498 and the mixtured on *C. gayana* showed the best shoot P absorption was caused by higher P content and shoot dry weight than another treatment. P

Table 4. The effect of phosphate-dissolve bacteria on the production of *S. splendida* and *C. gayana* grass

Treatments	SDW of *S splendida* (g/pot)	RDW of *S splendida* (g/pot)	SDW of *C gayana* (g/pot)	RDW of *C gayana* (g/pot)
Control	7.50	2.64	3.94	0.22
RJM.30.2	6.95	2.79	5.28	0.43
FT.3.2.	6.96	2.45	3.81	0.25
FT.3.3.	8.48	2.90	4.93	0.24
B8016495	6.53	2.68	4.73	0.30
B8016498	6.38	2.84	4.79	0.33
Combination	6.19	2.74	4.39	0.40

Table 5. The effect of phosphate-dissolve bacteria toward the pH and P level of soilof *S. splendida* and *C. gayana* grass

Treatments	pH of *S splendida*	P Level of *S splendida* (ppm)	pH of *C gayana*	P Level of *C gayana* (ppm)
Control	4.76	29.27	4.64	27.63
RJM.30.2	4.78	29.75	4.62	28.84
FT.3.2.	4.62	29.11	4.69	26.62
FT.3.3.	4.83	29.64	4.83	29.06
B8016495	4.94	28.42	4.69	27.99
B8016498	4.77	30.91	4.59	27.36
Combination	4.71	29.16	4.82	26.51

Table 6. The effect of phosphate solubilizing microorganism for P percentage of shoot and root, P absorption of *S. splendida* grass

Treatments	% P of *S splendida shoot*	% P of *S splendida root*	P Absorption of *S splendida* (mg/pot)
Control	0.016^{d}	0.015	0.160^{d}
RJM.30.2	0.020^{c}	0.018	0.197^{c}
FT.3.2.	0.021^{c}	0.020	0.201^{c}
FT.3.3.	0.037^{a}	0.017	0.369^{a}
B8016495	0.019^{c}	0.021	0.188^{c}
B8016498	0.022^{b}	0.025	0.221^{b}
Combination	0.029^{b}	0.019	0.240^{b}

Values bearing different letters at the same column differ significantly (P<0.05)

Table 7. The effect of phosphate-dissolve microorganism on the P percentage of shoot and root, P absorption of *C. gayana* grass

Treatments	% P of *C gayana shoot*	% P of *C gayana root*	P Absorption of *C gayana* (mg/pot)
Control	0.019^{b}	0.032^{d}	0.084^{b}
RJM.30.2	0.035^{a}	0.048^{a}	0.231^{a}
FT.3.2.	0.022^{b}	0.033^{d}	0.096^{b}
FT.3.3.	0.033^{a}	0.044^{b}	0.182^{a}
B8016495	0.028^{b}	0.044^{b}	0.147^{a}
B8016498	0.018^{b}	0.035^{c}	0.100^{b}
Combination	0.037^{a}	0.034^{c}	0.162^{a}

Values bearing different letters at the same column differ significantly (P<0.05)

absorption enhanced 131.25% when compared with control on *S. splendida* and 73.68% on *C. gayana.* The height of P content was caused by solubilizing of PSB. Chen et al. (2006) reported the use of phosphate solubilizing microorganism as inoculants increased the P uptake by plants. P-solubilizing activity of these strains was associatedwith the release of organic acids and a drop in the pH of the medium. HPLC analysis detected different kinds of organic acids, namely: citric acid, gluconic acid, lactic acid, succinic acid, propionic acid.

Phosphate solubilizing mechanism

Some bacterial species have mineralization and solubilization potential for organic and inorganic phosphorus (Hilda and Fraga, 2000; Khiari and Parent, 2005). Phosphorus solubilizing activity was determined by the ability of microbes to release metabolites such as organic acids, which through their hydroxyl and carboxyl groups chelated the cation bound to phosphate, the latter being converted to soluble forms (Sagoe et al., 1998). Phosphate solubilization took place through various microbial processes/mechanisms including organic acid production and proton extrusion (Surange et al., 1995; Dutton and Evans, 1996; Nahas, 1996). In this research organic acids exudated by FT.3.3. were acetic acid as much as 10.05 ppm and oxalic acid as much as 37.86 ppm. Inorganic P was solubilized by the action of organic and inorganic acids secreted by PSB in which hydroxyl and carboxyl groups of acids chelated cations (Al, Fe, Ca) and decreased the pH in basic soils (Kpomblekou and Tabatabai, 1994; Stevenson, 2005). The PSB dissolved the soil P through production of low molecular weight organic acids mainly gluconic and keto gluconic acids (Goldstein, 1995; Deubel et al., 2000). The release of root exudates such as organic ligands could also alter the concentration of P in the soil solution (Hinsinger, 2001). Organic acids produced by PSB solubilize insoluble phosphates by lowering the pH, chelation of cations and competing with phosphate for adsorption sites in the soil (Nahas, 1996).

Solubilization of Fe and Al occured via proton release by PSB by decreasing the negative charge of adsorbing surfaces to facilitate the sorption of negatively charged P ions. Proton release could also decrease P sorption upon acidification which increased $H_2PO_4^-$ in relation to HPO_4^{2-} having higher affinity to reactive soil surfaces (Whitelaw, 2000). Carboxylic acids mainly solubilize Al-P and Fe-P (Henri et al., 2008; Khan et al., 2007) through direct dissolution of mineral phosphate as a result of anion exchange of $PO4^{3-}$ by acid anion, or by chelation of both Fe and Al ions associated with phosphate (Omar, 1998). Carboxylic anions replaced phosphate from sorption complexes by ligand exchange (Otani et al., 1996; Whitelaw, 2000) and chelated both Fe and Alions associated with phosphate, releasing phosphate available for plant uptake after transformation. Ability of organic acids to chelate metal cations was greatly influenced by its molecular structure, particularly by the number of carboxyl and hydroxyl groups. Phosphorus desorption potential of different carboxylic anions lowered with decrease in stability constants of Fe- or Al-organic acid complexes (log *K*Al or log *K*Fe) in the order: citrate>oxalate>malonate/malate>tartrate>lactate>gluconate>acetate>formiate (Ryan et al., 2001).

Microorganism produced organic acid by catabolisme glucose process in tricarboxilate acid cycle (Kreb's cycles) that constituted glycolisis reaction sequel. Organic acid constituted subtrat to process anabolisme in sintesis amino acid. It was predicted that genetic reflection effect, phosphates dissolving microorganism resulted in rich organic acids and a portion gets cell issued diffusions because of balance reactions.

Conclusions

The best phosphate solubilizing microorganism on acid soil were FP.3.2., FP.3.3., B8016495 and B8016498. Phosphate solubilizing microorganism could not yet increase shoot and root dry weight production on *S. splendida* and *C. gayana*, but has shown increasing P shoot and root content and P uptake.

On grass *S. splendida* the best isolate that gets to increase P shoot and root content and P uptake was FT.3.3. On grass *C. gayana*was the best isolate that got to increase P shoot and root content and P uptake which were RJM.30.2 and FT.3.3. Organic acids exudated by FT.3.3 were oxalic and acetic acid as phosphate dissolution mechanism.

References

Börling K, E Otabbong and E Barberis. 2001. Phosphorus sorption in relation to soil properties in some cultivated Swedish soils. Nutrient Cycling in Agroecosystems. 59:39-46.

Chen YP, PD Rekha, AB Arun, FT Shen, W-A Lai and CC Young. 2006. Phosphate solubilizing bacteria from subtropical soil and their tricalcium phosphate solubilizing abilities. Applied Soil Ecol. 34:33–41.

Ezawa T, SE Smith and FA Smith. 2002. P metabolism and transport in AM fungi. Plant Soil. 244:221-230.

Deubel A, Gransee and W Merbach. 2000. Transformation of organic rhizodeposits by rhizoplane bacteria and its influence on the availability of tertiary calcium phosphate. J. Plant Nutr. Soil Sci. 163:387-392.

Deubel A and W Merbach. 2005. Influence of microorganisms on phosphorus bioavailability in soils. In: Buscot Fand A Varma (Eds.), Microorganisms in Soils: Roles in Genesis and Functions. Springer, Berlin Heidelberg. Pp. 177-191.

Dutton VM and CS Evans. 1996. Oxalate production by fungi: Its role in pathogenicity and ecology in the soil environment. Can. J. Microbiol. 42:881-895.

Furukawa J, N Yamaji, H Wang, N Mitani, Y Murata and K Sato. 2007. An aluminum-activated citrate transporter in barley. Plant Cell Physiol. 48:1081-1091.

Goldstein AH. 1995. Recent progress in understanding the molecular genetics and biochemistryof calcium phosphate solubilization by Gram-negative bacteria. Biol. Agri. Hort. 12:185-193.

Gyaneshwar P, GN Kumar, LJ Parekh and PS Poole. 2002. Role of soil microorganisms in improving P nutrition of plants. Plant and Soil. 245:83-93.

Hao X, CM Cho, GJ Racz and C Chang. 2002. Chemical retardation of phosphate diffusion in an acid soil as affected by liming. Nutr. Cycl. Agroecosys. 64:213-224.

Henri F, NN Laurette, D Annette, Q John, M Wolfgang, E François-Xavier and N Dieudonné. 2008. Solubilization of inorganic phosphates and plant growth promotion by strains of *Pseudomonas fluorescens* isolated from acidic soils of Cameroon. African J. Microbiol. Res. 2:171-178.

Hilda R and R Fraga. 2000. Phosphate solubilizing bacteria and their role in plant growth promotion. Biotech. Adv. 17:319-359.

Hinsinger P. 2001. Bioavailability of soil inorganic P in the rhizosphere as affected by root induced chemical changes: a review. Plant Soil. 237:173-195.

Hue NV, GR Craddock and F Adams. 1986. Effect of organic acids on aluminium toxicity in subsoils. Soil Sci. 128: 321-326.

Khan MS, A Zaidi and PA Wani. 2007. Role of phosphate solubilizing microorganisms insustainable agriculture. A review. Agron. Sustain. Dev. 27:29-43.

Khiari L and LE Parent. 2005. Phosphorus transformations in acid light-textured soils treatedwith dry swine manure. Can. J. Soil Sci. 85:75-87.

Kpomblekou and Tabatabai. 1994. Effect of organic acid sonelease of phosphorus from phosphate rocks. Soil Sci. 158:442-453.

Ma JF. 2000. Role of organic acids in detoxification of aluminium in higher plants. Plant Cell Physiol. 41(4):383 –390.

Marschner H. 1995. Mineral Nutrition of Higher Plants. 2nd Edition. Academic Press Limited. London.

Matsumoto H. 1991. Biochemical mechanism of the toxicity of aluminium and the sequestration of aluminium in plant cells. p 825-838. In. Plant-Soil interactions at low pH.

Mehrvarz S, MR Chaichi and HA Alikhani. 2008. Effects of phosphate solubilizing microorganisms and phosphorus chemical fertilizer on yield and yield components of barely (*Hordeum vulgare* L.). American-Eurasian J. Agric. Environ. Sci. 3(6):822-828.

Nahas E. 1996. Factors determining rock phosphate solubilization by microorganism isolated from soil. World J. Microb. Biotechnol. 12:18-23.

Omar SA. 1998. The role of rock-phosphate-solubilizing fungi and vesicular–arbuscular mycorrhiza (VAM) in growth of wheat plants fertilized with rock phosphate. World J. Microbiol. Biotechnol. 14:211-218.

Otani T, N Ae and H Tanaka. 1996. Phosphorus (P) uptake mechanisms of crops grown in soilswithlow P status. II. Significance of organic acids in root exudates of pigeonpea. Soil Sci. Plant Nutr. 42:553-560

Ryan PR, E Delhaize and DL Jones. 2001. Function and mechanism of organic anionexudation from plant roots. Annl. Rev. Plant Physiol. Plant Mol. Biol. 52:527-560.

Sagoe CI, T Ando, K Kouno and T Nagaoka. 1998. Relative importance of protons and Solution calcium concentration in phosphate rock dissolution by organic acids. Soil Sci. Plant Nutr. 44:617-625.

Salehrastin N. 1999. Biological Fertilizers, Soil and Water Research Institute of Iran. Scientific J. Soil and Water. 12(3):35-42.

Sasaki T, Y Yamamoto, B Ezaki, M Katsuhara, SJ Ahn and PR Ryan. 2004. A wheat gene encoding an aluminum-activated malate transporter. Plant J. 37:645-653.

Shen H, XL Yan, M Zhao, SL Zheng and XR Wang. 2002. Exudation of organic acids in common bean as related to mobilization of aluminum and iron-bound phosphates. Env. Exp. Bot . 48:1-9.

Somers E, J Vanderleyden and M Srinivasan. 2004 Rhizosphere bacterial signaling: a love parade beneath our feet. Crit. Rev. Microbiol. 30:205-240.

Stevenson FJ. 2005. Cycles of Soil: Carbon, Nitrogen, Phosphorus, Sulfur, Micronutrients. JohnWiley and Sons, New York.

Sudara B, V Natarajan and K Hari. 2002. Influence of phosphorus solubilizing bacteria on the changes in soil available phosphorus and sugarcane yields. Field Crops Res. 77:43-49.

Surange S, AG Wollum, N Kumar and CS Nautiyal. 1995. Characterization of *Rhizobium* from root nodules of leguminous trees growing in alkaline soils. Can. J. Microbiol. 43:891-894

Whitelaw MA. 2000. Growth promotion of plants inoculated with phosphate solubilizing fungi. Adv. Agron. 69:99-151.

Yang ZM, M Sivaguru, WJ Horts and H Matsumoto. 2001. Aluminum tolerance is achieved by exudation of citric acid from roots of soybean (*Glycine max).* Physiol. Plant. 110:72-74.

Zhao XR and QM Lin. 2001. A review of phosphate—dissolving microorganisms. Soil Fertilizer. 3:7–11.