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Increasing potassium (K) release from K-containing minerals in the presence of insoluble phosphate by bacteria

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Abstract

Introduction: Phosphorus and potassium are major essential macronutrients for biological growth and development. Application of soil microorganisms is one approach to enhance crop growth. Some bacteria are efficient in releasing K and solubilizing P from mineral sources but their behavior was not studied more in presence together.

Materials and methods: In this study the ability of seven bacterial strains, including *Pseudomonas putida* P13, *P. putida* Tabriz, *P. fluorescens* Tabriz, *P. fluorescens* Chao, *Pantoea agglomerans* P5, *Azotobacter* sp. and *Bacillus megaterium* JK3 to release mineral K from muscovite and biotite with application of insoluble ($Ca_3(PO_4)_2$) or soluble (Na_2HPO_4) P-sources was investigated. Nutrient Broth was used to prepare an overnight culture of bacteria to inoculate in Aleksandrov medium, which was used to study the dissolution of silicate minerals. It should be mentioned that Aleksandrov medium was used to determine the amount of released P from tricalcium phosphate (TCP) while muscovite was added to the medium as a sole source of potassium. Concentration of P was determined spectrophotometrically by ammonium-vanadate-molybdate method and K was determined by flame photometry.

Results: The insoluble P-source led to a significantly increased released K into assay medium (66%), and the net release of K from the biotite was significantly enhanced. Among bacterial strains, the highest mean of released K was observed with *P. putida* P13 which released more K (27%) than the control. The amounts of released K from micas in the presence of insoluble and soluble phosphate by *P. putida* P13 were 8.25 and 4.87 mg/g, respectively.

Discussion and conclusion: Application of insoluble phosphate could increase K release from mica minerals. The enhanced releasing of mineral K might be attributed to the release of organic acids from the bacteria, a mechanism which plays a pivotal role in solubilizing phosphate from inorganic source of phosphate.

Key words: Insoluble phosphate, potassium solubilizing bacteria, *Pseudomonas*, soluble potassium

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Introduction

Phosphorus (P) and potassium (K) are major essential macronutrients for biological growth and development. However, the concentrations of soluble P and K in soil are usually very low, and a major proportion of P and K in soil are present as insoluble rocks, minerals and other deposits (1). Inspite of that, these sources constitute the biggest reservoirs of P and K in soil because, under appropriate conditions, they can be solubilized and become available for plants (1).

The development of intensively managed agriculture has led to the consumption of increasing amounts of K so that low K supply has become an important yield limiting factor in agriculture (2). However, more than 98% of potassium in soil exists in the form of silicate minerals which K cannot be directly absorbed by plants. Potassium and other minerals can be released when these minerals are weathered. Some microorganisms can play a role in releasing K from minerals (2). Production of organic acids and acidic polysaccharides by microorganism is the mechanism by which K is released. Microorganisms play a central role in the natural P and K cycle. There are considerable populations of P- solubilizing or K-solubilizing bacteria in soil and in plant rhizospheres (3 & 4). Bacteria of the genera *Pseudomonas*, *Agrobacterium*, *Bacillus*, *Rhizobium* and *Flavobacterium* have been tested for their ability to solubilize inorganic phosphate compounds, such as tricalcium phosphate, hydroxyapatite and rock phosphate (1 & 5). Potassium solubilizing bacteria were found to release K, silicon and aluminum from insoluble minerals (6). Numerous studies have documented the release of K during the degradation of silicate minerals by bacteria (7- 10) and isolation and identification of K-releasing bacteria has been reported (4, 2, 11- 13). It was postulated that the reactions responsible for microbially promoted K solubilization may involve acidolysis, enzymolysis, capsule absorption and complexation by extracellular polysaccharides (14).

It seems that releasing K from K-containing minerals depends on the type of bacteria, kind of minerals, composition of media and many other factors. In the present study, the potential of seven bacterial species to release K from K-rich minerals was investigated. The major objective of the research was to determine the effect of P sources (soluble or insoluble) on the release of mineral K from biotite and muscovite micas.

Materials and methods

Minerals: Muscovite and biotite were obtained from Zamanabad (Hamadan, Iran) and Qharabagh (Urmia, Iran) Mica Ores, respectively. Micas sheets were powdered to the size <0.5 mm. Owing to the high content of K in mica minerals, soluble K was removed before adding the minerals to Aleksandrov medium (12) by washing with 0.1 M HCl and distilled water. Mica powder (0.4 g) was rinsed in 30 ml of 0.1 M HCl and shaked for 30 min, then the supernatant was removed after centrifuging at 4100 g for 5 min. Further washing was

done with 30 ml of water by shaking for 30 min. The mica powder was then dried at 80ºC (4). This pre-treatment should not significantly alter the surface chemistry of the minerals. The content of K in various forms was measured and is shown in Table 1. Soluble K was extracted with dH_2O. Potassium was also extracted from the micas with 1 N NH_4OAc and 1 N HNO_3 by shaking on a reciprocal shaker for 30 min (15). The NH_4OAc extract gave an estimate of the most available fraction of K (soluble and exchangeable K), while extraction with HNO_3 contains nonexchangeable K as well (11). All K in the extracts was determined by flame photometry (Flame photometer 410, Corning).

Bacterial strains and growth condition: *Pseudomonas putida* P13 and *Pantoea agglomerans* P5 were obtained from the Green Biotech company (Tehran, Iran), while *P. fluorescens* Chao, *P. fluorescens* Tabriz, *P. putida* Tabriz, *Azotobacter* sp. and *Bacillus megaterium* JK3 were taken from laboratory of soil biology, University of Tabriz (Tabriz, Iran). Nutrient Broth was used to prepare an overnight culture of bacteria to inoculate in Aleksandrov medium (as described by Hu et al. 2006), which was used to study the dissolution of silicate minerals. Aleksandrov medium contained 0.5% glucose, 0.05% $MgSO_4.7H_2O$, 0.0005% $FeCl_3$, 0.01% $CaCl_2$, 0.2% potassium aluminium silicate (muscovite or biotite micas) and 0.2% phosphate source ($Ca_3(PO_4)_2$ (TCP) or Na_2HPO_4). The initial pH was adjusted to 7.

Experiment 1 (Plate assay): Phosphate solubilizing and K releasing efficiency of seven bacterial strains was determined using Aleksandrov medium in Petri plates containing 0.2% muscovite and 0.2% TCP as sources of K and P respectively. Three replicates of each treatment were included in a completely randomized design. Efficiency was measured based on the diameter of bacterial colonies and halo zone around the colonies. An inoculum of bacteria (10 µl) containing 10^8 cfu ml^{-1} was inoculated on the agar and the inoculated plates were sealed with Parafilm. Dot culture of bacteria was done with three replications. Petri plates were incubated at 26°C and monitored for halo formation for 7 days and the hydrolysis diameter was measured. Solubilizing efficiency was estimated using a solubilizing index (E) according to the following equation: E = (Diameter of hydrolysis zone /Diameter of bacterial growth) (13).

Table 1- Content of K in various forms of muscovite and biotite, before acid wash treatment.

Extractant	Muscovite (mg/kg)	Biotite (mg/kg)
dH_2O (soluble K)	2350	4300
1 N NH4OAc (soluble and exchangeable K)	2550	5000
1 N HNO3 (soluble, exchangeable and nonexchangeable K)	4525	17820

Experiment 2 (liquid assay): In order to quantify the amount of released K in liquid medium a factorial completely randomized design with three replications was used with three factors of (1) the seven bacterial strains plus an uninoculated control, (2) two phosphorus sources (insoluble $Ca_3(PO_4)_2$ and soluble Na_2HPO_4) and (3) two K-containing minerals (muscovite and biotite). Quantitative estimation of K solubilization was carried out in 100 ml Erlenmeyer flasks containing 25 ml Aleksandrov. Each flask was inoculated with 0.5 ml of bacterial inoculum (approximately 10^8 cfu ml^{-1}) (4). Autoclaved, uninoculated medium served as the control to which was added 0.5 ml of sterile NB. The flasks were incubated for 7 days at 26 °C on an incubator with 150 rpm. At the end of incubation 2 ml of cultures were diluted 1:5 (v:v) using dH_2O and centrifuged 4100 g for 4 min. The supernatants were used to assay the solubilized K.

It should be mentioned that Aleksandrov medium was used to determine the amount of released P from TCP while muscovite was added to the medium as a sole source of potassium. Concentration of P was determined spectrophotometrically by ammonium-vanadate-molybdate method and K was determined by flame photometry (16). In presence of V^{5+} and Mo^{6+}, orthophosphates form a yellow colored phosphor-vanado-molybdayte complex which shows an optimal absoption at wavelength 430 nm (16). In order to determine P, 2 ml of diluted supernatant mixed with 4 ml nitrovanadomolybdate reagent and 4 ml dH_2O. Standard series were made between 0 to 50 ppm P. After one hour, the absorption was measured at wavelength 430 nm with a spectrophotometer. To determine K, after dilution of the original diluted supernatant to 1/5, the K-emission is measured in an air-propane flame at wavelength 768 nm. A calibration curve was made with a standard series of 0-50 ppm K. The pH value was measured at the end of incubation with a pH meter.

Statistical analysis: Analysis of variance and mean comparison by Tukey's Significant Difference Test were carried out using MSTATC software. Values are given as means ± S.E. and differences were considered to be significant at the *P value*< 0.01 level.

Results

P solubilization in solid and liquid media: In the plate assay only three strains produced a clear zone and highest ratio of clear zone to the diameter of colony (1.47) was for *Azotobacter* sp. which was followed by *P. putida* P13 and *P. fluorescens* Tabriz ratios of 1.40 and 1.39, respectively. A strong positive correlation (r = 0.76; *P value*< 0.01) was found between this ratio in solid medium and soluble-P concentration in liquid medium.

The solubilization of TCP in Aleksandrov liquid medium by different strains was accompanied by a significant drop in pH to 4.4 from an initial pH of 6.8–7.0 after one week. The soluble-P concentration in the medium, ranged between 67.2 and 348.8 mg l^{-1} with

variations among different bacterial strains (Fig. 1). In the control treatment 71.5 mg l^{-1} soluble-P was detected as well and no drop in pH was observed. The greatest P solubilization was recorded by *P. putida* P13 (348.4 mg/l) followed by *Azotobacter* sp., *P. putida* Tabriz and *P. fluorescens* Tabriz respectively. Among the isolates, the lowest concentration of soluble-P (67.2 mg/l) was observed in the cultures of *Bacillus megaterium* JK3. Statistically a strong negative correlation (r = -0.77; *P value*< 0.001) between pH and soluble-P concentration was observed (Table 4). Results showed that there is no difference between other strains (*Pantoea agglomerans* P5, *P. fluorescens* Chao and *Bacillus megaterium* JK3) and unioculated liquid medium of Aleksandrov.

K releasing under soluble or insoluble source of P: Potassium release was significantly (*P value*< 0.001) influenced by bacterial strains, type of K-containing mineral and P sources. The effect of seven strains on biotite and muscovite (as the sole K source) dissolution in Aleksandrov medium is shown in Table 2 and Table 3. The experiments indicated that there was considerable enhancement of K release in the bacterial treatment in the culture fluid with minerals as the sole K source.

The influence of P source (TCP or sodium phosphate) on releasing K is shown in Table 2. It can be seen that when TCP was added to the medium, the content of K in solutions inoculated with the bacterial strains were superior than those media contained sodium phosphate. In presence of TCP the highest released K is obtained by *P. putida* P13 (8.25 mg/g), which is 4.87 mg/g in the presence of sodium phosphate. In this condition, strain P13 compared with control increased content of K in solution by 12.8 and 62.5%, respectively (Table 2).

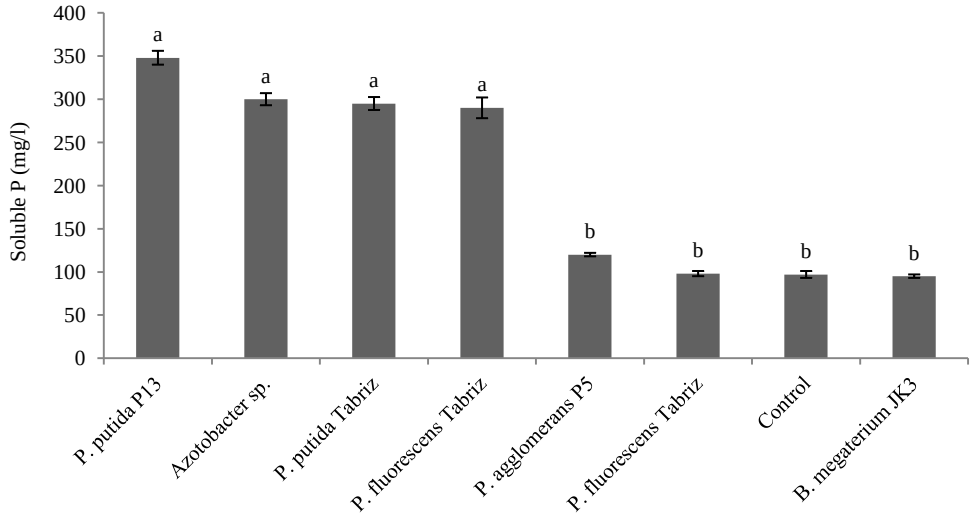

Fig. 1- Effect of bacterial strains on solubilizing of P from tricalcium phosphate (*Pseudomonas putida* P13, *P. putida* Tabriz, *P. fluorescens* Tabriz, *P. fluorescens* Chao, *Pantoea agglomerans* P5, *Azotobacter* sp., *Bacillus megaterium* JK3).

Table 2- Effect of source of phosphate (soluble or insoluble) on releasing K by bacterial strains (*P value*< 0.001).

Strains	$Ca_3(PO_4)_2$	Na_2HPO_4
	released K (mg/g)	
Pantoea agglomerans P5	5.750 ± 0.92 de	4.562 ± 0.67 efg
Pseudomonas putida P13	8.250 ± 0.95 a	4.875 ± 1.02 defg
P. fluorescens Chao	5.500 ± 0.62 de	3.625 ± 0.80 gh
P. putida Tabriz	5.312 ± 1.20 def	4.062 ± 0.75 fgh
P. fluorescens Tabriz	7.437 ± 1.37 ab	4.000 ± 1.12 fgh
Azotobacter sp.	6.000 ± 1.12 cd	3.875 ± 0.60 gh
Bacillus megaterium JK3	6.062 ± 0.75 bcd	3.875 ± 0.60 gh
Control (unioculated)	7.312 ± 1.07 bc	3.000 ± 0.45 h

Table 3- Effect of type of Micas on releasing K by bacterial strains (*P value* < 0.05).

Strains	Muscovite	Biotite
	released K (mg/g)	
Pantoea agglomerans P5	4.187 ± 0.37 ef	6.125 ± 0.65 bc
Pseudomonas putida P13	5.375 ± 1.42 cde	7.750 ± 1.35 a
P. fluorescens Chao	3.687 ± 0.85 f	5.437 ± 0.67 cd
P. putida Tabriz	3.500 ± 0.37 f	5.875 ± 0.72 c
P. fluorescens Tabriz	4.187 ± 1.27 ef	7.250 ± 1.52 ab
Azotobacter sp.	3.875 ± 0.65 f	6.000 ± 1.12 c
Bacillus megaterium JK3	4.187 ± 0.85 ef	5.750 ± 0.97 c
Control (unioculated)	4.437 ± 1.65 def	5.875 ± 1.92 c

Table 4- Correlation coefficient between P and K solubility and pH and H/C.

Parameters	P Solubility	K Solubility
Halo zone/Colony Diameter	0.76^{**}	0.62^{**}
pH	-0.77^{**}	-0.22^{ns}

** Significant at the 1% level, * significant at the 5% level, ^{ns} non-significant

Table 3 shows that amount of released K in liquid by strains, is affected by the type of micas. When biotite was added as a sole source of K in Aleksandrov liquid medium, released K values were higher. The highest value was obtained for *P. putida* P13 (7.75 mg/g) an increase around 31.9% in comparison to the control. The released K in the culture liquid with biotite was 1.44 times as much as the culture liquid with muscovite for P13 strain.

Results showed a positive correlation (r= 0.62; *P value*< 0.01) between growth of strains in solid Aleksandrov media (the ratio of clear zone/colony diameter) and K release in liquid media statistically. Furthermore, there was a weak correlation between pH decreasing and released K (r= -0.22; *P value*< 0.07) in liquid medium (Table 4).

Discussion and conclusion

At first, the ability of 7 different bacterial strains were checked in solid and liquid Aleksandrov medium containing muscovite (0.2%) and TCP (0.2%) as sole sources of K and P respectively. It was found good correlation (r = 0.76) between clear zone produced by grown bacteria in solid media and amount of solubilized P in liquid media. Released P from TCP in liquid media negatively correlated with

reduction of pH in assay medium (r= -0.77). Jiqiang and coworkers (17) showed that P and K releasing ability were significantly different among the potassium releasing bacteria (PRB). They concluded that 22% of PRB (40 isolated bacteria form purple soils) had the ability to release both P and K minerals while 29% of the strains had P releasing ability only. They noted that P solubilizing and K releasing capability were positively related to decreasing of pH value during culture.

It is generally accepted that the mechanism of mineral phosphate solubilization by PSB strains is associated with the release of low molecular weight organic acids (18), which through their hydroxyl and carboxyl groups chelate the cations bound to phosphate, thereby converting it into soluble forms (19). Nevertheless, acidification does not seem to be the only mechanism of solubilization, as the ability to reduce the pH in some cases did not correlate with the ability to solubilize mineral phosphates (20). However, P-solubilization is a complex phenomenon, which depends on many factors such as nutritional, physiological and growth conditions of the culture (21). But in this experiment, evidence was found to support the role of pH decreasing in mineral phosphate solubilization. Grudev (22) conducted an experiment which indicated that K can be released from silicate minerals and he proposed that the formation of mucilaginous capsules consisting of exopolysaccharides by the bacteria enhanced mineral dissolution. Vainberg et al. (23) also proposed that dissolution of minerals was caused by the formation of organic acids in the culture media. However, there is a small number of experimental evidence to support these hypotheses.

Potassium release from the minerals was obviously affected by pH, optical density of bacterial culture (OD), types of minerals and the strains used. In this experiment K release was influenced by the source of P in the medium. When TCP was used as a source of P, the amount of released K in solution was 1.62 times higher than that of sodium phosphate. The mechanism of potassium released from minerals, is still not clear. May be production of bacterial acids or chelants are agents to release K from potassium containing minerals. However, releasing potassium from K-bearing minerals was enhanced by plant root under P deficiency (15). Wang and coworkers (15) reported that P-starvation led to a significant increase in K concentration in ryegrass, maize and pak-choi, when the crops grew in feldspar and gneiss. The enhanced mobilization of mineral K might be attributed to the release of organic acids from the plant roots. In our experiment may be the same mechanism (production of organic acids) has the role in releasing K from micas under P deficiency when TCP as an insoluble source of P was added in Aleksandrov liquid medium.

Soil potassium can be released by PRB from silicate minerals. The results showed that bacteria have different potassium releasing ability for two types of mica. Among the bacterial strains maximum value was for *P. putida* P13. To have a

comparison with other bacteria, *B. mucilaginosus* is a well-known bacterium from point of K releasing. *Bacillus mucilaginosus* has been applied as a biological K fertilizer in some countries for many years and there have been some reports that the bacterium can dissolve K from soil or minerals (2, 4, 24- 25). The released K from mica after inoculation of *B. mucilaginosus* in liquid Medium A was reported 44.5 mg/l in comparison with that of control 26.8 mg/l by Liu et al. (4).

The mechanism by which *B. mucilaginosus* releases K or other elements from silicate minerals are complicated. Yakhontova et al. (26) proposed that the intensity of degradation of silicate minerals by the bacterium depended on the structure and chemical composition of the mineral. Liu et al. (4) indicated that leaching K^+ and SiO_2 from silicate minerals by *B. mucilaginosus* occurs as a result of participation of both exopolysaccharides and organic acids.

Bertsch et al. (27) reported that most of potassium in soil exists in the form of silicate minerals. The potassium can be available to plant when the minerals are slowly weathered or solubilized. Potassium and other minerals are released with different speeds from the silicate minerals. Due to high content of mica minerals in soils, these kind of minerals were used in this study and results showed that potassium in the biotite was most easily released than that of muscovite. The discrepancy between two minerals may be due to the differences in their crystal lattices, the structure and chemical composition of the mineral (26).

According to the results, among bacterial strains *P. putida* P13 was the most efficient in releasing K from micas particularly from biotite. Based on the results, releasing K from K-containing minerals was affected by the source of phosphate in liquid medium when TCP as an insoluble source of P was added to the medium released K in liquid phase increased in comparison soluble source of phosphate. It seems that the same mechanism has the function in releasing P and K from TCP and Micas, respectively.

It is a first report of potassium releasing ability of *P.putida* P13, which is known as PSB and is used as a biofertilizer in Iran. However, more experiments need to be done especially in pot and field experiments to study the role of this strain in K nutrition of crops.

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افزایش رهاسازی پتاسیم از کانی‌های پتاسیم‌دار در حضور فسفات نامحلول توسط باکتری‌ها

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چکیده

مقدمه: فسفر و پتاسیم عناصر غذایی ضروری پرمصرف برای رشد و توسعه سیستم‌های زنده محسوب می‌شوند. کاربرد و استفاده ریزجانداران خاک یکی از راه‌های افزایش تأمین این عناصر و رشد محصول هستند. برخی از باکتری‌ها در رهاسازی پتاسیم و انحلال فسفر از منابع معدنی کارایی لازم را دارا هستند اما رفتار آن‌ها در حضور هم‌زمان این دو منبع کمتر بررسی و مطالعه شده است.

مواد و روش‌ها: در پژوهش حاضر، توانایی ۷ سویه باکتریایی شامل: *Pseudomonas putida* P13، *Pantoea agglomerans* P5، *P. fluorescens* Chao، *P. fluorescens* Tabriz، *P. putida* Tabriz، *Azotobacter* sp. و *Bacillus megaterium* JK3 در رهاسازی پتاسیم از منابع کانی‌های پتاسیم‌دار موسکوویت و بیوتیت در حضور منابع فسفر نامحلول (تری‌کلسیم فسفات) و محلول (دی‌سدیم فسفات) ارزیابی شد. برای تهیه کشت شبانه باکتری‌ها از محیط NB برای تلقیح به محیط کشت الکساندروف استفاده شد. در محیط کشت الکساندروف انحلال کانی‌های سیلیکاته (موسکوویت و بیوتیت) بررسی شد. انحلال فسفر از منبع تری‌کلسیم‌فسفات توسط باکتری‌ها نیز به روش آمونیم- وانادات- مولیبدات از طریق اسپکتروفتومتری تعیین و پتاسیم آزادشده در محلول از طریق فلیم‌فتومتر اندازه‌گیری شد.

نتایج: استفاده از منبع فسفر نامحلول به طور معناداری میزان پتاسیم آزاد شده در محیط سنجش را افزایش داد (۶۶ درصد) و پتاسیم آزادشده از بیوتیت بیشتر از موسکوویت بود. در میان باکتری‌ها بیش‌ترین پتاسیم آزادشده در حضور باکتری *P. putida* P13 به دست آمد که نسبت به شاهد (۲۷ درصد) پتاسیم بیشتری آزاد کرد. مقادیر پتاسیم آزادشده توسط این سویه در حضور منابع فسفر نامحلول و محلول به ترتیب ۸/۲۵ و ۴/۸۷ میلی‌گرم بر گرم بود.

بحث و نتیجه‌گیری: استفاده فسفات نامحلول سبب شد تا آزادسازی پتاسیم از منابع کانی‌های میکا افزایش یابد. این افزایش شاید در نتیجه تولید و آزادسازی اسیدهای آلی از باکتری‌ها باشد، سازوکاری که نقش اساسی در انحلال فسفات از منابع معدنی فسفات بازی می‌کند.

واژه‌های کلیدی: فسفات نامحلول، باکتری‌های حل‌کننده پتاسیم، *سودوموناس*، پتاسیم محلول

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* نویسنده مسؤول مکاتبات

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