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Biology and Fertility of Soils
Cooperating Journal of International Society of Soil Science

ISSN 0178-2762

Biol Fertil Soils
DOI 10.1007/s00374-012-0763-5

Springer

Biol Fertil Soils
DOI 10.1007/s00374-012-0763-5

SHORT COMMUNICATION

Phosphate desorption from the surface of soil mineral particles by a phosphate-solubilizing fungus

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Received: 7 September 2012 / Revised: 14 November 2012 / Accepted: 27 November 2012

Abstract High phosphate (Pi) sorption in soils is a serious limiting factor for plant productivity and Pi fertilization efficiency, particularly in highly weathered and volcanic ash soils. In these soils, the sorbed Pi is so strongly held on the surfaces of reactive minerals that it is not available for plant root uptake. The use of phosphate-solubilizing microorganisms (PSM) capable of Pi desorption seems to be a complementary alternative in the management of these soils. The aim of this study was to evaluate the effectiveness of the soil fungus *Mortierella* sp., a known PSM, to desorb Pi from four soil minerals differing in their Pi sorption capacity. The fungus was effective in desorbing Pi from all tested minerals except from allophane, and its desorption depended on the production of oxalic acid. The effectiveness of the fungus to desorb Pi was ranked as montmorillonite > kaolinite > goethite > allophane. The quantity of desorbed Pi increased by increasing the amount of sorbed Pi. The Pi sorption capacity expressed as $P_{0.2}$ value (amount of P required to increase a solution P concentration up to 0.2 mg L^{-1}) was a good indicator of the effectiveness of *Mortierella* sp. to desorb Pi from soil minerals.

Keywords *Mortierella* · Phosphorus · Montmorillonite · Kaolinite · Goethite · Allophane

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Introduction

Phosphorus sorption is a process by which bioavailable phosphate (Pi) is removed from a soil solution and retained on the surface of soil minerals (Do Carmo Harta and Torrent 2007). Phosphate sorption is particularly strong on iron and aluminum hydrous oxides (e.g., goethite) that predominate in the highly weathered soils of the tropics (Jackman et al. 1997; Onweremadu 2007). In soils derived from volcanic materials, allophane, ferrihydrite, and goethite are responsible for the strong Pi sorption of these soils (Shoji et al. 1993; Jackman et al. 1997; Vistoso et al. 2012).

Many organic acids are effective in either reducing Pi sorption or desorbing Pi (Guppy et al. 2005), and, according to Hue (1991) and Bolan et al. (1994), their effectiveness in decreasing soil Pi sorption could be ranked as tricarboxylic > dicarboxylic > monocarboxylic acids. Organic acids such as citric, oxalic, gluconic, and malic, among others are produced by roots (Kirk et al. 1999; Radersma and Grierson 2004; Nwoke et al. 2008; Corrales et al. 2007; Li et al. 2009) and soil microorganisms (Gyaneshwar et al. 2002; Reddy et al. 2002; Welch et al. 2002; Chen et al. 2006; Pandey et al. 2006; Puente et al. 2009; Bashan et al. 2012). Therefore, soil microorganisms may desorb Pi from the surfaces of reactive particles of soils as reported by He and Zhu (1998); Hoberg et al. (2005), and He et al. (2002).

The phenomenon of Pi desorption by organic anions is widely accepted as a mechanism to enhance Pi availability in the rhizosphere, and this occurs onto soil surface by ligand exchange (Trolove et al. 2003; Sato and Comerford 2006; Bashan et al. 2012). However, the concentration of these organic acid/anions is relatively low in most soils because they can be precipitated with free ions (e.g., Al^{3+}, Fe^{3+}, Ca^{2+}), sorbed on the surface of soil minerals, and/or used as carbon sources by soil microorganisms (Jones et al. 2003). Thus, competent rhizosphere microflora capable of

Published online: 15 December 2012

producing organic acids or anions may play an important role in the management of Pi deficiency in high Pi-sorbing soils. He et al. (2002) found that a bacterium identified as *Moraxella* sp. was capable of desorbing Pi from the surface of goethite, montmorillonite, and kaolinite.

The hypothesis tested was that the effectiveness of a phosphate-solubilizing microorganism (PSM) may desorb Pi from the surface of soil mineral particles, but such Pi desorption is controlled by the Pi sorption capacity (measured as the amount of P required to increase soluble P concentration up to 0.2 mg L^{-1}) and the extent of saturation of Pi sorption sites in these mineral particles. The objective of this study was to determine the effectiveness of the fungus *Mortierella* sp., a known phosphate-solubilizing fungus, in desorbing Pi from the surfaces of soil reactive particles (allophane, goethite, kaolinite, and montmorillonite) widely differing in their Pi sorption capacity.

Material and methods

Fungal inoculum

The fungus *Mortierella* sp. is a known effective PSM that was originally isolated from an Andisol of Hawaii (Osorio and Habte 2001) and has been cultured on yeast mannitol agar (YMA) medium and stored on YMA slants at 4 °C. For this study, the fungus was multiplied in Petri dishes on YMA medium for 3 days at 28 °C. Then its mycelia were removed with a sterile loop and suspended in sterile deionized water and shaken by hand until the clumps were dispersed. The fungal suspension contained 4×10^5 colony-forming units of *Mortierella* sp. per milliliter.

Soil minerals

Samples of four standard minerals (allophane, goethite, kaolinite, and montmorillonite) were provided by Dr. Rollin Jones from the Soil Mineralogy Lab of the University of Hawaii. The sample of allophane was originally collected in Hawaii; the sample of goethite was obtained from Biwabik, Minnesota; the sample of kaolinite was collected from Bath, SC (Ward's Natural Science Establishment Inc., Rochester, NY); and the sample of Montmorillonite was collected from Columbia, MO (Department of Geology, University of Missouri). These minerals were used because they are commonly found in soils and exhibit a wide range of P sorption capacity. For instance, allophane is predominant in volcanic ash soils (Andisols); goethite and kaolinite are commonly found in highly weathered soils (Ultisols and Oxisols); and montmorillonite is predominant in Vertisols and Mollisols.

Preliminary Pi sorption isotherms

The samples of the minerals were passed through a 0.5-mm aperture sieve. Then, Pi sorption isotherms were constructed in triplicates following the procedure developed by Fox and Kamprath (1970). Briefly, aliquots of soil minerals (2 g, dry basis) were transferred into 50-mL plastic centrifuge tubes, and then 20-mL of 0.01 M $CaCl_2$ solution and graded amounts of dissolved KH_2PO_4 were added. To avoid microbial activity, particularly Pi uptake, the tubes and their contents were autoclaved (120 °C, 0.1 MPa for 30 min). Later, centrifuge tubes and their contents were horizontally shaken (twice a day, 30 min each time) in a reciprocal shaker for 7 days to reach equilibrium of Pi concentration. After this incubation, soil mineral suspension were centrifuged at $2{,}500\times g$ for 15 min, and the supernatant was filtered through a Whatman No. 42 filter paper followed by filtration through a Millipore membrane filter (0.45 μm). Solution Pi concentration in the filtrates was determined using the molybdate-blue method (Murphy and Riley 1962).

Microbial Pi desorption experiments

Based on the Pi sorption isotherms, amounts of KH_2PO_4 in 0.01 M $CaCl_2$ solution were mixed with 2 g of each mineral sample in 50-mL centrifuge tubes to reach the following solution P concentrations: 0.05, 0.1, and 0.2 mg/L (Table 1). Suspension pH was adjusted to 6.0 with 0.1 M NaOH. The tubes were shaken as explained above. Then, the solution was removed by centrifugation at $2{,}500\times g$, and the minerals were washed three times with deionized water. The Pi remaining on the soil minerals was considered as adsorbed. After that, the minerals were oven-dried at 60 °C for 2 days.

At the end of the drying treatment, each tube received 19 mL of a nutrient solution containing 1.0 g NaCl, 0.2 g $CaCl_2\ 2H_2O$, 0.4 g $MgSO_4\ 7H_2O$, 1.0 g NH_4NO_3, and 10.0 g glucose per liter. The sole source of P was the Pi adsorbed on the surfaces of the minerals. Then, the tubes and their contents were autoclaved (120 °C, 30 min, 0.1 MPa). *Mortierella* sp., at the concentration mentioned

Table 1 Amount of P added (in milligrams per liter) to soil minerals to achieve four target solution P concentrations

Initial solution P (mg L^{-1})	Soil minerals			
	Montmorillonite	Kaolinite	Goethite	Allophane
0.002–0.006	0 (0.002)	0 (0.003)	0 (0.006)	0 (0.002)[a]
0.05	238	26	143	3,009
0.1	289	51	183	3,578
0.2	340	94	224	4,146

[a] Values in parentheses are initial solution P (in milligrams per liter) found in soil minerals and soils without KH_2PO_4 addition

Table 2 Significant *P* values of ANOVA tests for solution Pi concentration as a function of the initial P level and the inoculation with the fungus *Mortierella* sp. in four soil minerals

Source	Montmorillonite	Goethite	Kaolinite	Allophane
Initial P level (A)	<0.0001	<0.0001	<0.0001	<0.0001
Inoculation (B)	<0.0001	<0.0001	<0.0001	NS
A × B	<0.0001	<0.0001	<0.0001	NS
CV (%)	10.7	15.9	11.5	10.7

CV coefficient of variation, *NS* not significant

above, was aseptically transferred into the tubes at the rate of 1 mL per Erlenmeyer. Tubes not inoculated with the fungus received 19 mL of the nutrient solution and 1 mL of sterilized deionized water. The tubes were continuously shaken on a reciprocal shaker (at 100 cycles per minute) for 6 days at 25 °C.

At the end of the incubation period, the suspensions were centrifuged at 2,500×*g* for 15 min, and the supernatant was filtered through a Whatman no. 42 filter paper followed by filtration through a Millipore membrane filter (0.45 μm). Solution Pi concentration in the filtrates was determined using the molybdate-blue method (Murphy and Riley 1962). The presence of Pi in the solution was considered an evidence of Pi desorption. The effectiveness of *Mortierella* sp. to desorb Pi was calculated by subtracting Pi desorbed measured in the absence of the fungus from that measured in its presence; this difference was designated as microbially desorbed Pi. The microbial Pi desorption efficiency was calculated based on this difference over the amount of P sorbed. On the other hand, an aliquot of 100 μL from the serial dilution 10^{-2} (prepared from the pellets) was aseptically transferred onto a selective YMA medium that contained streptomycin sulfate (500 μg L^{-1}), benomyl (75 μg L^{-1}), and cycloheximide (100 μg L^{-1}). The fungus tolerates both fungicides at these concentrations (Osorio 2008). Then, Petri dishes were incubated at 28 °C for 4 days, and colony-forming units (CFU) were counted.

Experimental design and data analysis

Each mineral was used in a separate experiment. The treatments consisted of a factorial combination (4×2) of four levels of sorbed Pi (corresponding to four solution Pi concentrations) and two levels of inoculation (with or without

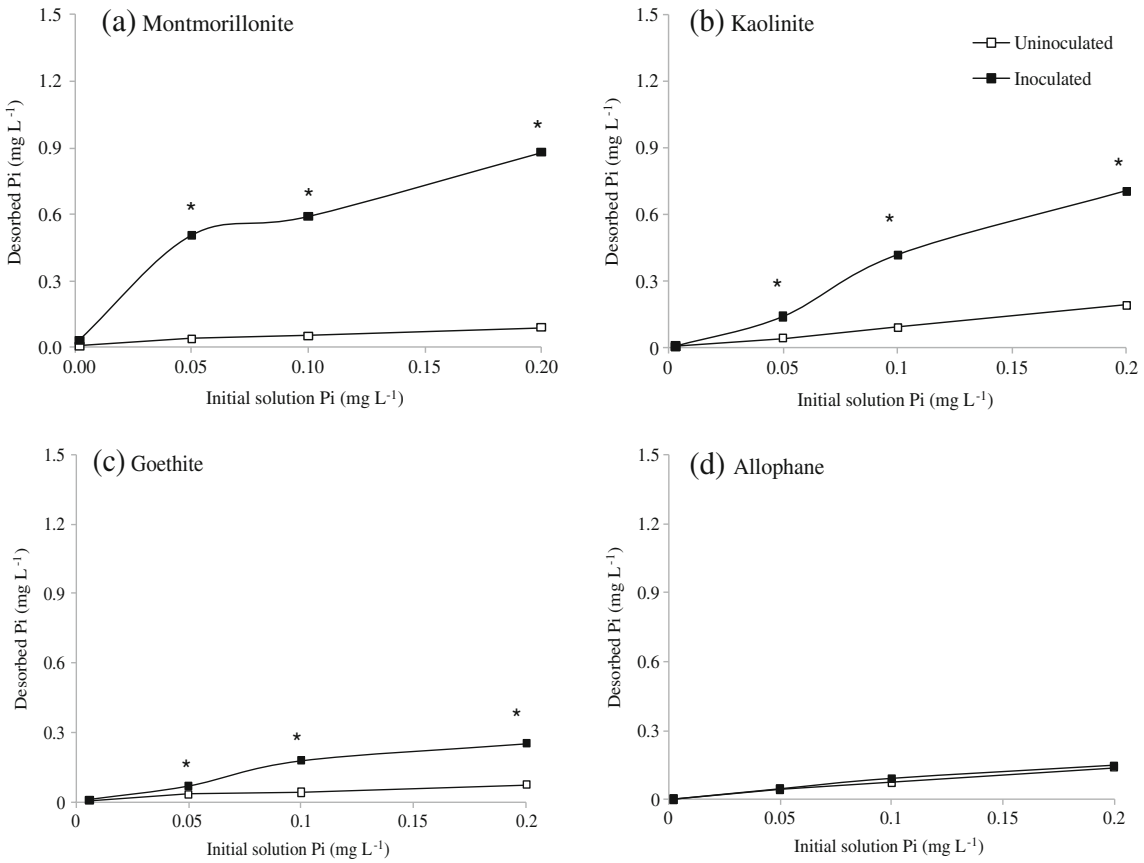

Fig. 1 Concentration of P in solution (in milligrams per liter) desorbed from four soil minerals as a function of four initial solution P concentrations and inoculation with *Mortierella* sp. (*black squares*, inoculated; *white squares*, uninoculated). The *asterisk* means that the two data at a given initial P level are significantly different (LSD test, $P \leq 0.05$)

Mortierella sp.). Treatments were arranged in completely randomized design with three replicates per treatment. Data were subjected to analysis of variance (ANOVA), least significant difference (LSD) tests, regression, and correlation (*P* value of 0.05) to detect treatment effects. For this purpose, the software Statgraphics Plus version 4.0 (Statpoint, Inc.; Herdon, Virginia) was used.

Results and discussion

In all minerals, except in the allophane, *Mortierella* sp. was capable of increasing solution P concentration significantly (Table 2). However, the effect of the fungus on the concentration of Pi in the solution depended on the amount of Pi initially sorbed on the surface of minerals (Fig. 1a–d).

In the case of allophane, *Mortierella* sp. did not increase solution Pi at any level of sorbed Pi (Fig. 1a). The very high Pi-sorbing capacity of the mineral was apparently responsible for the ineffectiveness of the fungus to desorb Pi. In contrast, *Mortierella* sp. inoculation increased Pi concentration in solution in all other tested soil minerals, except when Pi was not added to them prior to incubation (Fig 1b–d). The magnitude of Pi desorption by *Mortierella* sp. increased by increasing the amount of sorbed Pi (higher saturation of Pi-sorbing sites).

The effectiveness of *Mortierella* sp. in desorbing Pi from the soil minerals followed the order montmorillonite > kaolinite > goethite > allophane.

The capacity of *Mortierella* sp. to desorb sorbed Pi was demonstrated in three of the four tested minerals (Fig. 1). Generally, the effectiveness of the fungus to desorb Pi depended on the Pi-adsorbing capacity of minerals, which can be expressed as the $P_{0.2}$ value (the amount of P required to increase soluble P concentration up to 0.2 mg L^{-1}). The capacity of *Mortierella* sp. to desorb Pi is correlated (r= 0.87–0.98) with the initial level of soluble Pi (as an indicator of the amount of Pi adsorbed on the mineral surface), and it is inversely associated with the Pi sorption capacity of the mineral (Table 3). Therefore, the fungus was ineffective to desorb Pi from the surface of allophane, and probably it may also be ineffective in soils whose mineralogy is dominated by allophane (Andisols).

The capacity of *Mortierella* sp. in releasing Pi from soil minerals can be useful for the fungus as well as other

Table 3 Desorption of P from four soil minerals and microbial P desorption efficiency as a function of initial P concentration, P adsorbed, and P sorption saturation level

Mineral	Initial soluble P (mg L^{-1})	P adsorbed (mg kg^{-1})	P sorption saturation[a] (%)	P desorbed Uninoculated (mg kg^{-1})	P desorbed Inoculated (mg kg^{-1})	Microbial P-desorbed[b] (mg kg^{-1})	Microbial P desorption efficiency[c] (%)
Montmorillonite	0.003	–	–	0.06	0.32	0.26	–
	0.05	238	61.9	0.38	5.1	4.72	1.98
	0.1	289	75.1	0.52	5.9	5.38	1.86
	0.2	340	88.4	0.89	8.8	7.91	2.33
	Microbial P desorbed (mg kg^{-1})=1.4913+34.7863 (initial soluble Pi) [r=0.87; P value<0.001]						
Kaolinite	0.002	–	–	0.04	0.07	0.03	–
	0.05	26	6.8	0.43	1.40	0.97	3.73
	0.1	51	13.3	0.91	4.17	3.26	6.40
	0.2	94	24.4	1.92	7.04	5.12	5.45
	Microbial P desorbed (mg kg^{-1})=0.0009+26.641 (initial soluble Pi) [r=0.98; P value<0.001]						
Goethite	0.006	–	–	0.07	0.34	0.27	–
	0.05	143	37.2	0.34	0.67	0.33	0.23
	0.1	183	47.6	0.41	1.78	1.37	0.75
	0.2	224	58.2	0.74	2.53	1.79	0.80
	Microbial P desorbed (mg kg^{-1})=0.0701+9.2899 (initial soluble Pi) [r=0.92; P value<0.001]						
Allophane	0.002	–	–	0.016	0.022	0.006	–
	0.05	3009	60.2	0.45	0.47	0.02	0.001
	0.1	3578	71.6	0.74	0.92	0.18	0.005
	0.2	4146	82.9	1.40	1.49	0.09	0.002
	Microbial P desorbed was not significant in allophane						

[a] P sorption saturation = (P adsorbed/maximal P sorption capacity) × 100

[b] Microbial P desorbed = P desorbed in inoculated treatment − P desorbed in uninoculated treatment

[c] Microbial P desorption efficiency = (microbial P desorbed/P adsorbed) × 100

organisms, including plants and mycorrhizae. This phenomenon may, in part, explain the synergistic interaction of this fungus and the mycorrhizal fungus *Glomus aggregatum* to stimulate Pi uptake and growth of *Leucaena leucocephala* in a highly weathered Oxisol of Hawaii with moderate Pi-sorbing capacity (Osorio and Habte 2001).

The increasing Pi desorption of *Mortierella* sp. by increasing Pi concentration on the mineral may depend on the fact that the strength with which Pi is adsorbed on the mineral surfaces decreases by increasing the saturation of the adsorptive surfaces with Pi (Do Carmo Harta and Torrent 2007). The same probably occurred in several studies conducted with PSM in soils presumably dominated by kaolinite and montmorillonite (Asea et al. 1988; Kucey 1988; Kucey and Leggett 1989; Omar 1998; Peix et al. 2001).

Probably *Mortierella* sp. desorbed Pi by releasing oxalic acid/oxalate (Osorio and Habte 2009; Osorio 2011). It is very well known that organic acids/anions can desorb Pi from sorbing sites (Welch et al. 2002; Jones et al. 2003; Trolove et al. 2003; Ramirez and Osorio 2005; Jara et al. 2006; Sato and Comerford 2006), and their effects in highly weathered soils and in volcanic ash soils containing comparable minerals to those used in the current study have been studied by Hue (1991), Bolan et al. (1994), and Sato and Comerford (2006). The ability of microorganisms to produce organic acids/anions occurs in the rhizosphere whereby carbonaceous compounds are released by roots (Amos and Walters 2006; Le Bayon et al. 2006; Marschner et al. 2006; Corrales et al. 2007; Zhang et al. 2011). Once the root exudates are released, they are metabolized by rhizosphere microorganisms leading to the production of organic acids/anions (Gyaneshwar et al. 2002). Although this excretion appears to be genetically determined (Rodriguez et al. 2000, 2006), environmental conditions such as Pi deficiency or high ammonium concentration could trigger or enhance the production of organic acid/anions (Whitelaw 2000; Chen et al. 2006; Vyas et al. 2007).

The efficiency of *Mortierella* sp. to desorb Pi depended on the mineral and the initial P concentration (Table 3). The highest efficiency in desorbing P was detected in kaolinite (3.73–5.45 %), followed by montomorillonite (1.86–2.33 %) and then by goethite (0.23–0.8 %). In general, this efficiency increased with the increase of the amount of P sorbed. This tendency is comparable with that reported by He et al. (2002). On the other hand, in allophane, the P desorption efficiency was extremely low (0.001–0.005 %), which is consistent with the strong P sorption of this soil mineral.

Counts of the fungus were detected only when minerals were inoculated; the lowest counts were found when Pi was not added ($3.0–4.0\times10^3$CFU g^{-1} of mineral), likely due to the low available Pi (0.002–0.006 mg L^{-1}) that limited the fungal growth. The two highest level of initial soluble Pi (0.1 and 0.2 mg L^{-1}) produced the highest counts. This effect was higher in montmorillonite and kaolinite ($8.8–18.4\times10^3$CFU g^{-1}) than in goethite and allophane ($4.9–7.6\times10^3$CFU g^{-1}). The number of CFU were correlated with the initial soluble Pi concentration (r=0.67, P value= 0.004) and, particularly, with the final Pi concentrations (r=0.96, P<0.0001).

The results presented here indicate the potential effectiveness of *Mortierella* sp. in increasing soil Pi bioavailability by desorbing it from surfaces of soil minerals. We have also established the effectiveness of *Mortierella* sp. in dissolving Pi from rock phosphate (Osorio and Habte 2001, 2009). The effectiveness of this fungus in increasing soil solution Pi by dissolving rock phosphate and desorbing sorbed Pi may play an important role in Pi-deficient soils, particularly in the tropics where the high Pi sorption capacity of soils constrains plant productivity.

Conclusions

This paper showed that (1) the fungus *Mortierella* sp. was effective in desorbing Pi adsorbed on the surface of soil minerals; (2) the effectiveness of the fungus in doing so depended on the Pi sorption capacity on the soil mineral, and this followed the order montmorillonite > kaolinite > goethite > allophane; and (3) the efficiency of the fungus in desorbing Pi increased by increasing the amount of Pi adsorbed on the surface of the mineral.

Acknowledgments We want to thank COLCIENCIAS for the financial support provided to N.W. Osorio during his stay at the University of Hawaii.

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