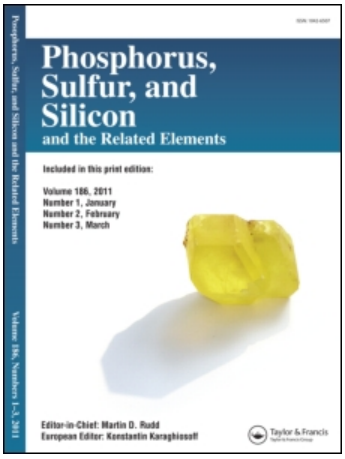

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MOBILIZATION OF P AND CD FROM ROCK PHOSPHATES BY RHIZOSPHERIC MICROORGANISMS (PHOSPHATE-DISSOLVING BACTERIA AND ECTOMYCORRHIZAL FUNGI)

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Abstract Some rock phosphates used as fertilizers or to produce fertilizers contain cadmium and have been suspected to increase trace element content in soils. The bioavailability (immobilization, solubilization) of Cd and P from three rock phosphates (from West Africa and East USA) has been investigated, with phosphate-dissolving microorganisms (two ectomycorrhizal fungi *P. tinctorius* and *S. granulatus* and a bacterium *Agrobacterium* sp.) in pure culture and in pine (*Pinus sylvestris* L.) rhizosphere. In pure culture the fungi accumulated more Cd than the bacterium. In pine rhizosphere, the uptake of P and Cd by the plant increased with bacterial inoculation. The bioavailability of Cd from the different rock phosphates was less related to their Cd content than to their cristallinity and solubility, and was relatively higher than P one.

INTRODUCTION

All plant roots are associated with microorganisms using root exudates as source of carbon and energy. Most of them are also associated with symbiotic fungi to form mycorrhizas which enhance the uptake of phosphorus by the host plant[1,2]. A great number of soil microorganisms, bacteria and fungi, have been reported as solubilizers of water-insoluble inorganic phosphates. Ectomycorrhizal fungi have also shown their ability to solubilize insoluble mineral phosphates[3,4]. Some rock phosphates used as fertilizers or to produce fertilizers contain cadmium and have been suspected to increase trace element content in soils. We have investigated the bioavailability (immobilization, solubilization) of Cd and P from these rock phosphates with phosphate-dissolving microorganisms. The comparative ability of bacterial and (ectomycorrhizal) fungal strains to mobilize P and Cd from different rock phosphates have been tested in pure culture and in plant rhizosphere.

MATERIAL AND METHODS

A phosphate-dissolving bacterium has been isolated from a pine rhizosphere on agar plate containing tricalcium phosphate and identified as *Agrobacterium* sp.. A 24 hour-old culture on nutrient broth (DIFCO) was centrifuged and rinsed twice in distilled sterile water. A suspension of this culture was added as inoculum to a flask containing 1.2 l of nutrient medium and 20 g of rock phosphate in dialysis bags. The ectomycorrhizal fungi *Pisolithus tinctorius* (Pers.) Coker and Couch and *Suillus granulatus* (Garbaye, INRA, Nancy Champenoux, France) were grown in flasks in liquid Pachlewski medium supplemented with 1 g of rock phosphate in a dialysis bag. Detailed growth conditions and inoculations were previously described[4]. Three replicates were performed for each treatment and three controls were kept uninoculated. The flasks were incubated in the dark at 24°C for 4 weeks.

Three rock phosphates from different origins and with different chemical compositions and surface properties were used (Table 1).

Pine seeds were disinfected with 30% H_2O_2 for 30 minutes. Young germinated seedlings were transfered to glass containers and were inoculated or not with a bacterial suspension (1 ml containing 10^7 bacterial cells / container). Then they were grown in axenic conditions for 3 weeks in liquid nutrient medium. P and Cd were determined in the solutions (P and Cd solubilization) and, after acid digestion (65% nitric acid), in bacterial, fungal and plant biomass (P and Cd immobilization) using ICP or ICP-MS. Mobilization of P or Cd is defined as the total quantity of the element released from the phosphates (solubilized in the medium + immobilized in the biomass).

TABLE 1: Phosphorus, cadmium content and surface properties of the rock phosphates (particle size : 100 to 250 µm)

	East USA	West Africa A	West Africa B
P_2O_5 (%)	29.4	35.5	36.4
P_2O_5 formic*	72.8	28.3	19.7
P_2O_5 citric**	70 à 85	30 à33	32
Cd (ppm)	37.5	93.7	53.7
Cd/P	$2.9\ 10^{-4}$	$6.0\ 10^{-4}$	$3.4\ 10^{-4}$
Specific area (m^2/g)	22.5	6.5	14.3

*: AFNOR method, NFU 42-201
**: ASC=absolute citric solubility

RESULTS

Mobilization of P and Cd by microorganisms in pure culture:
Agrobacterium sp. in pure culture released 0.8 to 1 % of phosphorus and 3 to 14 % of cadmium from the three rock phosphates after 15 days. The amount

of solubilized phosphorus but especially of cadmium was higher with the rock phosphate from East USA, although its content in Cd was lower, than with the rock phosphates from W. Africa (Fig. 1). The bioavailability of P and Cd varied with the microorganisms. The mobilization (total release) of P from the rock phosphates by the fungus was about the same than with the bacteria but the fungi accumulated Cd (8,9 and 28,1 µg/g dry weight after 15 days) much more than the bacteria (3,6 µg/g d.w).

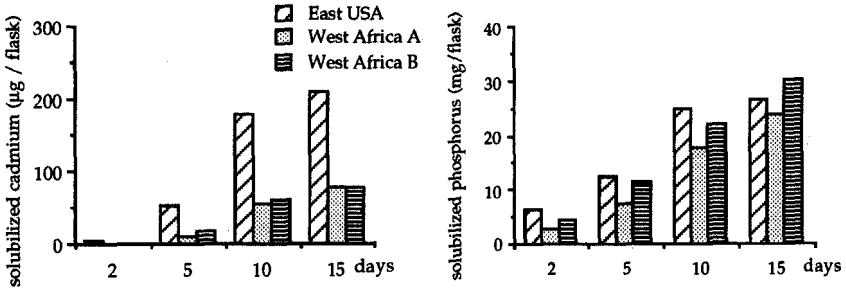

FIGURE 1 Solubilization of P and Cd from three rock phosphates by *Agrobacterium* sp.

The pH decreased very rapidly in these pure culture assays (Fig. 2). *Agrobacterium* sp. released citric acid in the medium in presence of the three phosphates. The quantity of mobilized Cd was well correlated with the citric acid production (Fig. 2)(r^2=0.91).

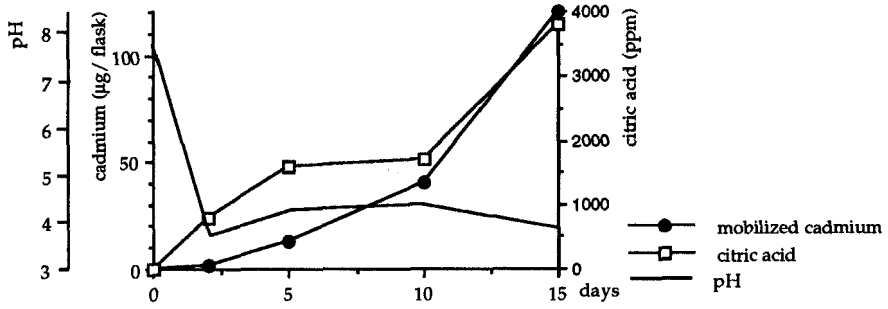

FIGURE 2 Acidification of the medium, release of citric acid and mobilization of cadmium from East USA rock phosphate by *Agrobacterium* sp.

Mobilization of P and Cd from rock phosphates in pine rhizosphere:
In pine rhizosphere, *Agrobacterium* sp. increased plant growth, and P and Cd uptake (Table 2). No decrease in pH was observed in that case. The uptake of Cd by non-inoculated plant roots and furthermore by roots

inoculated with the bacteria was larger with East USA rock phosphate than with the W. Africa one.

TABLE 2 Influence of bacterial inoculation (*Agrobacterium* sp.) on pine (*Pinus sylvestris* L) growth and immobilization of P and Cd from rock phosphates.

rock phosphate origin	East USA		West Africa 1	
	sterile plant	plant +bacteria	sterile plant	plant +bacteria
pH	7.6	7.7	7.7	7.6
plant biomass (mg d.w. /container)	91	212	71	162
P (mg/g) in the plant	0.3	1.2	0.1	1.8
Cd (µg/g) in the plant	2.3	4.8	0.4	0.6

DISCUSSION AND CONCLUSION

The bioavailability of P and Cd from rock phosphates varies with the microorganisms and with the rock phosphates. With the phosphate-dissolving bacterium mobilization of P and Cd could be related in these experimental conditions to the production of citric acid. In pine rhizosphere, the uptake of P and Cd by the plant increased with bacterial inoculation but the pH was not modified and other mechanisms to be further studied are involved. With the ectomycorrhizal fungi, it is interesting to notice that cadmium accumulation is higher than with the bacterium, which could suggest some specific binding mechanism or a better biosorption and/or bioaccumulation ability by fungal constituants, that will be further investigated. The bioavailability of Cd was less related to its content in the phosphates than to their cristalinity and solubility. East USA rock phosphate, which has a higher citric and formic solubility, was more solubilized by the microorganisms than the other ones. Comparing the P and Cd contents in the rock phosphates, Cd seemed to have a higher relative bioavailability than P.

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