Evaluation of the P-solubilizing activity of soil microorganisms and its sensitivity to soluble phosphate

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ABSTRACT

Microbial solubilization of hardly soluble mineral phosphates in soil is an important process in natural ecosystems and in agricultural soils. Regulation of the P-solubilizing activity by the presence of soluble phosphates in medium was determined. For this reason we decided to test a number of soil bacteria showing a high P-solubilizing activity for its sensitivity to the presence of soluble dihydrogen potassium phosphate in medium. At these studies, the direct determination of the solubilized phosphate in medium was masked by the presence of relatively high concentrations of soluble phosphate added. Therefore, we have modified the method, determining the residual tricalcium phosphate. The effect of soluble phosphate in medium on the P-solubilizing activity of rhizosphere isolates and strains of *Rhizobium* were tested in liquid cultures with the addition of various concentrations of soluble KH_2PO_4. The medium was filtered after incubation and the remaining tricalcium phosphate was separated by filtration. Filter papers with the remaining tricalcium phosphate were hydrolysed with 2N H_2SO_4. Phosphorus was determined spectrophotometrically. The P-solubilizing activity was expressed as a difference between the tricalcium phosphate added and its remainder after the incubation. These results fully confirmed that there exist the strains, whose P-solubilizing activity is inhibited and other strains, whose P-solubilizing activity is not inhibited or is inhibited very little in the presence of soluble phosphate. The use of our adapted method was much more suitable for this type of experiments.

Keywords: soil phosphorus; solubilization of phosphate; P-solubilizing microorganisms

Microbial solubilization of hardly soluble mineral phosphates in soil is an important process in natural ecosystems and in agricultural soils. In spite that soils usually contain a high amount of total phosphorus, its availability to plants is very low and it is often a limiting factor of the plant growth. In addition to traditional methods of mineral phosphate fertilization, microbial P solubilization may increase the availability of phosphates in arable soils. Besides of that, phosphorus applied to soil in mineral phosphate fertilizers is transformed (from 70–90%) to hardly available compounds and the effectivity of its uptake by plants is relatively low (Goldstein 1986, Domey 1987). Microbial P-solubilization can, therefore, improve the effectivity of mineral P fertilization.

As it was reported earlier, number of soil bacteria posses mineral phosphate solubilizing activity (Yahya and Al-Azawi 1989, Mikanová and Kubát 1994). P-solubilizing activity was also found in symbiotic nitrogenous bacteria (Mikanová and Kubát 1999). However, it was also shown that the P-solubilizing activity of microorganisms is affected by the presence of soluble phosphates in medium. Goldstein and Liu (1987) have shown that mineral phosphate solubilizing activity is genetically coded in a gene cluster on plasmids of microorganisms possessing this activity. They also transferred this gene cluster to *Escherichia coli* strain that had not shown P-solubilizing activity before and could demonstrate the transferred gene expression in the transgenic *E. coli* strain. They have also found that the gene expression and mineral phosphate solubilizing activity of bacteria is affected by the presence of soluble phosphate in medium (feed back regulation). Regulation of the P-solubilizing activity by the presence of soluble phosphates in medium was also shown in other organisms. Chhonkar and Subba-Rao (1967) determined the P-solubilizing activity of various kinds of fungi in medium containing soluble KH_2PO_4. Although the fungi showed a high P-solubilizing activity in medium without soluble phosphate, it was completely inhibited in medium containing soluble phosphate. Tiwari et al. (1989) have found a higher effect of the inoculation of plants by P-solubilizing bacteria in soils deficient in available phosphorus. Many other authors (Menge et al. 1978, Thomson et al. 1986, Amijee et al. 1989) demonstrated that the colonization of plant roots with VAM fungi was inhibited by higher mineral phosphate fertilization. In spite of many hypotheses, however, the mechanism of the colonization has not been yet sufficiently explained.

In our earlier work (Mikanová et al. 1997) we have also shown that there are differences not only in the P-solubilizing activity among the tested bacteria, but also in their sensitivity to the presence of soluble phosphates in medium. This feature is certainly important in the selection of the suitable bacteria strains for practical applications. For this reason we decided to test a number of soil bacteria showing a high P-solubilizing activity for its sensitivity to the presence of soluble dihydrogen potassium phosphate in medium. In these studies, the direct determination of the solubilised phosphate in medium was masked by the presence of relatively high concen-

Table 1. The rest of insoluble phosphorus (mg $P.l^{-1}$) in medium (with the various concentrations of soluble KH_2PO_4) after incubation

Isolate	Concentration of soluble P in medium before incubation					
	0 mmol $P.l^{-1}$	5 mmol $P.l^{-1}$	10 mmol $P.l^{-1}$	15 mmol $P.l^{-1}$	20 mmol $P.l^{-1}$	25 mmol $P.l^{-1}$
39	0	0	0	0	0	1.85
287	6.61	7.61	11.83	8.13	13.27	12.76
24	1.79	16.53	17.11	19.23	19.41	18.65
88	12.75	15.01	17.73	22.12	18.19	21.79
262	13.09	16.64	17.73	21.95	23.29	26.35
10	19.61	31.82	27.22	31.23	30.49	29.81
22	26.09	31.78	26.17	28.60	29.23	27.19
15	27.31	31.20	31.70	32.41	34.25	36.42
Control	40.00	40.00	40.00	40.00	40.00	40.00

trations of soluble phosphate added. We have, therefore, modified the method, determining the residual tricalcium phosphate. The indirect method of the P-solubilizing activity determination was shown to be more suitable in our studies.

MATERIAL AND METHODS

The liquid medium contained glucose 10.0 g, asparagin 1.0 g, K_2SO_4 0.2 g, $MgSO_4.7\,H_2O$ 0.4 g, yeast autolysate 0.2 g in 1 litre of distilled water. 30 ml of this medium was transferred into 250 ml flasks and 0.02 g $Ca_3(PO_4)_2$ was added to all of them. Analyses were performed in three replicates and average values are presented in tables. Except of the control variants, the medium was then amended with different doses of the soluble dihydrogen potassium phosphate. The flasks were then sterilised and inoculated with 1 ml suspension of the tested microorganisms and incubated for 7 days at 28°C. The medium was filtered after incubation and the remaining tricalcium phosphate was separated by filtration. Medium was poured onto the filter, rinsed with hot distilled water (in order to remove slime and soluble phosphates). Filter papers with the remaining tricalcium phosphate were dried for 15 minutes at 105°C and afterwards hydrolysed with 2N H_2SO_4 for 18 hours. The solution was filtered again and 2 ml of the filtrate was topped up to 100 ml with distilled water. Phosphorus was determined spectrophotometrically according to method described by Murphy and Riley (1962). The P-solubilizing activity was expressed as a difference between the tricalcium phosphate added and its remainder after the incubation.

RESULTS AND DISCUSSION

The effect of soluble phosphate in medium on the P-solubilizing activity of eight rhizosphere isolates from the collection of Research Institute of Crop Production was tested in liquid cultures with the addition of various concentrations of soluble KH_2PO_4 (Table 1). These isolates have shown a high P-solubilizing activity in previous tests. The concentration of phosphorus in uninoculated

Table 2. The rest of insoluble phosphorus (mg $P.l^{-1}$) in medium (with the various concentrations of soluble KH_2PO_4) after incubation by *Rhizobium leguminosarum*, *Rhizobium trifolii* a *Sinorhizobium meliloti*

Strains		Concentration of soluble P in medium before incubation					
		0 mmol.l^{-1}	5 mmol.l^{-1}	10 mmol.l^{-1}	15 mmol.l^{-1}	20 mmol.l^{-1}	25 mmol.l^{-1}
D558	*R. trifolii*	14.30	19.58	22.42	23.88	31.66	33.66
D659	*R. trifolii*	14.30	17.70	20.44	23.20	28.76	38.00
D481	*R. trifolii*	14.98	16.26	21.42	23.84	30.78	35.70
D561	*R. leguminosarum*	17.11	25.09	28.64	33.28	33.91	39.44
D663	*R. trifolii*	17.98	22.50	22.66	26.38	32.04	36.96
D661	*R. trifolii*	18.78	22.96	24.92	29.32	32.40	34.62
D600	*R. leguminosarum*	20.15	25.59	27.89	37.67	39.09	40.06
D662	*R. trifolii*	22.25	26.81	28.94	31.07	36.09	40.79
D562	*R. leguminosarum*	23.54	30.11	30.03	32.87	36.59	42.32
D163	*S. meliloti*	31.08	32.12	33.40	34.54	35.92	35.80
D4	*S. meliloti*	31.10	34.80	36.30	34.30	30.90	36.45
D55	*R. leguminosarum*	31.49	34.17	36.38	34.16	35.17	38.39
Control		40.00	40.00	40.00	40.00	40.00	40.00

control corresponds to the amount of hardly soluble calcium phosphate – 40 mg.l^{-1} added.

It can be seen from the Table 1 that all of tested isolates posses P-solubilizing activity. Six of these isolates solubilized more than half of added tricalcium phosphate. All of tested isolates (except 39) were repressed under the presence of soluble phosphate. The isolate number 39 dissolved all of added tricalcium phosphate and its P-solubilizing activity was not inhibited by added accessible phosphate. The isolate number 24 was inhibited the most markedly and it was inhibited already at the level 5 mmol P.ml^{-1}.

In submersion cultures with various levels of soluble phosphate 12 strains *Rhizobium* and *Sinorhizobium* were also tested (Table 2). From strains of *Rhizobium trifolii* (D558, D659 and D481), that showed high P-solubilizing activity (25–26 mg P were solubilized from 40 mg P added), only the activity of the strain D 558 was not completely inhibited even at concentration 25 mmol P.l^{-1}. The activity of other strains is already strongly negatively influenced at concentration 25 mmol of soluble phosphorus per liter or the solubilization is stopped completely. Five of tested strains stopped the dissolving of phosphates at the concentration 20 mmol.l^{-1}. Only the strain D600 stopped solubilization at concentration 15 mmol P.l^{-1}.

These results fully confirmed that there exist the strains, whose P-solubilizing activity is inhibited and other strains, whose P-solubilizing activity is not inhibited or is inhibited very little in the presence of soluble phosphate. The use of our adapted method was much more suitable for this type of experiments.

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ABSTRAKT

Hodnocení P-solubilizační aktivity mikroorganismů a její citlivosti na přítomnost rozpustných fosfátů

P-solubilizační aktivita půdních mikroorganismů je významnou vlastností, která může být využita pro uvolnění minerálního fosforu z půdní zásoby. Dosud používaná přímá metoda stanovení rozpuštěného fosforu z těžko rozpustných fosfátů (např. trikalcium fosfátu) v kultivačním médiu, zvláště při vyšší koncentraci rozpustných fosfátů v médiu, nebyla dostatečně citlivá. Měřená P-solubilizační aktivita, která se u testovaných mikroorganismů pohybovala řádově v desítkách mg na litr, se při vyšších dávkách rozpustného fosforečnanu pohybovala v rozmezí chyby analytického stanovení. Z toho důvodu jsme vypracovali modifikovanou metodu, pomocí níž P-solubilizační aktivita byla stanovena nepřímo, jako zbytkový nerozpuštěný fosfát v kultivačním médiu. S využitím této metody byla studována P-solubilizační aktivita půdních mikroorganismů a regulace této aktivity přítomností různé koncentrace rozpustného P v médiu. Vybrané mikroorganismy byly kultivovány v tekutých živných médiích obsahujících trikalcium fosfát a zvyšující se koncentrace rozpustného dihydrogen fosforečnanu draselného. Po inkubaci bylo médium filtrováno a zbylý nerozpustný fosfát byl hydrolyzován 2N H_2SO_4 a jeho množství bylo stanoveno spektrofotometricky. Rozdíl mezi množstvím nerozpustného P před inkubací a po inkuba-

ci byl použit jako měřítko P-solubilizační aktivity. Byly zjištěny podstatné rozdíly mezi stupněm inhibice jednotlivých mikroorganismů při různých koncentracích rozpustného fosfátu v médiu. Výsledky potvrdily vhodnost použité metody pro hodnocení P-solubilizační aktivity mikroorganismů a její citlivosti na přítomnost rozpustných fosfátů v médiu.

Klíčová slova: půdní fosfor; solubilizace fosfátů; P-solubilizující mikroorganismy

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