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SOLUBILIZATION OF ROCK PHOSPHATE BY *RHIZOBIUM* AND *BRADYRHIZOBIUM*

A. K. HALDER, A. K. MISHRA, P. BHATTACHARYYA,[1] AND P. K. CHAKRABARTTY*

Department of Microbiology, Department of Chemistry,[1] Bose Institute, 93/1, A. P. C. Road, Calcutta 700 009, India

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The potential of strains of *Rhizobium* and *Bradyrhizobium* to solubilize rock phosphate was evaluated in vitro. Almost all organisms tested effectively solubilized rock phosphate and lowered the medium pH. The presence or absence of $(NH_4)_2SO_4$ made little difference in the solubilization of rock phosphate. Among the strains, *Rhizobium leguminosarum* biovar *viceae* BICC635 was the most effective solubilizer. Maximum solubilization of phosphate and acid production was achieved after 3 days of incubation. The strain produced 2-ketogluconic acid in the culture medium, the primary cause of rock phosphate solubilization. Increasing the phosphate status of the medium had little effect on the extent of dissolution of Purulia rock phosphate. Adding calcium as $CaCl_2$, $CaCO_3$ and $Ca(OH)_2$ reduced the phosphate solubilization from phosphate rocks. The results indicated that pH, per se, is of less importance in phosphate solubilization. EDTA increased the extent of rock phosphate solubilization, possibly by chelation of the calcium ions that the solubilization produced.

Most of the essential plant nutrients, including phosphorus, remain in insoluble form in soil. A large portion of inorganic phosphates applied to soil as fertilizer is rapidly immobilized soon after application and becomes unavailable to plants (*10*). Applying phosphate solubilizing microbes to soil as biofertilizer may alleviate this problem by solubilizing these immobilized products. Application of phosphatic rocks, if solubilized by microbes, may help to increase the phosphate level of soil. It is estimated that almost 40 million tons of phosphatic rock deposits are present in different states of India (*23*). These materials should provide a cheap source of phosphate fertilizer for crop production in India. Although, several soil bacteria (*9, 12, 13, 18, 21, 25*), cyanobacteria (*23*) and fungi (*1, 2, 8*) have the property of solubilizing different inorganic and rock phosphates, little attention has been

* Address reprint requests to: Dr. P. K. Chakrabartty, Department of Microbiology, Bose Institute, 93/1, A.P.C. Road, Calcutta 700 009, India.

devoted to the part played by root-nodule bacteria. These bacteria fix atmospheric nitrogen in nodules of leguminous plants and are used extensively as biofertilizers (*4*). The objective of the present investigation was to assess the ability of strains of the root nodule bacteria *Rhizobium* and *Bradyrhizobium* to solubilize rock phosphates. We also studied the effects of several other factors on the extent of rock phosphate solubilization.

MATERIALS AND METHODS

Organisms. The *Rhizobium* and *Bradyrhizobium* strains used in these studies were obtained from several sources (Table 1). The strains were maintained by routine transfer on yeast extract mannitol agar (*28*).

Media and culture conditions. The composition of the ammonium sulphate yeast extract glucose (AYG) medium used to study rock phosphate solubilization was (per liter) as follows: glucose, 20 g; $(NH_4)_2SO_4$, 1 g; $MgSO_4 \cdot 7H_2O$, 0.5 g; yeast extract, 0.2 g; $FeCl_3$, 2 mg; $MnSO_4 \cdot H_2O$, 4 mg. Powdered phosphate rock

Table 1. *Rhizobium* and *Bradyrhizobium* strains tested.

Organism	Source[a]
Rhizobium leguminosarum biovar *viceae* SU 391	CSIRO
Rhizobium leguminosarum biovar *viceae* BICC 601	BI
Rhizobium leguminosarum biovar *viceae* BICC 635	BI
Rhizobium leguminosarum biovar *phaseoli* CC 511	CSIRO
Rhizobium leguminosarum biovar *phaseoli* CC 365	CSIRO
Rhizobium leguminosarum biovar *trifolii* CC 224	CSIRO
Rhizobium meliloti SU 47	CSIRO
Rhizobium meliloti SU 216	HAU
Rhizobium meliloti CC 169	CSIRO
Rhizobium meliloti U 45	CSIRO
Rhizobium meliloti BICC 604	BI
Rhizobium loti NZP 2213	NZP
Rhizobium sp. (*Cicer arietinum*) TAL 621	NifTAL
Rhizobium sp. (*Cicer arietinum*) BICC 630	BI
Rhizobium sp. (*Cicer arietinum*) BICC 632	BI
Rhizobium sp. (*Aeschynomene aspera*) BICC 608	BI
Bradyrhizobium japonicum CC 709	CSIRO
Bradyrhizobium sp. (*Cicer arietinum*) 27A8	Nitragin
Bradyrhizobium sp. (*Cicer arietinum*) 27A14	Nitragin
Bradyrhizobium sp. (*Cicer arietinum*) 27A15	Nitragin
Bradyrhizobium sp. (*Cicer arietinum*) TAL 385	NifTAL
Bradyrhizobium sp. (*Cicer arietinum*) TAL 619	NifTAL
Bradyrhizobium sp. (*Cicer arietinum*) IC 2099	ICRISAT

[a] CSIRO: Division of Plant Industry, CSIRO, Canberra, Australia; BI: Department of Microbiology, Bose Institute, Calcutta, India; HAU: Haryana Agricultural University, Haryana, India; NZP: Department of Microbiology and Genetics, Massey University, Palmerston North, New Zealand; NifTAL: NifTAL Project and MIRCEN, University of Hawaii, Paia, Hawaii, U.S.A.; Nitragin: Nitragin Company, Milwaukee, WI, U.S.A.; ICRISAT, ICRISAT, Patancheru, P. O., A. P., India.

(~240 mesh size) at 2 g per liter was added as a source of insoluble phosphate. In one experiment, yeast extract glucose (YG) medium was used where all the other ingredients of the AYG medium remained the same with the omission of $(NH_4)_2SO_4$ and the level of yeast extract was increased to 0.4 g per liter. The pH of the media was adjusted to 6.8 before autoclaving. The flasks were inoculated with cells of a stationary phase culture at 4% and incubated for 3 days on a rotary shaker at 28°C.

Estimation of phosphorus. Contents of the culture flasks at the end of incubation period were centrifuged at 15,000 × *g* for 15 min to remove biomass and unsolubilized matter. Then the soluble phosphate, expressed as equivalent phosphorus (P), was determined (*6*) as a measure of the extent of solubilization of rock phosphate.

Identification of organic acids. Organic acids produced in the cultures were identified by paper chromatography and comparison with authentic samples. The chromatograms were developed in a solvent system of butanol : acetic acid : water (2 : 1 : 1). Spots were detected by spraying with glucose-aniline and heating at 125°C for 5 min (*27*) or by spraying with aniline phthalate (*11*).

Organic acids were extracted after passing the spent culture through Dowex 50W-X4 column. The effluent was concentrated by lyophilization and extracted five times with *n*-butanol. The butanol was evaporated to dryness in a rotary vacuum evaporator to produce a solid compound. The melting point and IR spectrum of the compound were also studied.

Composition of phosphate rocks. The phosphate rocks used as sources of high grade and low grade phosphatic materials were obtained from Pyrites, Phosphates and Chemicals Ltd. (India) were as follows: P_1 (black Mussoorie phosphate rock), P_3 (red Mussoorie phosphate rock), P_5 (pale blue Purulia phosphate rock) and P_6 (yellowish grey Purulia phosphate rock) (*24*).

The composition of those four phosphate rocks, as determined by the method of Shapiro and Brannock (*26*), is given in Table 2. Purulia phosphate rocks (PPR), P_5 and P_6, respectively contained 34.8 and 32.7% P_2O_5 and were sources of high grade phosphate. On the other hand, Mussoorie phosphate rocks (MPR), P_1 and P_3 contained respectively 18 and 26% P_2O_5 and were sources of low grade phosphate. In the media containing either P_1, P_3, P_5 or P_6 phosphate rock at 0.2%,

Table 2. Percent chemical composition of phosphate rocks used in this study.

	P_2O_5	LOi[a]	CaO	MgO	SiO_2	RO[b]
MPR-P_1	17.0	14.1	48.9	2.3	13.6	3.6
MPR-P_3	26.2	10.4	40.8	1.6	2.5	18.5
PPR-P_5	34.8	5.1	50.8	2.9	2.5	3.6
PPR-P_6	32.7	11.2	51.2	2.3	1.6	1.0

[a] LOi signifies loss due to ignition which includes CO_2, water and other volatiles.
[b] RO includes all other oxides such as Al_2O_3, MnO, Fe_2O_3, FeO, TiO_2, Na_2O, K_2O, *etc.*

the total phosphorus equivalents added were 152, 228, 303 or 285 μg/ml respectively, if solubilized completely.

RESULTS

The results of phosphate solubilization from the phosphatic rocks by strains of *Rhizobium* and *Bradyrhizobium* in AYG medium after 3 days of incubation are presented in Table 3. The pH of the culture media after autoclaving was near the neutral point. After incubation, the end pH was determined to find out whether solubilization of P was accompanied by production of acid. The data show that the pH of all of the cultures decreased appreciably after 3 days of bacterial growth and the magnitude of the changes depended upon strains, indicating that all the bacteria could solubilize the rock phosphates. The metabolic activity of the bacteria was indicated by the production of acid in the media during the incubation period. In certain instances there was no detectable P in spent culture medium. This was possibly due to only a small amount of solubilization of phosphate and exhaustion of the solubilized P to support bacterial growth. However, to calculate the amount of P solubilized, we considered only the contents of the spent media minus that of the uninoculated control. From Table 3 it is apparent that the solubilization of rock phosphate by strains of *Rhizobium* and *Bradyrhizobium*, with the exception of BICC 635, ranged from nil (BICC 630) to over 7% (CC 169) from P_1, nil (CC 511) to over 5% (CC 169) from P_3, 1 (SU 216) to over 39% (BICC 630) from P_5 and from 2 (U 45) to over 15% (BICC 608) from P_6. BICC 635 solubilized more than 63, 17, 39 and 53% of P from P_1, P_3, P_5 and P_6 respectively. Strain BICC 630 which solubilized only a negligible quantity of P from MPR was as effective as strain BICC 635 in releasing P from P_5 rock. Table 3 also shows that solubilization of P from phosphate rocks by strains of *Bradyrhizobium* was comparable to that by the strains of *Rhizobium*. The strains of *Bradyrhizobium* also produced acid in the medium and lowered the pH in AYG medium. It might be argued that the use of ammonium salt as a nitrogen source in the AYG medium for bacterial metabolism produced acid by a proton exchange mechanism (*22*). The acid solubilized the rock phosphates. In order to investigate this, the bacteria were grown in YG medium where $(NH_4)_2SO_4$ of the AYG medium was left out and the level of yeast extract as source of nitrogen was increased to 0.4 g per liter. Table 4 presents the results of P solubilization from P_6 in YG medium by strains of *Rhizobium* and *Bradyrhizobium*. The data attest to the fact that even in this medium lacking ammonium salts, comparable amounts of P were released by the strains, although, the final pH of the respective cultures was slightly higher than those in the AYG medium.

Since, *R. leguminosarum* biovar *viceae* BICC 635 proved to be the highest solubilizer of all the strains studied and since P_6 proved to be a good source of P, further investigations were carried out to characterize the process of P solubilization from only P_6 with the strain BICC 635. The kinetics of solubilization of P and the

Table 3. Solubilization of rock phosphate by root-nodule bacteria on ammonium sulfate yeast extract glucose medium after 3 days, and end pH of cultures.

Strains	P_1		P_3		P_5		P_6	
	% Soluble P	pH	% Soluble P	pH	% Soluble P	pH	% Soluble P	pH
Rhizobium leguminosarum biovar *viceae* SU 391	4.60	5.25	4.38	4.77	4.29	4.69	5.96	4.68
Rhizobium leguminosarum biovar *viceae* BICC 601	0	4.27	3.50	4.37	4.95	4.43	7.71	4.37
Rhizobium leguminosarum biovar *viceae* BICC 635	53.33	3.06	17.54	3.02	39.60	3.06	53.30	3.06
Rhizobium leguminosarum biovar *phaseoli* CC 511	2.63	5.28	0	5.14	3.63	4.37	4.21	4.41
Rhizobium leguminosarum biovar *phaseoli* CC 365	3.94	5.86	3.51	4.75	2.97	4.70	3.51	4.67
Rhizobium leguminosarum biovar *trifolii* CC 224	3.29	6.11	4.39	4.70	2.64	4.68	3.51	4.76
Rhizobium meliloti SU 47	0.66	4.02	2.63	4.76	1.98	4.64	3.85	4.92
Rhizobium meliloti SU 216	2.63	4.98	2.63	5.30	1.65	5.49	3.15	5.25
Rhizobium meliloti CC 169	7.23	3.97	5.26	4.56	5.28	4.53	7.36	4.45
Rhizobium meliloti U 45	5.26	3.55	1.32	4.27	2.31	4.29	2.16	3.90
Rhizobium meliloti BICC 604	0.66	4.34	1.75	5.06	2.64	5.34	2.46	5.58
Rhizobium loti NZP 2213	3.29	5.34	4.39	3.96	4.95	4.04	7.72	4.23
Rhizobium sp. (*Cicer arietinum*) TAL 621	1.32	4.06	2.63	4.04	5.28	4.07	8.07	4.15
Rhizobium sp. (*Cicer arietinum*) BICC 630	0	4.06	0.88	4.10	39.60	4.56	13.33	4.37
Rhizobium sp. (*Cicer arietinum*) BICC 632	3.94	3.77	3.95	3.85	11.22	3.94	14.03	4.03
Rhizobium sp. (*Aeschynomene aspera*) BICC 608	3.94	3.77	3.95	3.90	10.56	4.06	15.43	3.98
Bradyrhizobium japonicum CC 709	0.66	6.70	1.75	4.38	2.64	4.41	2.80	4.45
Bradyrhizobium sp. (*Cicer arietinum*) 27A8	0.66	4.08	3.07	4.18	3.63	4.18	4.91	4.25
Bradyrhizobium sp. (*Cicer arietinum*) 27A14	1.97	3.99	3.50	4.18	3.63	4.15	6.66	4.20
Bradyrhizobium sp. (*Cicer arietinum*) 27A15	1.32	3.97	3.94	4.15	4.29	4.17	6.66	4.17
Bradyrhizobium sp. (*Cicer arietinum*) TAL 619	0	4.08	2.63	4.19	3.30	4.19	6.31	4.24
Bradyrhizobium sp. (*Cicer arietinum*) TAL 385	0	3.96	3.07	4.13	3.96	4.19	7.02	4.23
Bradyrhizobium sp. (*Cicer arietinum*) IC 2099	0	4.00	3.07	4.35	2.64	4.33	4.21	4.38

Table 4. Solubilization of Purulia rock phosphate (P_6) by root-nodule bacteria grown on yeast extract glucose medium after 3 days, and end pH of cultures.

Strains	% Soluble P	pH
Rhizobium leguminosarum biovar *viceae* SU 391	6.66	4.59
Rhizobium leguminosarum biovar *viceae* BICC 601	5.96	4.85
Rhizobium leguminosarum biovar *viceae* BICC 635	47.02	3.13
Rhizobium leguminosarum biovar *phaseoli* CC 511	3.16	4.31
Rhizobium leguminosarum biovar *phaseoli* CC 365	4.21	4.53
Rhizobium leguminosarum biovar *trifolii* CC 224	4.91	4.52
Rhizobium meliloti SU 47	4.21	5.31
Rhizobium meliloti SU 216	3.51	5.10
Rhizobium meliloti CC 169	4.21	4.93
Rhizobium meliloti U 45	1.40	5.08
Rhizobium meliloti BICC 604	3.85	5.01
Rhizobium loti NZP 2213	8.77	4.41
Rhizobium sp. (*Cicer arietinum*) TAL 621	1.40	6.70
Rhizobium sp. (*Cicer arietinum*) BICC 630	12.63	4.01
Rhizobium sp. (*Cicer arietinum*) BICC 632	13.33	3.98
Rhizobium sp. (*Aeschynomene aspera*) BICC 608	16.14	3.96
Bradyrhizobium japonicum CC 709	2.16	5.32
Bradyrhizobium sp. (*Cicer arietinum*) 27A8	5.96	4.21
Bradyrhizobium sp. (*Cicer arietinum*) 27A14	8.07	4.05
Bradyrhizobium sp. (*Cicer arietinum*) 27A15	6.31	4.32
Bradyrhizobium sp. (*Cicer arietinum*) TAL 619	5.26	4.45
Bradyrhizobium sp. (*Cicer arietinum*) TAL 385	5.61	5.31
Bradyrhizobium sp. (*Cicer arietinum*) IC 2099	2.80	4.87

corresponding values of culture pH in AYG medium at different time intervals are shown (Fig. 1). It appears that as the mean pH decreased the level of solubilized P increased linearly till the 3rd day of cultivation. A yield of 285 μg of P per ml of culture, a theoretical expectation upon complete solubilization of the rock phosphate, was never achieved. Expressed in terms of percentage, only about 64% of solubilization of P was achieved after 13 days of incubation and further incubation did not improve the extent of solubilization.

To identify the major organic acids produced by the strain BICC 635 culture, filtrates were analyzed by paper chromatography. A single spot of organic acid with an *Rf* value of 0.37 appeared upon spraying with glucose-aniline reagent. The acid was identified as 2-ketogluconic acid by comparison with authentic sample and other criteria. The spot fluoresced blue-white in UV-light and gave a red color when sprayed with aniline phthalate. The compound after isolation showed a strong band at γ_{max}^{KBr} 1745 cm^{-1} in the IR spectrum, indicating that it was 2-keto acid. The compound had a melting point of 169°C and a superimposable IR spectrum with authentic 2-ketogluconic acid.

The role of the organic acid in the solubilization of P from the phosphate rock P_6 was evaluated. Organic acid was extracted from the cultures of BICC 635

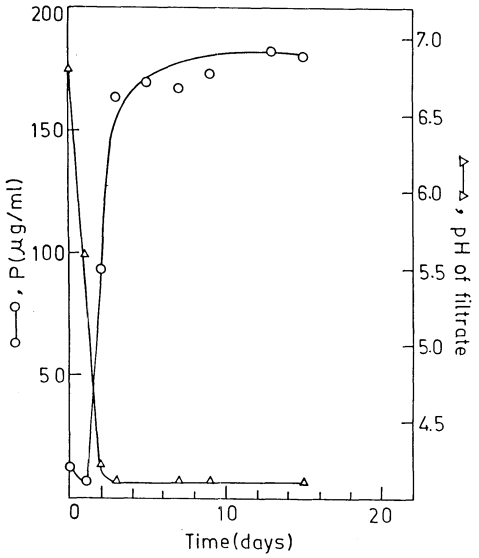

Fig. 1. Kinetics of solubilization of phosphate and acid production by *Rhizobium leguminosarum* biovar *viceae* BICC 635.

The strain was cultured in ammonium sulphate-yeast extract-glucose medium containing 0.2% Purulia phosphate rock (P_6). Release of phosphate, expressed as equivalent phosphorus, was measured at different time intervals.

separately grown in AYG and YG media and used for phosphate solubilization in corresponding fresh medium containing P_6 incubated for 3 days. As compared to 53 and 47% solubilization of P from P_6 in AYG and YG media by the bacteria, the isolated organic acids from the cultures solubilized almost equivalent amounts of P, 50 and 37%, respectively.

To study the effect of soluble P status on the solubilization of rock phosphate, soluble P as Na_2HPO_4 in increasing concentrations was added to the AYG medium and the level of solubilized P was quantitated in the culture of BICC 635 after 3 days of incubation. For the purpose, total soluble P in the cultures was estimated and from this value the amount of P added was subtracted (Fig. 2). The data show that the extent of solubilization from P_6 was largely unaffected by the initial soluble P status of the culture medium. Solubilization varied from 47 to 52%, expressed as a percentage of the total P in the rock material added.

Adding increasing amounts of calcium as $CaCl_2$ to the cultures of BICC 635 in the AYG medium made little difference in the culture pH after 3 days of growth from that of the control culture with no $CaCl_2$ added. With an increasing concentration of $CaCl_2$ there was a gradual decrease in the extent of solubilization of P from P_6 after 3 days of incubation (Fig. 3). The dissolution of P was reduced from 54% to only 19% at the highest addition of $CaCl_2$. Adding calcium as $Ca(OH)_2$ sharply increased the mean pH, which was inhibitory to bacterial

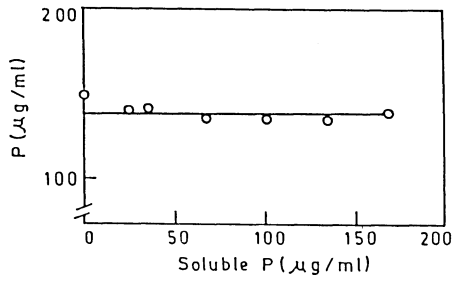

Fig. 2

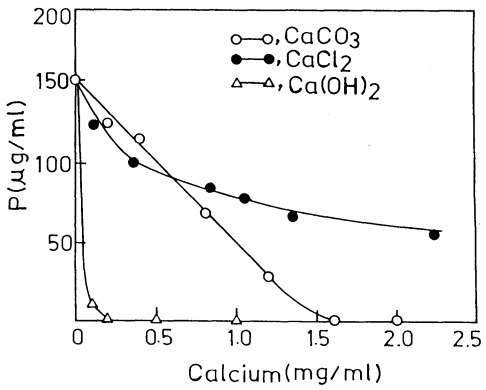

Fig. 3

Fig. 2. Effect of soluble phosphate supplement on phosphate solubilization from Purulia phosphate rock (P_6) in cultures of *Rhizobium leguminosarum* biovar *viceae* BICC 635.

Soluble phosphate as Na_2HPO_4 was added to the AYG medium. Release of phosphate, expressed as equivalent phosphorus, was measured after 3 days.

Fig. 3. Effect of calcium supplement on phosphate solubilization from Purulia phosphate rock (P_6) by culture of *Rhizobium leguminosarum* biovar *viceae* BICC 635.

Calcium in increasing levels was added to the AYG medium. Release of phosphate, expressed as equivalent phosphorus, was measured after 3 days.

growth, and there was little or no P solubilization in media of such high pH (Fig. 3). Adding increasing amounts of $CaCO_3$ resulted in a higher pH of the cultures after 3 days of incubation as compared to control cultures. The extent of P solubilized was reduced from 54% to an insignificantly low level at the highest addition of $CaCO_3$ (Fig. 3).

Adding disodium salt of ethylenediamine tetraacetic acid (EDTA) neutralized to pH 7.0 to 3 day old cultures of BICC 635 in AYG medium and further incubation of the cultures for another 24 h showed that increasing concentration of EDTA increased the solubilization of P from rock phosphate (Fig. 4). Adding neutralized EDTA to a final concentration of 5 mg per ml to a 5 day old culture gradually increased the solubilized P upon incubation for the next four days. The P reached a plateau when more than 80% of the P from P_6 was released (Fig. 5).

DISCUSSION

Bacteria belonging to the genera *Bacillus* and *Pseudomonas* are known to bring about dissolution of insoluble phosphatic compounds (*2, 3, 10, 13, 21, 25*). Our data show that root-nodule bacteria also have the potential to degrade phosphatic rocks and to solubilize phosphate. The inoculant cultures behave differently in four different phosphate rocks as revealed from the end pH of the cultures and the extent

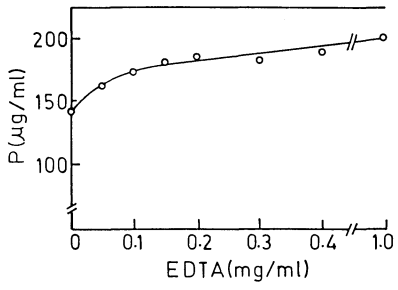

Fig. 4

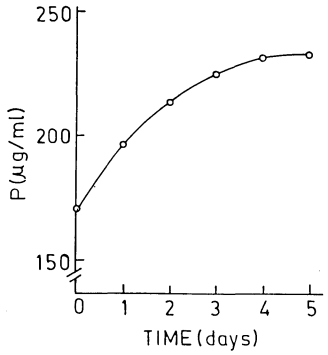

Fig. 5

Fig. 4. Effect of adding EDTA on phosphate solubilization from Purulia phosphate rock (P_6) in cultures of *Rhizobium leguminosarum* biovar *viceae* BICC 635.

The strain was allowed to grow in the presence of rock phosphate for 3 days in AYG medium when neutralized EDTA was added to the cultures in increasing concentrations. Soluble phosphate, expressed as equivalent phosphorus, was measured in the culture filtrate after incubation for another 24 h.

Fig. 5. Kinetics of solubilization of phosphate by *Rhizobium leguminosarum* biovar *viceae* BICC 635 upon addition of EDTA to a 5 day old culture.

The strain was allowed to grow in the AYG medium containing 0.2% Purulia phosphate rock (P_6) for 5 days when neutralized EDTA was added to the cultures to a final concentration of 5 mg per ml. Release of soluble phosphate, expressed as equivalent phosphorus, was then measured at time intervals.

of P solubilization (Table 3). This is probably due to the difference in the chemical makeup of the rock phosphates (Table 2) used. The extent of solubilization of P from the rock phosphates by the root-nodule bacteria is very comparable to that by other bacteria reported. The taxonomic grouping of the strains is not reflected in their P solubilization property. However, *Rhizobium leguminosarum* biovar *viceae* BICC 635 was superior to all the other root-nodule bacteria studied.

All the organisms produced acid and lowered the medium pH and it appeared that the acids produced were responsible for the P solubilization. It has been suggested that the microorganisms which decrease the pH during their growth are efficient P solubilizers (*1*, *12*, *21*). Chromatographic analysis of organic acid produced by strain BICC 635, the most efficient phosphate solubilizer, revealed only one spot of acid, which was identified as 2-ketogluconic acid. During phosphate solubilization, however, not all strains of root-nodule bacteria produced the same organic acid (data not shown). It has been suggested, that in phosphate solubilization, the nature of organic acid produced is more important than the quantity (*1*, *18*). There was no large difference in the solubilization of P from phosphate rock P_6 and lowering of medium pH in the presence or in the absence of $(NH_4)_2SO_4$ in the media containing glucose and yeast extract. This indicated that $(NH_4)_2SO_4$ is

probably of less importance for phosphate solubilization, or separate mechanisms are involved for phosphate solubilization in the presence or absence of NH_4 in the media. The in vitro experiment using extracted organic acid also indicated that there is no large difference in the rock phosphate solubilizing capacity of the organic acid produced in the AYG and YG media by BICC 635 and the values were close to those observed in in vivo bacterial cultures. This indicates that organic acid is the primary factor in rock phosphate solubilization.

The data of Fig. 1 apparently indicate that the mean pH determined the extent of rock phosphate dissolution. But it has been shown that the supply of protons, which depends on both pH and titratable acidity, determines the extent of rock phosphate solubilization rather than pH alone (*15*).

The dissolution of rock phosphate was influenced only to a small extent by the P status of the medium. The extent of dissolution remained practically constant throughout the range of P added to the medium (Fig. 2). This is consistent with the results of other studies carried out in soil (*19*, *20*).

The large effect of increasing the amount of calcium on the dissolution of rock phosphate contrasts sharply with the minor effect of added P. The decrease in the extent of dissolution of P_6 at the lowest addition of $Ca(OH)_2$ (Fig. 3) could be accounted for by the mean pH which was close to neutrality after 3 days of culture. Further addition caused the pH to be highly alkaline, which inhibited the growth of the bacteria resulting in little solubilization. Adding $CaCO_3$, on the other hand, provided resistance to the shift of medium pH towards the acidic side normally caused by the bacterial production of acid. The data (Fig. 3) show that adding increasing concentrations of calcium as $CaCO_3$ caused a linear decrease in the extent of P solubilization and at 4 mg calcium equivalent of $CaCO_3$ the end pH of the culture was close to neutrality and the level of P solubilization was practically nil. The data apparently indicate the role of acid for P solubilization. However, the large effect of adding $CaCl_2$ on the dissolution of rock phosphate seems to rule out the effect of pH only. Adding the $CaCl_2$ caused little change in the pH of the culture media as compared to the control. Figure 3 shows that there is a large decrease in the dissolution of P on addition of $CaCl_2$ and at the highest concentration of $CaCl_2$ added, the decrease is more than 63%. This indicates a role of calcium activity in the dissolution of P from phosphate rocks. Studies of Wilson and Ellis (*29*) involving soil solution suggested the calcium activity as an important factor controlling the rate and extent of dissolution of rock phosphate.

When EDTA is added, calcium activity in the dissolution of rock phosphate probably becomes of less importance due to its chelation. Increased calcium chelation would lead to further dissolution of rock phosphate by lowering the concentration of free calcium in the medium.

Many studies on the liberation of P directly from phosphate rock (PR) to soil have shown that the liberation is determined to a large degree by the properties of the soil. Agronomic effectiveness of PR is influenced by the chemical and physical properties of PR as well as soil properties. The rate of PR dissolution is low and

soil pH, exchangeable Ca and total Ca, all increase in soil samples treated with PR, while surface adsorption of P by oxides of Al and Fe is favored (*5*, *7*). As time goes by the accumulated adsorbed P may gradually form compound precipitates in the soil. More PR dissolves in acidic soil than in alkaline or neutral soils (*17*) and PR dissolution increases linearly with decreasing soil pH as also with high P retention (*16*). However, low soil pH may reduce plant growth. An increase in the available P in the soil solution increases the agronomic effectiveness of PR but not by the extent of PR dissolution since soil with a high P retention capacity is likely to restrict the utilization of P dissolved from PR. For soils where there are major leaching losses of P due to the very low P sorption to the soil, PR application may be highly suitable even though only a minor amount of it may dissolve (*14*). For soils with a moderate P sorption capacity where retention of released P is high, application of metal chelator may help to keep metal P in soil solution.

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