EXPLORING THE PHOSPHATE SOLUBILIZING CAPACITY OF SOIL BACTERIA THROUGH THE APPLICATION OF ^{32}P RADIOISOTOPE TECHNIQUES AND X-RAY DIFFRACTION METHOD

G.I. Jumaniyazova, T.S. Tillayev, K.S. Takhtobin, M.Kalonov

Institute of Microbiology, Institute of Nuclear Physics,
Academy of Sciences Republic of Uzbekistan/ Tashkent

Introduction

One of global ecological problems of agriculture is the problem "over phosphatization" of soils [1]. Till now process of biological transformation of phosphorus in soil poorly studied, the optimum methods of its regulation are not detected, in this connection, annually to agriculture the large damage is put. Only of 10 %-25 % of phosphorus, introduced by the way fertilizers to acquire by plants, the other main part, as a result of chemical changes in soil, transforms in insoluble, hard to reach for plants forms. It demands new deposits of fertilizers and, thus, there is an accumulation in soil of insoluble compounds of phosphorus ("over phosphatization" of soils). The situation is aggravated by vast application of complex fertilizers, keeping apart from phosphorus nitrogen and potassium, that entailed an accumulation and excess one at lack of other elements. Such unbalance influence both on quality of agricultural production and on a harvest as a whole.

It is known, that the part of soil bacteria is capable to participate in decomposing insoluble phosphoric compounds, secreting an acids and enzymes [2]. Soil bacteria have symbiotic relationship with roots systems of plants (rhizosphere) and other microorganisms, they augment the contents of solvable phosphorus in soil, which is easy assimilate by plants. It increases efficiency of other kinds of fertilizers, keeping nitrogen, the potassium and as a whole leads to favourable, balanced composition of soil [3]. The methods with application of an isotope of phosphorus-32 allow to study processes of mobilization and immobilization of soil phosphorus, quantitatively to evaluate a role of different strains of bacteria and have large theoretical and practical value [4].

The aim of our investigations was to isolate the phosphate solubilizing bacteria from cotton and sugar-beet rhizosphere and elaborate on the basis of application of an isotope Ð-32 a method of a quantitative assessment of capacity of soil bacteria strains to acquire phosphorus from insoluble phosphoric compounds of soil. On the basis of a designed method we are going to conduct targeted selection of the strains with the high characteristics in term of creation effective biofertilizers boosting fertility of soils.

2. Materials and methods

2.1 Research the capacity of endemic bacteria strains to decompose insoluble compounds of phosphorus in soil.

The strains was isolated on selective mediums, which are contained as an alone source of phosphorus the nucleic acid (DNA) and mineral phosphate - tricalcium phosphate or ground phosphate [5].

Composition of mediums (in g /L):

Medium 1	Medium 2	Medium 3
Peptone-10	Glucose-20	Saccharose - 10
NaCL - 0.5	NaCL - 0.2	(NH4)2 SO4 - 1
DNA-0.2%	Mg SO4 -tracks	MgSO4 - 0.5

Agar - 20	Fe SO4- tracks	KCL - 0.5
Water - 1 L	Ca 3(PO4)2 - 5	CaCL2 - 0.5
	Agar-20	NaCL - 0.1
	Water - 1 L	Ground (phos)-10
		Agar-20
		Water -1 L

Sowing made in Petri cups by introducing 1 ml of soil suspension diluted from 100 up to 1000000. The cups filled up by molten and cooling down to 45 grades medium, carefully mixed with a soil suspension and after a cooling placed in the thermostat and incubate within 48-72 hours at the temperature of 28-30 grades. After incubation the colonies, around of which appear the clear zones of phosphate dissolution, were selected. Such colonies was sowing on a slant agar with medium similar in which one were grown, previously having measuring diameter of a zone of phosphate dissolution. Then the bacteria was sowing on a fluid medium with phosphate to study the capacity of them to dissolve water-insoluble P205.

On medium 1 with nucleic acid we have selected two strains from a genus Bacillus with the highest characteristics - BM -1 and BMVP-11. On medium 2 and medium 3 with tricalcium phosphate and ground phosphate we have selected 2 strains from a genus Bacillus - BP- 700 and Bac. Sp.-701.

Capacity of bacteria to mobilize phosphate determined by a Sergeev`s method [6]. The experience conducted in conical flasks, where introduced 100 ml of a medium and phosphoric compounds at the rate of 0,2 % P205. The sterile mediums infected with culture of bacteria and incubated in the thermostat at 28-30 grades during 15, 30 and 60 days. Solvable mineral phosphorus determined at the end of each term with the help spectrophotometer.

2.2 The use of ^{32}P radioisotope techniques for research the capacities of endemic bacteria to decompose insoluble compounds of phosphorus in soil.

Further, after these preliminary experience, the researches with application of radioactive phosphorus Ð-32 were conducted. To investigate the behavior of phosphorus in soil it is necessary to receive those insoluble compounds (keeping a radioactive label phosphorus-32) to which one transforms at depositing by the way of fertilizers. According to the literary data the final compound in which phosphorus transforms at depositing to soil is water insoluble tricalcium phosphate-Ca3(PO4)2 [1,2].

Previously screened through the screen 0,20ìì sample of soil (300g) placed in conical flask, then have added distilled water by a volume 800 ml and 18 mCi of radioactive phosphorus Ð-32 (H3PO4), carefully have mixed. After four weeks the score the soil was washed by water and utilized for the subsequent experiments. Allowing a short half-life time of radioactive phosphorus Ð-32 (14,5 days) and with the purpose of the coordination on time of a beginning and end of the next experiment, it is necessary each time to prepare a new portion of radioactive soil.

The strains of the endemic bacteria 1, 11, 700, 701 grew during night in fluid medium 1. One ml (10^6 of cells) of each strain was introduced in Petri caps with radioactive soil , and one cup abandoned for the control (i.e. soil without bacteria). The associative sample contained 1 ml of night culture from each strain (10^6 of cells). After one week took from each cup by the special probe the sample of soil in quantity 1 gram for measurements after washing from it by water the radioactive phosphorus, which one, as it was supposed, was freed by bacteria from the bound forms of insoluble compounds of soil in free, i.e. water soluble. The samples of soil in quantity 1

gram was filled up by 5 ml of a distilled water and put on the magnetic stirrer at 5 hours, after that samples centrifuged and took 20 mkl of a liquid on measurement of counting per minute (cpm).

2.3 Application of X-ray diffraction method for study of soil mineral structure transformation by endemic bacteria strains.

Theoretically possible and experimentally obtained types of orthophosphates and related to him compounds are characterized by ratio of number of cations and anions M/n = 1. Cations and the anions in these compounds in many respects predetermine their crystalline constitution, and also their stability in an environment [7]. One of ways of research the compounds having a crystalline constitution is a X-ray diffraction method (roentgen-phase analysis method)[8]. In the basis of this method lies the fact, that each crystalline structure have a definite pattern of reflects on a diffraction picture of sample [9]. In practice some ways of roentgen-phase analysis of drugs will be used, from which one most eligible for definition of a mineralogical structure of soils are the methods of the internal standard and ratio of intensities of analytical lines. The nature of a method of the internal standard consists in the adding to an investigated drug precise known quantity of reference matter. As the mineralogical structure of soil is combined and have some crystalline phases, the method of ratio the intensities of analytical lines was applied for definition of each of them.

The measurements conducted on a X-ray diffractometer DRON-3Ì with radiation ÑuÊà (1.54178 À). Samples of soils for research by this method was washed by water to remove all soluble compounds in order to eliminate their different masking effects. After washing out the soil was dried, grinding up and screened through the screen with cells 0.20ìì. On the analysis following samples were taken:

1. control (washed soil);
2. soil + Ca3(PO4)2 ;

3à. sample 2 + associate of the strains 1,11,700,701 after 15 days of an incubation;

3á. same (3à) only in 30 days of exposure.

Samples of soil for measurement placed in the special quartz cell (28 mm diameter and depth of 0,5 mm). Weight of a sample was 100 mg.

3. Results And Discussion

3.1 Research the capacity of endemic bacteria strains to decompose insoluble compounds of phosphorus in soil.

As a result of the conducted laboratory experiments the direct dependence between quantity mobile P2O5, speed of a breeding of bacteria and duration of an incubation was established. The mineralization of organic phosphate (nucleic acid) went in an interval pH for the strain BM-1 from 5,0 up to 5,1, and for the strain ÂÌVP-11 from 4,6 up to 4,8.

The dissolution of tricalcium phosphate by the strain ÂÐ-700 went in range pH 6,2 - 6,3, and for the strain Bac. Sp.-701 - in an interval 5,5 -6.5.

The data, introduced in a Tab. 1 show, that the strain Bacillus megaterium BM-1 for 60 day of experience has freed 50 mg P2O5 from nucleic acid, and strain Bacillus megaterium var. Phosphaticum BMVP-11 - up to 78 mg P2O5 for 60 day of experience, that in 3,9-6 times (accordingly) exceeds the control (without bacteria).

Table 1

Day\ Strain	BM-1	BMVP-11	control
5	15	16	15

15	23	45	16
30	45	60	17
60	50	78	16

Table 2

Day/Strain	BP-700	Bac.sp.701	control
5	15	16	15
15	25	32	16
30	32	50	15
60	55	70	16

From the data, introduced in a Tab. 2 it is visible, that the strain Bacillus polymyxa, BP-700 for 60 day of experience has freed up to 55 mg P2O5 from tricalcium phosphate, and strain Bacillus sp.701 - up to 70 mg, that in 4,2 -5,3 times exceeds the control (culture medium without bacteria).

The conducted experiments demonstrate, that the strains of bacteria BM-1, ÂÌVP-11, ÂÐ-700 and Bac. Sp.-701 are able to decompose the insoluble compounds of phosphorus in the soil.

3.2 The use of ^{32}P radioisotope techniques for research the capacities of endemic bacteria to decompose insoluble compounds of phosphorus in soil.

Observed more intensive radioactive decay in samples of soil with bacteria contrasted to control (Table 3) may be explained by presence last and exerting influence on the contents of radioactive phosphorus in soil. As the phosphorus was in soil by the way of insoluble compounds, it is necessary to relate the detected phenomenon to capacity of the strains to extract phosphorus from them.

Table 3

Strains\ Date\ cpm	09 07	19 07	25 07	08 08
BM-1	2304	2208	1296	816
BMVP-11	2184	2088	1224	912
BP-700	2712	2490	1890	870
Bac.sp.-701	2814	2447	2082	852
ass.700, 701	2083	2054	1632	858
ass.1,11,700,701	2118	2062	1518	876
control (without bac.)	2327	1853	935	263

Less intensive radioactive decay as contrasted to by control for all investigated strains is observed. In this experiment already it is possible quantitatively to assess the capability of the different strains of endemic bacteria in matching one another to decompose the insoluble compounds of phosphorus in soil and to select the strains which have the highest characteristics. Under identical launching conditions, naturally, that the strains, most capable of the given function, will have higher count.

3.3 Application of X-ray diffraction method for study of soil mineral structure transformation by endemic bacteria strains.

The outcomes of X-ray crystallographic analysis of soils are submitted that the addition to the washed soil of insoluble tricalcium phosphate Ca3(PO4)2 all mineralogical structure of soil varies, in particular, there is an increase of percentage of a silicon oxide at 2,74 , calcium carbonate - on 1,23 , tricalcium phosphate - on 0,32 , hydromica - on 0,04 , muscovite - on 0,01 and reduction of percentage of adamant - on 0,42, sodium feldspar - on 1,15, calcium feldspar - on 1,04, kaolinite - on 0,4, chlorite - on 0,08, dolomite - on 0,36, meritmeryllonite - on 0,37 and plaster rocks - on 0,44. At addition in soil of associative culture of bacteria (strains 1,2,700 and 701) in 30 days of experience the increase of percentage of a silicon oxide - on 1,33 , calcium carbonate - on 2,51, kaolinite - on 0,38; a muscovite - on 0,95. The percentage of the majority of minerals has decreased under addition of bacteria, namely in 30 day: adamant - on 0,91; potassium feldspar - on 0,61; sodium feldspar - on 1,11; tricalcium phosphate - on 0,28; hydromica - on 0,27; a chlorite - on 1,03; dolomite - on 0,46; meritmeryllonite - on 0,18; a plaster rock - on 0,02. It is probably, the introduction of the bacterial strains in soil has resulted in transformation of a mineralogical structure and reallocating of minerals - both in the party of increase one, and in the party of reduction other. The main result is the reduction percentage of insoluble tricalcium phosphate under effect of bacteria, that indicates their capacity to decompose the insoluble compounds of phosphorus in soil.

In general, three independent methods applied in researches, namely:

à) a chemical method of testing of concentration of phosphorus in medium,

b) a designed method of testing of the strains of endemic bacteria with the help of radioactive phosphorus,

c) X-ray diffraction analysis method of a mineral structure of soil have confirmed capacity of the selected strains of endemic bacteria to decompose insoluble compounds of phosphorus in soil.

Now scientists of the majority of developed countries were approved in judgement on impossibility of extraction of phosphorus from insoluble compounds of soil by chemical methods for needs of farms. The look of the majority scientists is reversed in the party of biotechnological ways, which one are intensively designed by way of usage endemic bacteria of soil for each local region of a planet. It is supposed hereafter to conduct researches of different soils and to select the strains with the highest characteristics with the purpose of creation of cheap biofertilizers.

Table 4 **Results of analyses by X-ray diffraction method (% from total)**

1	Mineral	Formula	1	11	3a	3б
1.	Silicon oxide	Si02	37.72	40.46	41.00	41.79.
2.	Calcium carbonate	CaC03	31,31	32,14	35,18	34,65
3.	Adamant	Al 2O3	4,04	3,62	3,62	2,71
4.	Potassium feldspar	K20*AL203*6Si02	5,02	5,05	5,19	4,43
5.	Sodium feldspar	Na20*Al203*6Si02	5,37	4,22	8,50	3,11
6.	Tricalcium phosphate	Ca3(PO4)2	0,69	1,01	0,56	0,73

7.	Calcium feldspar	Ca0*Al2 03*2Si02	3,06	2,02	3,25	1,48
8.	Kaolinite	Al2 03*2Si02*2H20	2,66	2,26	1,75	2,64
9.	Muscovite	K2 0*3Al2 03*6Si02*2H20	1,85	1,96	2,37	2,91
10.	Hydromica	4Si02*1.5Al2 03*0.25K20*0.25Na20*nH2 0	1,27	1,31	0,62	1,04
11.	Chlorite	3Si02*Al203*5.5Mg0*0.5Fe 203*5H20	1,50	1,42	0,87	0,39
12.	Dolomite	CaMg(CO3)2	3,23	2,97	2,69	2,51
13.	Meritmeryllo nite	3.5Si02*0.66Al203*0.5Mg02 *0.1Ca0*5.4H20	1,44	1,07	1,00	0,89
14.	Plaster rocks	CaS04*2H20	0,91	0,47	0,37	0,45

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