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Studies on phosphate solubilizing actinomycetes isolated from rhizospheric soil of medicinal plants

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Abstract
Phosphorus is one of the major essential macronutrients for biological growth and development. Some bacteria and other microorganisms have the ability to solubilizing inorganic phosphate as a plant macronutrient. Using this ability to utilize inorganic phosphate, researchers try to produce such actinomycetes from rhizospheric soil of different plants, which solubilize phosphate itself using microorganisms. The different medicinal plant has varied phosphate solubilizing activity. The current study showed that actinomycetes of rhizospheric soil of medicinal plant *Tinospora cordifolia* highest capacity to utilize phosphate and actinomycetes from rhizospheric soil of *Curcuma longa* has least phosphate solubilizing activity. The present study provides great interest in organic farming by reducing chemical fertilizer as a source of phosphate.

Keywords: solubilizing, isolated, soil, medicinal, plants

Introduction
As their name reflects (in Greek, “atkis" means ray and "mykes" means fungus), this shares some morphological features with fungi. Actinomycetes belong to the order Actinomycetales, a division of gram-positive bacteria characterized by a high genomic G+C content. Actinomycetes species are well-known saprophytic bacteria that decompose organic matter (Fox and stackebrandt 1987) [1], especially biopolymers such as lignocellulose, starch, and chitin in soil (Buchanan *et al* 1984) [2]. Several actinomycetes have characteristic features such as mycelial growth that culminates in sporulation, they also possess the ability to biosynthesize wide verities of antibiotics as secondary metabolites (Hillel 1991) [3]. The agroindustry shows a marked interest in actinomycetes as a source of proactive compound (Hellmann *et al* 1997) [4] and (Tanaka and Omura 1993) [5].

Actinomycetes are filamentous bacteria that represent the high properties of soil microbial biomass and have the capacity to produce a wide variety of antibiotics and extracellular enzymes (Behal 2000) [6]. Several strains of actinomycetes have been found to protect plants against plant disease (Franklin *et al*. 1989) [7]. The habitat of actinomycetes corresponds to its behavior characteristics. Actinomycetes are saprophytes, another has for a decomposing organism which means it grows best in moist moderate to the tropical atmosphere. This bacterium is also a heterotrophy, which means it draws energy from dead and decomposing animal matter (Crawford *et al*. 1993) [8].

Medicinal plants are the important recourse for isolating endophytic actinomycetes, which can induce secondary metabolites of very important value. The importance of medicinal plants in the treatment of various types of ailments Ayurveda and other traditional medicine practices. Those several types of plants are covered worth acting. Mycetes, it is known that not all plant contains entophytic actinomycetes. The symbiotic existence of endophytic actinomycetes in medicinal plant and their role in deciding the medicinal value of plants due to the production of various bioactive compounds is worth probing. Relevance of screening medicinal plants is an important approach to explore and exploit new entophytes (C.L. Doumbou, M.K. Salove 2001).

The main role of actinomycetes in the field of agriculture is to degrade all sorts of organic substances like cellulose, polysaccharide, protein, fats, and organic acids, etc. Organic substances added to soil are first attacked by bacteria and fungi and later by actinomycetes because they are slow in activity and growth than bacteria and fungi (Bashan 1998) [10].

Five medicinal plants have been chosen for the present study that is *Alam sativum*, *Tinospora cordifolia*, *Tridax procumbans*, *Curcuma longa*, and *Envicostema auxillare* locally named as Korphad, Gulvel, Jakhamjudi, Halad, and Nay respectively.

Materials and Method
Five medicinal plants namely Alam sativum, Tinospora cordifolia, Tridax procumbans, Curcuma longa, and Envicostema auxillare collected from Nanded and nearby places were selected for the present study. The plants were uprooted and soil transferred in sterile polythene bags. The soil samples were brought to the laboratory and stored at 40°C till further processing.

Isolation of Actinomycetes
The soil samples were serially diluted in sterile distilled water before use for isolation. 1 gm of each soil sample was suspended in 10 ml sterile distilled water, mixed vigorously, and used for preparing serial dilutions ranging from 10-1 to 10-9. Suitable dilution range (10-4 to 10-7) of each set was selected. 0.1 ml of each dilution was spread on the surface of sterile starch casein agar plated supplemented with 50 µg/ml nystatin. The plates were incubated at 30°C for 7 days for the appearance of colonies. After incubation, morphologically distinct, well-isolated colonies of actinomycetes were picked up and transferred on sterile slants of SCA.

Screening

The screening was performed in two ways; one is quantitative screening and the other is qualitative screening.

Qualitative screening

Glasswares and Picovasky agar medium are sterilized at 15 lbs pressure for 20 minutes by the use of an autoclave. Picovasky media is poured in Petri plates. Morphologically distinct, well-isolated colonies are transferred on a picovasky agar medium in the form of a spot at four quadrants of the plate. Then Petriplate is placed for incubation at room temperature for 7 days. After incubation observes changes on picovasky agar medium.

Quantitative screening

Glassware and picovasky broth are sterilized with the help of an autoclave. 15 ml of picovasky medium is transferred in 25 ml of the beaker. 59 well-isolated colonies are vigorously mixed in different 59 conical flasks of 25 ml capacity. These conical flasks are placed for incubation at room temperature for 7 days. After the incubation period observed changes occurred in a conical flask and record observation. After incubation, a picovasky medium of 59 different isolates is centrifuged. After the centrifugation procedure supernatant was collected and the pellet was discarded. Check the optical density of supernatant for determining how much concentration of phosphate was solubilized by actinomycetes isolates.

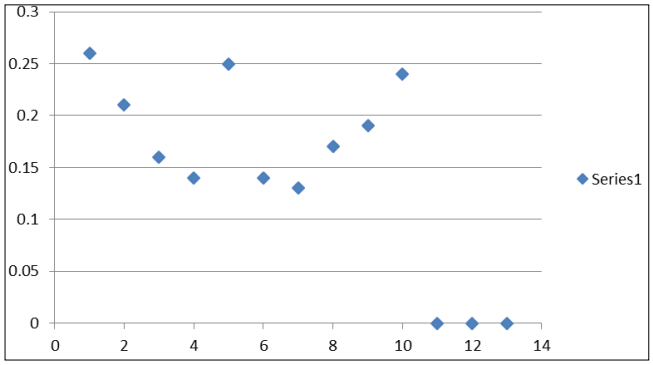
A sample is having the color and colloidal impurities, remove them by adding a spoonful of activated charcoal and filtering. Now add 2 ml of ammonium molybdate solution and 5 drops of stannous chloride reagent. The blue color is appearing in presence of phosphate. Take the optical density readings at 690 nm on a spectrophotometer using a distilled water blank, with the same amount of chemical. Reading on the spectrophotometer should be taken after 5 minutes but before 10 minutes of the adding of the last reagent. Find out the concentration of phosphate with the help of a standard curve. Prepare the standard curve in the range of 0.0 to 1.0 mg/L of PO4- p at the interval of 0.1.

Results

Phosphate solubilizing activity of actinomycetes was determined in different medicinal plants as in *Alam sativum*, *Curcuma longa*, *Tinospora cordifolia*, *Tridax procumbans*, and *Envicostema auxillare* (Table and graph 1-4). Comparing the highest phosphate solubilizing concentration of actinomycetes as a model to decide the highest phosphate solubilizing activity. *Curcuma longa* (Halad, 0.40 mg/L) has the highest concentration to solubilize inorganic phosphate from rhizospheric soil as a plant macronutrient. *Envicostema auxillare* (Nay, 0.26 mg/L) has the least concentration of phosphate solubilization among the five medicinal plants. *Tridax procumbans* (Kolsan) and *Alam sativum* (Korphad) have the same (0.38 mg/L) phosphate solubilizing activity. But along with the phosphate solubilizing activity, actinomycetes isolates were observed in rhizospheric soil, which is responsible for the solubilization process is seen highest (13 isolates) in *Curcuma longa*, *Alam sativum*, and *Tinospora cordifolia*. *Tridax procumbans* has moderate (11 isolates) actinomycetes isolates and *Envicostema auxillare* has the least (10 isolates) actinomycetes isolates observed.

Table 1: Phosphate solubilizing activity of rhizospheric soil from *Curcuma longa.*

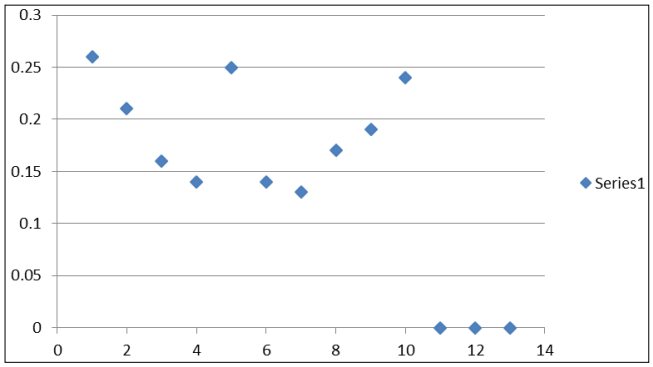
Sr. no.	Isolates	O.D at 690nm	Conc. from a stand. graph (In mg/L)
1	T_1	1.02	0.29
2	T_2	1.27	0.37
3	T_3	0.96	0.27
4	T_4	0.82	0.24
5	T_5	1.14	0.40
6	T_6	0.78	0.22
7	T_7	0.74	0.21
8	T_8	0.69	0.20
9	T_9	0.10	0.03
10	T_{10}	1.09	0.31
11	T_{11}	1.32	0.37
12	T_{12}	0.57	0.16
13	T_{13}	0.54	0.15



Graph 1: Phosphate solubilizing activity of rhizospheric soil from *Curcuma longa.*

Table 2: Phosphate solubilizing activity of rhizospheric soil from *Tinospora cordifolia*.

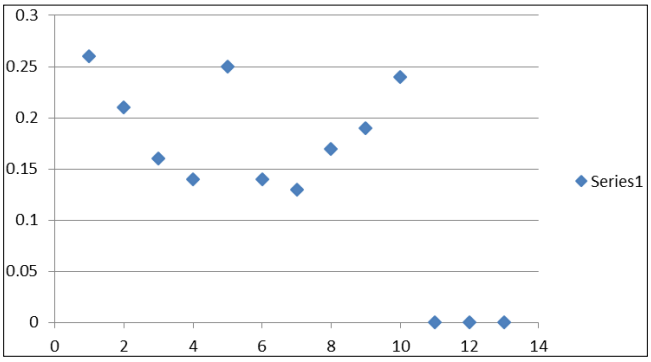
Sr. no.	Isolates	O.D at 690 nm	Conc. from stand. graph (mg/L)
1	G_1	0.61	0.17
2	G_2	1.30	0.37
3	G_3	0.35	0.10
4	G_4	0.44	0.13
5	G_5	0.47	0.14
6	G_6	0.94	0.27
7	G_7	1.21	0.35
8	G_8	1.28	0.34
9	G9	1.14	0.33
10	G_{10}	1.26	0.36
11	G_{11}	1.27	0.36
12	G_{12}	0.77	0.22
13	G_{13}	0.52	0.15



Graph 2: Phosphate solubilizing activity of rhizospheric soil from *Tinospora cordifolia*.

Table 3: Phosphate solubilizing activity of rhizospheric soil from *Tridax procumbans.*

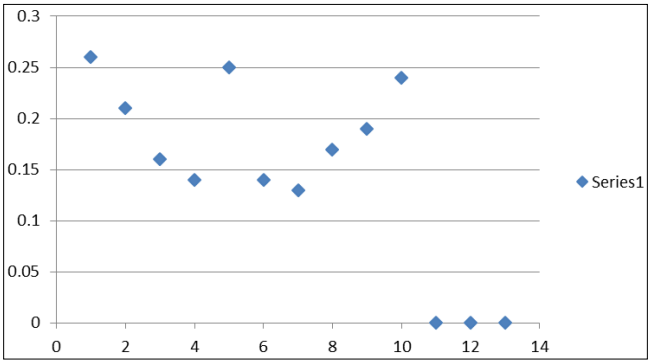
Sr. no.	Isolates	O.D at 690 nm	Conc. from a stand. Graph (mg/L)
1	K_1	0.55	0.16
2	K_2	0.43	0.12
3	K_3	1.10	0.31
4	K_4	1.32	0.38
5	K_5	0.92	0.26
6	K_6	1.19	0.34
7	K_7	1.24	0.35
8	K_8	1.28	0.37
9	K_9	1.26	0.36
10	K_{10}	1.25	0.36
11	K_{11}	1.10	0.32



Graph 3: Phosphate solubilizing activity of rhizospheric soil from *Tridax procumbans.*

Table 4: Phosphate solubilizing activity of rhizospheric soil from *Alam sativum.*

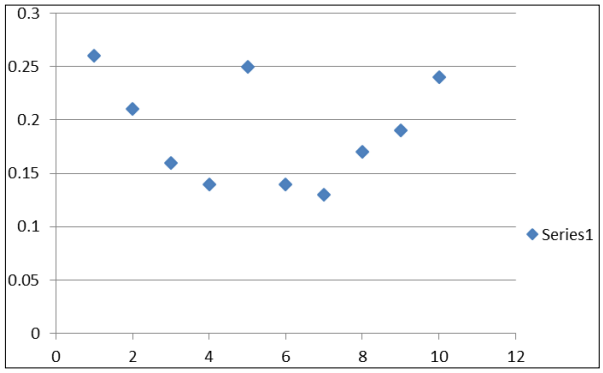
Sr. no.	Isolates	O.D at 690 nm	Conc. from a stand. graph (mg/L)
1	A_1	1.21	0.35
2	A_2	0.51	0.15
3	A_3	1.32	0.38
4	A_4	0.96	0.28
5	A_5	0.98	0.26
6	A_6	0.47	0.14
7	A_7	1.00	0.29
8	A_8	1.27	0.36
9	A_9	1.30	0.37
10	A_{10}	1.09	0.31
11	A_{11}	0.51	0.15
12	A_{12}	0.71	0.21
13	A_{13}	0.55	0.16



Graph 4: Phosphate solubilizing activity of rhizospheric soil from *Alam sativum.*

Table 5: Phosphate solubilizing activity of actinomycetes from rhizospheric soil from *Envicostema auxillare.*

Sr. no.	Isolates	O.D at 690 nm	Conc. from a stand. graph (mg/L)
Blank	-	Auto zero	
1	N_1	0.90	0.26
2	N_2	0.73	0.21
3	N_3	0.55	0.16
4	N_4	0.50	0.14
5	N_5	0.89	0.25
6	N_6	0.48	0.14
7	N_7	0.46	0.13
8	N_8	0.57	0.17
9	N_9	0.66	0.19
10	N_{10}	0.83	0.24



Graph 5: Phosphate solubilizing activity of actinomycetes from rhizospheric soil from *Envicostema auxillare.*

Discussion

The phosphate solubilizing activity of actinomycetes is varied from isolate to isolate and plant to plant. Each actinomycete isolate has its unique phosphate solubilizing activity. The ability of phosphorus or phosphate solubilization was maximum in the rhizospheric soil sample of *Curcuma longa*, which is determined as 0.40 mg/L. So actinomycetes from Curcuma longa utilize a maximum inorganic or insoluble form of phosphate from the soil to fulfill the phosphate requirements of the plant.

The actinomycetes from the rhizospheric soil sample of *Envicostema auxillare* have the least ability to utilize inorganic phosphate as a plant macronutrient. *Alam sativum* and *Tridax procumbans* soil samples have the same phosphate solubilizing activity. Also, actinomycetes isolates that utilize inorganic phosphate are varied from plant to plant. The highest isolates are seen in the rhizospheric soil sample of *Curcuma longa*, *Tinospora cordifolia*, and *Aloe Vera*. Moderate isolates were seen in *Tridax procumbans* and the least isolates are found in *Envicostema auxillare*.

Conclusion

Curcuma longa has been cultivated as a commercial in some regions of India. So, the requirement of chemical fertilizer containing phosphate is in less amount as compared to other medicinal plants studied. It is due to its rhizospheric soil sample contain a high number of actinomycete isolates that solubilize inorganic phosphate. *Envicostema auxillare* requires a high amount of chemical fertilizer containing phosphate due to its soil sample has less number of actinomycete isolates which is mainly responsible for the inorganic phosphate solubilization process. Rhizospheric soil sample from *Tridax procumbans* and *Alam sativum* has

a moderate number of actinomycete isolates, which use moderate inorganic phosphate as compared to *Curcuma longa* and more than *Envicostema auxillare*.

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