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Research Article

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Isolation and Screening of Phosphate Solubilizing *Streptomyces* sp. from Rhizosphere Soil of Maize

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Abstract

Soil fertility and plant nutrition require an adequate management of essential macronutrient such as phosphorus (P), which is mandatory for plant development. In the present study, screening of phosphate solubilizing *Streptomyces* sp. was carried out. 18 *Streptomyces* spp. were isolated and purified on starch casein nitrate agar from rhizosphere soil of maize. These isolates screened for phosphate solubilizing activity on Pikovskaya medium which contains tricalcium phosphate as a sole Phosphorous source. Out of 18 isolates, 4 isolates were showed the activity of phosphate solubilization. Among them S-13 isolate showed good activity. *In vitro* seedling tests were carried out by using four different varieties of seeds such as Chilli, Wheat, Maize and Sorghum. All the S-13 treated seeds showed good growth parameters compared to control treated with distilled water. Hence, *Streptomyces* spp. are promising strains for the implementation of efficient biofertilization strategies to improve soil fertility and plant yield under Phosphorus fertilization.

Keywords: *Streptomyces* spp., Phosphate solubilization, Pikovskaya medium, Rhizosphere soil.

Introduction

Next to nitrogen, Phosphorus is the most essential element in plant nutrition. It is crucial where all the key metabolic functions of plants are carried out, including photosynthesis, energy transmission, signal transduction, respiration, and nitrogen fixation in legumes (Khan *et al.,* 2017).

Although phosphorus exists often in soils in both organic and inorganic forms, its availability is limited since it primarily takes the form of insoluble compounds. Average soil contains roughly 0.05% P, however due to weak solubility and its fixation in soil, only 0.1% of the total P is accessible to plants (Zhou *et al*. 1992). The primordia of a plant's reproductive components must be laid down during the early stages of plant growth, when there must be an appropriate supply of phosphorus. It serves a crucial function in boosting root ramification and strength, giving plants to grow and also the ability to withstand illness. Additionally, it aids in the development of seeds and the early maturity of crops like legumes and grains. Plant size and development are significantly hampered by phosphorus (P) supply issues or deficiencies. About 0.2% to 0.8% of the dry weight of the plant is made up of phosphorus (Sharma *et al*. 2013).

One of the key groups in the microbial community, the actinomycetes, invade the rhizosphere soil and make up the majority of the microbial population (Javed *et al.* 2020). As Plant Growth-Promoting Rhizobacteria (PGPR), they invade the rhizosphere and promote plant growth by supplying nutrients and phytohormones, reducing stress, and inhibiting pathogens. Actinomycetes in the rhizosphere can promote the development of maize and soybean sprouts. They display traits that encourage plant development, such as the solubilization of phosphate, the formation of indole-3-acetic acid (IAA), and nitrogen fixation. (Wahyudi *et al.* 2021; Sari *et al.* 2021).

The ability of actinomycetes to synthesize different organic acids and to provide an acidic environment is crucial for the release of bound phosphate. Acid phosphatase and alkaline phosphatase are two phosphatases involved in the solubilization of phosphate. While bacteria are the main producers of alkaline phosphatase, plants, bacteria, and fungus are also known to create acid phosphatase. Phosphatase enzyme function is essential for the enzymatic hydrolysis of soil organic P. (Wan *et al.* 2020).

In the present study, we screened the *Streptomyces* sp. S-13 for solubilization of phosphate and *In vitro* seedling tests was carried out on four varieties of seeds.

Materials and Methods

Isolation and Screening of *Streptomyces* sp. for phosphate solubilizing activity

A total of 18 *Streptomyces* spp. were isolated from rhizosphere soil of maize. Starch Casien Nitrate agar media was used for the isolation and preservation of *Streptomyces* isolates for further studies. All the 18 isolates were screened for phosphate solubilizing activity. The isolates were spot inoculated on Pikovskaya's medium having pH (6.8-7.8) containing 0.5% of tricalcium phosphate as sole phosphorus sources for selective screening of the *Streptomyces* sp. which have the ability of release inorganic phosphate from tricalcium phosphate producing clearing zone around the *Streptomyces* colonies on the Pikovskaya's medium. The phosphate solubilization index was calculated according to the formula (Khadem *et al.* 2019).

Solubilization Index (SI) = colony diameter + clearing zone diameter colony diameter / colony diameter

Preparation of inoculum

Fermentation

The isolate showed the highest zone on Pikovskaya medium was grown on 250 ml Erlenmeyer flask contains 100 ml of SCN broth medium. The flask was inoculated with *Streptomyces* isolate S-13 and incubated at 28 ± 2^{o} C for 7-9 days. After incubation, the broth was filtered through whatmann filter paper and cell free suspension was used for seed treatment for the phosphate solubilizing activity. The purified isolate S-13 was grown on SCN broth for 7 to 9 days at 30±2^{o} C. The spore suspension was adjusted to 10^{6} spores /ml (Arusha *et al*. 2017).

In vitro seedling test

Seed inoculation with the isolate S-13

For *In vitro* seedling test, four varieties of seeds were selected ie, chilli, wheat, maize and sorghum. Healthy seeds of four varieties were selected and washed 4-5 times repeatedly with sterile distilled water. Again all the seeds were washed with 75% alcohol. Repeated washing was done by sterile distilled water to remove the dust and other contaminants. The sterilized seeds were soaked in the culture broth of the isolate S-13 for 1 hour. The seeds were treated with sterile distilled water for control. All the treated seeds were placed onto previously sterilized wet blotting paper and kept at 28^{o} C. The seeds were supplied with 2 ml of sterile distilled water for 2-3 days for germination under dark conditions. After one week, the effect of S-13 isolate on root length, shoot length and number of lateral roots were measured (Hamdali *et al*. 2012).

Results

In vitro Screening of *Streptomyces* spp. for Phosphate solubilizing activity

All the 18 *Streptomyces* isolates were subjected for phosphate solubilization tests and 4 isolates showed positive result.

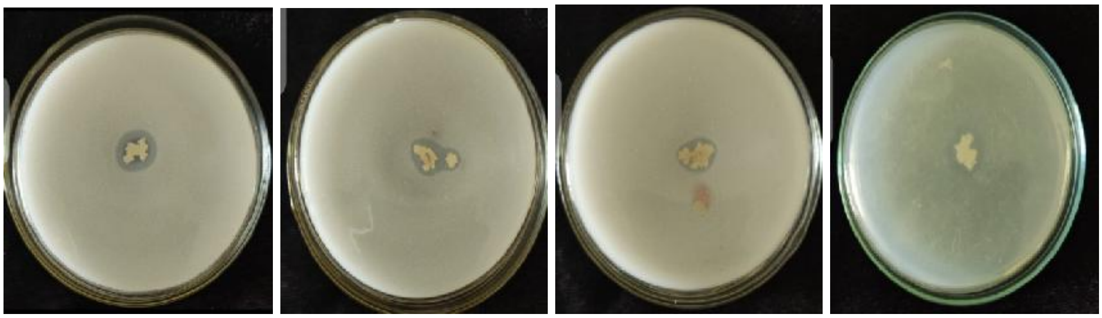
In vitro screening of *Streptomyces* isolates for phosphate solubilization activity was performed by inoculating all the 18 isolates on Pikovskaya agar medium and incubated for 30±2° C for 7-9 days and measured the zone around the colonies. Isolate S-13 showed highest zone among 18 isolates (Table 1).

Table 1 : *In vitro* screening of *Streptomyces* isolates for solubilization of phosphate

Isolate No.	Phosphate solubilizing activity
S-1	+
S-2	++
S-3	-
S-4	-
S-5	-
S-6	-
S-7	-
S-8	-
S-9	-
S-10	-
S-11	-
S-12	-
S-13	+++
S-14	-
S-15	-
S-16	+
S-17	-
S-18	-

‘- ‘ Negative, ‘+’ Positive, ‘++’ Good, ‘+++’ Very good

Among 18 isolates 4 isolates were showed zone around the colony after incubation on Pikovskaya media. Whereas the isolate S-13 showed highest zone among 4 isolates and very least producer was S-16 (0.115±0.021 mm) (Fig 1, Table 1).



S-13 S-2 S-1 S-16

Figure 1: Zone diameter of *Streptomyces* isolates

Isolates S-13 showed highest zone diameter (0. 424 mm) compared to other isolates and least zone producer was S-16 (0.115 mm) (Fig. 2).

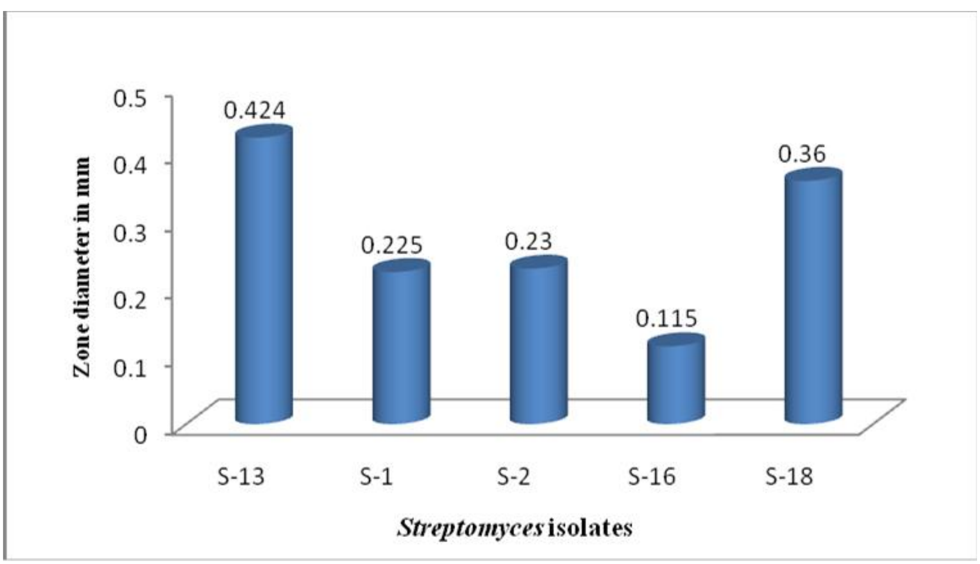


Figure 2: Zone diameter of *Streptomyces* isolates for solubilization of phosphate

All the 18 isolates were tested for solubilizing phosphate.

Phosphate solubilizing activity for each isolate was screened by inoculating the isolates on Pikovskaya agar medium. and incubated at room temperature (27±2 °C) for 5 days. After incubation clear zone was formed around the isolates, was considered as positive test for the phosphate solubilization. The phosphate solubilization index of S-13 was found to be 1.923 mm. Each test was conducted in triplicates (Fig. 3).

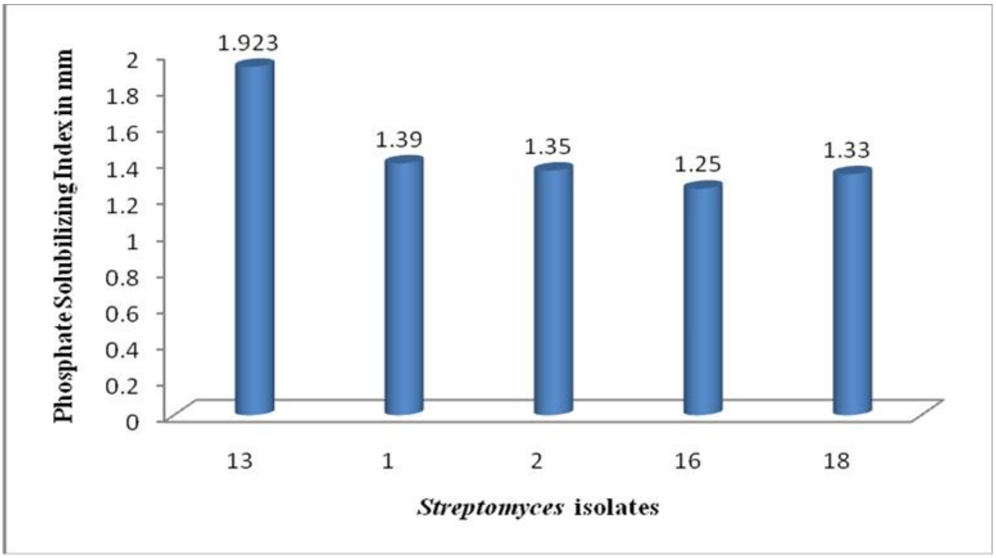
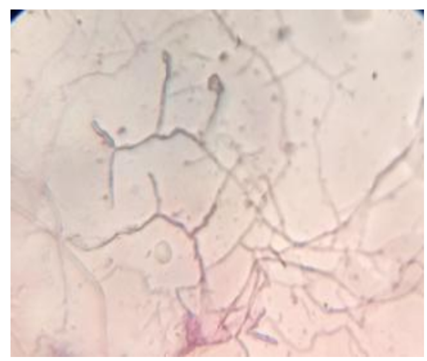


Figure 3: Phosphate solubilizing index of *Streptomyces* isolates

S-13 on SCN media



Microscopic view (100X) of S-13

Figure 4: Morphology of the isolate S-13

In vitro seedling test of *Streptomyces* sp. S-13

In vitro seedling test was carried out with seeds of Chilli, Wheat, Maize and Sorghum. The seeds were treated with seven days old cell-free culture suspension of the potent isolate S-13 and control was treated with distilled water for 7 days. The seeds treated with S-13 were showed highest plant growth promoting traits compared to control (Fig. 5).

The significant effect of plant growth promotion of chilli seedlings by the culture filtrate of the isolate S-13 showed substantial increase in root length (1.61±0.268cm) and shoot length (2.7±0.560cm). The seedling vigour index was found to be (251.3±0.136) (Table 2, Fig. 5).

The effect of plant growth promotion activity of wheat seedlings by the culture filtrate of the isolate S-13 showed increase in root length (6.3±0.55cm) and shoot length (6.05±0.16cm). The seedling vigour index was found to be (213.125±0.036) (Table 2, Fig. 5).

The effect of plant growth promotion activity of maize seedlings by the culture filtrate of the isolate S-13 showed increase in root length (11.6±0.56cm) and shoot length (8.6±1.69cm). The seedling vigour index was found to be (215.2±0.016) (Table 2, Fig. 5).

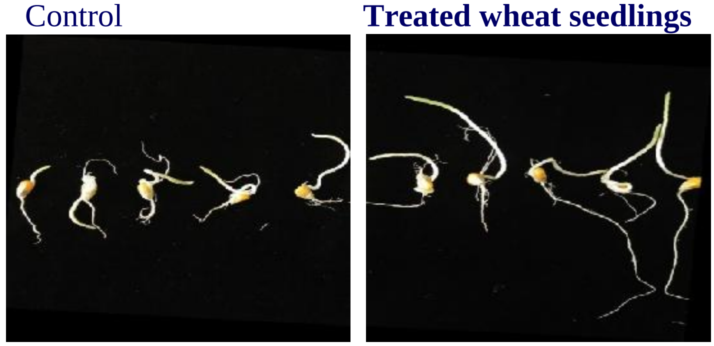
The effect of plant growth promotion activity of sorghum seedlings by the culture filtrate of the isolate S-13 showed increase in root length (5.8±0.28cm) and shoot length (16±1.41cm). The seedling vigour index was found to be (302±0.013) (Table 2, Fig. 5).

Table 2: Effect of culture filtrate treatment on seeds germination and vigour index

Seeds		Root length (cm)	Shoot length(cm)	Vigour index
Chilli	Control	0.35±0.070	1.78±0.091	121.52±0.102
	Treatment	1.61±0.268	2.7±0.560	251.3±0.136
Wheat	Control	3.6±0.84	3.3±0.30	91.125±0.033
	Treatment	6.3±0.55	6.05±0.16	213.125±0.036
Maize	Control	5±1.41	4±4.24	125±0.014
	Treatment	11.6±0.56	8.6±1.69	215.2±0.016
Sorghum	Control	2.8±0.42	8.5±2.12	219±0.015
	Treatment	5.8±0.28	16±1.41	302±0.013

Control **Treated chilli seedlings**

Control **Treated wheat seedlings**



Control **Treated maize seedlings**

Control **Treated sorghum seedlings**

Figure 5: *In vitro* seedling tests of Chilli, Wheat, Maize and Sorghum

Discussions

Actinomycetes are widely distributed in many natural habitats including various soil, fresh water habitat, marine habitat, organic matter and found as a plant root colonizer. A total of 18 *Streptomyces* spp. were isolated from rhizosphere soil of maize collected from agriculture field. All the 18 isolates were screened for phosphate solubilizing activity on Pikovskaya media. Out of 18 isolates, four isolates were positive for Phosphate solubilization and showed zone around the colonies. Among the four, isolate S-13 was showed highest zone (0.242 cm) compared to other isolates (Fig. 2).

One of the essential minerals that affect plant metabolism and growth is phosphorus. Because phosphorus is a limited natural resource, it poses a new problem for global sustainable development (Amri M.F. *et al*. 2021; Chaiharn *et al*. 2018). Because there is not enough phosphorus available to support plant development (Chen *et al*. 2021). Deficiency of P in soil results in reduction of food production since all plants require an adequate supply of P for its growth and development. Even though synthetic phosphate fertilizer has played some major roles in enhancing crop production, its excessive use has cause negative effect, where it has been found to damage the environment, destruct soil fertility, and food chain, also seriously affect the human health (Hanane *et al*. 2008).

The effectiveness of seedling growth is clearly seen in plant height, root length, biomass, and other morphological growth parameters. Phosphorus-solubilizing bacteria stimulate the growth of Chinese fir seedlings in terms of plant height, root length, and biomass in the roots, stems, and leaves. This might be as a result of the PSB strains creating organic acid and extracellular phosphates which help to dissolve the insoluble phosphate in the soil and increase the amount of accessible phosphate (Parekh *et al.* 2018; Bashan *et al*. 2019). The PSB inoculation greatly increased plant height, ground diameter, and biomass. This result may be related to the strains' ability to produce organic acids such gluconic, formic, and citric acids (Pereira *et al*. 2021).

By reducing the amount of insoluble phosphate in the soil and fixing nitrogen, PSBs enhance the levels of nitrogen and phosphorus in the soil (Li, H. *et al.*2020; Rezakhani *et al*. 2020). PSB releases additional nutrients in addition to solubilizing and mineralizing P from insoluble molecules (Oteino *et al.* 2019; Prodhan, *et al*. 2019).

The isolate S-13 was subjected for *In vitro* seedling tests. A total of ten seeds of four varieties were selected for each treatment. The seeds used were chilli, wheat, maize and sorghum. The growth parameters of all four varieties of seedlings after inoculation were significantly greater than those of the control group. The vigour index was also found to be higher in S-13 treated seeds compared to the control. Similar results were obtained with the treatments inoculated with *Streptomyces griseorubens* BC10 and *Nocardiopsis alba* BC11 in greenhouse conditions (Chen *et al*. 2021).

The actinomycete isolates' determined phosphate solubilization index and solubilized amounts of P are generally comparable with the levels of acidity (pH values) produced in their cultures. This suggests that the primary source of solubilization may be organic acids created by the actinomycete isolates as they fermented sugars in the medium. Since phosphate solubilization raises the concentration of soluble phosphate, which is necessary for plant development, it is suggested that the phosphate-solubilizing actinomycetes may be employed as plant growth promoters (Hamdali *et al*., 2012).

Conclusion

In conclusion, PSB could be used as biological agents instead of chemical fertilizers for agro forestry production to reduce environmental pollution and increase the yield. Plant growth and development are significantly hampered by phosphorus (P) supply issues or deficiencies. About 0.2% to 0.8% of the dry weight of the plant is made up of phosphorus. P is typically applied to soil as a chemical fertilizer to meet the nutritional needs of crops, but its synthesis is an energy-intensive process that has long-term effects on the ecosystem in the form of eutrophication, soil fertility loss, and carbon footprint. Moreover, plants can utilize only a tiny quantity of this P as 75-90% of supplied P is precipitated by metal-cation complexes, and soon becomes fixed in soils. Due to these environmental problems, sustainable crop P feeding methods are being sought after. Phosphate-solubilizing microorganisms (PSM) have been considered as best eco-friendly means for P nutrition of crop.

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Conflicts of interest: The authors declare no conflict of interest.

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