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In-vitro Phosphate Solubilization by Native Phosphate Solubilizing Microbes in Soils of Northern Region of India

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ABSTRACT

The investigations were conducted at Sardar Vallabhai Patel University of Agriculture and Technology, Meerut, Uttar Pradesh, India during March 2019 to January 2020 for isolation and characterization and identification of phosphate solubilizing microbes (PSM) in soils of northern region of India. Soil samples (0-30 cm) were collected from four locations *i.e.* KVK, Lohaghat, SVPUAT, Meerut, College of Agriculture field, SVPUAT, Meerut and KVK, Bulandshahar covering Uttar Pradesh and Uttarakhand states of India and kept in refrigerator for analysis. The isolation of two rhizobial bacteria isolates (S_1 and S_2) in conjunction with the two-phosphate solubilizing efficient fungal isolates (F_1 and F_2) was carried out by the serial dilution method. The study of their morphological and microscopic characters, together with Solubilization Efficiency (SE), and Solubilization Index (SI) were also assessed. Finally, the biochemical tests were run according to their nature that was gram-negative rod by using KB002 TM HI Assorted Biochemical Test Kit (for Gram-negative rods). Between the two bacterial isolates was S_1 was showing more solubilization on Pikovskaya agar (PVK) compared to S_4 on the 5th day of observation, while S_4 gave better results on the 8th-day solubilization observation. The biochemical tests on bacterial isolates were showing disagreement between the two, proving them as completely different isolates. In the case of fungi, the F_2 isolate was showing better SE and SI values compared to the F_1 isolate.

KEYWORDS: Phosphate enrichment, microbial population, phosphorus solubilization, soil fertility

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1. INTRODUCTION

The scientists and farmers in developing countries like India needs better crop production so that they can sustain food needs of growing population (Fatima et al., 2021), up to 50% increase in crop production can be achieved by supplying nutrients using chemical fertilizers but these in long term causes soil salinity, soil quality loss as well as environmental degradation (Lin et al., 2019). Due to increasing demand of agricultural crops the need of P nutrient for crop replenishment had been increased (Shah et al., 2019). Every crop needs phosphorus for energy generation, signal transduction, biosynthesis of macromolecules, respiration in plants and nitrogen fixation in leguminous crops (Khan et al., 2010, Qu et al., 2019), but the main challenge for the crop plants is in obtaining phosphorus from soil because of fixed P forms and less reserves of available phosphorus in it (Gupta et al., 2020, Elias, 2016). Tropical and sub-tropical soils are suffering with P-deficiency due to its fixation (Bortoluzzi et al., 2015, Fink et al. 2016, Koutika et al., 2016, Hong and Lu, 2018). The phosphate precipitation after fertilization is the most dangerous phenomena causes P-fixation, which is about 80–90% from the phosphate fertilizer supplied thereby P nutrient gets precipitated as a metal cation complex in soils (Hoosbeek, 2000, Prajapati and Pattanayak, 2019) only 20% of the applied P can be taken up during 1st year by the crops (Cao et al., 2020). In intensive agriculture practices plants are supplied by more inorganic P fertilizers, which are costly and even non-environment friendly (Intani et al., 2018; Azzi et al., 2017). According to Brady and Weil (2002) phosphorus concentration in the soil ranges from 0.001 to 1 mg l^{-1} that is much lower than the concentration of any other plant nutrient present. Hence, there is a need to solubilize unavailable soil phosphates to plant available forms with the help of microbes (Bar-Yosef et al., 1999). The Phosphate solubilizing microbes (PSMs) are of fundamental importance in the sphere of agriculture. These PSMs are heterotrophic microbes (bacteria and fungi) which are ubiquitous (Awais et al., 2017) and can easily convert insoluble phosphatic compounds into a plant-available orthophosphate form by releasingP from its chelating cation partner (Ca, Fe, and Al) using several organic acids. The PSMs are diversified in their soil actions, they help in plant growth promotion in stress as well as in non-stressed environment, solubilize other nutrients like potassium, promote nitrogen fixation, remediate heavy metal contaminates soils, and also regulate the production of useful plant enzymes like cytokinins, gibberellins, and auxins (Rawat et al., 2020).

The sources of phosphate in soils are primary and secondary minerals together with some organic compounds. The rhizobacteria and fungi like VAM facilitates the mobilization of different forms of phosphates by various mechanisms (Wang et al., 2012). The growing PSMs reduce soil pH due to production of organic acids (Collavino et al., 2010, Prabhu et al., 2019). The population of phosphate solubilizing bacteria is 1–50% and fungi are 0.1-0.5% respectively out of total soil microbes (Souza et al., 2015). All these above mechanisms help convert tricalcium phosphate to mono or dicalcium phosphates that enhances their availability for plants (Khan et al., 2014).

Under *in-vitro* conditions, the identification of phosphate solubilizing organisms is done by analyzing the size of halo zone (clear-zone) produced by the identified microbe either bacteria or fungi (Chung et al., 2005). The zone was developed due to the production of organic acids produced by the microbial agents on Pikovskaya agar (Pikovskaya, 1948a). Therefore, the objective of the current study is to isolate, characterize and identify the P solubilizing microbes from various soils.

2. MATERIALS AND METHODS

The investigations were conducted at Sardar Vallabhai Patel University of Agriculture and Technology, Meerut, Uttar Pradesh, India during March 2019 to January 2020.

2.1. Site description

Soil samples were collected from four locations (Appendix a) i.e. KVK, Lohaghat, Distt. Champawat, Uttarakhand (UK.)80°10'E longitude, 29°60' N latitude (Soil-1)HRC, SVPUAT, Meerut (U.P.)77°69'E longitude, 29°09'N latitude(Soil-2), College of Agriculture field, SVPUAT, Meerut (U.P.)77°69'E longitude, 29°08' N latitude (Soil-3), and KVK, Bulandshahar (U.P.) 77°78'E longitude, 28°28'N latitude (Soil-4), India for isolation of microbes. About 500 g of soil sample was taken from 0–30 cm of the soil depth aseptically by keeping it into sterile polyethylene. The samples were collected at different time slots but kept in the air tight sterile polythene under refrigerator for analysis.

2.2. Method of isolation

The isolation of phosphate solubilizing bacteria and fungi was done by the serial dilution method (Kurniawan, 2021). In this process, 2 g of soil sample was taken in test-tube having 10ml. distilled water and diluted the soil from 10^{-1} concentration till the desired dilution level (10^{-3} to 10^{-5}) is achieved. At dilution level between 10^{-3} to 10^{-5}, about 1ml. of suspension was taken and spread on to the media plates (King's B, PVK, and PDA) for obtaining a mixed culture of microorganisms. The microbes solubilizing P (bacteria and fungi) were isolated using Pikovskaya (PVK) agar media (Pikovskaya, 1948b) for observing the phosphate solubilization *in-vitro*. The media consists of yeast extract

0.5 g l^{-1}, dextrose 10.0 g l^{-1}, $Ca_3(PO_4)_2$ 5 g l^{-1}, $(NH_4)_2SO_4$ 0.5 g l^{-1}, KCl 0.2 g l^{-1}, $MgSO_4$ 0.1 g l^{-1}, $MnSO_4$0.0001 g l^{-1}, $FeSO_4$ 0.0001 g l^{-1} and agar 15.0 g l^{-1}. The bacterial and fungal colonies form a clear halo zone surrounding them which helps in the identification of PSM.

The pure culture preparation of bacterial and fungal cultures was done using King's B and PDA media respectively. The King's B media was made for maintaining the pure culture of bacterial isolates and it 20.0 g l^{-1}, proteose peptone 20.0 g l^{-1}, K_2HPO_4 1.5 g l^{-1}, $MgSO_4.7H_2O$1.5 g l^{-1}, $C_3H_8O_3$(Glycerol) 15 ml l^{-1}. The preparation of PDA media consists of agar 20.0 g l^{-1}, potato 250 g l^{-1}, $C_6H_{14}O_7$ (Dextrose) 20.0 g l^{-1}. The isolated microorganisms can be further checked for their phosphate solubilizing ability on PVK medium by analyzing Solubilization index (SI) and Solubilization Efficiency (SE) using the formula (Edi Premono et al., 1996).

Solubilization Index (SI)=D/d

Solubilization Efficiency (SE)=(D-d)/2

Where,

d= bacterial/fungal smear zone distance

D= Total distance (bacterial smear zone distance+clear zone)

The identification of microbial isolates was done by analyzing colony morphology and microscopic characteristics of the screened microorganisms. Microscopic analysis of microbial isolates was done following the gram staining technique. Further confirmation of the gram's test was done following Rhu's test on the respective isolates.

The bacterial smear was spread on the slide and was flooded with crystal violet for one minute. The excess dye was poured off and washed gently with distilled water. The smear was then exposed to gram's iodine solution for one minute, wash it with tap water, and drain carefully. Wash the slide with 95% alcohol for 30 seconds. Again wash it with tap water to avoid decolorization. Finally, counterstain with 0.25% safranin for 30 seconds and wash, drain, blot the slide. Observe the slide with the help of a microscope. The fungal identification was done using transparent adhesive tape (Sellotape) mount on the slides. The tape to which fungal structures got attached was placed over a drop of the staining solution (lactophenol cotton blue) over the slide and was observed under the microscope.

Further, for the analysis of bacterial isolates, 10 biochemical tests (Appandix b) for bacterial identification were performed for their diversity analysis. The tests performed were oxidase test, catalase test, nitrate-reducing test, urease, citrate utilization test, lysine decarboxylation test, ornithine decarboxylation test, phenylalanine deamination test, H_2S production test, carbohydrate utilization tests (glucose, adonitol, lactose, arabinose, and sorbitol utilization test). The oxidase test was done by using the reagent tetramethyl-p-phenylenediamine, which when comes in the contact of culture converted to deep purple-blue color, that indicates positive reaction and if no color change indicates a negative reaction. Additional tests were done using company Hi-media, KB002 TM HiAssorted Biochemical Test Kit which is having a combination of 10 biochemical tests for identification of Gram-negative rod bacteria. The soil pH was determined with the help of combined glass electrode pH meter with (1:2.5 w/v) soil water suspension method (Jackson, 1973).

3. RESULTS AND DISCUSSION

3.1. Isolation of different microbial isolates

The soils were taken from KVK, Lohaghat was slightly acidic in pH 5.80, while other samples were in the range of normal pH i.e. Soil-2 (7.13), Soil-3 (7.55), and Soil-4 (7.48). The variable bacterial and fungal isolates were observed from these four soil samples when inoculated over PVK media. Among various mixed cultures of microbes the organisms showing phosphate solubilization were mainly identified from each soil. The results of various macroscopic plate observations of mixed cultures from different soil samples are arranged in (Table 1).

It is clear from the results that the pure cultures of the P solubilizing bacteria were observed from soils Soil-1, Soil-3, and Soil-4. The pure culture of the bacterial isolate from Soil-1 sample and an isolate from the Soil-4 sample showed solubilization on PVK medium, however, the pure culture of the bacterial isolate from the Soil-3 sample didn't show any solubilizing effect. The solubilization effect of bacterial isolates on PVK medium was represented by the halo-zone formation (Gayathri et al., 2022; Waday et al., 2022 and Lal et al., 2022). Hence, bacterial isolate from soil sample Soil-1 and Soil-4 were named accordingly and selected for further P solubilization studies on PVK medium. The pure culture of the fungal isolate from Soil-1, Soil-2, and Soil-3 soil sample did not show considerable solubilization on PVK medium, thereby pure culture of the two fungal isolates, obtained from previous studies was used for further analysis. These two fungal isolates named F1 and F2 to be used for further P solubilization studies on PVK medium.

3.2. Microscopic study of different pure cultures of microbial isolates

The fungal and bacterial isolates were identified by colony morphology and microscopic characterization. A total of above four isolated microbial isolates (Bacteria and Fungi) were used for identifying their colony morphology and microscopic studies. The two bacterial isolates were maintained on King's B medium and two fungal isolates were maintained on PDA medium respectively. The isolates

Table 1: Macroscopic characterization of soil isolated bacteria and fungi on PVK media

Sl. No.	Soil Sources	Soil code	Macroscopic identification of various microbial colonies on PVK medium	Remarks
1.	KVK Lohaghat, Distt. Champawat, Uttarakhand (Uk.)	S_1	Fungal growth shows blackish cottony appearance. The bacterial colonies were as follows: White filamentous, flat colonies. White irregular, lobate colonies. White round entire flat colonies	Bacterial and fungal both the colonies were observed. Only a bacterial culture isolated from identified colonies shows phosphate solubilization on PVK media.
2.	HRC, SVPUAT, Meerut (U.P.)	S_2	The fungal colonies were white in color laden with black spores spreading over almost half of the petri-plate. Bacterial colonies were: Red pigmented round entire and flat colony. Most scattered ones were the white round entire flat colonies.	Fungal colonies dominated showing slight solubilization zones. None of the bacterial cultures showing phosphate solubilization.
3.	College of Agriculture field, SVPUAT, Meerut (U.P.)	S_3	Small white irregular fungal colony observed in the corner of the plate. Different bacterial colonies were observed as follows: White filamentous flat colony Yellow filamentous flat colony Red pigmented round entire and flat colony. Pale round entire flat colonies scattered. Pale round colony showing halo zone	The fungal colonies on side of the plate show slight solubilization. The pale round colony was showing solubilization halo zone was picked up for further testing.
4.	KVK, Bulandshahar (U.P.)	S_4	One fungal colony was also observed, its colony morphology was similar to *Aspergillus* spp. Multiple colonies of bacteria were observed, only one with greenish irregular lobate slimy bacterial colony was showing characteristic solubilization zone.	No fungal strain was showing solubilization. Bacterial colony identified to be showing solubilization zone.

were then screened based on their colony morphology and growth parameters such as colony form, color, elevation, margin, and fluorescence. The findings were supported by Gandhi et al., 2014; Awais et al., 2017 and Esikova et al., 2021. After gram staining and observation under a microscope, KOH Ryu's test was performed to confirm whether the isolate is gram-negative or positive. Isolate gives positive Ryu's test indicate a gram-negative bacterium as it produces a string when picked with a loop after addition of 3% KOH. The results are shown in (Table 2) and (Figure 1) and (Figure 2).

3.3. Screening of microbial isolates for phosphorus solubilization

The four microbial isolates were examined for their ability to solubilize phosphorus on Pikovskaya's agar medium (PVK) supplemented with tricalcium phosphate. The solubilization was observed and various readings were taken on the interval of 5th day and 8th day respectively. The experiment for analysis for each isolate was done under three replications. The colony diameter (d) and halo zone diameter (D) were noted for each replication, from this data Solubilization Efficiency (SE) and Solubilization index (SI) were calculated. The Quantitative analyses of isolates for P solubilization recorded during different intervals are present in Table 3 and Table 4 as well as in Figure 3 and Figure4. From the results of Solubilizing Efficiency (SE) it was clear that, among bacteria's and fungi, at 5th day and 8th day of solubilization the bacteria S_1 and fungi F_2 was showing better SE value, than the other two isolates. The results of Solubilizing Index (SI) slightly vary, among bacteria's and fungi, at 5th day and 8th day of solubilization the bacteria S_4 and fungi F_2 was showing better SI, than the other two isolates.

The similar findings were done by Qarni et al., 2021 and Ditta et al., 2017 for fungal isolates and Suleman et al., 2018 for bacterial isolates was done .

3.4. Biochemical testing of different bacterial isolates

Total of 10 biochemical tests were performed for testing of different bacterial isolates. The results for these biochemical tests were presented in (Table 5). Both the two isolates gave a positive test for citrate utilization. Isolate S_1 gave a positive test for lysine and ornithine utilization whereas S_4 isolate showed negative results. Urease test was positive for both

Table 2: Macroscopic and microscopic characteristics of bacterial and fungal isolates

Name of microbes	Colony Morphology	Microscopic Characters	Results
Bacteria			
S_1	White colored colonies in King's B media. Medium sized and irregular. No fluorescence under UV rays. Colony diameter <1 mm. Fully opaque. Flat colonies.	Gram negative, rod shaped	May be *Bacillus* sp.
S_4	Greenish color in King's B media. Colonies are medium sized and irregular. Gives greenish-yellow fluorescence (Fluorescein) under UV rays. Colony diameter <1mm. Smooth (fresh isolation); Mucoid (When slime layer is formed). Translucent to opaque.	Gram negative, rod shaped	May be *Pseudomonas* sp.
Fungi			
F_1	Colonies growing rapidly on PDA media showing white floccose initially, later spread on the plate rapidly and quickly. The spores turned black coloured and hence the black coloured colonies started appearing. The reverse side of the plate shows white to pale yellow pigments. Colonial diameter 15-17 mm and increases with time.	Conidia were small, brownish black to greenish in colour. Hyphae were septate with rough brown and smooth, colourless long conidiophores having distinctive conidial heads.	The fungi may belongs to *Aspergillus* spp.
F_2	Colonies were initially showing bright orange colour pigmentation then turn dark green with time, showing velvety appearance. The colony diameter was about 5-10 mm. The fungus was filamentous and on the back side red colour pigmentation appears on PDA media.	Conidia were found in longer chains, green coloured and transparent. Hyphae septate hyaline. Penicillate evolved conidophores, phaibides produced in groups from branched metulae giving brush like appearance.	The fungi may belongs to *Penicillium* spp.

Table 3: Measurement of phosphorus solubilization of different microbial isolates on PVK medium at 5th day

Sl. No.	Isolate code	d		D		Halo zone (D-d)	SE (D-d)/2	SI (D/d)
		Mean	SD	Mean	SD			
1.	S_1	6.61	±0.49	11.11	±0.70	4.50	2.25	1.68
2.	S_4	4.67	±0.71	8.22	±0.57	3.56	1.78	1.76
3.	F_1	10.72	±0.57	13.72	±0.53	3.00	1.50	1.28
4.	F_2	6.61	±0.60	14.72	±0.67	8.11	4.06	2.23

isolates. Both isolates S_1 and S_4 showed a positive result for the Phenylalanine deamination test. Nitrate reductase activity was observed in S_4 but not in the S_1 isolate. Isolate S_4 gave a positive result for the H_2S production test and isolate S_1 showed negative results. In the carbohydrate utilization test, the glucose utilization test was positive for both isolates. Sorbitol and adonitol utilization tests were negative for both isolates. Isolates S_1 utilized lactose, whereas isolates S_4 utilized arabinose sugars. Results show that isolate S_1 and S_4 were gram-negative rods but gave completely different results for the biochemical analysis. This observation indicates that S1 and S4 are completely different isolates form each other.

The isolate S_1 was approximately identified for its genus based on the study of Sadiq et al., 2013 and Bashir et al., 2018 as the biochemical tests conducted by him on his isolates was almost similar to the ones conducted by us. The results of S1 isolate for Catalase, Urease and Citrate utilization was positive while Oxidase and H_2S production test was negative. These results coincided with the results by Sadiq et al., 2013 and Bashir et al., 2018 for all *Bacillus* isolates. Hence, the isolate S_1 may be of *Bacillus* sp.

For the isolate S_4 biochemical test results were coincided with the findings of Hi-media, KB002 TM HiAssorted

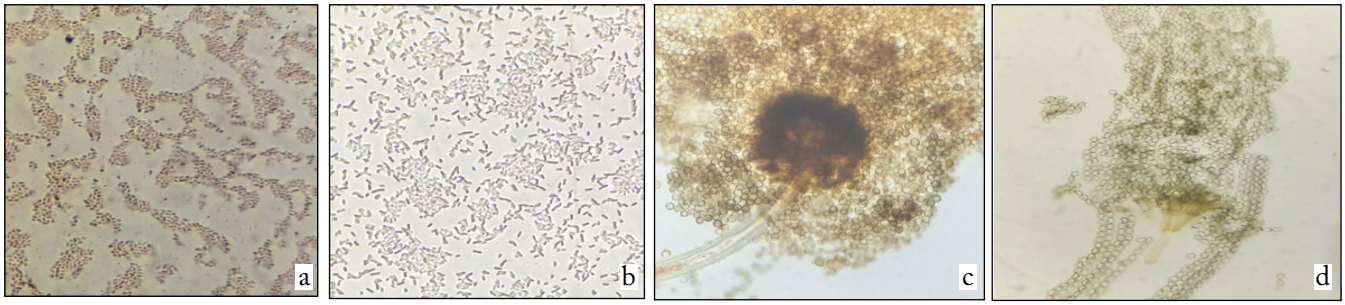

Figure 1: Microscopic images of Bacteria and Fungi. a. S_1, b. S_4, c. F_1, d. F_2

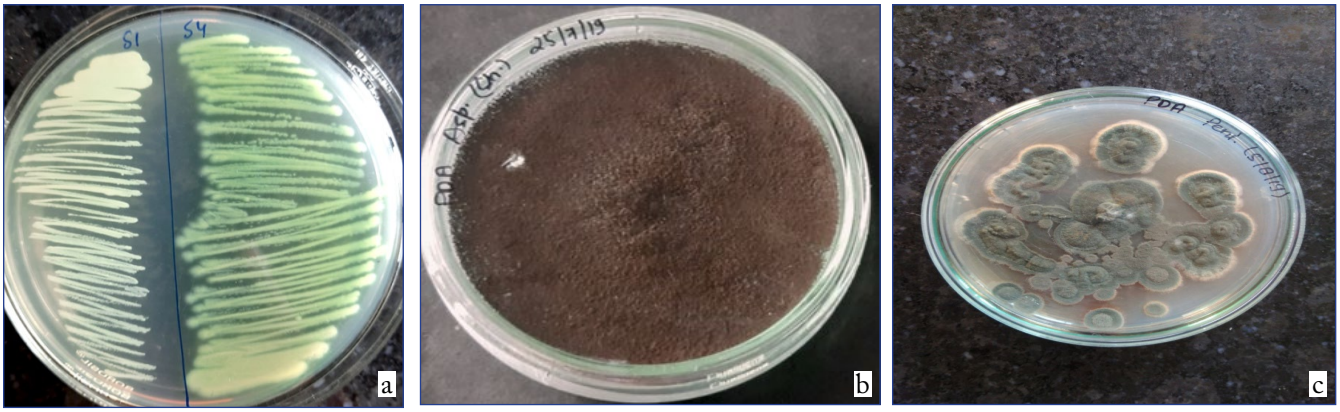

Figure 2: Colony morphology of Bacteria and Fungi. a. S_1 and S_4, b. F_1, c. F_2

Table 4: Measurement of phosphorus solubilization of different microbial isolates on PVK medium at 8th day

Sl. No.	Isolate code	d		D		Halo zone (D-d)	SE (D-d)/2	SI (D/d)
		Mean	SD	Mean	SD			
1.	S_1	9.39	±0.86	17.06	±0.88	7.67	3.83	1.82
2.	S_4	4.83	±0.50	10.39	±0.93	5.56	2.78	2.15
3.	F_1	17.50	±0.75	22.78	±0.57	5.28	2.64	1.30
4.	F_2	7.50	±0.61	15.72	±0.57	8.22	4.11	2.10

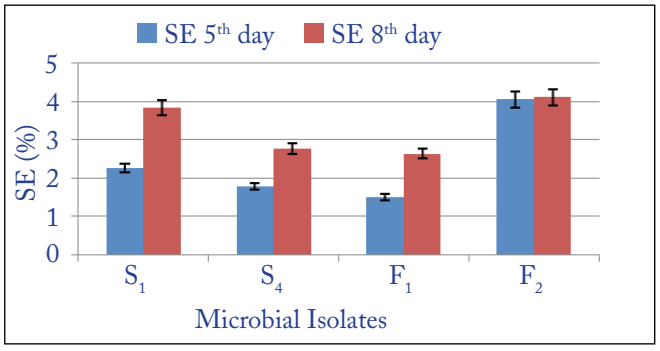

Figure 3: Solubilizing efficiency (SE) of microbial isolates

Biochemical Test Kit (for Gram-negative rods), (Naz and Bano, 2010) and Ahemad, 2012. The test results shows positive results of S_4 isolate are catalase, oxidase, arabinose, glucose, urease and citrate utilization while the negative tests were lysine, ornithine, adinol, actose and sorbitol tests. Almost similar observations were written in KB002 TM HiAssorted Biochemical Test Kit results as well as in research of (Naz and Bano, 2010) and Ahemad, 2012 for *Pseudomonas*. The morphological characterstics like cell shape (rods) and colony morphology (mucoid, smooth margins) as described by (Naz and Bano, 2010) and Ahemad, 2012 for *Pseudomonas* is similar to the once described for stain S_4. Hence, the isolate may be of *Pseudomonas spp*.

In Indian rhizospheric, agricultural soils a higher population of phosphate solubilizing microbes is commonly found (Panda et al., 2016). Therefore, different soils from agricultural fields were taken for this experimental study for the isolation of phosphate solubilizing microbes. As there were so many colonies identified over the plate after spreading of dilution solution of all four soil samples, but the potent bacterial isolates were limited in number. They

Table 5: Biochemical analysis for 14 tests of 02 different bacterial isolates (S_1 and S_4)

Sl. No.	Tests	S_1	S_4
1.	Citrate utilization	+	+
2.	Lysine utilization	+	–
3.	Catalase Test	+	+
4.	Ornithine utilization	+	–
5.	Urease	+	+
6.	Phenylalanine deamination	+	+
7.	Nitrate reduction	–	+
8.	H2S production	–	+
9.	Glucose utilization	+	+
10.	Adonitol utilization	–	–
11.	Lactose utilization	+	–
12.	Arabinose utilization	–	+
13.	Sorbitol utilization	–	–
14.	Oxidase	–	+

were selected based on their solubilizing efficiency (SE) in solid media (Panda et al., 2016). The bacterial cultures from two, out of four soil samples were identified as P solubilizer, none of the potential phosphate solubilizing fungal cultures was obtained from any soil samples being tested. Similar illustrations for the isolation of microbial isolates from soil were made by (Tallapragada and Seshachala, 2010, Mukherjee and Dutta, 2019).

The morphological differentiation of these isolates was based on their features seen in their colony morphology, as the color of the colony, pigmentation, shape of colony. The colony morphology of microscopically gram-negative rods i.e. S_1 and S_4 were quite dissimilar from each other. S1 was having fully opaque, flat, white colonies while S_4 was opaque to translucent, smooth, and greenish colonies on King's B media.

The colonial morphology of fungal isolates F_1 gave a black dense colonial growth on the front side of PDA plate with white color on the backside. Its microscopic analysis reflected long conidiophores with terminal smooth sac-like structure as well as the round black conidia which were arranged in chains over the sac. Based on this morphological and microscopic analysis the fungal isolate F1 appears to be *Aspergillus* species. The fungal isolates F_2 was showing grey and yellow color colonies on the front side of the PDA plate while orange on the backside and microscopically, branched conidiophores and phialides were observed in the microscope giving a brush-like appearance, the conidia were globular, greenish, and smooth. The results of identification have coincided with the results of Pandey et al., 2020; Sane and Mehta, 2015; Houbraken et al., 2020 and Pangging et al., 2022 as they also had found the same morphological characters in their fungal isolates (*Aspergillus* spp. and *Penicillium* spp.).

The solubilization of isolates was observed at incubation under the temperature range of 28°C to 32°C for bacteria, the same temperature for incubation was taken by Gaind and Gaur, 1991. The fungal isolates were incubated at the temperature of 25–28 C as also suggested by Elias et al., 2016. The biochemical tests for the two gram-negative bacterial isolates showing positive results for citrate utilization, catalase test, urease, phenylalanine deamination, and glucose utilization test while other tests were either or positive for the two bacteria's. The variability for the gram-negative biochemical tests was also observed in the results of Chung et al., 2005 his results of biochemical tests for gram-negative rods were showing positive catalase tests while the other test observations were variable. This proves that the bacterial isolates were dissimilar to each other. The solubilization done by the isolates was confirmed due to the formation of the halo zone on PVK media. As said by Darmwall et al., 1989 the halo zone forms when the solubilization of insoluble phosphates due to acidification either by proton extrusion or organic acid secretion. On estimating the phosphate solubilizing efficiency and solubilization index it was observed that the *Penicillium spp.* (Fungi), was showing better solubilization as compared with two bacterial isolates. The fact was supported by Sanjhotha et al., 2011 that the fungal isolates have more efficiency to solubilize inorganic phosphates than bacteria. The solubilization observations were made by recording the halo zone diameter, which shows that the halo zone increases with the duration of incubation (Sanjhotha et al., 2011). The solubilization on PVK media of bacterial and fungal isolates is present in (Figure 4).

Among the bacterial cultures, S_4 was showing better solubilization than S_1 which was determined by the higher SE and SI of the S_4 isolate. The phosphate Solubilizing Index (SI) values of S_1 and S_4 bacterial isolates vary from 1.68 to 2.15, quite similar results were presented by Mukherjee

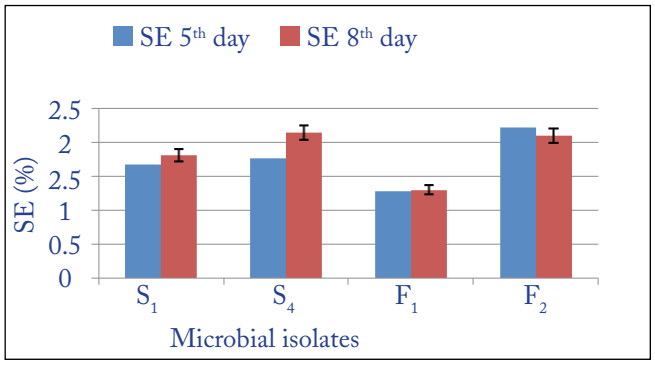

Figure 4: Solubilizing Index (SI) of microbial isolates

and Dutta, 2019. The 9 bacterial isolates showed a variation of SI values from 1.04 to 2.85. The best isolate S_4 showed an SI value of 2.15 and the same was observed in Mukharjee's CP6 isolate showing an SI value of 2.85. Chena and Liu, 2019 identified 5 bacterial inoculants having SI ratio of 1.50 on inorganic phosphate inoculated media. The solubilizing efficiency (SE) of S4 inoculant, in tricalcium phosphate media, was 21.5% on the 8th day of observation which was highest among bacterial isolates. Chena and Liu, 2019 reported that his bacterial isolate (S32) had shown 24.09% solubilizing efficiency (SE) in tricalcium phosphate [Ca_3 $(PO_4)_2$] media. Elias et al.,2016 isolated and characterized, the phosphate solubilizing fungi belong to *Aspergillus* spp. and *Penicillium* spp. their solubilizing indexes (SI) were in the range of 1.10 to 3.05. Sane and Mehta, 2015 had also observed the maximum SI 3.5 by *Aspergillus* fungi followed by *Penicillium* which shows SI in the range of 3 to 3.3.

The utilization of fixed inorganic phosphorus, into a more usable form, can be done by efficient PSMs biofertilizers (Zhu et al., 2012). The present study on the different types of soil isolates shows potential phosphate solubilizers in them. Soil-1 being an acidic soil shows higher phosphate fixing potential due to isolate S_1 which may be of *Bacillus sp.*, compared to isolate S_4 (*Pseudomonas sp.*) from Soil-4. The phosphate solubilizing fungi *Aspergillus* spp. and *Penicillium* spp. also showing higher solubilizing efficiency and could be used for further testing for P solubilization under soil conditions. Hence these microbes can be further tested for their efficiency as potent bio inoculants after soil application which is an eco-friendly and yield-enhancing strategy for agriculture.

4. CONCLUSION

The acidic soil (Soil-1) having isolate S_1 may be of *Bacillus* spp. showed higher phosphate fixing potential compared to *Pseudomonas* spp. (S_4) isolated from Soil-4. The phosphate solubilizing fungi *Aspergillus* spp. and *Penicillium* spp. also showed higher solubilizing efficiency. These soil isolated microbes are potential phosphate solubilizers. Hence, these can be further tested for their efficiency as potent bio-inoculants after soil application which can be an eco-friendly and yield-enhancing strategy for agriculture.

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