Microbial Efficacy of Phosphate Solubilization in Agro-Saline Soils of Various Areas of Sindh Region

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ABSTRACT

Microorganisms are the most prominent entities for solubilization of phosphate in various soils of different areas of Sindh Province including Tando Muhammad Khan, Tando Allah Yar, Nawabshah, Rato Dero-Larkana, Shikarpur and Umer Kot. These soils, having varying concentrations of chemicals, different climatic conditions, pH and varying numbers of microorganisms for PSA (Phosphate Solubilization Activity). This presentation shows the isolation of different fungi and bacteria capable Psa including fungi (Fusarium sp. Aspergillus sp. Penicillium sp. and Rhizopus sp.) and bacteria (Bacillus sp. Pseudomonas sp. and Arthrobacter sp.). From the observations, it was revealed that fungi Aspergillus sp. and Bacillus sp. showed greater phosphate solubilization activity as compared to other fungi and bacteria showing 60 and 53.33% Psa (Phosphate Solubilizing Activity) respectively.

Key Words: **Phosphorous and its Types, Phosphate Solubilizing Bacteria and Mold Fungi, Acid Production, Plant Growth, Saline Soil.**

1. INTRODUCTION

Soil is the natural body consisting of layers (soil horizons) of mineral constituents of variable thicknesses, which differ from the parent materials in their morphological, physical, chemical, and mineralogical characteristics [1]. Phosphorus is the second most important, expensive source of nutrient required for the increased yield of the plants. In soils the availability of phosphorous is low or unavailable and found only in the form of insoluble phosphates and hence cannot be utilized by the plants. Its availability is limited due to the production of oxides and hydroxides of aluminum and iron in acid soils and calcium in alkali soils [2-6]. Many chemical phosphate containing fertilizers show adverse effects on soil when used and easily precipited as insoluble form that indicates the [2,7-9].

Many soil fungi and bacteria are reported to have the ability to solubilize the inorganic phosphate. The PSMs (Phosphate Solubilizing Microorganisms) play magnificent role in supplementing phosphorous to the plants allowing a sustainable use of phosphate fertilizers for plant growth [6,10-9]. Mainly the PSMs are

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concentrated in the rhizosphere of the plants and increase the phosphate up-take by plants under field conditions and in pot experiments [10-12]. Filamentous fungi and bacteria were important soil flora used for phosphate solubilization in soil [4,13].

PSMs are ubiquitous in soils in soils and support the plant growth in a convenient manner [14]. Number of workers reported different PSMs such as Pseudomonas striata, Aspergillus awamori, Penicillium bilaji [15], Bacillus megaterium, Aspergillus niger, Penicillium oxalicum [16], Penicillium citrinum [2] and many workers found the acid production during the phosphate solubilization process [17-22]. The other workers reported the increased crop growth and yield by several mechanisms like lowering of pH by acid production, ion chelation and exchange reactions in the growth environment [23].

2. AIMS AND OBJECTIVES

The essence of this work is:

(i) To isolate the microorganisms having greater phosphate solubilization activity.

(ii) To observe the specific time duration (days/ hours) for the phosphate solubilization process by the test isolates.

2.1 Materials and Method

Eighty five soil samples were collected from 20cm depth from the cultivated lands (near salinity areas) of wheat, sugar cane, banana and rice. One (01) ml of the soil samples were separately poured directly on the surface of Potato dextrose and Nutrient agar plates and spread by glass spreader, incubated at room temperature for 1-2 weeks and 24-48 hours for the growth of fungi and bacteria respectively. The bacteria isolated from soil were identified according to the Bergey's manual for the characterization of Genera (Species characterization will be published in next issue of this article).

Isolates were transferred to PVK (Picovskayas) medium [24] with slight modification as $MnSO_4$ 0.05, $FeSO_4.7H_2O$ 0.05 were added along with the tricalcium phosphate 0.2, glucose 13, $(NH_4)SO_4$ 0.5, NaCl 0.2, $MgSO_4.7H_2O$ 0.1, KCL 0.2, Yeast extract 0.5, Agar-Agar 20 g/L of distilled water, pH 7.2 for phosphate solubilizing activity by liquid phase and disc diffusion methods. The total percentage of fungal and bacterial isolates was determined by standard method as % of microbial isolates = Growth of isolates in number of media plates / total no of 100 soil samples.

3. DETERMINATION OF PHOSPHATE SOLUBILIZING ACTIVITY

3.1 Liquid Phase Method

The grown cultures of microbial isolates were subcultured separately in PVK broth medium for 24, 48, 72 hours and 1 week for the growth of bacterial and fungal isolates. After a week fungal mycelia were removed and the solution was used for further process. Ten (10) ml of the each culture were centrifuged at 12000 rpm for 15 minutes, 5 ml of the supernatants were poured into 20 ml of AB-DTPA (Ammonium Bicarbonate Diethylene Triamine Penta Acetic Acid) extracting solution. The samples were shaken for 15 minutes at 180 cpm (Alam, et al. [2]). Later the phosphorous was estimated by the Vanadomolybdo Phosphoric acid method of (Jackson, [25]) where 5 ml culture medium, 5 ml colour reagent and a mixture of (ammonium molybdate 50, ammonium vanadate 0.2 g/l, concentrated HNO_3 2.5 ml and HNO_3 was added. The same experiment was repeated

with the phosphorous standards of 5-30 ppm. Optical density (of two replicates) was determined at 100 rpm, 430 nm after 24, 48 and 72 hours and every day till two weeks for phosphate solubilization by bacterial and fungal isolates and the concentration of phosphorous was obtained in µg/ml.

3.2 Disc Diffusion Method

Discs of 6mm were prepared from Whattman filter paper and sterilized in autoclave at 121ºC, 15 lbs, 20 minutes, later dried in oven at 50ºC for 30 minutes. Using sterile forceps the discs were dipped separately into the broth culture and kept in the center of the surface of PVK agar. The plates were incubated at 37ºC for 24-72 h and at room temperature for 1 week for the appearance of halo zone of Psa by test bacterial and fungal isolates.

3.3 Determination of Percentage of Psa by Microbial Isolates [26]

4. RESULTS AND DISCUSSION

- Eighty five saline soil samples were collected from various areas of cultivated lands of different cities of Sindh Province. Table 1 shows the total percentage of isolates of all soil samples collected from various regions of Sindh Province as Aspergillus sp. (71.76%), Penicillium sp. (12.94%), Fusarium sp. (9.41%), Rhizopus sp. (5.88%), Bacillus sp. (78.82%), Pseudomonas sp. (11.76%), and Arthrobacter sp (9.41%), which are the important for Psa. From the observations it was also revealed that fungi especially Aspergillus sp. showed increasing Psa and reached at maximum peak after 9 days and showed halo zone after 9 days of incubation as compared to the other fungi. On the other hand Bacillus sp. showed maximum peak and halo zone after 48 hours as compared to the other bacteria.

- The observations (Figs. 1-2) of Psa of fungi revealed 0.622, 0.545, 0.444, 0.348 µg/ml after 12 days and bacteria 0.401, 0.277, 0.173 µg/ml after 48 hour. The results of disc diffusion method as shown in Table 2 indicated that Aspergillus sp. has the highest halo zone of 3.6mm giving 60% Psa after 9 days and Bacillus sp. indicated that it has 1.6mm halo zones showing 53.33% Psa after 48 hour due to the greater enzymatic activity. This observations revealed that the soil samples

TABLE 1. DETERMINATION OF THE PERCENTAGE OF VARIOUS FUNGI AND BACTERIA ISOLATED FROM 85 SALINE SOIL SAMPLES OF VARIOUS REGIONS OF SINDH PROVINCE

Microorganisms	Growth in 85 Soil Samples of Various Locations of Sindh Province	Total Percentage of Isolates of Various Locations of Sindh Province
Aspergillus sp.	61	71.76
Penicillium sp.	11	12.94
Fusarium sp.	08	09.41
Rhizopus sp.	05	05.88
Bacillus sp.	67	78.82
Pseudomonas sp.	10	11.76
Arthrobacter sp.	08	09.41

possess better environmental conditions for their growth, survival and osmotolerance of the test isolates. The variation in growth and Psa among the other isolates was also reported, which may be due to the variation in chemical composition of the soil and also due to the climatic changes. The increase in yield of the agricultural land and forests [27] and the availability of phosphorous in agricultural soils is in the insoluble form are the important factors.

- Numbers of workers reported both fungi and bacteria are PSMs (Phosphate Solubilizing Microorganisms) and found fungi as major PSMs having greater phosphate solubilizing activity [4,6]. Others reported that microorganisms are responsible for phosphatase activity and the acidic phosphatase play a significant role in solubilization of inorganic phosphate when soil pH ranges from acidic to neutral [6]. Our results

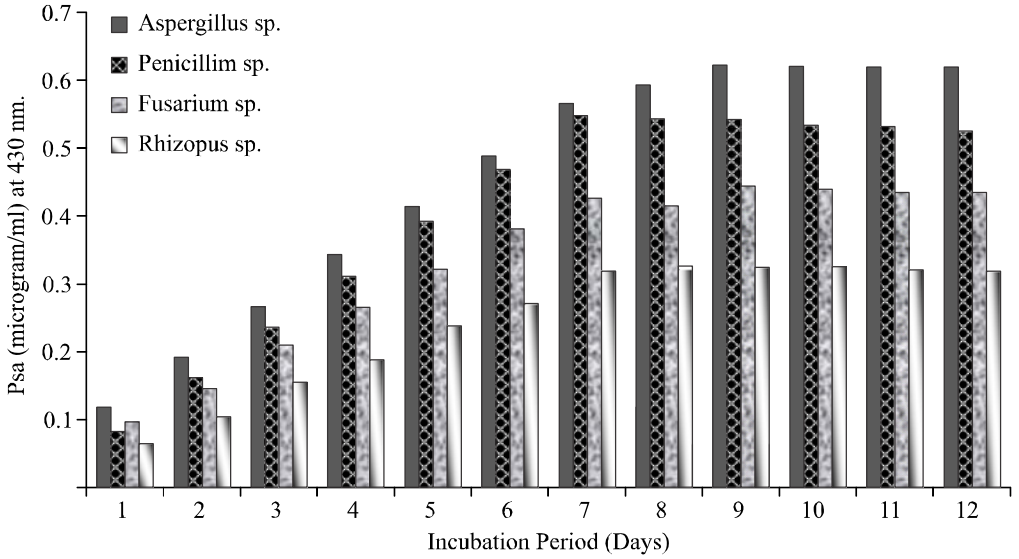

FIG. 1. DETERMINATION OF MAXIMUM PHOSPHATE SOLUBILIZING ACTIVITY BY FUNGAL ISOLATES AFTER 12 DAYS INCUBATION IN PVK BROTH, pH 7.2 AT ROOM TEMPERATURE, 430 NM

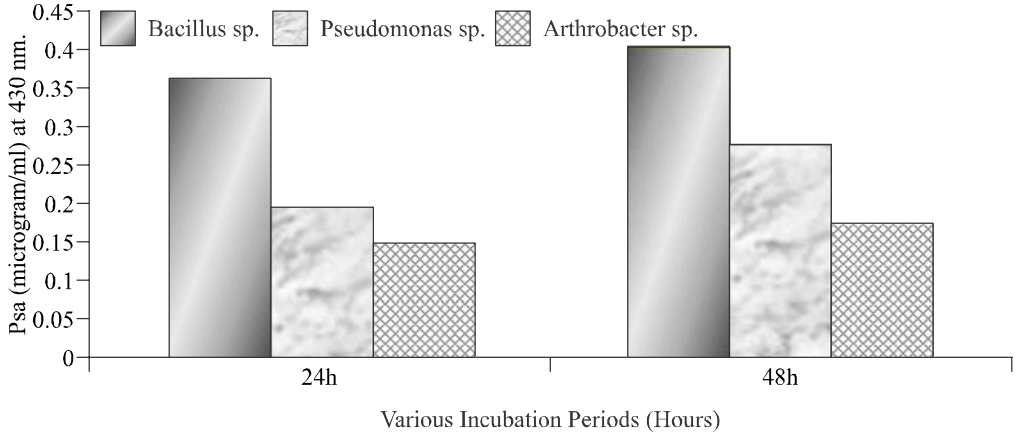

FIG. 2. DETERMINATION OF Psa BY BACTERIAL ISOLATES AT VARIOUS INCUBATION PERIODS (HOUSRS) IN MODIFIED PVK BROTH pH 7.2 AT 37°C, 100 RPM, 430NM. AFTER 48 HOUSRS

revealed the decrease in pH in PVK medium indicating the Psa by test isolates due to the microbial respiration, production of organic acids, microbial metabolites and proton and cation dissociation as reported by Nenwani, et al., [5], Nautiyal, et al., [26], Khalil, et. al. [28]. These acids have low molecular weight and are the sources of carbon and plant growth stimulator [4] such as oxalic acid, citric acid, gluconic acid, fumaric acid, acetic acid and succinic acid [2,29].

- In-vitro acid production in the test PVK broth is the increased phosphorous production (µg/ml) with in 8-9 days decreasing the pH of the medium from 7.2-6.5 and 7.2-6.9 by test fungi and bacteria respectively. Our results are in accordance of [30-32]. The disc diffusion method showed the halo zones of varying sizes (mm) produced by test isolates. Fungi showed larger zones than bacteria. Our results are well acquainted with [2,15, 33-36]. The production of halo zone indicates the Psa but sometimes PSMs loose ability to produce zone on sub culturing. Low values of bacterial solubilization may be due to their performance, which is severely influenced by environmental factors. This is why because PSMs may suffer from the stressed conditions especially salt concentration, pH, temperature, which may result in poor growth and survival [26] or this could be due to some damages occurred in the genetic constitution of bacterial isolates, which showed slow or retarded growth.

5. CONCLUSION

From the above observations, it is concluded that fungal isolates have the greater activity of phosphate solubilization (60%) than bacteria (53.33%). It is also concluded that Aspergillus sp. and Bacillus sp. that showed halo zone after 7 days and 72 hour time respectively as compared to other species of their respected groups. It is also concluded that Aspergellus sp. and Bacillus sp. are

TABLE 2. DETERMINATION OF HALOZONES OF PHOSPHATE SOLUBILIZATION BY VARIOUS FUNGAL AND BACTERIAL ISOLATES FROM 85 SALINE SOIL SAMPLES OF VARIOUS REGIONS OF SINDH PROVINCE

Isolates (Fungi)	Size of the Colony after 12 Days (cm)	Size of the Halozone after Every 4 Days up to 12 Days (mm)			Psa by Fungal Isolates (%)
Aspergillus sp.	06	2.1	2.8	3.6	60
Penicillium sp.	11	1.3	2.4	3.0	27.3
Fusarium sp.	08	00	1.0	1.7	21.25
Rhizopus sp.	08	00	0.7	1.4	17.5
Isolates (Bacteria)	Size of the Colony After 48 Hours (cm)	Size of the Halozone After Every 24 Hours up to 72 Hours (mm)			Psa by Bacterial Isolates (%)
Bacillus sp.	3	1.0	1.6	1.6	53.33
Pseudomonas sp.	2.6	0.8	1.0	1.2	46.15
Arthrobacter sp	2.6	00	0.7	1.0	38.40

the good candidates for phosphate solubilization in all test soil samples in their varying environments respectively.

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