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Research Article

Isolation and Evaluation

Isolation and Evaluation of Aspergillus niger as Phosphofungi from Rhizosphere Soil of Medicinal Plant to Supplement Phospho-biofertilizer

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In the present study, the phosphate-solubilizing fungus was isolated and identified as Aspergillus niger. Its 18S rRNA gene sequence was deposited at GenBank, NCBI (MN904862). For the study of the Phosphate solubilization capacity of the fungus, different rhizosphere soil samples were collected from medicinal plants. 40 fungal colonies were isolated after serial dilution and one fungal colony with high phosphate solubilization zone was selected and further tested. The fungus showed good results in different phosphate solubilization tests, SI (3.91), SE (290), and pH of the culture filtrate after the growth of the fungus decreased from 6.89 to 3.13 due to the production of organic acids. The colour changed from blue to yellow on the agar plate and red to yellow in the broth due to the acidic condition of the media during the growth of the fungus. Titrable acidity was measured at 32.8g/L and 420µg of P was estimated in culture broth by the Vanadomolybdate method. The fungus showed a positive result for siderophore production. 20µg of Indole Acetic Acid (IAA) was produced by the fungus and was estimated by the Salkowaski reagent method using a standard calibration curve. Due to the phosphate solubilization capacity and production of plant growth promoters of A. niger, can be recommended as a Phosphate solubilizer in an agricultural field.

Keywords: aspergillus niger, 18S rRNA sequence, organic acid, IAA, siderophore

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1. Introduction

Phosphorus (P) is a naturally occurring element and is one of 17 elements that are essential for plant growth (Nisha et al.2014). Research has documented that applying fertilizer phosphorus increases crop growth and yields on soils that are naturally low in phosphorus and in soils that have been depleted through crop removal. Crop fertilization represents the greatest use of phosphorus in agriculture today. It is necessary to supply this element through fertilizer. Unfortunately, the major portion of the applied phosphorus gets fixed in the soil and becomes unavailable for plant growth. In a greater part of the soil, phosphorus approximately 95 – 99% is present in the form of insoluble phosphates and cannot be utilized by the plant (Naik et al.2013). To increase the availability of phosphorus for plants, a large amount of fertilizer is applied to the soil. But a large proportion of fertilizer's phosphorus after an application is quickly transformed into an insoluble form (Omar 1998).

Phosphorus is the kingpin in Indian agriculture and occupies a unique position both in conventional as well as in alternative agriculture (KarunaiSelvi et al.2011). A huge number of microbial species are reported in soil, especially in the rhizosphere soil plays a significant role in P solubilization (Walpola and Yoon 2012). So the phosphate solubilizing microbes (PSM) convert these insoluble phosphates into soluble forms through a special mechanism. That is they carry out the process of acidification, chelation, exchange reaction, and production of gluconic acid (Stephen and Jisha 2011). The organic acids and inorganic acids from the microbes covert tri–calcium phosphates into the di – and monobasic phosphates, with the net result of enhanced availability of the element to the plants (Mahamuni 2012). The type of organic acids produced and their amounts differ with different microorganisms. The major mechanism of mineral phosphate solubilization is the action of organic acid synthesized by soil microorganisms and by the action of the phosphatase enzyme. Production of these organic acids results in the acidification of the microbial cell and its surroundings (Nisha et al. 2014).

The phosphorus solubilizers not only proving phosphorus to the plants but also facilitate the growth of plants by stimulating the efficiency of accelerating the accessibility of other trace elements and by synthesizing important growth-promoting substances (Mittal et al.2008), they are also known to produce the amino acid, vitamins, growth promoting substance like indole – 3 – acetic acid (IAA) and gibberellic acid which helps in better growth of plants (Nenwani et al. 2010), siderophore (Wani et al.2007b) and antibiotics (Lipping et al.2008) and protecting plants against soil-borne pathogens (Hamdali et al.2008). PSM includes various bacteria, fungi, and actinomycetes which help to convert insoluble phosphate into a simple and soluble form of phosphate. Among these soil bacteria belonging to the genera *Bacillus* and *Pseudomonas* and fungi belonging to the genera, *Aspergillus* and *Penicillium* are more common (Sharma 2007). The solubilization effect of PSM is generally due to the production of organic acids by these organisms. Antarikanonda et al.(1991) found that fungi are more active in solubilization phosphate than bacteria (Alam et al.2002).

In India, medicinal plants are used as a source of medicine since ancient times, their rhizosphere inhabitant various microbes having greater activity. Hence the present work was focused on the isolation of P-solubilizing fungi from the rhizosphere soil of medicinal plants with high P-solubilization capacity. It produced organic acids which are involved in P solubilization. The fungus, therefore, may be used as phosphate–solubilizing bio fertilizer in the agricultural field.

2. Materials and Methods

Collection of Rhizosphere Soil Samples

The rhizosphere soil samples were collected at 10 – 15cm depth of different medicinal plants from the dry deciduous forest of the Western Ghats regions of Shivamogga District. The collected soil samples were brought into the laboratory in sterile polythene bags aseptically and maintained at 4ºC for further use (Jain and Singh, 2015; Chatli et al. 2008).

Isolation of Phosphate Solubilizing Fungi

About 1g of soil sample was suspended in 9ml of sterilized 0.84% saline and serially diluted. Then dilutions (10^{-3}, 10^{-4}, and 10^{-5}) were plated on Pikovskaya's agar medium (Tri-calcium phosphate 5g, Glucose 10g, Ammonium sulfate 0.5g, Potassium chloride 0.2g, MgSO4 7H2O 0.1g,

MnSO4 7H20 trace, Ferrous sulfate trace, Yeast extract 0.5g, Distilled water 1L, Agar 20g and pH - 7.2) and incubated at room temperature for 7days. Plates were examined for solubilizing zone around the fungal colony. A colony showing solubilizing zone was picked and subcultured for further use (Jain and Singh 2015).

Characterization of Phosphate Solubilizing Fungi

Microscopic Characterization of PSF: Identification of PSF was done by lactophenol cotton-blue (LPCB) mounting technique. LPCB wet mount preparation is the most widely used method of microscopic observation of fungi. The specimen was stained with LPCB stain, a coverslip was placed above it and observed under the microscope at 40X magnification, and characters were noted by observing spore shape, spore size, spore arrangement, and arrangement of hyphae and identified by referring to the standard manuals (Aneja 2009; Subramanian 1983; Barnett 1975; Booth 1971).

Molecular Characterization of PSF by 18S rRNA Method: Isolated phosphate solubilizing fungi DNA was extracted by the CTAB method. The DNA concentration in the sample was estimated by recording absorbance at 260 and 280nm in a UV/ VIS spectrophotometer. Sequenced the 18S rRNA region, observed in gel documentation system, and amplified in PCR. Sequencing files obtained were in AB1 format viewed by using the software SeqScanner and the quality of the obtained sequence was observed through electropherogram peaks and analyzed the sequencing data using a BLAST server or servers related to specific databases.

Solubilization Index (SI)

The isolated fungal cultures were point inoculated on Pikovskaya's agar plates and incubated at room temperature for 7 days. The solubilization index was measured based on colony diameter and solubilization zone diameter formed around the colony of phosphate solubilizing fungi (Elias et al.2016; Verma and Ekka 2015). Using the formula,

$$SI = \frac{\text{Colony diameter} + \text{Halo zone diameter}}{\text{Colony diameter}}$$

% Solubilization Efficiency

The isolates were point inoculated on Pikovskaya's agar plates and incubated at room temperature for 7 days. % Solubilization efficiency was measured by using the following formula (Joseph and Jisha 2008).

$$\% SE = \frac{\text{Solubilization zone}}{\text{Diameter of the colony}} \times 100$$

Measurement of P^H

The isolates were grown in Pikovskaya's broth and incubated at room temperature for 7 days. Then after the culture filtrate was collected and the P^H of the culture filtrate was measured by a P^H meter as the final P^H. Before the inoculation of isolates into the broth, the P^H was recorded as the initial P^H. Uninoculated broth served as a control (Yasser et al.2014).

Qualitative Acid Production Assay

Qualitative Acid Production on Solid Media: Pikovskaya's agar plates were prepared by supplemented with Bromophenol blue indicator. These plates were point inoculated with each fungal culture and incubated at room temperature for 7 days (Chadha et al.2015).

Qualitative Acid Production in Broth: Sterilized Pikovskaya's broth supplemented with Bromocresol purple indicator was inoculated with fungal culture and incubated for 7 days at room temperature (Khan and Gupta 2015).

Quantitative Acid Production Assay (Titrable acidity)

The production of various organic acids from the PSF cultures results in acidification of the microbial cell and its surroundings leading to solubilize the insoluble phosphate and the amount of acid production was estimated by titration using alkali. The culture supernatant of each PSF culture was collected by centrifugation at 1000rpm for 10min. 50ml of culture supernatant was titrated against 0.1N NaOH solution with a few drops of phenolphthalein indicator. The titrable acidity was expressed in g/L (Khan and Gupta 2015).

Estimation of Phosphate

A culture supernatant from PSF was used to estimate the phosphate concentration.

PSF culture was inoculated in Pikovskaya's broth and incubated at room temperature for 7 days at 100rpm in an orbital shaker incubator. The culture filtrate was collected and centrifuged at 3000rpm for 30min. Estimation of phosphate in the supernatant was done by the Vanadomolybdate method and it was expressed in µg/ml. The amount of phosphate was calculated from a standard curve of KH2PO4. The absorbance of the developing yellow color was measured at 420 nm (Verma and Ekka 2015; Sahoo and Gupta 2014).

Screening of Siderophore Production

Siderophore production was detected by using a Chrome Azurol Sulfonate (CAS) assay. The medium contains an iron CAS-HDTMA (Hexadecyltrimethyl ammonium bromide) complex which is blue coloured. The presence of siderophore is indicated by decolourization of the blue-coloured ferric-dye complex, resulting in a yellow-to-orange halo around the colonies. 60.5mg of Chrome Azurol S was dissolved in 50ml of distilled water and mixed with 10ml of Iron solution (1mM Ferric chloride in 10mM Hydrochloric acid). While constantly stirring this solution was slowly added to HDTMA solution (72.9mg of HDTMA dissolved in 40ml of distilled water) and sterilized. The resultant dark purple liquid was added to sterile Pikovskaya's medium containing no Tricalcium phosphate to make CAS agar. Then the CAS agar plates were spot inoculated with PSF culture and incubated at room temperature for 7 days (Ghosh et al. 2017; Kotasthane et al. 2017).

Screening and Estimation of IAA Production

PSF culture was grown in potato dextrose broth supplemented with Tryptophan (1%). After complete growth, the culture filtrate was collected at 1000rpm for 10min. About 2ml of supernatant was mixed with 2 drops of orthophosphoric acid and 4ml of Salkwoski reagent (50ml of 35% perchloric acid, 1ml of Ferric chloride solution) and kept for incubation. The development of pink colour after 2h incubation at room temperature indicates indole acetic acid (IAA) production (Nenwani et al.2010). The concentration of IAA production by PSF was estimated by a standard graph taking the concentration of standard IAA on the X-axis and Optical Density (530nm) on Y – the axis (Pant and Agrawal 2014).

3. Results

Isolation Phosphate Solubilizing Fungi

A total of 38 rhizosphere soil samples of medicinal plants in the dry deciduous forest of the Western Ghats regions of Shivamogga District were collected for the isolation of phosphate-solubilizing fungi (Table 1). Among the soil samples, 40 fungal colonies were isolated and they were labeled as PSF1 to PSF 40 listed below in Table 1. Among the 40 isolates, 10 fungal colonies were selected as more phosphate solubilization, and they were selected and further evaluated.

Table 1: Isolation of PSF from Rhizosphere soil sample of medicinal plants

Sl No.	Plant Name	Culture Code	Sl No.	Plant Name	Culture Code
1	Datura fastuosa	PSF 1	21	Vinca rosea	PSF 21
2	Leucus aspera	PSF 2	22	Ocimum sanctum	PSF 22
3	Phyllanthus acidus	PSF 3	23	Phyllanthus emblica	PSF 23
4	Argemone mexicana	PSF 4	24	Amaranthus viridis	PSF 24
5	Achyranthus aspera	PSF 5	25	Alternanthera sessilis	PSF 25
6	Centella asiatica	PSF 6	26	Euphorbia hirta	PSF 26
7	Asparagus racemosus	PSF 7	27	Euphorbia heterophylla	PSF 27
8	Gymnema sylvestres	PSF 8	28	Ixora coccinea	PSF 28
9	Tinospora cordifolia	PSF 9	29	Mimosa pudica	PSF 29
10	Costus ingneus	PSF 10	30	Cassia occidentalis	PSF 30
11	Saraca asoca	PSF 11	31	Asclepias curassavica	PSF 31
12	Calotropis sp.	PSF 12	32	Bauhinia purpurea	PSF 32
13	Vitex nigundo	PSF 13	33	Momordica charantia	PSF 33
14	Holorrhena antidysenterica	PSF 14	34	Solanum xanthocarpum	PSF 34
15	Clitoria ternatea	PSF 15	35	Eclipta prostrata	PSF 35
16	Wrightia tinctoria	PSF 16			PSF 36
17	Santalum album	PSF 17	36	Brassica sp.	PSF 37
18	Azadirachta indica	PSF 18	37	Phyllanthus niruri	PSF 38
19	Eucalyptus sp.	PSF 19			PSF 39
20	Pongamia glabra	PSF 20	38	Phyllanthus sp.	PSF 40

Characterization of Phosphate Solubilizing Fungi

Microscopic characterization: Isolated PSF was identified based on its culture characteristics, morphology, spore arrangement, and structure of hyphae observed under the microscope and they were grouped as *Aspergillus*sp., *Penicillium* sp., *Fusarium* sp. and*Curvularia* sp. (Table 2).

Table 2: Isolation and identification of selected Phosphate solubilizing fungi

Sl No.	Plant name	Culture code	Culture
1	Argemone mexicana	PSF 4	Aspergillus sp.
2	Gymnema sylvestres	PSF 8	Aspergillus sp.
3	Calotropis sp.	PSF 12	Penicillium sp.
4	Vitex nigundo	PSF 13	Aspergillus sp.
5	Santalum album	PSF 17	Fusarium sp.
6	Vinca rosea	PSF 21	Aspergillus sp.
7	Phyllanthus emblica	PSF 23	Aspergillus sp.
8	Asclepias curassavica	PSF 31	Curvularia sp.
9	Phyllanthus niruri	PSF 38	Aspergillus sp.
10	Phyllanthus sp.	PSF 40	Aspergillus niger

Molecular Characterization by 18S rRNA Method: PSF 40 isolated from*Phyllanthus*sp. (Fig 1A) was selected based on its maximum phosphate solubilizing index and its molecular characterization was done by18S rRNA sequencing and confirmed the culture PSF 40 as *Aspergillus niger* (Fig 1C and 1D) and its 18S rRNA gene sequence (Fig 2) given below was deposited at GenBank, NCBI with reference code MN904862**.**

ACTTCCTTCCTGATCCGAGGTCAACCTGGAAAGAATGG
TTGGAAAACGTCGGCAGGCGCCGGCCAATCCTACAGA
GCATGTGACAAAGCCCCATACGCTCGAGGATCGGACGC
GGTGCCGCCGCTGCCTTTCGGGCCCGTCCCCCCGGAG
AGGGGGACGGCGACCCAACACACAAGCCGGGCTTGAG
GGCAGCAATGACGCTCGGACAGGCATGCCCCCCGGAA
TACCAGGGGGCGCAATGTGCGTTCAAAGACTCGATGAT
TCACTGAATTCTGCAATTCACATTAGTTATCGCATTTCGC
TGCGTTCTTCATCGATGCCGGAACCAAGAGATCCATTGT
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GGCACGGGCCCGGGGG

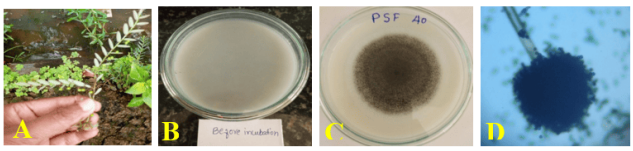

Figure 1: A: Medicinal plant (*Phyllanthus* sp.), B: Before incubation plate, C: *Aspergillus niger* (Pure culture plate) and D: Microscopic observation (40X)

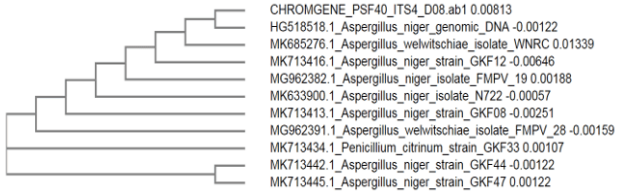

Figure 2: Phylogenetic tree constructed to compare *Aspergillus niger* (PSF40) with related species

Solubilization Index (SI) and % Solubilization Efficiency

The qualitative analysis of the phosphate solubilization potential of*Aspergillus niger*was measured*invitro*. The solubilization index and Solubilization efficiency of different PSF were found to range from 1.29 to 3.91 and 29.8 to 290 respectively. The fungus *Aspergillus niger* showed a solubilization index of 3.91 (Table 3) and a Solubilization efficiency of 290 (Table 3) (Fig 3).

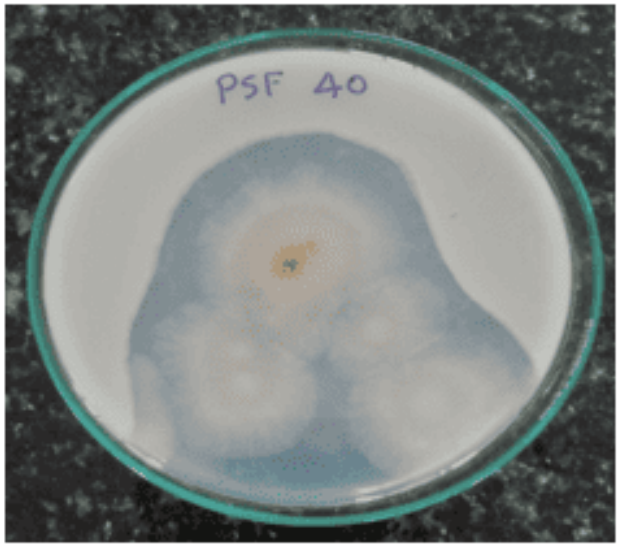

Figure 3: Solubilization zone arround the colony of *Aspergillus niger*

Measurement of pH

Decreased pH of 5.49 to 3.13 was recorded in different PSF from the initial pH of 6.89. The fungus*A. niger*reduced pH from 6.89 to 3.78 was recorded in the culture filtrate, after the period of incubation due to the production of organic acids (Table 3).

Qualitative Acid Production Assay

Due to the production of organic acids by*A. niger*reduction of pH in the culture media was observed. This was observed by using colour indicators, the colour change was observed in the media during the growth of the fungus. It showed colour change from blue to yellow on the agar plate when using Bromophenol Blue (Fig 4A). In the broth, colour change from red to yellow was observed while using Bromocresol purple as an indicator (Fig 4B).

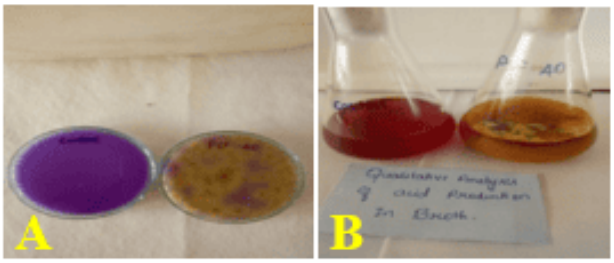

Figure 4: Assay of qualitative acid production (A: on solid media and B: in broth)

Quantitative Acid Production Assay (Titrable Acidity)

The titrable acidity of different PSF was found from 12 to 38.08g/L. The measure of the amount of acid present in the culture broth was titrated and recorded at 38.08g/L (Table 3).

Estimation of Phosphate

The concentration of phosphate in the culture filtrate of PSF was recorded from 360 to 20 µg. The concentration of the phosphate in the culture filtrate of *A. niger* was determined as 20µg (Table 3).

Table 3: Parameters of phosphate solubilization by selected P solubilizers

Sl. No	Culture Code	Culture	SI	SE	pH	TA (g/L)	Conc. of P (µg)
1	PSF 4	Aspergillus sp.	1.29	29.8	5.49	12	40
2	PSF 8	Aspergillus sp.	3.21	221	3.86	32.8	280
3	PSF 12	Penicillium sp.	1.49	49.5	5.28	13.73	60
4	PSF 13	Aspergillus sp.	1.33	33.6	5.17	12.4	40
5	PSF 17	Fusarium sp.	2.36	136.5	5.26	22.32	120
6	PSF 21	Aspergillus sp.	2.17	117.2	5.04	8.88	100
7	PSF 23	Aspergillus sp.	2.37	137.7	4.97	23.2	120
8	PSF 31	Curvularia sp.	1.34	34.1	5.43	10.56	40
9	PSF 38	Aspergillus sp.	3.61	261	3.47	34.4	360
10	PSF 40	Aspergillus niger	3.91	290	3.13	38.08	20

Screening for Siderophore Production

The formation of a pink halo around the colony on the blue CAS agar plate was observed after 7 days of incubation indicating siderophore production by Phosphate solubilizing fungus *A. niger* (Table 4) (Fig 5).

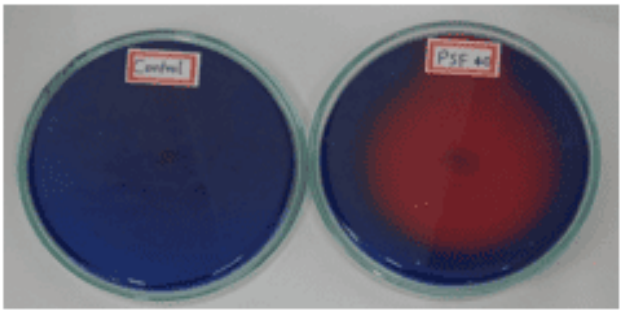

Figure 5: Siderophore production by *Aspergillus niger*

Estimation of Indole Acetic Acid (IAA)

The*A. niger*produced IAA was observed by the development of pink colour after the period incubation with the addition of the Salkowaski reagent. The produced amount of IAA was estimated at 20µg (Fig 6) by comparing it with the standard calibration curve (Table 4). The standard calibration curve was set up by determining the prepared different concentrations of IAA at 530nm by a colorimeter.

Table 4: Plant growth promoting activity of selected isolates

Sl. No	Culture Code	Culture	Siderophore production	Conc. of IAA (µg)
1	PSF 4	Aspergillus sp.	+	289
2	PSF 8	Aspergillus sp.	+	80
3	PSF 12	Penicillium sp.	+	20
4	PSF 13	Aspergillus sp.	+	200
5	PSF 17	Fusarium sp.	+	380
6	PSF 21	Aspergillus sp.	+	430
7	PSF 23	Aspergillus sp.	+	300
8	PSF 31	Curvularia sp.	+	340
9	PSF 38	Aspergillus sp.	+	430
10	PSF 40	Aspergillus niger	+	20

4. Discussion

Phosphorus is the kingpin in Indian agriculture, essential for the growth and development of plants and microorganisms. It is one of the essential mineral macronutrients, which are required for the maximum yield of agriculturally important crops and also it helps with plant growth and reproduction. Phosphorus occupies a major factor in stalk and stems length, flower and seed formation, crop maturity and production, nitrogen fixation in legumes, crop quality, and resistance to plant disease.

Soil microorganisms play a key role in soil P dynamics and the subsequent availability of phosphate to plants. The major mechanism of mineral phosphate solubilization is the action of organic acid synthesized by soil microorganisms and by the action of the phosphatase enzyme. While Chatli et al., (2008) collected the soil samples at a 15cm depth of rhizosphere and non-rhizosphere of *Salix alba* for isolation and characterization of phosphate solubilizing microorganisms. In our work also a total of 38 rhizosphere soil samples were collected from different medicinal plants and 40 phosphate solubilizers were isolated from samples followed by the serial dilution method. Among the 40 isolates, 10 fungal colonies were selected as good phosphate solubilization, and they were selected and further evaluated.

Screening of phosphate solubilizing fungi was done by qualitative analysis of isolates through solubilization index and efficiency. Earlier findings of Verma and Ekka (2015) have reported SI of 18 fungal culture strains ranging from 1.06 to 3.56, while Joseph and Jisha (2008) have reported 100 to 575 solubilization efficiency. Our work also followed the same method and solubilization index and efficiency were detected by recorded colony diameter and halo zone diameter. Jain and Singh (2015) have applying the inoculation of phosphate solubilizing fungi, decrease in pH was observed in a liquid medium ranging from 4.0 to 3.2 from initial pH of 7.5±0.2, in our work also, the results were correlated. While Yasser et al.(2014) have reported reduced pH of 4.80 – 5.4. The phosphate solubilizing fungi produce various organic acids to reduce the pH of the surroundings was tested by using indicators and the same result was observed in earlier findings of Chadha et al.(2015) and Khan and Gupta (2015) have also checked the ability of acid production through titrable acidity of 29 acidophilic fungal isolates were isolated and 5 isolates LAK-2, BS-1.6, CM-2, DR-1 and DR-2 showed good acid production. Verma and Ekka (2015) have recorded that the phosphate solubilization gradually increased ranging from 219.17µg/ml to 59.17µg/ml and the same results were recorded in our work showed a gradual increase in phosphate solubilization.

Not only providing P to the plants the PSF also facilitates the growth of plants by stimulating by synthesizing important growth-promoting substances including siderophore and indole acetic acid.

The results obtained in our work were highlighted by earlier reports of Ghosh et al. (2017), who have reported that isolates of *Trichoderma* showed siderophore production in CAS agar plate, *Trichoderma harzianum* produced a maximum percentage of siderophores than *T. viride*, *T. asperellum*, and *T. longibrachiatum.* Pant and Agrawal (2014) isolated 6 bacterial cultures and used them to test the estimation of IAA by the standard calibration curve.

5. Conclusion

Phosphorus is the second most important nutrient and is an essential element for plant growth and development. In nature, several bacterial and fungal species majorly solubility inorganic and organic form of phosphate compounds. Hence the present study deals with the isolation of a typical fungus from a rhizosphere soil sample and identified as *Aspergillus niger* by microscopic observation, of molecular identification 18S rRNA sequence. It shows good results in Phosphate solubilization in laboratory conditions. Also, it produces IAA and siderophore for plant growth and development. Due to its maximum Phosphate solubilization capacity under laboratory conditions, *Aspergillus niger* could be used for bio inoculums preparation as a phosphate solubilizer in an eco-friendly and profitable manner.

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Conflict of Interest: Not applicable.

Availability of Data and Materials: The 18s rRNA sequence of *Aspergillus niger* was deposited in the GenBank, NCBI under the Accession No. MN904862.

Authors Contribution: Corresponding author helps to complete the work by constant support and corrections made in manuscript.

References

1. Alam S, Khalil S, Ayub N, & Rashid M. (2002). *In vitro* solubilization of inorganic phosphate by phosphate solubilizing fungi (PSF) from maize rhizosphere. *International Journal of Agriculture and Biology, 4*(4), 454–458.

2. Aneja KR. (2007). *Experiments in microbiology, plant pathology and biotechnology*. New Dehli: New age International Publisher, pp. 157–162.

3. Antarikanonda P, Sassanarakit S, Amaret P, & Waiamai. (1991). *Biological system for improving availability of rock phosphate to plant*. in: Kinoshita, S. and Bhumiratana, A. (eds.), 612–19, Songkla, Thailand and Singapore.

4. Barnett HL. (1975). *Illustrated genera of imperfect fungi*, *2*, 1–225.

5. Booth C. (1971). Illustrated the genus *Fusarium*. *Common Wealth Mycological Institute,* pp. 1–237.

6. Chandha N, Prasad R, & Varma A. (2015). Plant promoting activity of fungal endophytes associated with Tomato roots from central Himalaya, India and their interaction with *Piriformspora indica*. *International Journal of Pharma and Bio Sciences, 6*(1), 333–343.

7. Chatli AS, Beri V, & Sidhu BS. (2008). Isolation and characterization of phosphate solubilizing microorganisms from the cold desert habitat of *Salix alba* Linn. in trans Himalayan region of Himachal Pradesh. *Indian Journal of Microbiology, 48*, 267–273.

8. Elias F, Woyessa D, & Muleta D. (2016). Phosphate solubilization potential of rhizosphere fungi isolated from plants in Jimma zone, Southwest Ethiopia. *International Journal of Microbiology, Article ID 5472601*, 1-11.

9. Ghosh S, Banerjee S, & Sengupta C. (2017). Bioassay, characterization and estimation of siderophores from some important antagonistic fungi. *Journal of Biopesticides, 10*(2), 105–112.

10. Hamdali H, Hafidi M, Virolle MJ, & Ouhdouch Y. (2008). Rock phosphate solubilizing Actinimycetes: Screening for plant growth promoting activities. *World Journal of Microbiology and Biotechnology, 24*, 2565–2575.

11. Jain P, & Singh D. (2015). Study on the role of phosphate solubilising fungi in phosphorus bioavailability and growth enhancement of potato. *Chemical Science Review and Letters*, *4*(13), 101–108.

12. Joseph S, & Jisha MS. (2008). Buffering reduces phosphate solubilizing ability of selected strains of bacteria. *American-Eurasian Journal of Agricultural and Environmental Science, 4*(1), 110-112.

13. KarunaiSelvi B, John Paul JA, Ravindran AD, & Vijaya V. (2011). Quantitative estimation of insoluble inorganic phosphate solubilization. *International Journal of Science and Nature, 2*(2), 292-295.

14. Khan R, & Gupta AK. (2015). Screening and optimization of organic acid producers from mine areas of Chhattisgarh region, India. *International Journal of Current Microbiology and Applied Science, 4*(2), 103-111.

15. Kotasthane AS, Agrawal T, Zaidi NW, & Singh US. (2017). Identification of siderophore producing and cynogenic fluorescent *Pseudomonas* and a simple confrontation assay to identify potential bio-control agent for collar rot of chickpea. *Biotechnology, 7*, 137.

16. Lipping Y, Jiatao X, Daohong J, Yanping F, Guoqing L, & Fangcan L. (2008). Antifungal substances produced by *Penicillium oxalicum* strain PY-1-potential antibiotics against plant pathogenic fungi. *World Journal of Microbiology and Biotechnology, 24*, 909–915.

17. Mahamuni SV, Wani PV, & Patil AS. (2012). Isolation of phosphate solubilizing fungi from rhizosphere of sugarcane & sugar beet using TCP & RP solubilization. *Asian Journal of Biochemical and Pharmaceutical Research, 1*(2), 237–244.

18. Mittal V, Singh O, Nayyar H, Kaur J, & Tewari R. (2008). Stimulatory effect of phosphate solubilizing fungal strains (*Aspergillus awamori* and *Penicillium citrinum*) on the yield of Chickpea (*Cicer arietinum* L. cv. GPF2). *Soil Biology and Biochemistry, 40*, 718-727.

19. Naik SK, Maurya S, Kumar R, Sadhna K, Gagrai S, Das B, Kumar S, & Bhatt BP. (2013). Inorganic phosphate solubilization by phosphate solubilizing fungi isolated from acidic soils, *African Journal of Microbiology Research, 7*(34), 4310–4316.

20. Nenwani V, Doshi P, Saha T, & Shalini R. (2010). Isolation and characterization of a fungal isolate for phosphate solubilization and plant growth promoting activity. *Journal of Yeast and Fungal Research, 1*(1), 009–014.

21. Nisha K, Padma Devi SN, Vasandha S, & Sunitha Kumari K. (2014). Role of phosphorous solubilizing microorganisms to eradicate p- deficiency in plants: A review. *International Journal of Scientific and Research Publications, 4*(7), 1–5.

22. Omar SA. (1998). The role of rock Phosphate Solubilising fungi and Vesicular Arbuscular Mycorrhiza (VAM) in growth of wheat plants fertilized with rock phosphate. *World Journal of Microbiology and Biotechnology, 14*(21), 1–8.

23. Pant G, & Agrawal PK. (2014). Isolation and characterisation of indole acetic acid producing plant growth promoting rhizobacteria from rhizospheric soil of *Withania somnefera*. *Journal of Biological and Scientific Opinion, 2*(6), 377–383.

24. Sahoo HR, & Gupta N. (2014). Evaluation of phosphate solubilizing potential of some endophytic fungi under solid and liquid state. *BMR Microbiology, 1*(1), 1–6.

25. Sharma AK. (2007). *Biofertilizers for sustainable agriculture*. (1st ed.). India: AGROBIOS, pp. 194–200.

26. Stephen J, & Jisha MS. (2011). Gluconic acid production as the principal mechanism of mineral phosphate solubilization by *Burkholderia* sp. (MTCC 8369). *Journal of Tropical Agriculture, 49*(1-2), 99-103.

27. Subramanian CV. (1983). *Hyphomycetes taxonomy and biology*. London: Academic Press, Vol. I and II, pp. 1-930.

28. Verma A, & Ekka A. (2015). Assessment of phosphate solubilizing potential of fungal isolates from soils of three blocks of Raipur, Chhattisgarh, India. *Journal of Ravishankar University-B, 28*(2), 44–50.

29. Walpola BC, & Yoon Min-Ho. (2012). Prospectus of phosphate Solubilizing microorganisms and phosphorus availability in agricultural soils: A review. *African Journal of Microbiology Research, 6*(37), 6600–6605.

30. Wani PA, Khan MS, & Zaidi A. (2007b). Synergistic effects of the inoculation with nitrogen fixing and phosphate solubilizing rhizobacteria on the performance of field grown chickpea. *Journal of Plant Nutrition and Soil Science, 170*, 283–287.

31. asser MM, Ahmad S, Mousa M, Massoud ON, & Nasr SH. (2014). Solubilization of inorganic phosphate by phosphate solubilizing fungi isolated from Egyptian soils. *Journal of Biology and Earth Sciences, 14*(4), B83-B90.