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EFFICIENCY OF *ASPERGILLUS TERREUS* TO SOLUBILIZE INSOLUBLE FORM OF PHOSPHORUS INTO SOLUBLE FORM UNDER VARIOUS CULTURAL CONDITIONS

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ABSTRACT

Phosphorous forms the second major nutrient required by the plants for its growth. It is supplied to the plants in the form of synthetic fertilizer which gets accumulated in the soil due its conversion into unavailable form. Transformation of unavailable form of phosphorus (P) to an available form becomes inevitable due to its physiological and biochemical role in plants. Since its deficiency results in the cessation of plant growth, supplementation of soluble form of phosphorus becomes essential. Fungus has the capability to convert insoluble form of phosphorus to soluble form through the secretion of organic acid as one of the solubilization mechanism. Thus in the present study, *Aspergillus terreus* was isolated from the rhizospheric soil sample of tomato field and was tested for its phosphorus solubilization efficacy by Broth assay method. The strain showed highest solubilization (7.5 mg/l) at the 10^{th} day of incubation with the pH drop of 4.0. Solubilization efficacy of the isolate was high due to the secretion of organic acid (gluconic acid) which was confirmed by HPLC method. Effect of various parameters such as Carbon sources, Nitrogen sources, pH and Temperature on the solubilization of phosphorus by *A. terreus* was also tested. The strain showed maximum solubilization in the presence of fructose as carbon source, ammonium sulphate as a nitrogen source, at the temperature of 30°C and at the pH of 7.0 in both qualitative and quantitative assays. Thus *A. terreus* shall be used as a viable Bioinoculant to eradicate P- deficiency in plants.

KEYWORDS: **Phosphorus, *Aspergillus terreus*, solubilization, growth parameters.**

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INTRODUCTION

Phosphorus (P) is an essential macronutrient required for the growth and productivity of plants. It plays an important physiological and biochemical role in crop plants. Phosphorus deficiency leads to chlorosis, necrosis and stunted growth in plants. In India, phosphorous content in an average soil is 0.05%. Only 0.1% from the total phosphorous is available to plants, rest of the phosphorous forms insoluble salt due to interaction between the soil's phosphorous and soils constitutes[1]. In order to restrict phosphorus deficiency in plants application of chemical fertilizer becomes conventional. Soluble forms of chemical fertilizer applied as soon also get precipitated into insoluble forms and becomes unavailable to plants. The utilization of microbial inoculants for crop production will form a sustainable eco-friendly approach and will replace the massive use of synthetic fertilizers. The rhizospheric microorganisms have the capability to solubilize insoluble phosphorus into soluble form and make them available to plants[2]. The phosphorus solubilizing fungi secreting organic acids have the capability to dissolve fixed form of phosphate to soluble forms and make them available to plants[3]. The production of organic acids seems to be the main mechanism of phosphate solubilization[4]. Thus Phosphate solubilizing fungi through the solubilization of phosphate promote a sustainable P nutrient supply to the plants results in the higher yield. Among the different fungal isolate, *Aspergillus* Sp. was found more efficient in phosphate solubilization and inoculation of this fungi as phosphate solubilizer significantly increase crop yield[5].The production of P solubilizing activity has been found to be highly dependent on the cultural conditions. Phosphate solubilization affected by many factors during the cultural conditions such as temperature, type of carbon and nitrogen sources, pH[6]. Thus the aim of this present investigation is to isolate *Aspergillus* sp. and to test its efficiency of phosphate solubilization under various cultural parameters.

MATERIALS AND METHODS

Aspergillus terreus was isolated from the rhizospheric soil samples of tomato field of Tirupur district, India and was tested for its phosphorous solubilization efficiency by growing them in Pikovskaya's medium contains 0.5% Tri Calcium Phosphate (TCP) as insoluble source. The formation of halo zone around the colony in the agar medium indicated its phosphorous solubilization efficiency was calculated by [7]. The isolate was identified using universal primer 18S rRNA and was deposited in the Genbank with the accession number as KT377251. Quantitative estimation of phosphorous, analysis of organic acid production using HPLC method and the effect of various cultural parameters such as carbon sources, nitrogen sources, pH and temperature on the solubilization of P by *Aspergillus terreus* was tested by following methods:

(i) *Quantitative estimation of P solubilizing ability of Aspergillus terreus*[8]

The P solubilizing potential of the *Aspergillus terreus* was assessed by growing the culture in Pikovskaya's broth supplemented with 0.5% Tri Calcium Phosphate (TCP). The culture was inoculated to 50 ml Pikovskaya's broth taken in 100 ml Erlenmeyer flask and incubated at room temperature. An uninoculated control was maintained. At 6^{th}, 8^{th} and 10^{th} days intervals the contents were filtered and centrifuged at 10,000 rpm for 10 min to remove cells and debris. The clear supernatant was analyzed for the phosphorous content and the pH drop in the growth medium was also tested using Elico pH meter.

(ii) Analysis of gluconic acid production by *Aspergillus terreus* using HPLC[9]

Aspergillus terreus was tested for its efficacy to produce gluconic acid was done by growing the culture in 50 ml of Pikovskaya's medium containing 0.5% of Tri Calcium Phosphate (TCP). After incubation of the culture for ten days at room temperature, 20 µl culture filtrate was injected in to HPLC using a separon SGX C18 column. Elution was performed with an isocratic consists of acetonitrile: water (30:70 v/v) with a flow rate of 1.0 ml/min at 210nm. Presence of gluconic acid in the culture filtrate was determined by comparing the retention time and peak area of the sample with the standard gluconic acid.

(iii) P-solubilizing ability of the *Aspergillus terreus* under different cultural conditions[10]

Effect of different carbon sources (Glucose, Sucrose, Maltose, Fructose and Lactose), nitrogen sources (Ammonium sulphate, Sodium nitrate, Potassium nitrate and Urea), temperature (20°C, 30°C and 40°C) and pH (5.0, 7.0 and 9.0) were studied to test the efficacy of *Aspergillus terreus* to solubilize insoluble form of phosphorus by both qualitative and quantitativte method.

RESULTS AND DISCUSSION

Phosphorus is an important macro-nutrient required for proper growth and development of plants. The soil constitutes about 0.5% of phosphorus in that only a minute amount is available to plants and rest remains as an insoluble form which cannot be used by plants[11]. In soil phosphorus present in the form of tricalcium phosphate, ferric phosphate and aluminium phosphate which cannot be utilized by plants due to its unavailable form[12]. Some organisms have the ability to solubilize this inorganic phosphate through the production of organic acids which results in the decrease of soil pH leading to the solubilization of P[13]. Phosphate solubilizing microorganisms are concentrated in rhizosphere for high proportions and they are metabolically more active than microorganisms from other sources[14]. In the present study, phosphorus solubilizing fungi was isolated from tomato field of Tirupur District, India using Pikovskaya's medium containing 0.5% Tri Calcium Phosphate (TCP) as insoluble source by the plate count method. The clear

zone forming fungi was isolated and was identified as *Aspergillus terreus* using 18S rRNA gene sequencing (Fig 1). *A. terreus* was used for further studies such as quantitative estimation of phosphorus (broth assay), determining the pH change of the medium, analysis of gluconic acid production using HPLC and to test its efficacy to solubilize P under various cultural conditions. The quantitative estimation of phosphate was carried out using Pikovskaya broth containing Tri Calcium Phosphate (TCP). The observations regarding the phosphate solubilized were done after 6^{th}, 8^{th} and 10^{th} day of incubation. The phosphorus solubilization by *A. terreus* was found maximum at the 10^{th} day of incubation (7.5 mg/l) (Fig 2). Phosphorus solubilizing activity was determined by the ability of microbes to release metabolites such as organic acids in which their hydroxyl and carboxyl groups chelate the cation bound to phosphate the latter being converted to soluble forms of phosphate[15]. During the solubilization of phosphorus reported that the tricalcium phosphate solubilization higher in *Aspergillus* sp. comparing to *Penicillum* sp[16]. Influence of *Aspergillus terreus* on the pH of the growth medium was studied between 6^{th}, 8^{th} and 10^{th} day interval. Drop in pH by *A. terreus* was observed to be 4 on the 10^{th} day (Fig 2). Phosphate solubilization was related to acid production that resulted in the pH drop of the culture medium[17]. The release of phosphate by *Aspergillus niger* in the liquid culture was associated with the reduction in the pH of the culture medium[13]. Organic acid production by the isolate plays a significant role in the acidification of the medium for the phosphorus solubilization[18]. Among the organic acid, gluconic acid seems to be the major organic acid involved in the solubilization mechanism. Thus in the present study, HPLC analysis of the cultural filtrates showed the presence of gluconic acid in the culture medium which indicated solubilization ability of the isolate (Fig 3). The gluconic acid produced by *Aspergillus niger* is an important factor for phosphate solubilization mechanisms[19].The acidification of medium was caused by the accumulation of gluconic acid[20].Phosphate solubilizing activity of *Aspergillus terreus* in the presence of various carbon (Glucose, Fructose**,** Sucrose, Maltose, Lactose), nitrogen (Ammonium sulphate, Sodium nitrate, Potassium nitrate, Urea), temperature (20°C, 30°C, 40°C) and pH (5, 7, 9) sources were evaluated. Production of organic acids was greatly related to the nature of carbon sources. A soluble phosphate level in the culture medium depends on the consumption of sugar by the fungus[21].Thus among the carbon sources tested fructose caused highest solubilization efficiency (184.72 %) and solubilization of phosphorus (13.50 mg/l) followed by glucose and other carbon sources (Table 1). Among the different carbon sources tested fructose, glucose, and starch enhanced solubilization more than other carbon sources due to the activity of *Aspergillus niger* resulting from consumption of sugar in the culture medium [21]. Phosphorus solubilizing efficiency of *A. terreus* using different nitrogen sources was tested. Among the nitrogen sources, the isolate showed maximium solubilization efficiency (129.96%) and available P (9.07 mg/l) in the presence of ammonium sulphate as N-source followed by all other nitrogen sources (Table 2). In much number of fungi were able to solubilize the phosphate only in the presence of ammonium sulphate as a nitrogen source[22].The effect of temperature on the efficiency of phosphate solubilization was studied and was found that maximum phosphorus solubilization was observed at 30°C by *A. terreus* (Table 3) in both plate (153.37%) and broth assay (11.47 mg/l). Different temperatures have been reported for solubilization, but most of them have found 25°C to 28°C to be the optimum temperature for phosphorus solubilzation [23]. 30°C is the best temperature for phosphate solubilization[24], [25]. The growth and solubilization activity were influenced by pH of the medium. The strain with maximum reduction in pH of the medium can have greater ability to solubilize phosphorus[26]. Thus the effect of different pH on the efficiency of phosphate solubilization by *A. terreus* were tested. The results recorded that the highest solubilization efficiency (167.86%) and solubilization (5.03 mg/l) of *A. terreus* was found at the pH 7 (Table 4). The maximum phosphate solubilization activity occurred in pH 7[25].

Figure 1
Phylogenetic tree based on 18S rDNA gene sequences comparison showing the position of Aspergillus terreus

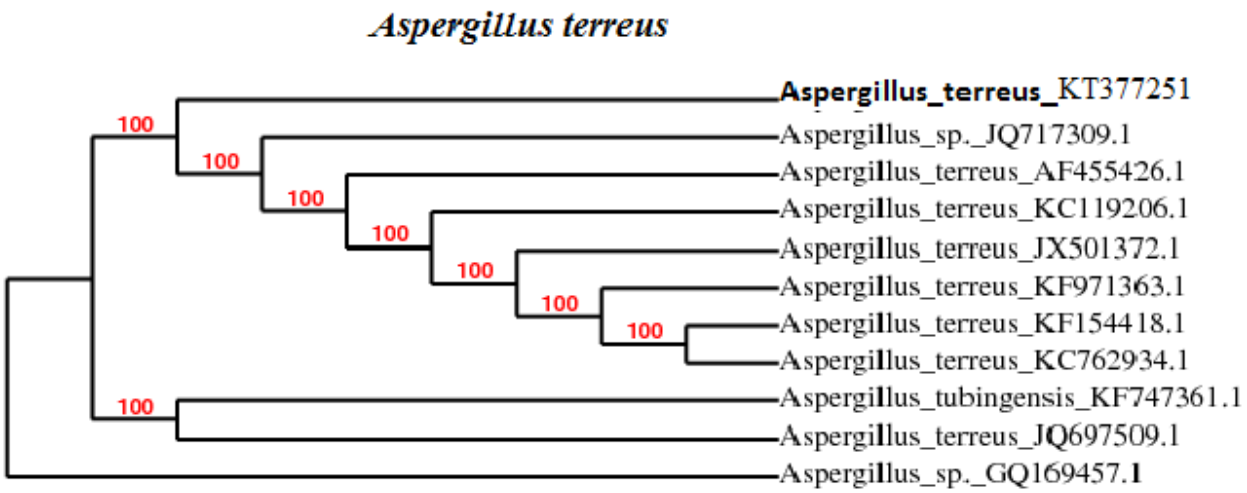
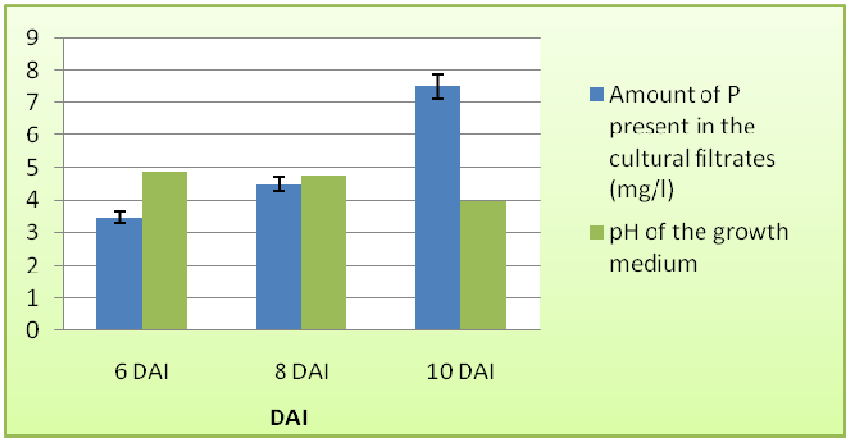
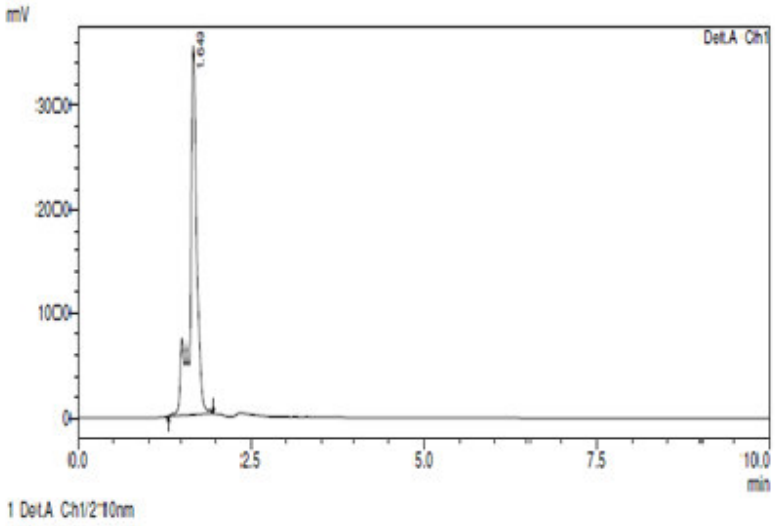


Figure 2
In vitro solubilization of insoluble phosphorus and the pH change of the medium by Aspergillus terreus



DAI-Days after Incubation

Figure 3
HPLC chromatograph depicting the peaks of gluconic acid secreted by Aspergillus terreus 3a. Gluconic acid standard



3b Aspergillus terreus

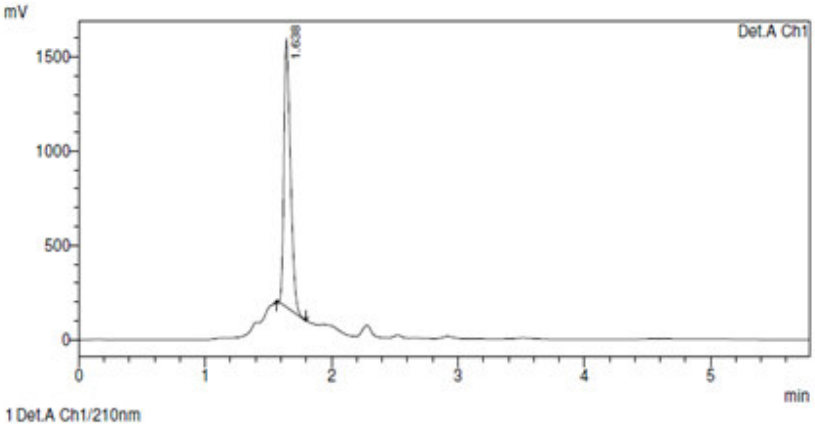


Table 1
Effect of different carbon sources on the P Solubilization by Aspergillus terreus

Carbon sources	Solubilizing Efficiency (%)	Amount of P solubilized (mg/l)
Glucose	144.34 ± 8.34[b]	12.03 ± 0.45[d]
Fructose	**184.72 ± 6.05[c]**	**13.5 ± 0.3[e]**
Sucrose	113.89 ± 2.41[a]	11.06 ± 0.40[c]
Maltose	144.81 ± 5.01[b]	10.06 ± 0.60[b]
Lactose	114.82 ± 3.21[a]	8.26 ± 0.25[a]
SEd	4.4344	0.3432
CD (0.05)	9.8804	0.7647
CD (0.01)	14.0544	1.0877

Values are mean ± SD of triplicate samples in each group

Table 2
Effect of different nitrogen sources on the P Solubilization by Aspergillus terreus

Nitrogen sources	Solubilizing Efficiency (%)	Amount of P solubilized (mg/l)
Ammonium sulphate	**129.96 ± 2.08[c]**	**9.07 ± 0.40[c]**
Sodium nitrate	108.58 ± 5.28[a]	6.13 ± 0.42[b]
Potassium nitrate	119.39 ± 1.05[b]	6.53 ± 0.25[b]
Urea	114.43 ± 0.97[b]	5.07 ± 0.31[a]
SEd	2.3916	0.2867
CD (0.05)	5.5150	0.06612
CD (0.01)	8.0248	0.9622

Values are mean ± SD of triplicate samples in each group

Table 3
Effect of different temperature on the P Solubilization by Aspergillus terreus

Temperature	Solubilizing Efficiency (%)	Amount of P solubilized (mg/l)
20°	145.98 ± 6.53[b]	6.5 ± 0.6[a]
30°	**153.37 ± 2.96[b]**	**11.47 ± 0.45[c]**
40°	123.6 ± 3.64[a]	9.45 ± 0.95[b]
SEd	3.7880	0.5709
CD (0.05)	9.2694	1.3970
CD (0.01)	14.0441	2.1166

Values are mean ± SD of triplicate samples in each group

Table 4
Effect of different pH on the P solubilization of Aspergillus terreus

pH	Solubilizing Efficiency (%)	Amount of P solubilized (mg/l)
5	119.74 ± 8.49[a]	4.09 ± 0.11[a]
7	**167.86 ± 8.52[c]**	**5.03 ± 0.35[b]**
9	153.37 ± 2.96[b]	4.06 ± 0.07[a]
SEd	5.8424	0.1757
CD (0.05)	14.2965	0.4299
CD (0.01)	21.6609	0.6513

Values are mean ± SD of triplicate samples in each group

CONCLUSION

It is concluded that *Aspergillus terreus*, can solubilize inorganic phosphate and it can enhance the P-fertility of the soil. The phosphorus solubilizing ability of *Aspergillus terreus* can be exploited further by using them as P bioinoculants for crop plantation.

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