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SCREENING OF PHOSPHORUS SOLUBILIZING ASPERGILLI FROM UNUSUAL HABITATS OF AGRA REGION

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ABSTRACT

A total of 42 isolates of 12 species of *Aspergillus* have been isolated from 40 soil samples of unusual habitats of Agra region. The most abundant and frequent Aspergilli include, *Aspergillus niger*, *A. flavus*, *A. terreus*, *A. nidulens*, which showed frequency in the range of 60-80%, out of the 42 isolates of these Aspergilli, 27 isolates showed varying phosphate solubilizing activity in Pikovskaya medium. Further, 18 isolates belonging to *Aspergillus clavatus*, *A. fumigatus*, *A. nidulans*, *A.niger*, *A. sydowii*, *A. terreus* and *A. ustus* showed more activity and were further screened for quantitative phosphate solubilizing activity. Three isolates of *A. niger* (KH-4, KH-6 and CH-2) showed very high levels of activity. The next best were isolate KH-1 of *Aspergillus fumigatus*, CH-3 of *Aspergillus nidulens* and CH-4 of *Aspergillus niger*. Two isolates of *Aspergillus ustus* showed minimum phosphate solubilization activity. This study revealed that three isolates of *Aspergillus niger* were most efficient phosphate solubilizers and can be used in the field as biofertilizers for increasing crop productivity.

KEYWORDS : Phosphorus solubilizing fungi, *Aspergillus niger*

Soils are excellent culture media for the growth of many kinds of micro-organisms. The soil micro-organisms include bacteria, fungi, protozoa, nematodes and some algae. A spoonful of fertile soil is said to contain more micro-organisms than total people in this world. Out of these, soil bacteria and fungi play pivotal role in various biogeochemical cycles and are responsible for the recycling of organic compounds (Molin and Molin, 1997). Fungi are important components of soil microbiota, typically forming more of the soil biomass than bacteria, depending on soil depth and nutrient conditions. About 25,000 different fungal species have been isolated from agricultural soils all over the world and since fungi interact with plant community by contributing to plant nutrition, soil structure and soil fertility, it is expected that farming practices will change the soil fungus flora.

The saprophytic fungi particularly Aspergilli and Penicillia represent the largest proportion of the fungal species in the soil and they perform a crucial role in the decomposition of plant debris containing structural polymers such as cellulose, hemicellulose, pectin and lignin, thereby contributing to the maintenance of the global carbon cycle. Similarly other substances of plant origin are decomposed and mineral cycling is accomplished. Thien and Myers (1992) indicated that by increasing soil microbial activities, bioavailability of phosphorus in a bioactive soil was remarkably enhanced. The fact that certain soil microbes are capable of dissolving relatively insoluble phosphatic compounds (Asea et al., (1988) has opened the possibility of inducing microbial solubilization of phosphate in the agricultural soils. Among the phosphate solubilizing micro-organisms, fungi have been reported to possess more ability to solubilize insoluble phosphate than bacteria (Nahas , 1996). Thus, in view of the importance of fungi as phosphate solubilizers, the present study was undertaken to screen isolates of *Aspergillus* species collected from soils of unusual habitats (forest, river basin, waste land and saline habitat) for their phosphate solubilizing ability, so as to explore the possibility of using them for plant growth improvement in degraded agricultural soils.

MATERIALS AND METHODS

Isolation and Identification of Soil Fungi

Soil samples were collected from four places viz., Chalesar forest, usar (saline) soil near Etmadpur, river basin near Dayalbagh and waste land near agricultural fields of Agra. In all 40 soil samples were collected in sterilized polythene bags. Fungi from these samples were isolated using soil plate method (Warcup,1950) using potato dextrose agar (PDA) and Czapek's dox agar (CDA) media. The plates were incubated at 28 ± 1^0C for 7 days in a B.O.D incubator.

After incubation period, slides of each fungus were prepared using cotton blue in lactophenol as mounting medium and observed under microscope. Identification of

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fungi was done based on morphological characters using the literature and descriptions given by Nagamani et al (2005). The different species of *Aspergillus* were identified following descriptions of Raper and Fennell (1965). Finally different isolates of *Aspergillus* species were maintained on PDA slants.

Phosphorus Solubilizing Activity

All the isolates of *Aspergillus* species were screened for primary phosphate solubilizing activity by allowing them to grow on Pikovskaya's agar medium for 10 days at 25^0C (Pikovskaya, 1948).The appearance of a transparent zone around the fungal colony indicated the activity of phosphate solubilizing fungus. After primary screening 18 isolates showing promising phosphate solubilizing activity were further studied for quantification of the phosphorus solubilizing activity.

For this purpose Erlenmeyer flasks (250ml) containing Pikovaskaya broth (50ml) with $Ca_3(Po_4)_2$ (5g/l having phosphate content of 20%) were sterilized. Control flasks were without any phosphate source. Spore suspension from 1 week old cultures of isolates of *Aspergillus* species were prepared in sterile distilled water containing Tween 80 (0.01%).The flasks were inoculated with 1 ml spore suspension of each test fungus individually. All the treatments and control flasks were incubated in triplicates in an incubator at 28 ± 2^0c for 12 days. After incubation, the contents of each flasks were used to determine the dry weight of mycelium, pH of culture filtrate and the amount of phosphate solublized following method of Durga and Paliwal (1961).The mycelial mat was removed from each flash and dried in hot air oven at 65^0C for 72 hours for calculating the dry weight.

RESULTS AND DISCUSSION

Perusal of table,1 indicates that a total of 12 species of *Aspergillus* have been isolated from soil samples of unusual habitats of Agra region. These include *Aspergillus amestelodarmi ,A clavatus ,A. chevalieri ,A. flavus ,A. fumigatus ,A. nidulens ,A. niger,A.ruber ,A. sydowii, A. terreus, A.ustus* and *A. versicolor* .The maximum number (12)species have been isolated from waste lands followed by forest soil (9) and the minimum number (4)of *Aspergillus* species were obtained from saline soils . In all 42 isolates of these *Aspergilli* were recognized on the basis of cultural characteristics out of these ,7 isolates of *Aspergillus niger* ,5 isolates each of

Table 1: Aspergilli isolated from different types of soils

S.N.	Fungal species	Forest soil	Ravine soil	Waste land	Saline soil	No. of isolate
1	*Aspergillus amestelodami*	+	__	+	__	3
2	*A. clavatus*	+	+	+	__	5
3	*A. chevalieri*	__	__	+	__	1
4	*A. flavus*	+	+	+	+	4
5	*A. fumigatus*	+	__	+	__	2
6	*A. nidulens*	+	+	+	__	5
7	*A. niger*	+	+	+	+	7
8	*A. ruber*	__	__	+	__	1
9	*A. sydowii*	+	__	+	__	3
10	*A. terreus*	+	+	+	+	5
11	*A. ustus*	+	__	+	__	4
12	*A. versicolor*	__	+	+	+	2
	Total	9	6	12	4	42

Table 2: Screening of fungi for their abitity to solubilize $Ca_3(Po_4)_2$ (Tricalcium phosphate) after 12 days in Pickovskaya broth

S.N.	Name of *Aspergillus sp.*	Isolate No.	Phosphate solubilized	pH	Dry of mycelium(mg)
1	*Aspergillus clavatus*	KH-2	56	4.50	0.198
		KH-5	62	4.20	0.203
2	*Aspergillus fumigatus*	KH-1	75	4.25	0.190
3	*Aspergillus nidulens*	KH-3	69	5.35	0.167
		DB-2	72	4.02	0.172
		CH-3	75	4.50	0.180
4	*Aspergillus niger*	KH-4	80	3.66	0.190
		KH-6	86	3.88	0.182
		DB-5	71	4.41	0.243
		CH-2	81	5.81	0.191
		CH-4	75	3.82	0.184
5	*Aspergillus sydowii*	CH-1	53	4.66	0.210
		DB-3	56	5.21	192
6	*Aspergillus terreus*	KH-3	69	3.82	0.182
		CH-2	70	3.95	0.190
		DB-1	72	4.21	0.195
7	*Aspergillus ustus*	KH-1	46	5.92	0.185
		DB-1	50	5.66	0.212

KH= Keatham; DB= Dayalbagh; CH=Chalesar

A.clavatus, *A. nidulens* and *A. terreus*, 4 isolates each of *A. flavus* and *A. ustus*, 3 isolates each of *A. amestelodami a*nd *A. sydowii*, 2 isolates each of A. fumigatus and *A. versicolor* and 1 isolate each of *A. chevalieri* and *A. ruber* were obtained. The most abundant and frequent *Aspergilli* were *A.niger*, *A. flavus*, *A. terreus* and *A. nidulens* , which showed frequency in the range of 60-80%.

Primary screening of 42 isolates of *Aspergillus* species for Phosphate solubilizing activity in Pikovskaya's medium showed varying phosphate solubilizing activity of 27 isolates as determined on the basis of transparent zone around fungal colony (Pikovskaya, 1948). Out of these , 18 isolates showing promising activity were further screened for their phosphate solubilizing ability in Pikovskaya broth (Table, 2) .The minimum activity (46%) was shown by isolate KH-1 of *Aspergillus ustus* while the maximum phosphate solubilizing activity (86%) was noted for isolate KH-6 of *Aspergillus niger.* It is interesting to note that isolates of *Aspergillus niger* showed phosphate solubilizing activity in the range of 71 ot 86% which was quite significant as compared to other *Aspergillus* species. Other species which showed more than 70% activity include *Aspergillus fumigatus* , *A. nidulens* and *A. terreus* . Isolates of *Aspergillus ustus* (KH-1 and DB-1) showed 46 and 50 per cent activity respectively , which was minimum among the species of *Aspergillus* screened for this purpose. Thus, it can be concluded that *Aspergillus niger* , *A. fumigatus*, *A. nidulens* and *A. terreus* can be used for phosphate solubilization and increasing growth and productivity of Agricultural crops .

Phosphorus is one of the macro elements required as a plant nutrient and is second only to nitrogen in importance. About 95% of phosphorus in the soil is in an insoluble state and cannot be utilized as such by plants. Further, any application of phosphorus as a chemical fertilizer quickly results in its accumulation as an insoluble form. Phosphate solubilizing micro- organisms have been reported to occur in different environmental niches and are abundant in rhizosphere of plants (Chen et al ., 2002: Tiwari et al.,1993) have reported that *Aspergillus* and Penicillium species have a greater potential in the solubilization of inorganic phosphates. Chakraborty et al., (2008) reported that three isolates of *Aspergillus niger* showed high levels of phosphate solubilising activity .In a study carried out to determine the role of dual application of vesicular arbuscular mycorrhiza and *Aspergillus niger* on the growth of tea seedlings , it was found that when both were inoculated in soil mixed with rock phosphate , a significant increase in growth was noted (Bora et al., 2003). In another study, it was reported that *A. niger* was one of the most efficient phosphatase producing fungi among other PSFs screened. It was also noted that the reduction of the pH of the medium was maximum with *A. niger* isolate, which efficiently hydrolyzed different compounds (mono and hexa) of organic phosphours (Tarafdar et al., 2003). Our data also indicated that isolates of *A. niger* have more potential to solubilize the inorganic phosphates as compared to other species .Further, we have also noted decrease in pH of medium (3.81-3.88) in isolates of *A. niger* showing more than 80% activity.

In conclusion, it can said that among the various PSF isolates obtained from soil, most efficient phosphorus solubilizers are species of *Aspergillus*, particularly *Aspergillus niger*, which is also having antimicrobial activity. The strains showing more phosphate solubilizing activity can be used in the field as efficient biofertilizers .

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