IOSR Journal of Environmental Science, Toxicology and Food Technology (IOSR-JESTFT)
e-ISSN: 2319-2402,p- ISSN: 2319-2399.
Volume 1, Issue 1, PP 29-36
www.iosrjournals.org

Isolation, Screening and Assessment of Phosphate Solubilizing Efficiency of Some Fungal Isolates of Raipur, Chhattisgarh

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Abstract: *In the present study total of fifty six fungi were isolated fromrhizospheric soil of paddy plants. Out of 56, thirty were screened as phosphate solubilizersupon inoculation in Pikovskaya's Agar medium and were grouped into six genera Arthrinium, Aspergillus, Chaetomium, Curvularia, Fusarium and Penicillium. The solubilization efficiency of screened isolates was evaluated by calculating solubilization index (SI) that ranged from 1.06 to 2.29. Fungi having SI ≥ 1.45 were selected for quantitative assay in broth amended with tricalcium phosphate (TCP) and rock phosphate (RP) as insoluble phosphorus sources. All the isolates solubilized TCP more efficiently than RP by decreasing the medium pH. Gradual increase in phosphate solubilzationfrom both the sources i.e. TCP and RP was recorded by increasing incubation periods. Aspergillus niger 8 (RAS4), Aspergillus niger 13 (RABO1) and Penicilliumpur purogenum Stoll 4 (RABO6) showed higher efficiency for phosphate solubilization therefore, these strains can be exploited as biofertilizers for sustainable agriculture.*

Keywords: *Fungi, Solubilization index, Tricalcium phosphate, Rock phosphate, Biofertilizer.*

I. Introduction

Phosphorous (P), second most essential macronutrient is required by the plants for vital functions. Most of the soils are deficient in phosphorus containing small fraction (0.05%) of total phosphorus of which only 0.1% is available to plants (Achalet al., 2007). Phosphorus deficiency in soil restricts plant growth that lead to regular application of chemical phosphatic fertilizers for achieving optimum yields (Shenoy and Kalagudi, 2005). However, a very small fraction of added phosphorus is taken by plants and the rest is precipitated as salts of Al, Fe and Ca (Gyaneshwaret al., 2002). Regular and excess application of synthetic phosphatic fertilizers poses a series of environmental problems such as eutrophication (Chang and Yang, 2009; Kang et al., 2011) and also resulted in intensemining of phosphorus containing minerals i.e. rock phosphate (RP), a non-renewable natural resource (Singh and Reddy, 2011). According to the estimate of Gilvert (2009) phosphorus reservoirs could be exhausted soon by 2060.

Agricultural soils have sufficient phosphorus pool to sustain maximum crop yields throughout the world for about 100 years (Walpola and Yoon, 2012). Therefore it is necessary to provide some economical and eco-friendly alternatives to make better use of rock phosphate and to activate the insoluble phosphates from different soil types (Carpenter, 2008). Use of phosphate solubilizing microorganisms (PSMs) capable of transforming insoluble phosphorus to soluble form is expected to conquer problems related with phosphate fertilization and pollution control (Mehta et al., 2013; Kauret al., 2014). PSMs includefungi (Khan et al., 2010), bacteria (Oveset al., 2013)and actinomycetes (Hamdaliet al., 2012). Phosphate solubilizing bacteria (PSB) out-numbers phosphate solubilizing fungi (PSF) i.e. bacteria constitute 1-50% and fungi 0.1 to 0.5% among the whole microbial population in soil (Chen et al., 2006). However it has been reported that the phosphate solubilization efficiency offungi is found to be greater than bacteria (Akintokunet al., 2007).PSMs assimilate phosphorus from insoluble sources bythe production of organic acids, the principle mechanism (Khan et al., 2010) and also by the secretionof phosphatases (Aseriet al., 2009) and phytases (Vassilevet al., 2007). PSMs have the potential to be used as biofertilizers. Inoculation of these organisms in several crops such as legumes (Manivannanet al., 2012), vegetables (Islam et al., 2014), cereals (Patilet al., 2012), oilseeds (Malviyaet al., 2011), and forest trees (Dash et al., 2013) resulted in improved plant growth and yield. Since large population of Chhattisgarh state is dependent on agriculture the present investigation is aimed to isolate some fungal strains that may have high efficiency for phosphate solubilization.

II. Materials And Methods

1.1 Sample collection: Soil samples were collected from rhizosphere of paddy plantation from different sites of Arangblockof Raipur district of state Chhattisgarh. Samples were collected in polythene bags, transported to laboratory and stored in refrigerator for further processing. Soil samples were separated from roots, air dried at room temperature, crushed, sieved and collected in separate polythene bags.pH of the samples were recorded using pH meter.

1.2 Isolation: Suitable dilutions prepared in 0.85% saline were spread on plates containing Potato Dextrose Agar (PDA) medium. The plates were kept for incubation at 28±2ºC for 5-7days. Fungal colonies were subcultured several times on PDA plates till the appearance of pure cultures. The isolates were stored in refrigerator on PDA slants for further studies.

1.3 Screening: The isolates were screened by inoculating on plates containing Pikovskaya's Agar (PKA) medium (Pikovskaya, 1948) amended with 0.5% tricalcium phosphate (TCP) as insoluble phosphate source and were incubated at 28±2ºC for 5 days. Fungal colonies with clear halozone around them were screened as phosphate solubilizers.

1.4 Identification: The fungal cultures were identified on the basis of colony characteristics and microscopic examination (Ellis, 1971;Barnett and Hunter, 1998; Gilman, 2008). Some of the fungal isolateshave been sent and deposited to NFCCI for identification.

1.5 Qualitative phosphate solubilization assay: Fungal suspensions were prepared in sterile saline (0.85% NaCl). 10µl suspension of each isolateswere inoculated in plates containing PKA mediumand incubated at 28± 2ºC for 5 days. Phosphate solubilizing potential was determined by calculating solubilization index (SI) using following formula (Premonoet al., 1996):

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

1.6 Quantitative phosphate solubilization assay: The flasks containing 100 ml Pikovskaya's broth amended with 0.5% of rock phosphate and 0.5% tricalcium phosphate were inoculated with 1 ml fungal suspension of each isolates and incubated at 28±2ºC for 5, 7 and 9days along with uninoculated control. The pH of the broth was adjusted to 7.00±0.03 using pH meter. After incubation periods cultures were filtered using Whatman no. 42 in order to record change in pH and concentration of released phosphorus in the filtrate.The pH was measured using pH meter and soluble phosphorus concentration was determined by vanado-molybdate method (APHA, 1999).

III. Results And Discussions

1.1 Isolation and screening: Total 56 fungi were isolated from rhizospheric soils of paddy plantation and 30 were screened as phosphate solubilizers based on appearance of clear halozone on Pikovskaya's agar medium (**Table 1**). The screened isolates were identified and grouped into six genera based on their colony characteristics and microscopic examinations (**Table 2**).Aspergillus niger was found to be the dominant group followed by Penicillium sp. and other species of Aspergillus. The similar results were highlighted by Mahamuniet al. (2012) and Deepaet al. (2010).

Table 1: Phosphate solubilizing fungi from rhizospheric soil of paddy plantation along with soil pH

Sr. No.	Soil sample code*	Soil pH	No. of isolates along with code	No. of screened isolates along with code
1.	RAS	7.46	10(RAS1-RAS10)	07 (RAS1, RAS3-RAS7, RAS9)
2.	RAA	7.04	11(RAA1-RAA11)	06 (RAA1, RAA2, RAA5, RAA6, RAA8, RAA11)
3.	RAB	7.03	09(RAB1-RAB9)	04 (RAB1, RAB2, RAB3, RAB7)
4.	RASP	6.32	07(RASP1-RASP7)	01 (RASP2)
5.	RABO	5.61	08(RABO1RABO8)	07 (RABO1-RABO7)
6.	RAK	5.10	11(RAK1-RAK11)	05 (RAK1, RAK4-RAK6, RAK11)

*First alphabet- District name first letter, Second alphabet- Block name first letter, Third alphabet- Village name first letter.

1.2 Qualitative assay: The solubilization indices of different isolates ranged from 1.06 to 2.29 (**Table 2**). Fungal strains isolated from sugarcane and sugar beet rhizosphere showed SI in range of 1.13 to 1.59 (Mahamuniet al., 2012). Alamet al. (2002)reported SI of the fungal strains isolated from maize rhizospherethat ranged from 1.53 to 1.80.

Table 2: Solubilization index for different fungal isolates (Data are means of three replicates±SE)

Sr. No.	Code	Name of the fungal isolates	Solubilization index		
			A* (mm)	B† (mm)	SI
1.	RAS1	PenicilliumpurpurogenumStoll3	33.00±1.53	14.67±0.88	2.25±0.65
2.	RAS3	Aspergillusniger7	53.33±0.33	35.33±0.88	1.50±0.03
3.	RAS4	Aspergillusniger8	51.00±0.00	35.00±0.00	1.45±0.00
4.	RAS5	Penicilliumsp. aff. P. capsulatumRaper& Fennell	26.33±0.88	16.33±0.88	1.61±0.03
5.	RAS6	Penicilliumsp. aff. P. citreonigrumDierckx 1	45.33±0.33	28.67±0.33	1.57±0.01
6.	RAS7	Curvulariasp.	42.67±0.33	33.33±0.88	1.27±0.02
7.	RAS9	ChaetomiumglobosumKunze ex Fr.	30.67±0.33	17.00±0.57	1.80±0.04
8.	RAA1	Aspergillusniger9	63.33±1.20	45.67±0.33	1.38±0.03
9.	RAA2	Aspergillussp.	34.67±0.88	22.33±0.67	1.55±0.01
10.	RAA5	Aspergillusflavus	44.00±0.57	40.67±1.45	1.08±0.02
11.	RAA6	Penicilliumsp.	29.33±1.20	17.67±0.67	1.65±0.05
12.	RAA8	Chaetomiumsp	37.33±1.67	28.33±0.33	1.31±0.05
13.	RAA11	Aspergillusversicolorgr.	16.33±0.33	11.33±0.88	1.45±0.08
14.	RAB1	Aspergillusniger10	78.00±0.57	69.00±0.57	1.12±0.008
15.	RAB2	Aspergillusniger11	79.33±0.33	59.00±0.57	1.34±0.01
16.	RAB3	Aspergillusniger12	64.66±0.33	60.67±0.67	1.06±0.01
17.	RAB7	Penicilliumsp. aff. P. citreonigrumDierckx 2	38.33±0.33	20.67±0.33	1.85±0.02
18.	RASP2	Penicilliumsp.	50.00±0.57	38.67±0.33	1.29±0.02
19.	RABO1	Aspergillus niger13	65.67±0.33	44.00±0.57	1.48±0.02
20.	RABO2	Aspergillus sp.	36.33±0.88	32.33±0.67	1.12±0.01
21.	RABO3	Aspergillu ssp.	58.00±0.57	45.67±0.33	1.26±0.006
22.	RABO4	Fusarium sp.	37.33±0.33	33.33±0.33	1.11±0.02
23.	RABO5	Fusarium sp.	50.33±0.33	38.00±0.57	1.32±0.02
24.	RABO6	Penicillium purpurogenumStoll 4	64.33±0.33	28.00±0.57	2.29±0.03
25.	RABO7	Aspergillus fumigatus	51.00±0.57	41.67±0.67	1.21±0.01
26.	RAK1	Aspergillus niger14	60.67±0.33	50.67±0.33	1.19±0.01
27.	RAK4	Aspergillus niger15	60.33±0.33	48.67±0.33	1.23±0.008
28.	RAK5	Penicillium sp.	35.00±0.57	27.67±0.33	1.26±0.03
29.	RAK6	Arthrinium phaerospermumFuckel	22.67±0.33	15.33±0.33	1.47±0.05
30.	RAK11	Penicilliumsp. aff. P. pseudostromaticumHodges & Warner	33.33±0.33	25.67±0.33	1.29±0.01

Where *= Colony diameter + Halo zone diameter, † = Colony diameter

1.3 Quantitative assay: Thirteen fungal isolateshaving SI ≥1.45 were assayed for the quantitative determination of soluble phosphates.The concentration of soluble phosphate gradually increased from 5th to 9th day of incubation.The concentration of soluble phosphate from TCP and RP ranged from 78.33µg/ml to 218.33µg/ml and from 4.17µg/ml to 100.83µg/ml respectively after 9 days of incubation. The maximum solubilization from TCP was observed by RAS4 (218.33µg/ml) followed by RABO6 (206.67µg/ml) and RABO1 (173.33µg/ml) after 9 days of incubation (**Figure. 3)** while maximum solubilization from RP was recorded by RABO6 (100.83 µg/ml) and RABO1 (100.83 µg/ml) followed by RAS3 (52.5µg/ml) and RAA2 (42.5µg/ml) after 9 days of incubation **(Figure. 4**). The steep decline in pH of TCP and RP amended broth were observed from 5.79 to 3.02 and 6.88 to 2.99 respectivelyindicating the production of acids (**Figure. 1 and Figure. 2**). All the thirteen isolates solubilized TCP more efficiently compare to RP which may be due to low phosphorus content and the complex structure of rock phosphate. This result was found to have similarity with the findings of Gupta et al. (2010). Initial decline in pH was associated with low phosphate solubilization but with further incubation pH was found to be increased in several cases with increase in solubilization. These results showed similarities with findings of Alamet al. (2002) and Kang et al. (2002) indicating that organic acid production is not the only mechanism of phosphate solubilization. Lower pH values were recorded during RP solubilization than TCP which was in accordance with the findings of Nahas (1996).

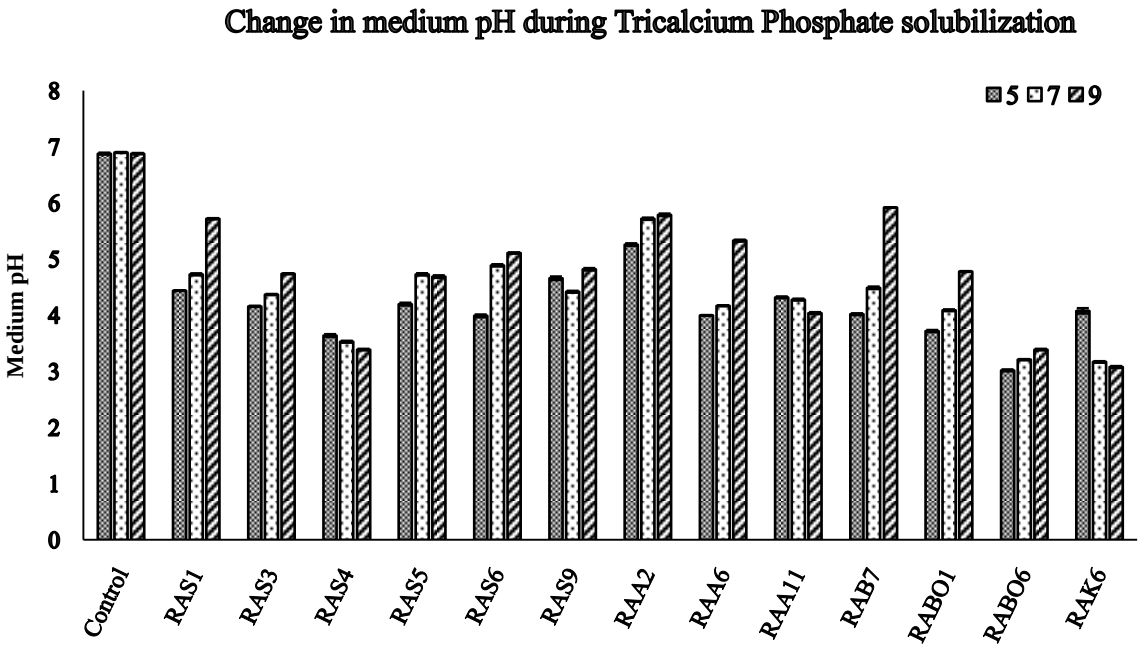

Figure.1: Change in medium pH during TriCalcium Phosphate solubilization after 5th, 7th and 9th days of incubation (Data are means of three replicates±SE).

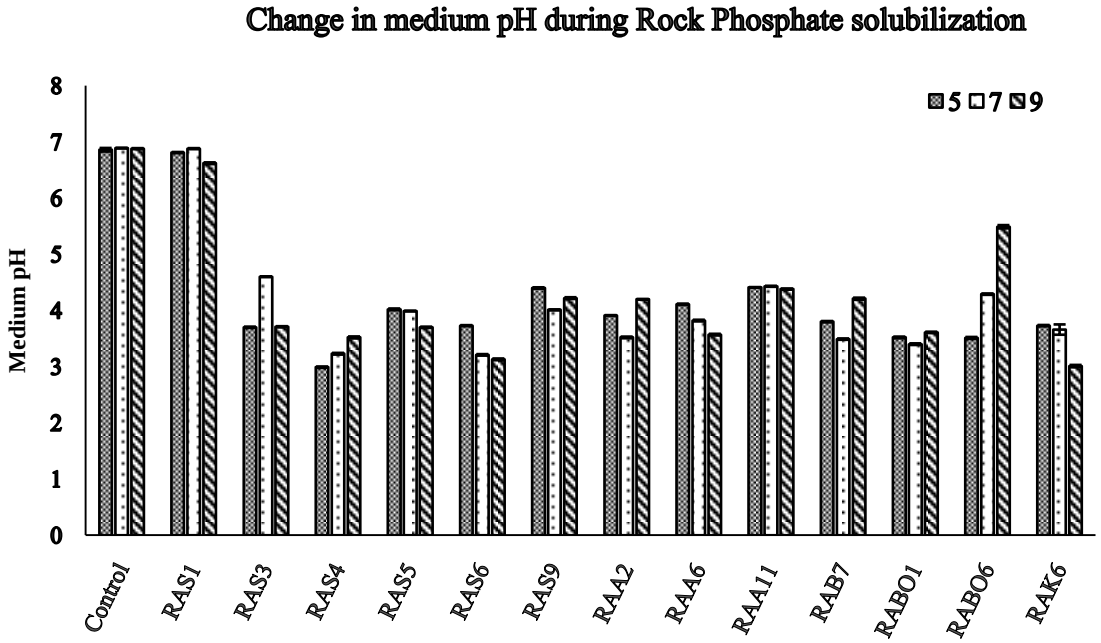

Figure.2: Change in medium pH during Rock Phosphate solubilization after 5th, 7th and 9th days of incubation (Data are means of three replicates±SE)

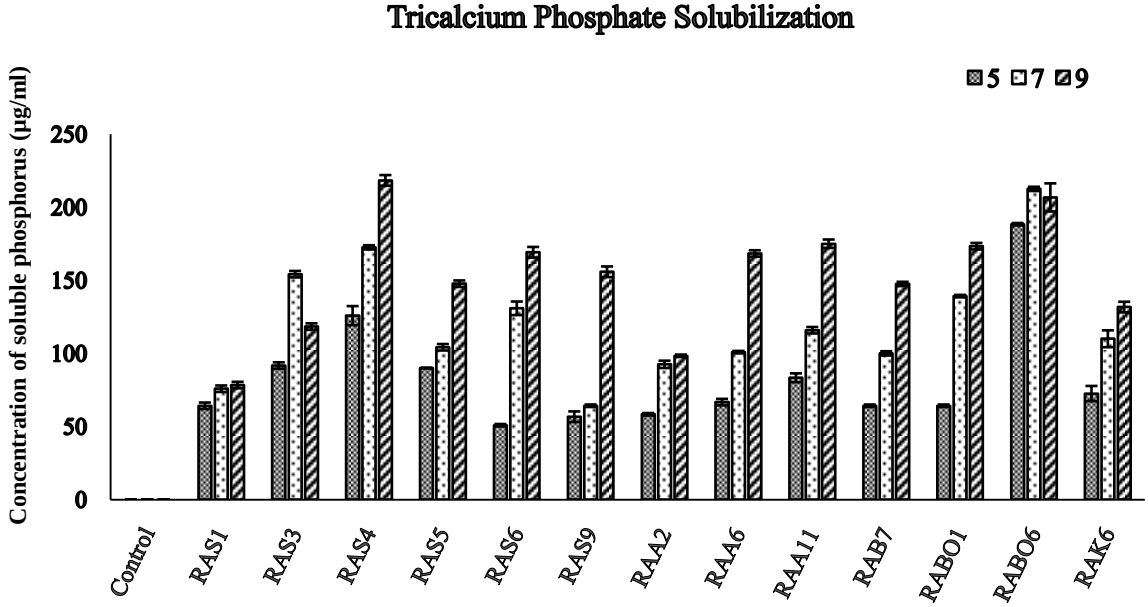

Figure.3: Histogram representing concentration of soluble phosphate during TriCalcium Phosphate solubilization after 5th, 7th and 9th days of incubation (Data are means of three replicates±SE)

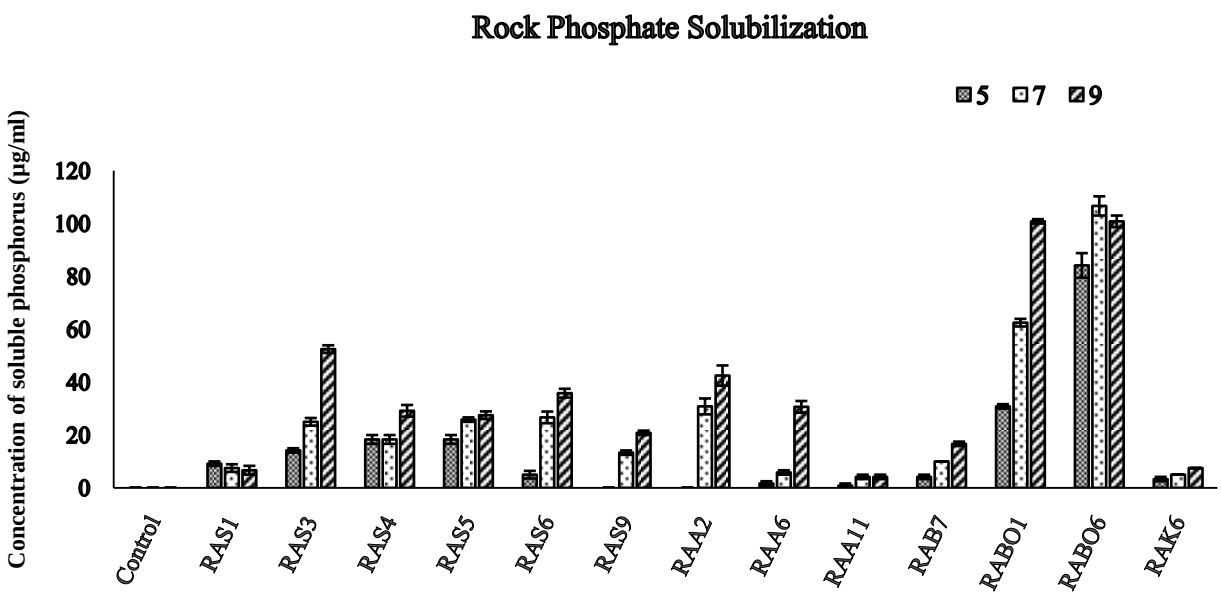

Figure4: Histogram showing concentration of soluble phosphate during Rock Phosphate solubilization after 5th, 7th and 9th days of incubation (Data are means of three replicates±SE)

IV. Conclusions

This study reveals that wide varieties of phosphate solubilizing fungi are present in the vicinity of paddy plants but the dominant strains are Aspergillus and Penicillium. The solubilization potential for TCP is higher than RP by all the isolates. Penicillium purpurogenum Stoll 2 (NFCCI-3788) can be used as potential phosphaticbio fertilizersfor promoting growth of different crop plants because of its higher solubilization efficiency for both (TCP and RP) the insoluble phosphate sources. Experiments on nursery and field conditions are required for its bio inoculant effects for sustaining maximum crop yields.

Acknowledgements

We gratefully acknowledge University Grant Commission, New Delhi for their financial support as BSR-Fellow to one of us (AV) and also to the head of the department for his constant support and laboratoty facilities.

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