Rubber Science, **30**(3): 262-268, 2017 RESEARCH ARTICLE

ISOLATION AND SELECTION OF EFFICIENT PHOSPHOFUNGI FROM RUBBER PLANTATIONS

Kochuthresiamma Joseph, Shaji Philip and Geetha Jose

Rubber Research Institute of India, Kottayam-686 009, Kerala, India

Received: 06 July 2017 Accepted: 10 November 2017

Joseph, K., Philip, S. and Jose, G. (2017). Isolation and selection of efficient phosphofungi from rubber plantations. *Rubber Science*, **30**(3): 262-268.

Twenty five phosphofungal isolates from rubber (*Hevea brasiliensis*) plantations were evaluated for solubilzation of ferric phosphate, aluminium phosphate, tricalcium phosphate fertilizer grade rock phosphate and acid phosphatase activity. Among the twenty five, seven isolates showed solubilization of phosphates and fertilizer grade rock phosphate and belonged to *Aspergillus* spp. and *Penicillium* spp. and was selected for further studies. These selected isolates were further evaluated for its beneficial effect on growth of rubber seedlings raised in root trainer cups. Among the seven, one isolate, *Penicillium* spp. (Pf 11) was further evaluated for growth improvement in root trainer plants in combination with 25, 50 and 100 per cent of the recommended dose of fertilizer and compared with uninoculated plants with 25, 50 and 100 per cent of fertilizer. The plants inoculated with Pf 11 at 50 per cent fertilizer showed higher girth, height and root development followed by inoculation with Pf 11 at 25 per cent fertilizer level. Girth of plants in treatment with Pf 11 at full fertilizer was lower than the treatment with 25 and 50 per cent fertilizer application in combination with inoculation. Highest population of inoculated phospho fungi was at 25 per cent fertilizer application and was reduced as the fertilizer levels increased. An efficient phosphofungus (*Pencillium* spp.) could be isolated from the rubber plantations and upon inoculation in rubber seedlings recorded better growth at 50 per cent of the recommended level of fertiliser.

Key words: *Hevea brasiliensis, Penicillium* spp., Phosphofungi, Phosphorus solubilization

INTRODUCTION

Phosphorus (P) is one of the important macro elements for both plants and microorganisms. Phosphatic fertilisers applied in soil are rapidly fixed as iron and aluminium phosphates in acid soils or as calcium phosphate in neutral and alkaline soils and get precipitated strongly on the surface of the soil particles. These reactions reduce the available P in soil solution (Bray and Kurtz, 1945; Buckman and Brady, 1969; Bear, 1976). Major share of the soils in the traditional rubber growing tract are low in available P due to the high fixation of applied P as hydroxides of iron and aluminium (Karthikakuttyamma *et al*., 1991; NBSS&LUP, 1999; Joseph, M., 2016). A large proportion of the total P in rubber growing soils exists in organic form (Prasannakumari *et al*., 2008). Soil microorganisms including bacteria, fungi, actinomycetes and arbuscular mycorrhiza have the ability to solubilize the precipitated phosphates, converting them into soluble forms that are available to plant which is a less expensive and environment friendly approach (Coutinho *et al*., 2012). Microbial

Correspondence: Shaji Philip (E-mail: shaji@rubberboard.org.in)

solubilization of insoluble phosphates is by different mechanisms, such as acidification, chelation and ion-exchange reactions (Delvasto *et al.*, 2006). Kucey (1983) found that fungi in contrast to bacteria retained their P solubilising activity for many years and are able to traverse distances more easily. The important genera of phosphate solubilising fungi (PSF) are *Aspergillus* and some species of *Penicillium* (Saber *et al.*, 2009). Phosphate solubilising fungi do not lose the P dissolving activity upon repeated sub culturing under laboratory conditions (Kucey, 1983). In the present study, *Penicillium* and *Aspergillus* isolates solubilising insoluble phosphates were isolated from rubber growing soils from different locations and assessed for their P solubilizing capacity and the isolate with higher phosphate solubilizing ability was further evaluated for their efficiency in improving the growth of rubber seedlings.

METERIALS AND METHODS

A total of 417 fungi from 36 different locations under rubber plantations in North Kerala and South Karnataka were isolated and tested for phosphate solubilization. Selection of phosphate solubilizing fungi was carried out using apatite agar medium. A pinpoint inoculation of fungal strains was placed on the centre of plates under aseptic conditions. They were incubated at 28 ± 2 °C for four days with continuous observation for colony diameter. The P solubilizing fungi were detected by the formation of clear halo around their colonies (Fig. 1). The phosphate solubilising efficiency of the isolates was compared by per cent solubilising efficiency (SE %) (Nguyen *et al.*, 1992) of the isolates inoculated in apatite agar medium as

$$\frac{\text{Solubilisation diameter}}{\text{Growth diameter}} \times 100.$$

Twenty five phosphofungi selected based on solubilisation efficiency using apatite agar

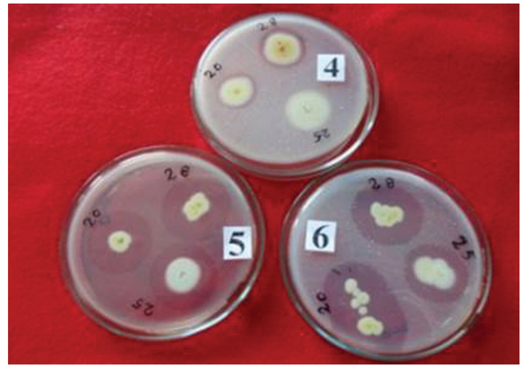

Fig 1. Solubilization of phosphate (4), ZnO (5) and $ZnCO_3$ (6)

were further tested for solubilzation of other forms of phosphate namely ferric, aluminium and tricalcium phosphates and fertilizer grade rock phosphate in Pikovskaya's broth as described by Subba Rao (1982). The acid phosphatase activity of the selected phosphofungi was estimated as per Eivazi and Tabatabai (1977). The isolates were also screened for their ability to solubilise zinc in mineral salt agar medium (Desai *et al.*, 2012).

Based on the different tests carried out under *in vitro* conditions, seven phosphofungal

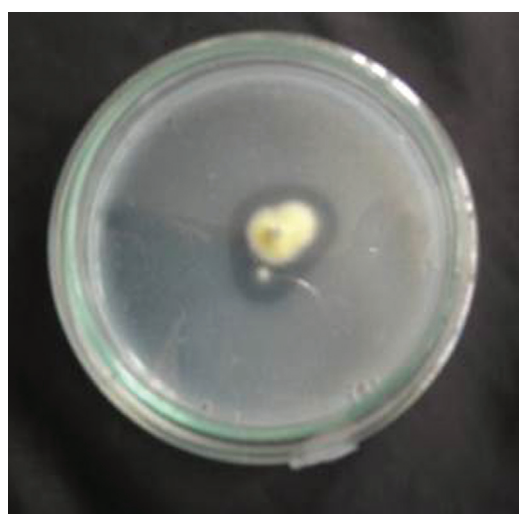

Fig 2. Phosphofungi (Pf11) with zone of P solubilization

isolates were selected multiplied and inoculated on rubber seedling at the time of transplanting to root trainer to evaluate their growth promoting efficiency in comparison with un inoculated control and full fertilized (standard recommended dose) control. The standard recommended dose is weekly application of 100 ml solution containing 0.1 per cent N and P and 0.05 per cent K and 0.01 per cent Mg. The experiment was designed in CRD with 32 replications. Cow dung slurry was applied to all the plants at fortnightly interval for two months. Fifty per cent of fertilizers were applied after two months. The girth and height of rubber seedlings were recorded after six months growth. The number of buddable plants and budding success were also recorded. Three plants from each treatment were uprooted for recording volume and fresh weight of roots. Population of the inoculated fungi in the potting mixture in all the treatments were carried out by phosphate solubilization in apatite agar medium.

From the seven, four isolates were selected and further evaluated by inoculating to rubber seedling at the time of transplanting to root trainers at 50 per cent level of fertilizer. Full level of fertiliser and 50 per cent of fertilisers alone applied plants were maintained as controls. The various growth parameters were recorded periodically and one isolate (Pf 11) showing more beneficial effect on plant growth was selected. This selected phosphofungi (Pf 11) was again evaluated for growth promoting efficiency at 25 per cent, 50 per cent and full fertiliser application keeping uninoculated plants with the corresponding levels of fertilizer application as controls. Microbial culture application was carried out fortnightly intervals and fertiliser at weekly intervals. The girth and height of rubber seedlings were monitored periodically.

RESULTS AND DISCUSSION

Out of the 417 fungal isolates collected from soils under rubber plantation in North Kerala and South Karnataka, 50 were showing phosphate solubilizing efficiency. Clear zones were formed around the colonies after five to seven days of incubation on solidified apatite medium supplemented with tricalcium phosphate indicating phosphate-solubilizing ability of the fungal isolates. Many of them did not retain their efficiency upon sub culturing. Forty isolates were found solubilizers of ZnO and 33 of them were found solubilizers of $ZnCO_3$. From the collection of isolates, twenty five isolates were selected based on their solubilising efficiency in apatite agar plates for further evaluation. The selected isolates belonged to *Aspergillus* spp. and *Penicillium* spp.

Table 1. **Comparative beneficial activities of selected phosphofungi**

Selected isolates	SE (%)			Acid phosphatase activity
	Phosphate	ZnO	$ZnCO_3$	(µg of PNP released mL^{-1})
Pf 5 (*Gongronell*a sp)	112	120	263	35.6
Pf 8 (*Penicillium* sp)	175	388	350	48
Pf 10 (*Aspergillus* sp)	140	122	316	41.8
Pf 11 (*Pencillium* sp)	124	211	412	46.5
Pf 12 (*Aspergillus* sp)	109	121	375	32.2
Pf 13 (*Pencillium* sp)	144	133	-	50.7
Pf 17 (*Penicillium* sp)	114	130	-	37.6

Table 2. **Solubilization of different phosphates by selected phosphofungi**

Selected Isolates	$FePO_4$		$AlPO_4$		Ca_3PO_4		Rock phosphate	
	P released (µgmL^{-1})	pH	P released (µgmL^{-1})	pH	P released (µgmL^{-1})	pH	P released (µgmL^{-1})	pH
Pf 5	630	3.4	370	4.8	500	4.9	420	4.9
Pf 8	680	3.4	340	4.0	340	4.4	420	4.7
Pf 10	620	3.6	340	4.2	490	4.3	570	4.8
Pf 11	660	3.5	340	3.8	570	4.3	390	4.4
Pf 12	800	3.3	320	4.2	690	4.2	460	4.5
Pf 13	660	3.3	330	4.2	460	4.3	560	4.6
Pf 17	710	3.2	390	3.7	630	4.2	190	4.8
Control		4.8		5.7		5.3		5.5

Most of the selected phosphate solubilizers were also solubilizing zinc.

The solubilisation efficiency of seven selected isolates are presented in Table 1. The solubilisation efficiencies (SE) ranged from 109 to 175 per cent for phosphate, 120 to 388 per cent for ZnO and 263 to 412 per cent for $ZnCO_3$. The acid phosphatase activity of the isolates ranged from 32.2-50.7 µg of PNP released mL^{-1} of culture broth (Table 1). Acid phosphatases and phytase secretions by microorganisms also have an important role in phosphate solubilisation (Aseri *et al.*, 2009). All the phosphofungal isolates tested showed solubilisation of ferric, aluminium and tricalcium phosphate and fertilizer form of rock phosphate. Phosphate solubilisation was accompanied by lowering of pH (Table 2) suggesting production of organic acids by fungi (Fankem *et al.,* 2006). Among the twenty five, seven isolates were selected for evaluation of the plant growth promoting efficiency.

Table 3. **Growth of root trainer plants inoculated with phosphofungi**

Selected Isolates	Height (cm)	Girth (mm)	Shoot dry wt.(g)	Budding success (%)
Pf5	109.7	8.4	19.3	89
Pf8	113.4	8.9	21.0	86
Pf10	109.1	9.2	24.0	93
Pf11	114.3	8.8	22.0	93
Pf12	112.5	9.5	24.7	74
Pf13	108.4	8.8	25.0	89
Pf17	90.7	8.8	20.7	92
50% fertilizer	116.5	9.2	17.3	92
Control	101.5	8.2	16.0	64.0
CD (P=0.05)	7.4	0.8	6.2	

Table 4. **Effect of phosphofungi inoculation on root parameters**

Treatment	Feeder root vol. (cc)	Tap root vol. (cc)	Feeder root wt. (g)	Tap root wt. (g)
Pf 5	6.7	17.3	5.9	14.7
Pf 8	5.3	14.7	5.5	15.3
Pf 10	7.3	18.	7.0	15.3
Pf 11	3.3	18.7	4.7	16.7
Pf 12	6.0	24.3	4.3	22.0
Pf 13	2.7	16.0	3.6	14.0
Pf 17	6.0	18.7	5.3	20.7
50% fertilizer	2.7	10.0	1.6	11.3
Control	2.0	10.0	1.1	9.3
CD (P=0.05)	2.6	5.5	1.7	4.1

The growth of rubber seedlings raised in the root trainer cups inoculated with the

Table 5. **Effect of selected phosphofungi at 50 per cent fertilizer application**

Treatment	Height (cm)	Girth (mm)	Budding success (%)	Inoculant count
Pf 8	117.9	12.0	89	9 x 10^4
Pf10	118.5	11.5	89	18 x 10^4
Pf11	128.8	12.2	100	14 x 10^4
Pf12	118.1	11.6	71	9 x 10^4
50% fertilizer	119.1	10.6	86	-
100% fertilizer	110.8	9.9	94	-
CD (P=0.05)	13.78	1.1		

seven selected phosphofungal isolates is given in Table 3 and 4. Initially no fertilizer other than cow dung slurry was applied to the plants and they showed yellowing. Hence, 50 per cent fertilizers were applied from two months of planting onwards. The seedlings in root trainer treated with Pf 11 showed highest buddability and budding success. Root volume and fresh root weight were highest for the plants treated with Pf 10. Four isolates gave comparable growth of the plants to full fertiliser applied plants in root trainers.

The four selected fungi were further evaluated in root trainer plants by applying 50 per cent fertilizer from the beginning of the experiment and the results are given in Table 5. Inoculation with phosphofungal isolates gave more girth of the plants than full fertilizer applied plants in root trainers. Among the four phosphofungi studied, Pf 11 inoculated plants showed higher girth and height. Root volume and root fresh weight were highest for the plants treated with Pf 11. The population of introduced fungi were higher in the treated root trainers. Further evaluation of the selected phosphofungi Pf 11 (*Pencillium* spp.) at different levels of fertilizer application is shown in Table 6. The plants inoculated with Pf 11 at 50 per cent fertilizer showed highest girth, height and root development and was followed by the treatment of fungal inoculation combined with 25 per cent level of fertilizer. The inoculated plants showed more growth than uninoculated plants at different levels of fertilizer application. Plants inoculated with phospho fungi at full fertilizer level showed less growth than those plants with 25 and 50 per cent fertilizer application combined with phospho fungi inoculation.

Morphological studies of the fungi from potting mixture showed the survival of the inoculated phosphofungi. Highest population of inoculated phosphofungi was at 25 per cent fertilizer application and was reduced as the fertilizer levels increased (Table 7). In apatite agar medium the isolates showed very clear zones of phosphate solubilisation.

Table 6. **Effect of inoculation with selected phosphofungi isolate (Pf 11) on the growth of rubber plants**

Treatment	Height (cm)	Girth (mm)	Root vol. (cc)	Root wt. (g)
Pf 11+25 % fertilizer	125.3	10.8	24.0	20.5
Pf 11+50% fertilizer	159.9	12.4	25.7	28.6
Pf 11+100% fertilizer	96.3	9.5	23.7	20.6
25% fertilizer alone	118.8	10.5	24.7	19.3
50% fertilizer alone	135.2	10.8	11.8	19.0
100% fertilizer alone	96.3	9.2	13.3	10.8
(P=0.05)	13.8	1.4	7.2	7.1

Table 7. **Microbial population in the potting medium**

Treatment	Total bacteria	Phosphobacteria	Total fungi	Phosphofungi
Pf 11+25% fertilizer	$63x10^5 g^{-1}$	$8x10^5 g^{-1}$	$302x10^5 g^{-1}$	$162x10^5 g^{-1}$
Pf 11+50% fertilizer	$14x10^5 g^{-1}$	$4x10^5 g^{-1}$	$132x10^5 g^{-1}$	$61x10^5 g^{-1}$
Pf 11+100% fertilizer	$10x10^5 g^{-1}$	$2x10^5 g^{-1}$	$40x10^5 g^{-1}$	$13x10^5 g^{-1}$
25% fertilizer alone	$29x10^5 g^{-1}$	$3x10^5 g^{-1}$	$15x10^4 g^{-1}$	-
50% fertilizer alone	$25x10^5 g^{-1}$	$2x10^5 g^{-1}$	$12x10^4 g^{-1}$	-
100% fertilizer alone	$12x10^5 g^{-1}$	$1x10^5 g^{-1}$	$18x10^4 g^{-1}$	-

The use of phosphate-solubilizing fungi is a promising strategy in the management of P fertilization, as it solubilizes the fixed form of soil P. In agreement with several published reports, the present study confirms high phosphate solubilizing potential of common soil fungal strains *Aspergillus* and *Penicillium* as reported by Fenice *et al.* (2000) and Reyes *et al.* (2002). In the case of *Penicillium*, gluconic, glycolic and malic acids are responsible for rock phosphates solubilization (Vassilev *et al.*, 1996; Whitelaw *et al.*, 1999). In addition to phosphate solubilization under laboratory conditions, plant growth promotional ability of *Aspergillus* and *Penicillium* in pot and field experiments have also been reported by several authors (Saber *et al.*, 2009; Singh and Reddy, 2011; Sane and Mehta, 2015). Similar effects were observed during the present study when this isolate was applied in combination with rock phosphate. This study revealed that application of rock phosphate in soil along with this selected phosphate solubilizing fungus can increase plant growth, and is a promising candidate in the P management in soils.

REFERENCES

Aseri, G.K., Jain, N. and Tarafdar, J.C. (2009). Hydrolysis of organic phosphate forms by phosphatases and phytase producing fungi of arid and semi-arid soils of India. *American-Eurasian Journal of Agricultural and Environmental Science*, **5**: 564-570.

Bear, F.E. (1981).*Chemistry of the soil*, Indian Edition, Oxford & IBH Publishing Co., Calcutta. 515p.

Bray R.H. and Kurtz, L.T. (1945). Determination of total organic and available forms of phosphorous in soils. *Soil Science*, **59**: 39-45.

Buckman, H.O. and Brady, N.C. (1969). *The Nature and Properties of Soils.* Macmillan, New York, 653 p.

Coutinho, F.P., Felix, W.P. and Yano-Melo, A.M. (2012). Solubilization of phosphates *in vitro* by *Aspergillus* spp. and *Penicillium* spp. *Ecological Engineering*, **42**: 85-89.

Delvasto, P., Valverdeb, A., Ballestera, A., Igualb, J.M., Muñoza, J.A. and Gonzáleza, F. (2006). Characterization of brushite as a re-crystallization product formed during bacterial solubilization of hydroxyapatite in batch cultures. *Soil Biology and Biochemistry*, **38**: 2645-2654.

Desai, S., Kumar, P. G., Sultana, U., Pinisetty, S., Ahmed, M.H.S.K., Amalraj, L.D.E. and Reddy, G.(2012). Potential microbial candidate strains for management of nutrient requirements of crops. *African Journal of Microbiology Research*, **6**(17): 3924-3931.

Eivazi, P. and Tabatabai, M.A. (1977). Phosphatase in soil. *Soil Biology and Biochemistry*, **9**: 167-172.

Fankem, H., Nwaga, D., Deubel, A., Dieng, L., Merbach, W. and Etoa, F.X. (2006). Occurrence and functioning of phosphate solubilizing microorganisms from oil palm tree (*Elaeis guineensis*) rhizosphere in Cameroon. *African Journal of Biotechnology*, **5**: 2450-2460.

Fenice M., Seblman, L., Federici, F. and Vassilev, N.

(2000). Application of encapsulated *Penicillium* variabile P16 in solubilization of rock phosphate. *Bioresource Technology*, **73**: 157-162.

Joseph, M. (2016). Rubber growing soils of India: An over view. *Rubber Science*, **29**(2): 119-139.

Karthikakuttyamma, M., Nair, A.N.S., Krishnakumar, A.K., Potty, S.N. and Mathew, M. (1991). Important inorganic P fractions in rubber growing soils. *Indian Journal of Natural Rubber Research*, **4**(1): 72-76.

Kucey, R.M.N. (1983). Phosphate solubilizing bacteria and fungi in various cultivated and virgin Alberta soils. *Canadian Journal of Soil Science*, **63**: 671-678.

NBSS and LUP (1999). Resource Soil Survey and Mapping of Rubber Growing Soils of Kerala and Tamil Nadu. *National Bureau of Soil Survey and Land Use Planning* (Indian Council of Agricultural Research), Nagpur, India, 295 p.

Nguyen, C., Yan, W., Tacon, F.L. and Lapeyrie, F. (1992). Genetic variability of phosphate solubilising activity by monocaryotic and dicaryotic mycelia of the ectomycorrhizal fungus,*Loccaria bicolour* (Maire). *Plant and Soil*, **143**: 193-199.

Prasannakumari, P., Joseph, M. and Nair, N.U. (2008). Organic phosphorous status of the major soil series under rubber cultivation in South India.*Natural Rubber Research*, **21**(1&2): 98-103.

Reyes, I., Bernier, L. and Antoun, H. (2002). Rock phosphate solubilization and colonization of maize rizosphere by wild and genetically modified strains of *Penicillium rugulosum*. *Microbial Ecology*, **44**: 39-48.

Saber, W.I.A., Ghanem, K.M. and El-Hersh, M.S. (2009). Rock phosphate solubilization by two isolates of *Aspergillusn iger* and *Penicillium* sp. and their promotion to mung bean plants. *Research Journal of Microbiology*, **4**: 235-250.

Sane, S. A. and Mehta, S. K. (2015). Isolation and evaluation of rock phosphate solubilizing fungi as potential bio-fertilizer. *Journal of Biofertilizer and Biopesticides*, **6**: 156.

Singh, H. and Reddy, M.S. (2011). Effect of inoculation with phosphate solubilizing fungus on growth and nutrient uptake of wheat and maize plants fertilized with rock phosphate in alkaline soils. *European Journal of Soil Biology*, **47**: 30-34.

Subba Rao, N.S. (1982). *Biofertilizers in Agriculture*. Oxford and IBH Publishing Co., New Delhi, 182 p.

Vassilev, N., Fenice, M. and Federici, F. (1996). Rock phosphate solubilization with gluconic acid produced by immobilized *Penicillium* variabile P16. *Biotechnology Techniques*, **10**: 585-588.

Whitelaw, M.A., Harden, T.J. and Helyar, K.R. (1999). Phosphate solubilisation in solution culture by the soil fungus *Penicillium radicum*. *Soil Biology and Biochemistry*, **31**: 655-665.