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Review on Phosphate solubilizing micro-organisms

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Abstract

Biofertilizer can provide an economically viable support to small and marginal farmers for realizing the ultimate goal of increasing productivity. Biofertilizer are low cost, effective and renewable source of plant nutrients to supplement chemical fertilizers. Microorganisms, which can be used as biofertilizer, include bacteria, fungi and blue green algae. These organisms are added to the rhizosphere of the plant to enhance their activity in the soil. Sustainable crop production depends much on good soil health.

Introduction

Phosphate solubilizing micro-organisms (PSM) solubilize insoluble forms of inorganic phosphorus and also mineralize organic forms of it and progress the availability of phosphorus to the plants. It is reported that phosphate solubilizing microorganisms of plant rhizosphere are more effective than others from the same soil. High quantity of phosphate solubilizing microorganism is concentrated in rhizosphere and they are metabolically more active than microorganisms from other sources [1]. Phosphate solubilizing organisms dissolve the fixed mineral phosphate and make it available to plants [2,3].

Isolation and Screening

It should be noted that filamentous fungi are among the most active and studied solubilization agents and a typical process for RP solubilization in submergd (single batch, shake-flask) fermentation conditions involves glucose based media and is performed for 7-20 days [4, 5, 6,7, 8].

Filamentous fungi are broadly used as producers of organic acids [9] and in particular *Aspergillus niger* and some *Penicillium* species have been tested in fermentation system or inoculated directly into soil in organize to solubilize 13 rock phosphate [10, 11]. [12] found that all the isolates of *Aspergillus tubingensis* and *A. niger* isolated from rhizospheric soils were found to be competent of solubilizing all the natural forms of rock phosphates. [13] reported the optimum incubation period and the optimum level of rock phosphate for a Phosphate Solubilizing Fungus (PSF), *Aspergillus niger* BCCF.194, isolated from tropical acid soils. They conducted a simple, effective, and environmentally sound process to improve P availability of phosphate rocks to crops by Phosphate Solubilizing Fungus. *Aspergillus niger*, *A. terreus* and *Penicillium digitatum* were isolated from rhizosphere soil and identified based on their colony morphology and spore structure by [14]. [15] isolated some phosphate solubilizing fungi from rhizosphere soil which were identified as *Aspergillus flavus*, *A. nidulans*, *A. terreus*, *A. niger* and *Penicillium liliacinum*. [16] isolated fungal cultures associated with legume root nodules. Among them, isolates of *Aspergillus niger*, *A. flavus*, *A. nidulans*, *A. terreus*, *Aspergillus* sp. and *Penicillium lilacinum* solubilized insoluble tricalcium phosphate. [17] isolated hydroxy-apatite and rock phosphate dissolving bacteria (Gram +ve and Gram –ve rods and cocci), fungi (*Aspergillus*, *Penicillium* and *Rhizopus)* and actinomycetes (*Micromonospora* and *Nocardia*) from soil samples of Bihar on carrot-extract agar.[18] investigated phosphate solubilizing potential of 48 fungi isolated from Delhi and Ludhiana soils in culture medium supplemented with tricalcium phosphate and calcium phosphate.

Jhamarkotra and Maton Rock phosphates by [19]. They also studied their phosphate solubilizing ability. [20] isolated Idaho rock phosphate and calcium phosphate dissolving bacteria and fungi from southern Alberta soils. He also explained that fungi as better phosphate solubilizers than bacterial isolates. [21] solated phosphate solubilizing fungal cultures namely *Aspergillus awamori* and *A. niger* which were identified based on their colony characteristics and spore structure.

Numerous microorganisms, especially those associated with roots, have the capacity to increase plant growth and productivity [22, 23]. There are numerous soil microorganisms beneficial for plants growing in an ecosystem, but are not well studied. [24] isolated 36 fungal species from soil were tested for their ability to solubilize rock phosphate (RP) in agar plates. *Aspergillus niger* and *Penicillium citrinum*, shown high activity. [25] explained the presence of fungi in nature, their diversity, their interaction with plant and their role in plant growth inhibition. [26] was screen out the seasonal and depth wise variations in soil fungal population in paddy field at Thanjavur district of Tamilnadu. The dominating species recorded was *Aspergillus*. Most of the phosphate solubilizing fungi were isolated from the rhizosphere of various plants [27, 28, 29]. [30] was revealed that four hundred isolates obtained from soil samples collected from forest, river basin, agricultural fields and rhizosphere of plantation crops of North Bengal. Among the screened isolates, ninety showed phosphate-solubilizing activity and dominating species was *Aspergillus.* [31] isolated phosphate-solubilizing actinomycetes from orchid soils using modified Pikovskaya medium. [32] identified phosphate solubilizing fungal cultures viz., *Aspergillus awamori* and *A. niger* based on their colony color, colony morphology and spore structure. [33] isolated phosphate solubilizing fungal cultures from different samples of rhizosphere of wheat, gram, garden soils and composed materials. [34] isolated one hundred forty five fungi from Kanchanaburi, Thailand of which thirty strains showed phosphate solubilization.

According to [35] phosphofungi belong mainly to the *Aspergillus* and *Penicillium* genera, and several strains have been identified that support the nutrient concentrations and yield of vegetables under both greenhouse and field conditions. [36] isolated *Penicillium thomii*, *Penicillium restrictum*, *Penicillium sp*., *Penicillium albidium*, *Gliocladium roseum*, *Myrothecium roridum*, *Penicillium frequentans*, *Penicillium jensenii* and *Eupenicillium javanicum* from the volcanic soils of southern Chile. [37] isolated three fungi *Aspergillus niger*, *A. fumigatus* and *Penicillium pinophilum* from the rhizosphere of peas, faba bean, wheat and kidney bean grown in Ismailia and South Sinai Governorates. [38] isolated one fungal and thirteen bacterial strains from the rhizosphere of black and white mangroves.

Different bacterial and fungal strains were isolated from the rice field soil of Bhubaneswar, Orissa, India. Out of all bacterial and fungal isolates only two fungi showed significant zone of phosphate solubilization after 5 days of (PVK) incubation, medium supplemented with calcium phosphate. The fungal strains were identified as *Aspergillus fumigatus* and *Penicillium* sp. based upon their colony and microscopic characteristics [39]. [40] isolated phosphate solubilizing fungi viz. *Aspergillus* spp., *Fusarium* spp. and *Penicillium* spp. and bacteria viz. *Bacillus subtilis* and *B. megatherium* from rhizosphere of wheat, orange, gram, jowar, onion, tomato, cabbage, tuar, cabbage and sunflower from saline affected areas of Bidar, Karnataka.

Pseudomonas spp. from soil of Korea district and *Azospirillum* spp., *Bacillus subtilis* and *Streptococcus* spp. from soil of Raigarh district of Chhattisgarh have been isolated by [41 a & b]. [42] isolated six promising strains of phosphofungi namely *Penicillium albidium*, *P. frequentans*, *P. restrictum*, *P. thomii*, *Penicillium* sp. and *Gliocladium roseum* on Martin medium (rose bengal-streptomycin agar) amended with calcium phosphate or calcium phytate as the P source from volcanic soils of southern Chile. [43] isolated twelve fungal isolates from rhizosphere soil extracts from eight cinnamon species and three pepper species on Pikovskaya (PVK) medium. These isolates were further tested for their insoluble phosphate solubilization activity in two liquid media: PVK medium and rock phosphate medium. Three fungal isolates exhibiting the highest efficiencies in phosphate solubilization were identified as *Trichoderma virens*, *Aspergillus* sp. and *Penicillium oxalicum* based on the micro-morphological and molecular characteristics. *Aspergillus* sp. showed the maximum phosphate solubilization followed by *P. oxalicum* and *T. virens*. [44] was describe laboratory experiments to isolate, screen out and select the efficient P-solubilizing fungal isolates from maize rhizosphere in the districts of northern Karnataka. Total 21 P-solubilizing fugal strains were isolated and were screened for phosphate solubilizing potential on pikovasakya's agar and broth. *Aspergillus* sp. [M-10(1)] showed highest P-solubilizing activity.

Similarly, two phosphate solubilizing fungi *Talaromyces auranticus* (TalA-JX04) and *Aspergillus neoniger* (AspN-JX16) were isolated from the rhizosphere soil of moso bamboo (*Phyllostachys edulis*) by [45]. [46] also isolated PSF from the rhizosphere soils of cash-crops such as tomato, cotton, sunflower, black gram, green gram, chilly, okra, sorghum, brinjal and red gram. Total twenty PSF were screened based on their solubilization zone formation activity. This isolate was identified on the basis of morphological, biochemical and microscopic

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examinations. Out of twenty PSF, nine were identified as *Aspergillus flavus*, seven as *A. niger* and four as *Penicillium notatum*. [47] isolated four phosphate solubilizing bacteria NBRI0603, NBRI2601, NBRI3246 and NBRI4003 from the rhizosphere of chickpea and reported that among the four strains; NBRI2601 was the most competent strain in terms of its potential to solubilize phosphorus in the presence of 10% salt and pH 12 at 45^o C temperature.

Ten phosphate solubilizing bacteria and three fungi were isolated from the maize rhizosphere using Pikovskya's medium by [48]. [49] isolated 36 strains of phosphate solubilizing bacteria of which ten isolate was identified as genus *Bacillus*, nine as genus *Rhodococcus*, seven as genus *Arthrobacter*, six as genus *Serratia* and each as genera *Chryseobacterium, Delftia, Gordonia* and *Phyllobacterium* after confirming their ability to solubilize significant quantity of tricalcium phosphate in the medium by secreting organic acids. [50] was isolated strains of PSB & PSF and confirmed their phosphate solubilizing activity on Pikovskaya's medium. [51] isolated phosphate solubilizing strains of *Trichoderma* sp. and *Penicillium* sp. from forest tree rhizosphere of pinus, deodar, bamboo, guava and oak on Selective Medium.

Assessment of phosphate solubilization

[52] was isolated phosphorus solubilizing *Aspergillus tubingensis* and two strains of *Aspergillus niger* which showed higher phosphate solubilization capacity when grown in presence of 2 % rock phosphate. [53] calculated the efficiency of mangrove phyllosphere fungi in solubilization of tricalcium phosphate and rock phosphate in liquid culture and found the better efficiency of *Aspergillus* sp. over *Penicillium* sp.[54] experienced phosphate solubilizing potential of three fungal strains *Aspergillus niger* strain BHUAS01, *Trichoderma harzianum* and *Penicillium citrinum* strain BHUPC01 using Pikovskaya's broth containing tricalcium phosphate. *Aspergillus niger* showed maximum amount of soluble phosphate after 6 days of incubation. [55] isolated 138 Phosphate solubilizing fungi from the sugarcane and sugar beet rhizosphere soil of Western Maharashtra region, on the basis of clear zones formation on Pikovskaya's agar medium and solubilization index. They reported *Aspergillus* as dominating species. [56] were isolated 23 different phosphate-solubilizing bacteria and 35 different phosphate-solubilizing fungi in the rhizospheric and non-rhizospheric soils from Madhya Pradesh and Karnataka. The bacterial isolates were belonging to *Pseudomonas, Xanthomonas, Bacillus, Aerococcus, Alteromonas, Erwinia and Enterobacter* and fungal isolates was identified as *Aspergillus* and *Penicillium*. [57] isolated 56 fungai from rhizospheric soil of paddy field plants. Fungi isolated were belonging to *Arthrinium, Aspergillus, Chaetomium, Curvularia, Fusarium and Penicillium* sp. Among these *Aspergillus niger* 8 (RSA4), *Aspergillus niger* 13 (RAB01) and *Penicillium purogenum* (RAB06) showed higher efficiency for phosphate solubilization.

There are many reports of phosphate solubilization capacity by fungi. [58] reported maximum phosphate solubilization by three strains of *Aspergillus niger* (78.63%, 56.32%, 52.65%) followed by *Penicillium* sp. (46.32%). [59] was tasted the potential of *Aspergillus* sp. PS104 to solubilize rock phosphate *in vitro* with decrease in pH up to 2.48. They also reported the role of citric acid and phosphatase in the phosphate solubilization mechanism. [60]

isolated *Aspergillus niger* from mangrove sediment and determine their phosphate solubilization competence. Various Carbon and nitrogen sources are added in PVK broth medium, maximum solubilization is 443µg/ml and 468µg/ml were recorded with glucose and ammonium supplementation. Optimum pH and temperature for maximum solubilization was 7.0 and 30°C respectively.[61] were isolated *Aspergillus* sp. and *Penicillium* from the rice field soil of Bhubaneswar, Orissa, India, and observe that phosphate solubilization was related to pH decrease caused by growth of fungus in medium added with glucose as carbon source. *Aspergillus* sp. solubilized 480 µg/ml of phosphorus, while *Penicillium* sp. solubilized 275 µg/ml of phosphorus from 0.5% tricalcium phosphate after 4 and 3 days of growth respectively**.** [62] demonstrated effect of different carbon and nitrogen sources on phosphate solubilization by *Aspergillus niger* F7. *A. niger* showed maximum significant solubilization of tricalcium phosphate in Pikovskaya broth containing glucose as carbon source followed by glycerol, maltose, sucrose at 21 days of incubation [63]. [64] investigated the effect of nitrogen source on the solubilization of rock phosphate by *Mortierella* sp. He was reported that more dissolution is observed in the presence of ammonium chloride and ammonium nitrate than potassium nitrate. [65] was screen out 47 fungal isolates for phosphate solubilization on solid medium containing hydroxyapatite (HA). These isolates were recognized as *Penicillium* or its teleomorphs. All the isolates were assessed for solubilization of Idaho rock phosphate in broth culture. *Penicillium bilaiae* strain RS7B-SD1 was establish to be most effective in mobilizing P after 7 days followed by *Penicillium simplicissimum*, five strains of *Penicillium griseofulvum*, *Talaromyces flavus*, two unidentified *Penicillium* spp. and newly isolated strain of *Penicillium radicum* (KC1-SD1). Additionally, they also evaluated the RP solubilization, biomass production and solution pH for three fungal strains namely *P. bilaiae* RS7B-SD1, *P. radicum* FRR4718 and *Penicillium* sp. 1 KC6-W2. In between them *P. bilaiae* RS7B-SD1 showed the highest RP solubilization activity per unit of biomass produced. [66] compared the solubilization activity of three different sources of phosphorus (tricalcium phosphate-PC, aluminium phosphate-AP and phosphorite-PP) by different fungal isolates namely *Talaromyces flavus* (S73), *T. flavus* var *flavus* (TM), *T. helices* (L7b), *T. helices* (N24), *Penicillium janthinellum* (PJ) and *P. purpurogenum* (POP). They also considered the possible mechanisms involved in the solubilization process. The type and concentration of organic acids formed by each isolates varied according to the source of available P. The medium containing PC showed highest proportion of gluconic acid, while in the media added either with AP or PP showed that of citric and valeric acids.

[67] investigated the phosphate solubilization potential of two fungal isolates *Aspergillus* sp. and *Penicillium* sp. in both solid and liquid medium. Presence of clear halo zone after five days of incubation indicated their phosphate solubilization ability. Czapek Dox liquid medium containing rock phosphate was used for quantitative estimation of soluble phosphate concentration. Lowering in medium pH was recorded during the study. Phosphate solubilization was connected to decrease in pH caused by the growth of fungus. The rock phosphate was solubilized upto 61.6%. One more study, *A. niger* showed maximum phosphate solubilization followed by *Penicillium citrinum* and *Trichoderma harizanum* [68]. [69] demonstrate tricalcium phosphate and rock phosphate

solubilization by different fungal strains. They reported the percent phosphorus solubilization that ranged from 34.2-58.0% for tricalcium phosphate and 16.6-36.6% for rock phosphate.

A further study revealed that, fifteen varieties of fungal species were isolated from the Arecanut husk waste; of these nine isolates showed phosphate solubilization ability and seven showed lignolytic property. The zone of appearance of phosphate solubilization on Pikovskaya's medium *Aspergillus terreus*, was medium in *Botrytis cenerea* and very low in *A. niger* (Strain-2) and Unidentified-3. On the other hand, the lignolytic activity shows that the zone of clearance was higher in *Gibberella fujikuroi*, medium in *A. niger* (Strain-1) and very low in *A. flavus*. The phosphate solubilization effectiveness was also tested in Pikovskayas liquid medium and was found to be higher in Unidentified-2, medium in Unidentified-1 and very low in *A. niger* (Strain-2). The fungal isolate *A. niger* (Strain-1) showed highest growth rate based on total biomass yield followed by Unidentified-2 and Unidentified-1 after 12 days incubation periods [70]. [71] were also seperated phosphate solubilizing fungi from teff rhizosphere soil. Fungi were identified using lactophenol cotton blue staining and Biolog microstation.

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