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Screening of Some Microbial Isolates from Soil Samples for Solubilization of Inorganic Phosphate

Ekundayo E. A.[1*], Ogunnusi T. A.[1], Ogunmefun O. O.[1], Alegbe M. O.[1] and Oso A. O.[2]
[1]Department of Biological Sciences, Afe Babalola University, Ado-Ekiti, Nigeria
[2]Department of Chemical Sciences, Afe Babalola University, Ado-Ekiti, Nigeria
esttydayo2010@yahoo.com

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Abstract

Thirty two bacterial and six fungal strains isolated from different soil samples collected from the Federal University of Technology, Akure and Afe Babalola University, Ado Ekiti, Nigeria were qualitatively and quantitatively screened for phosphate solubilization ability using National Botanical Research Institute phosphate agar and broth containing tricalcium phosphate as the single source of phosphorus. The result of the qualitative screening showed that none of the isolates formed any halo zone which was indicative of phosphate solubilization. However, in liquid medium, fifteen bacterial isolates showed phosphate solubilization which was evident by decrease of pH in National Botanical Research Institute phosphate broth after 24, 48, 72, 96 and 120 hours. All the fungal isolates showed more efficiency in solubilization of phosphate with Aspergillus parasiticus solubilizing most of the phosphate in the medium.

Keywords: Microbial isolates, Phosphate solubilization, Halo zones, *Aspergillus parasiticus*.

Introduction

Phosphorus is an important micronutrient required for the growth as well as development of plants next to nitrogen. However, greater than 70-90% of phosphate fertilizers applied are fixed in soil making phosphorus unavailable for plant uptake thus increasing the phosphorus requirement of the plant[1]. Microorganisms such as bacteria and fungi found in the soil play essential roles in the biogeochemical cycling of phosphate in soil ecosystems.[2] Strains of *Pseudomonas* spp., *Bacillus* spp., *Enterobacter* spp. and endosymbiotic symbiotic nitrogen fixers such as *Rhizobium* spp. are effective phosphate solubilizers. *Bacillus megaterium*, *B. circulans*, *B. subtilis*, *B. polymyxa, B. sircalmous, Pseudomonas striata*, and *Enterobacter spp.* could be referred as the most important strains[3]. The soil fungi especially Ascomycota and Zygomycota examples of which include *Penicillium and Rhizopus* are also capable of solubilizing insoluble phosphates by secreting weak organic acids[4].

Inorganic phosphate solubilization by microorganisms occurs mainly by organic acid production either by lowering the pH, or enhancing chelation of the cation bound to phosphate. The lowering in pH of the medium suggests the release of organic acids by the phosphate solubilizing microorganisms[5, 6] via the direct oxidation pathway that occurs on the outer face of the cytoplasmic membrane[7]. These acids are the product of the microbial metabolism, mostly by oxidative respiration or by fermentation of organic carbon sources (e.g., glucose)[8, 9] or such organic acids can either directly dissolve the mineral phosphate as a result of anion exchange of phosphate by acid anion or can chelate Fe, Al and Ca ions associated with phosphate[10].

Pikovskaya suggested that microbes could dissolve non-readily available forms of soil phosphate and play an important role in providing phosphate to plants, numerous methods and media, such as Pikovskaya[11], bromophenol blue dye method[12] and National Botanical Research Institute phosphate (NBRIP) medium[13] have been proposed for the detection of phosphate solubilizing activity by microorganisms. Both bacterial and fungal strains exhibiting phosphate solubilising activity are detected by the formation of clear halo (a sign of solubilization) around their colonies[14].

Several studies have shown the potentials of different isolates of microorganisms to solubilize phosphate, however, there is no report on the use of microbial isolates from Afe Babalola University, Ado-Ekiti and Federal University of Technology in solubilization of inorganic phosphate. This current investigation was therefore conducted to determine the phosphate solubilizing potentials of microorganisms isolated from different soil samples.

Materials and Methods

The bacterial isolates used in this present investigation were *Bacillus sphaericus* SS1, *B. larvae* SS2, *B. siamensis* SS4, *B. firmus* SS5 , *Salimicrobium halophilum* SS 6, *B. endophyticus* NSS1, *Brevibacillus laterosporus* NSS2, *Photorhabdus temperta* NSS3, *Xenorhabdus japonica* NSS4, *Oligella ureolytica* NSS5, *Paenibacillus assamensis* NSS6, ,

Enterobacter taylore NSS7, *Paenibacillus alvei* NSS8, *Salimicrobium halophilum* NS1, *Paenibacillus apiaries* NS2 , *Paenibacillus cellulositrophicus* NS3 , *Lysinibacillus sphaericus* NS4, *Aeromanas popoffi* NS5, *Paenibacillus alvei* NS6, *Brevibacillus laterosporus* DS1, *Bacillus firmus* DS2, *Enterobacter dissolvens* DS3, *Bacillus larvae* DS4, *Paenibacillus curdlanolticus* DS5, *Tatumella ptyseos* DS6, *Rummeliibacilus pycnus* DS7, *Brevibacillus agri* CO1, *Paenibacillus lentimorbus* CO2, *Bacillus larvae* CO3, *Lysinibacillus sphaericus* CO4, *Viridibacillus neidei* CO5 and were provided from the Department of Biological Sciences, Afe Babalola University, Ado-Ekiti, Nigeria. The SS coded samples were obtained from treated sterile soils which were used for cultivation of okra seeds, NSS coded samples were obtained from treated non sterile soils, CO coded samples were untreated, NS coded samples were not used for planting while DS coded samples were obtained from dumpsite. The isolates were authenticated using standard microbiological techniques[15] and were stored in MacCartney bottles at 4^{o}C. The fungal isolates used include *Rhizopus* spp., *Mucor* spp., *Trichoderma* spp, *Aspergillus parasiticus* and *T. viride*. The insoluble phosphate (tricalcium phosphate) was collected from Department of Microbiology, Federal University of Technology, Akure, Nigeria.

Phosphate solubilisation test: The capability of the microbial isolates to solubilise phosphate in solid media was determined using National Botanical Research Institute phosphate solid media containing the following ingredients (g.L^{-1}): glucose, 10.0; tricalcium phosphate (TCP), 10.0; $MgCl_2.6\ H_2O$, 5.0; $MgSO_4.7H_2O$, 0.25; KCl, 0.2; $(NH_4)_2SO_4$, 0.1, agar agar, 15. Ten µL of each isolate was deposited in spot on the surface of the solid media in Petri dishes and was observed for clear zone around the colony for 5 days at 30°C which was measured with a meter rule according to the method of Nautiyal[13].

For solubilisation in liquid media, a 100 µL of 24 hour culture of each isolate was inoculated in tubes containing ten mL of National Botanical Research Institute phosphate (NBRIP) liquid medium for quantitative analysis of the solubilised phosphate. and incubated at 30°C for a time interval of 24 hours for 5 days. Centrifugation of the cultures was done at 3000 rpm for 20 minutes. The pH of the filtrate was determined using the method of Olsen and Sommers[16].

Results and Discussion

None of the bacterial isolates showed zone of inhibition on the National Botanical Research Institute phosphate solid medium. However, it was observed that some bacterial species were able to decrease the pH of the medium in the National Botanical Research Institute phosphate broth medium. The pH of *Paenibacillus lentimorbus* showed decrease after every 24 hour interval. At 24 hr the pH was recorded to be 6.2 while by 120th hr the pH had decreased to 5.4. In the case of *Lysinibacillus sphaericus* the pH was not constant indicating the organism could not decrease the pH of the medium as shown in Figure 1. Figure 2 shows that Br*evibacillus laterosporus* and *Enterobacter dissolvens* isolated from dumpsite soil showed decrease in pH. *B. Laterosporus* decreased from 5.7 (24 hr) to 5.2 (120 hr) and *E. dissolvens* decreased from 5.8 (24 hr) to 5.1 (120 hr). All the bacterial isolates coded as NS showed decrease in pH of the medium. They were all able to make the medium acidic as shown in Figure 3. Only *Brevibacillus laterosporus* showed decrease in the pH of the medium in NSS soil samples as can be seen in Figure 4. Also, only *Bacillus firmus* showed decrease in pH from 6.2 (24 hr) to 5.0 (120 hr) in bacteria coded as SS in Figure 5. The pH levels decreased from neutral to variables between 6.0 and 4.0 at different time intervals.

All the fungal isolates showed decrease in pH of the medium. *Mucor* spp. had a pH of 6.1 (24 hr) and decreased to 5.3 (96 hr). Comparing all fungal species, it was discovered that *A. parasiticus* appeared to be the best phosphate solubilizer because the pH decreased from 5.5 to 4.5 from 24hour to 120 hour of incubation as shown in Figure 6.

Although phosphate is abundant in several soils, it is one of the major nutrients limiting plant growth due to the formation of insoluble complexes[17]. As a result of this, there is need for application of soluble forms of inorganic phosphate is necessary for crop production . However, this leaches to the ground water and the resultant effect is eutrophication of aquatic systems[18].

The phosphate solubilizing activity of microbes results in production of organic acids, which through their carboxylic groups chelate the cations (mainly Ca) bound to phosphate converting them into the soluble forms[19,20]. None of the isolated microorganisms were able to solubilize phosphate in the soil medium. Deubel and Merbach[21] found out that only two out of eight different microbes tested for phosphate solubilisation capacity on calcium phosphate agar plates showed clear zone around their colony. Moreover, it was noted that the best strain in solubilizing the same phosphate source in liquid media was one of the strains which could not show clear zone on agar plates making solubilisation in solid medium a non reliable technique[13]. Cherif-Silini *et al.*[22] also observed that solid media are less sensitive than liquid media in the detection of the solubilization capacity and could be attributed to the low diffusion of the acids produced by microorganisms.

Fourteen bacterial isolates along with six of the fungal species were found to solubilize tricalcium phosphate supplemented in liquid media which was evidenced by reduction in pH of the medium. Most of the phosphate solubilizers in this investigation were found to be from the genus *Bacillus.* This finding is supported by an earlier report that most efficient and frequently encountered phosphate solubilizing bacteria belong to the genus *Pseudomonas* or the genus *Bacillus*[23,24,25]. Venkateswaran and Natarajan[26] reported *Pseudomonas* sp. and *Bacillus* sp. as dominant inorganic phosphorus compounds solubilizing microbes.

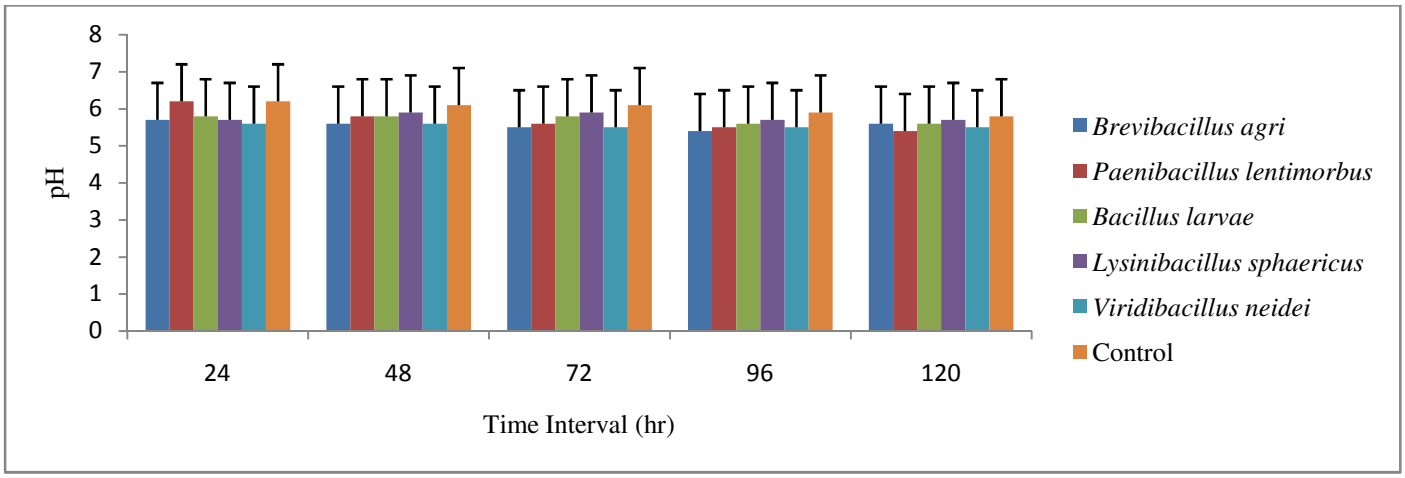

Figure -1
pH changes in bacterial isolates soil during incubation in NBRIP broth at different time intervals

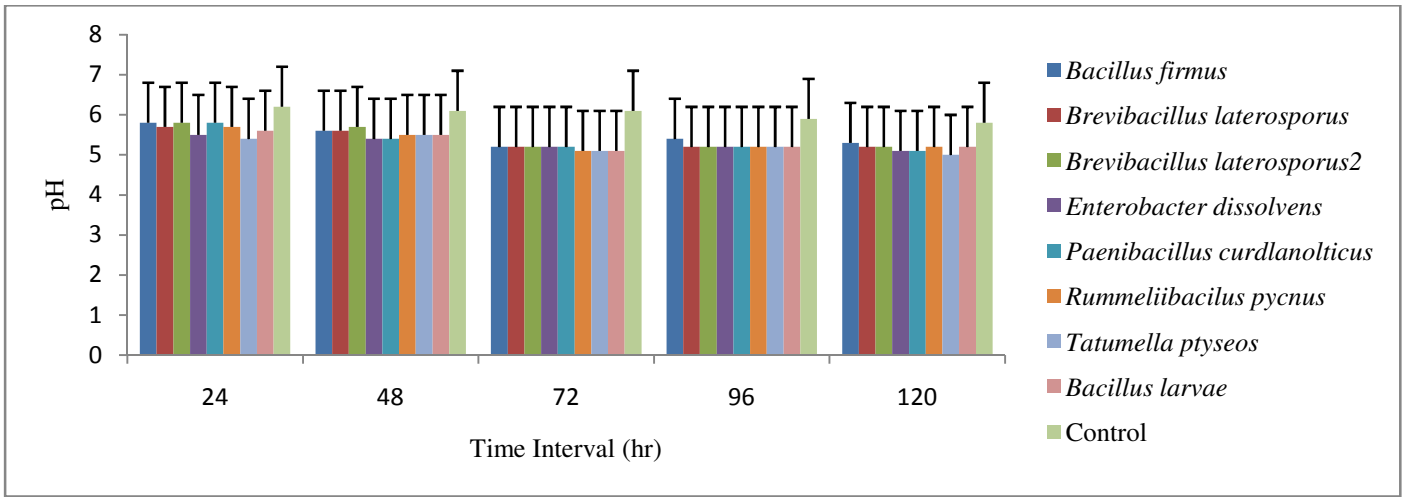

Figure-2
pH changes of DS coded bacterial isolates during incubation in NBRIP at different time intervals

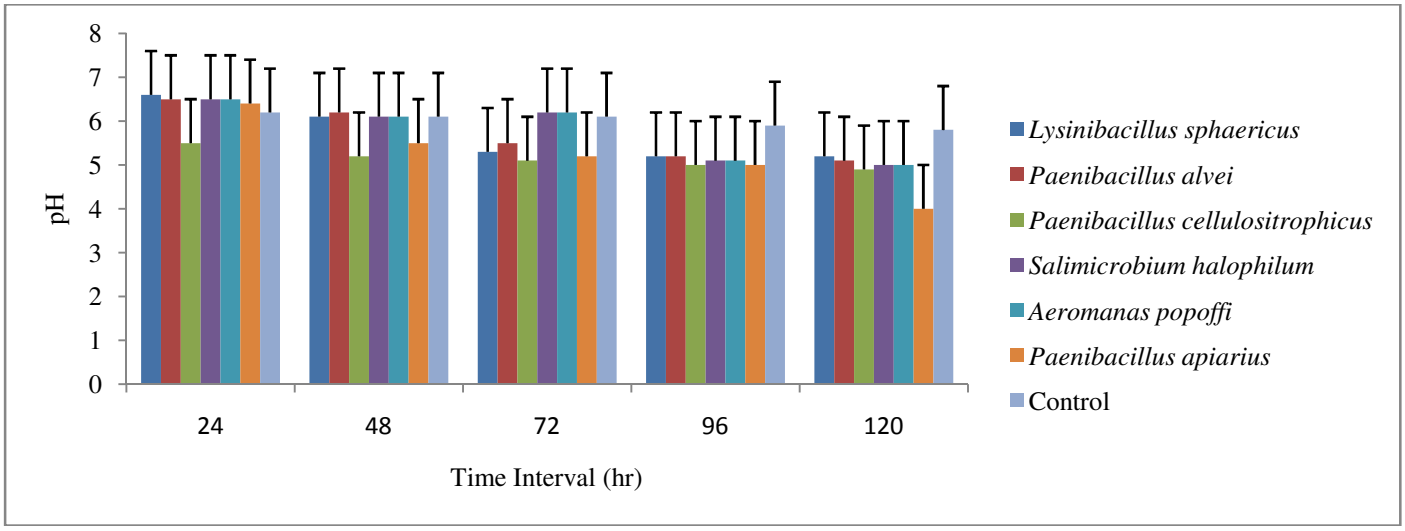

Figure -3
pH changes of NS coded bacteria during incubation in NBRIP broth at different time intervals

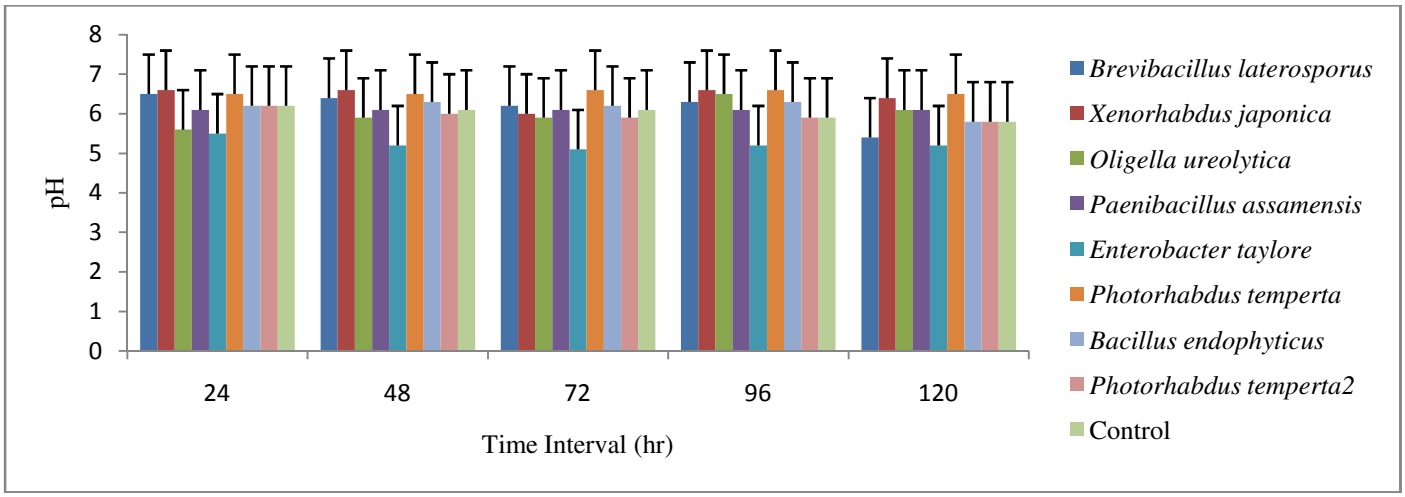

Figure-4
pH changes of NSS coded bacterial isolates during incubation in NBRIP broth at different time interval

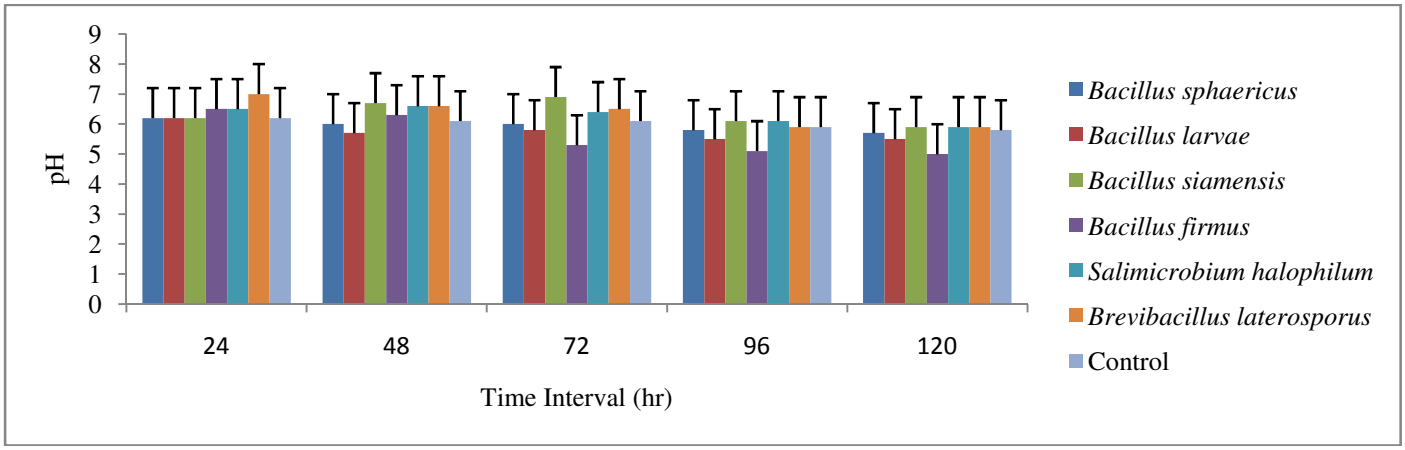

Figure -5
pH changes of SS coded bacterial isolates during incubation in NBRIP broth at different time intervals

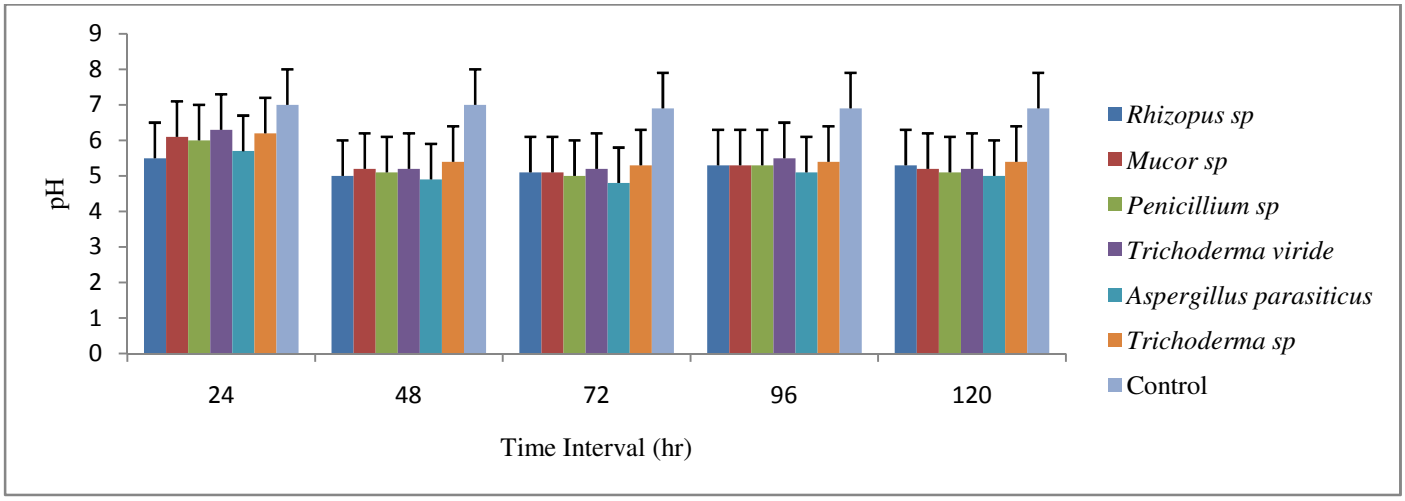

Figure-6
pH changes of fungal isolates at different time intervals

All the fungal isolates used in this investigation were capable of solubilizing the phosphate present in the media. These findings also correlate with the findings of Nahas *et al*.[27] who suggested that genus *Aspergillus* is a well-known phosphate solubilizing fungi. *Penicillium* sp. was also identified as efficient phosphate solubilizer[28].

The current study also evidenced that the liquid media inoculated with phosphate solubilizing microorganisms is accompanied by a reduction in pH leading to production of different organic acids by depending of the kind of microorganisms producing them[2+ , 29]. It has been suggested that microorganisms which tend to decrease the pH of the medium during growth are efficient phosphate solubilizers[19].

Conclusion

This study highlights the comparative phosphate solubilizing potential of different phosphate solubilizing organisms. It was found that all the isolates were capable of differentially utilizing 10 g.l-1 TCP in NBRIP broth. This was indicated by increase in acidity (decrease in pH) of the growth medium. The pH of the broth culture gradually decreased with the progress of incubation up to five days and thereafter increased. From this present study, it is recommended that the isolates able to solubilize phosphate can be used as bioinoculants which will increase the available phosphorus in soil and help to minimize the use of phosphate fertilizer application and to reduce environmental pollution and promote sustainable agriculture thereby improving the phosphate nutrition of crop plants.

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