INTRODUCTION

After nitrogen phosphorus is the second amongst the mineral nutrients those usually limit the growth of crops (Das et al., 2013). Phosphorus is essential for storage and transfer of energy, photosynthesis as well as biochemical and genetic activities of plant (Armstrong 1988; Theodorou and Plaxton, 1993). Major proportion of phosphate fertilizer is converted to insoluble form during application (Bardiya and Gaur, 1974; Singal et al., 1994). This conversion makes this important nutrient unavailable to the plants (Igual et al., 2001; Rodriguez and Fraga, 1999). Phosphate solubilizing microbes possess the characteristic to convert the fixed form of phosphorus to available form for plants to absorb phosphorus from soil (Rodriguez and Fraga, 1999). Several studies revealed that rhizobacteria particularly from genera Agrobacterium, Bacillus, Burkholderia, Enterobacter, Erwinia, Pseudomonas, Rhizobium, Mesorhizobium, Pantoea and Serratia are known to be highly efficient in solubilizing insoluble soil phosphate into soluble inorganic phosphate (Illmer and Schinner, 1995; Rodriguez and Fraga, 1999; Castagno et al., 2011). Several studies have been made to find out the ability of fungi; mainly of genus Aspergillus and Penicillium that solubilize various forms of phosphate (Seshadri et al., 2004; Wakelin et al., 2004). Several mechanisms have been reported that play an important role in phosphate solubilization like lowering of pH by acid production, ion chelation and exchange reaction by phosphate solubilizing microorganisms (Halder et al., 1991; Abd-Alla, 1994; Whitelaw, 2000; Goldstein, 1986).

The bioavailability of soil inorganic phosphorus in the rhizosphere is differing according to the plant species, nutritional status of soil and ambient soil conditions (Hinsinger, 2001). Burkholderia is gives good phosphate solubilizing activity than other microbial microflora present in that environment. Many bacteria of this genus have been isolated from soils (Coenye and Vandame, 2003). Burkholderia spp. can be found free living in the rhizosphere along with epiphytic or endophytic and obligate endosymbionts or phytopathogens (Janssen, 2006). Burkholderia spp. having many beneficial plant growth promoting activities such as production of IAA and siderophore for promoting the crop growth as well as for controlling the plant pathogens (Pandey et al., 2008). Aspergillus spp. is also consisting dominant species that are capable of phosphate solubilization, siderophore production as well as indole
acetic acid production (Wakelin et al., 2004; Nath et al., 2015; Milagres et al., 1999). For maximum phosphate solubilization by microorganisms, many authors have optimized the different cultural conditions such as incubation time (Chadha et al., 2015; Laxmi et al., 2015; Narveer et al., 2014), inoculum size (Onyia et al., 2013; Walpola and Yoon, 2013), pH (Maheswar and Sathiavani, 2012; Mardad et al., 2014), temperature (Walpola et al., 2014; Hefnawy et al., 2009), phosphate sources (Tallapragada and Seshachala, 2012; Saxena et al., 2013), carbon sources (Pallavi and Gupta, 2013; Aarab et al., 2015), nitrogen sources (Scervino et al., 2011; Arumanayagam and Arunmani, 2014)), TCP concentration (Hefnawy et al., 2002) and glucose concentration (Song et al., 2008).

In the present study incubation time, inoculum size, pH, temperature, phosphate sources, carbon sources, nitrogen sources, TCP concentration and glucose concentration were optimized to enhance phosphate solubilization by potent strains Aspergillus awamori and Burkholderia latens.

MATERIALS AND METHODS

Quantitative estimation of phosphate

Both fungal and bacterial strains were isolated from Bt-cotton rhizospheric soil and selected for optimization of phosphate solubilization. Phosphate solubilizing efficiency of Aspergillus awamori and Burkholderia latens was tested in PVK media with 0.5% tri calcium phosphate as phosphate substrate. Flasks were separately inoculated with 8% v/v (1.5 x 10⁷) of fungal and bacterial cultures and incubated on shaker at 28°C for 6 days at 100 rpm. After incubation the fermented broth were centrifuged at 10,000 rpm for 15 min, the pH of the supernatant was measured and dissolved phosphate concentration in supernatant was determined by Vanado-Molybdate method as described in APHA (1995), phosphate concentration was expressed in terms of µg ml⁻¹ of phosphate released in culture.

Effect of various cultural conditions on efficiency of phosphate solubilization by Aspergillus awamori and Burkholderia latens.

Different cultural conditions were optimized for maximum phosphate solubilization by Aspergillus awamori and Burkholderia latens.
Effect of incubation time

The study was carried out to optimize the incubation time for maximum phosphate solubilization. 100 mL of PVK broth was prepared, it was autoclaved at 121°C for 15 lb and maintained for 15 min. Then it was inoculated with 8% (v/v) inoculum of *Aspergillus awamori* and *Burkholderia latens*. The both flasks were incubated in shaker for 28°C at 100 rpm. After regular time interval (24 h) from 1 day upto 10 day for fungi and 7 days for bacteria. Then fermented broth was withdrawn, centrifuged and pH was checked. Dissolved phosphate concentration in supernatant was determined by Vanado-Molybdate method.

Effect of inoculum size

To determine the volume of inoculum required for maximum phosphate solubilization, PVK broths were inoculated with growing active pure culture of *Aspergillus awamori* and *Burkholderia latens*, inoculum size ranging from 4 to 10% (v/v) for fungi and 1 to 8% (v/v) for bacteria. Efficiency of phosphate solubilization was measured as described above.

Effect of temperature

To study the effect of different temperature on phosphate solubilization, the optimized parameter uptill were kept constant and the flasks were inoculated with both cultures separately and incubated at different temperatures *i.e.*, 24°C, 28°C, 30°C, 32°C, 36°C and 40°C for fungi and 24°C, 28°C, 32°C, 36°C and 40°C at 100 rpm. The amount of phosphate released was checked from Pikovskaya’s broth after incubation period.

Effect of pH

In the present study the effect of different pH ranging from 5.0 to 9.0 were studied for maximum phosphate solubilization. To check effect of different pH on phosphate solubilization, the optimized incubation time, inoculum size and temperature were keep constant for *Aspergillus awamori* and *Burkholderia latens*. The pH that gave maximum phosphate solubilization was utilized and exploited to optimize other parameters.
Effect of phosphate sources

After having optimized incubation time, inoculum size, temperature and pH, effect of various phosphate sources i.e., Ca₃(PO₄)₂, AlPO₄ and FePO₄ were used to study their effects on maximum phosphate solubilization by fungal and bacterial isolates. The optimized parameters up till now were kept constant for both isolates. The broth were inoculated, incubated and then checked for amount of phosphate released.

Effect of carbon sources

In the present study, different carbon sources i.e., sugar like glucose, sucrose, fructose, lactose and galactose were used to check their effect on phosphate solubilization. The optimized parameters were kept as for Aspergillus awamori and Burkholderia latens. The PVK broth were inoculated, incubated and then checked for amount of phosphate released.

Effect of nitrogen sources

For medium optimization study, various inorganic and organic nitrogen sources i.e., (NH₄)₂SO₄, NaNO₃, urea and casein were used to check their effect on phosphate solubilization. To check effect of various nitrogen sources on phosphate solubilization, the optimized parameters up till now were keep constant. The amount of phosphate released was checked from each flask after completion of incubation period.

Effect of different TCP concentration

After having optimized incubation time, inoculum size, temperature and pH, phosphate sources, carbon sources, nitrogen sources and effect of tri calcium concentration ranging from 0.3 to 0.8% (w/v) were used to check their effect on maximum phosphate solubilization by fungi and bacteria. The optimized parameters were kept as for Aspergillus awamori and Burkholderia latens. Efficiency of phosphate solubilization was measured as described above.

Effect of different glucose concentration

For medium optimization study, glucose concentration ranging from 0.5 to 3% was used to check their effect on maximum phosphate solubilization by both isolates. To
check the effect of different glucose concentration on phosphate solubilization, the optimized parameters uptill now were kept constant for both isolates. The amount of phosphate released was checked from each flask after completion of incubation period.

RESULTS AND DISCUSSION

Effect of Incubation time

![Graph showing the effect of incubation time on phosphate solubilization by A. awamori](image)

Figure 3.1 Effect of incubation time on phosphate solubilization by *A. awamori*

The incubation period plays a significant role in phosphate solubilizing activity of microorganisms. It varies from organism to organism. It may be in hours, days or sometime in weeks or more than that. *Aspergillus awamori* showed $561 \pm 7.47 \mu g \text{ mL}^{-1}$ of phosphate solubilization after 6 days in unoptimized condition. To investigate the effect of incubation time on phosphate solubilization, the samples were checked for phosphate solubilization at regular time interval (24 h), starting from 1 day upto 10 days. *Aspergillus awamori* showed optimum phosphate solubilization when it was in logarithmic phase of growth curve. *Aspergillus awamori* showed $589 \pm 5.85 \mu g \text{ mL}^{-1}$ of phosphate solubilization after 8 day, after then phosphate solubilization was slightly decreased (Figure 3.1). There are some evidences which showed that incubation time varies from genus to genus and species to species for optimum
phosphate solubilization. Chadha et al., (2015) reported that phosphate solubilizing fungi showed maximum phosphate solubilization after 8 day. Darmwal et al., (1991) who proved that *A. niger* was found to be the best phosphate solubilizer among several tested fungi and bacteria and maximum amount of phosphate solubilization was reached after 7 to 10 days. Hefnawy et al., (2002) mentioned that the maximum amount of uranium was released by *A. terrus* after 6 to 8 days. Laxmi et al., (2015) investigated that solubilization of dicalcium and tricalcium phosphate both were recorded maximum on the 8 day of incubation period by *Allochromatium spp.* GSKRLMBKU-01.

**Figure 3.2 Effect of incubation time on phosphate solubilization by B. latens**

*Burkholderia latens* showed 328.7 ± 7.13 µg mL⁻¹ of phosphate solubilization after 6 days in unoptimized condition. To check the effect of incubation time on phosphate solubilization, the sample were taken at regular time interval (24h), starting from one day upto after completion of incubation period. *Burkholderia latens* showed 396 ± 2.64 µg mL⁻¹ of phosphate solubilization on 3rd day, then after phosphate solubilization was slightly decreased upto 3.63 (Figure 3.2). Our finding is in good correlation with these reports. Mardad et al. (2013) reported that maximum phosphate solubilization of phosphate solubilizing bacteria was observed after 60 to 72h. Walpola et al., (2012a) investigated maximum phosphate solubilization after 2 to 3 days by *Burkholderia* species and then significant drop in phosphate solubilization
when incubation period progressed. Walpola and Yoon (2013) reported *Pantoea agglomerans* and *Burkholderia anthina* solubilized maximum phosphate after 2 to 3 days of incubation time. Narveer et al., (2014) investigated that maximum phosphate solubilization of *Bacillus spp.* was recorded after 3 to 4 days of incubation period.

**Effect of inoculum size**

![Figure 3.3](image1.png)

**Figure 3.3 Effect of inoculum size on phosphate solubilization by *A. awamori***

![Figure 3.4](image2.png)

**Figure 3.4 Effect of inoculum size on phosphate solubilization by *B. latens***
The inoculum size plays a significant role in phosphate solubilization, so there is needed for optimizing inoculum size for phosphate solubilization. To investigate the optimum inoculum size, the Pikovskaya’s broth was inoculated with 4.0 to 10.0% v/v inoculum of *Aspergillus awamori*, whereas for *Burkholderia latens* inoculum size ranging from 1 to 8% v/v. *Aspergillus awamori* showed 611 ± 8.18 µg mL\(^{-1}\) of phosphate solubilization and pH drop upto 2.45 by inoculum size of 5% (Figure 3.3) and *Burkholderia latens* solubilized 451.7 ± 7.57 µg mL\(^{-1}\) of phosphate solubilization and pH drop to 3.49 by inoculum size of 3% (Figure 3.4). Barroso et al., (2006) reported that 1.34 x 10\(^7\) spores mL\(^{-1}\) of *A. niger* was inoculated to solubilize CaHPO\(_4\) and AlPO\(_4\). Fankem et al., (2006) studied phosphate solubilization by inoculating approximately 1 to 2 x 10\(^7\) cell mL\(^{-1}\) of various soil isolates. Pradhan and Sukla (2005) studied that maximum phosphate was solubilized with 5% (v/v) spore suspension of *A. niger* and *Penicillium spp*. Walpola and Yoon (2013) reported that 10\(^8\) cell mL\(^{-1}\) of *Pantoea agglomerans* and *Burkholderia anthina* each were used for phosphate solubilization. Onyia et al., (2013) investigated that maximum phosphate solubilization was observed when cell size was 17.4 x 10\(^7\) cell mL\(^{-1}\).

**Effect of temperature**

![Figure 3.5 Effect of temperature on phosphate solubilization by A. awamori](image)

The temperature is considered as an important environmental parameter for optimization study of phosphate solubilizing microorganisms. For *Aspergillus awamori* temperature like 24°C, 28°C, 30°C, 32°C, 36°C and 40°C were selected for
phosphate solubilization. The optimum temperature for *Aspergillus awamori* was 30°C and phosphate solubilization was 643 ± 5.29 µg mL\(^{-1}\) and pH drop was 2.36 (Figure 3.5). Barroso *et al.*, (2006) investigated that optimum temperature for *A. niger* was 30°C. Wani *et al.*, (1979) reported that 30°C was optimum temperature for tri calcium phosphate solubilization by *Aspergillus awamori*. Illmer and Schinner (1992) investigated that maximum phosphate solubilization by *Pseudomonas* *spp.* and *Penicillium* *spp.* were observed at 25°C and 30°C respectively. Hefnawy *et al.*, (2009) reported that optimum incubation temperature for best phosphate solubilization by *A. niger* and *A. fumigatus* was approximately at 28°C. Hefnawy *et al.*, (2002) reported that *A. terrus* was able to solubilize 75% of uranium content of the ore at 30°C. For *A. niger* and *Penicillium* *spp.*, optimum temperature for phosphate solubilization was 30°C (Pradhan and Sukla, 2005).

**Figure 3.6 Effect of temperature on phosphate solubilization by *B. latens***

The optimum temperature is required for sufficient microbial growth and it is responsible for phosphate solubilization. Temperature like 24°C, 28°C, 32°C, 36°C and 40°C were tested for phosphate solubilization by *Burkholderia latens*. *Burkholderia latens* was maximum solubilized 539.3 ± 3.21 µg mL\(^{-1}\) of phosphate at 32°C and after optimum condition phosphate solubilization was decreased upto 3.37 (Figure 3.6). Walpola *et al.*, (2012a) reported that maximum tri calcium phosphate solubilization was observed at 30°C to 35°C by *Burkholderia* species. Walpola *et al.*, (2012a) report...
(2014) investigated that maximum phosphate solubilization of inorganic phosphate in NBRIP medium was recorded at 35°C temperature by *Klebsiella oxytoca*.

**Effect of pH**

![Figure 3.7 Effect of pH on phosphate solubilization by A. awamori](image)

The pH is plays very important role in phosphate solubilizing mechanisms for both bacteria and fungi. The range of pH 5.0, 6.0, 6.5, 7.0, 8.0 and 9.0 were selected to obtain maximum phosphate solubilization. For *Aspergillus awamori*, pH 6.5 was found optimum which showed $658.7 \pm 7.57 \mu g \text{ mL}^{-1}$ of phosphate solubilization and pH of the medium was highly decreased from 6.5 to 2.34 after completion of incubation period (Figure 3.7). However, acidification does not seem to be the only mechanisms of phosphate solubilization, as the ability to reduce the pH in some cases does not correlate well with the ability to solubilize mineral phosphates (Muleta, 2007). Wani *et al.*, (1979) found that for maximum solubilization of tri calcium phosphate in Pikovskaya’s medium, the best pH was around 6.0 for *Aspergillus awamori*. Walpola *et al.*, (2012b) reported that *Aspergillus awamori* bxq33110 was maximum phosphate solubilized at pH 7.0. Hefnawy *et al.*, (2009) investigated that maximum rock phosphate and tri calcium phosphate solubilization was observed at pH 6.5 and 7.0 by *A. niger* and *A. fumigates*. 

95
The range of pH values from 5.0 to 9.0 were selected to obtain maximum phosphate solubilization for *Burkholderia latens*. Nevertheless, it is generally believed that the production of organic acids, added to a steep drop in pH, is the main driving force for mobilization of mineral phosphates. For *Burkholderia latens*, pH 7.5 gave the maximum phosphate solubilization. *Burkholderia latens* solubilized 590.6 ± 5.68 µg mL\(^{-1}\) of phosphate and pH was decreased from 7.5 to 3.26 ± 0.10 (Figure 3.8). Sanjotha and Sudheer (2016) reported that around pH 7.5 to pH 8.0 was best for phosphate solubilization by three phosphate solubilizing bacteria. Walpola *et al.*, (2014) reported that maximum phosphate solubilization was observed at pH 7.0 by *Klebsiella oxytoca*. Shahab and Ahmed (2008) investigated that pH 7.0 was the most favourable for solubilization of zinc by phosphate solubilizing bacteria. Maheswar and Sathiyavani (2012) reported that maximum tri calcium phosphate solubilization of *Bacillus subtilis* and *Bacillus cereus* was observed at pH 7.0. Mardad *et al.*, (2014) investigated that maximum phosphate solubilization was reported at pH 7.0 by *Enterobacter spp.* and *Acinetobacter spp.* Walpola *et al.*, (2012a) reported that phosphate solubilization of *Burkholderia* species was ideal between pH 7.0 to pH 9.0. Narveer *et al.*, (2014) reported that maximum phosphate solubilization of *Bacillus* species was observed around pH 7 to pH 8.0.

**Figure 3.8 Effect of pH on phosphate solubilization by *B. latens***
Effect of phosphate sources

![Graph showing phosphate solubilization by A. awamori](image1)

**Figure 3.9 Effect of phosphate sources on phosphate solubilization by A. awamori**

![Graph showing phosphate solubilization by B. latens](image2)

**Figure 3.10 Effect of phosphate sources on phosphate solubilization by B. latens**

The phosphate solubilization is greatly dependent on phosphate source as substrate. *Aspergillus awamori* and *Burkholderia latens* were solubilized maximum phosphate with TCP compared to FePO₄ and AlPO₄. This may be probably due to the adaptive nature of the enzyme that is responsible for solubilizing Ca₃(PO₄)₂ (Banik and Dey,
1982). *Aspergillus awamori* solubilized 668 ± 7.23 µg mL⁻¹ TCP and pH dropped upto 2.35 ± 0.08 from 6.5 (Figure 3.9). In case of *Burkholderia latens* maximum TCP solubilization was 588.7 ± 9.07 µg mL⁻¹ and reduction of pH was from 7.5 to 3.31 (Figure 3.10). There was a very vast difference observed in phosphate solubilization between TCP to other phosphate sources. The majority of the phosphate solubilizing microorganisms mobilizes calcium phosphate complexes and only a few can solubilize iron phosphate and aluminium phosphate complexes. As a result of acidification of the surrounding medium, soluble orthophosphate ions can be readily released. More precisely, the organic acids secreted can either directly dissolve the mineral phosphate as a result of anion exchange of PO₄³⁻ by acid anion or can chelate both iron and aluminium ions associated with phosphate (Muleta, 2007). Saxena *et al.*, (2013) investigated that insoluble phosphates such as di calcium phosphate, tri calcium phosphate and hydroxyapatite were tested, TCP was highly solubilized closely followed by hydroxyapatite where as DCP was least solubilized. Tallapragada and Seshachala (2012) reported that TCP was the best phosphorus source for phosphate solubilization. Walpola *et al.*, (2012b) investigated that *A. awamori* highly solubilized phosphate using tri calcium phosphate as phosphate source compared to AlPO₄, FePO₄ and rock phosphate. Walpola *et al.*, (2012a) reported that solubilization of tri calcium phosphate in NBRIP medium was very high by *Burkholderia* species but solubilization of FePO₄ and AlPO₄ was very poor. Song *et al.*, (2008) reported that the TCP gave the most extensive solubilization of phosphate by *Burkholderia cepacia* DA 23, however it showed very poor solubilization of aluminium phosphate.
Effect of carbon sources

The carbon sources play a significant role in phosphate solubilization mechanisms. Acids production was really affected by the nature of carbon sources (Pradhan and Sukla, 2005). The various carbon sources were used e.g., glucose, fructose, sucrose, lactose and galactose to check their effect on phosphate solubilization by *Aspergillus awamori* and *Burkholderia latens*. In the present study, for both microorganisms glucose proved the best carbon source for phosphate solubilization.

**Figure 3.11 Effect of carbon sources on phosphate solubilization by A. awamori**

**Figure 3.12 Effect of carbon sources on phosphate solubilization by B. latens**
For *Aspergillus awamori* 637 ± 10.69 µg mL\(^{-1}\) of phosphate solubilization was observed with glucose and pH drop was 2.37. The remaining sugar sucrose, fructose, lactose and galactose showed 588.7 ± 9.07, 582 ± 10.92, 291 ± 6.24 and 186 ± 5.03 µg mL\(^{-1}\) of phosphate solubilization. There was some little difference in phosphate solubilization observed by glucose, sucrose and fructose but there was a vast difference in phosphate solubilization and pH observed by lactose and galactose (Figure 3.11). For *Burkholderia latens* glucose gave the best phosphate solubilization followed by fructose, galactose and lactose, where sucrose gave very low phosphate solubilization. Glucose gave 586 ± 6.08 µg mL\(^{-1}\) phosphate solubilization and pH drop was 3.31. Sucrose gave 219.3 ± 7.63 µg mL\(^{-1}\) phosphate solubilization by *Burkholderia latens* (Figure 3.12).

Rathore (2014) reported that glucose was the best carbon source for phosphate solubilization by *Aspergillus* species. Pradhan and Sukla (2005) investigated that glucose gave the highest solubilization of phosphate (433 µg mL\(^{-1}\)), followed by sucrose, xylose and galactose. Relwani *et al.*, (2008) showed that glucose and sucrose were the best sources for phosphate solubilization by *Aspergillus tubingensis*. On the other hand, Narsian and Patel (2000) reported that maximum phosphate solubilization by *Aspergillus aculeatus* was obtained using arabinose and glucose as carbon sources. Shahab and Ahmed (2008) investigated that glucose was the most favourable carbon source for zinc solubilization while lactose is the poor carbon source. Walpola *et al.*, (2014) reported maximum phosphate solubilization in the presence of glucose followed by fructose and galactose. Arumanayagam and Arunmani (2014) investigated that glucose was best carbon for phosphate solubilization of *L. Fraternal* and its MHB strains. Karunai and Ravindran, (2012) reported that *Bacillus subtilis* showed maximum TCP phosphate solubilization with glucose. Mardad *et al.*, (2014) investigated that most extensive solubilization of TCP was shown by *Enterobacter spp.*, using glucose followed by fructose and galactose. Walpola *et al.*, (2012a) reported glucose was the ideal source of carbon for solubilization of tri calcium phosphate by *Burkholderia spp.* followed by galactose however xylose was least favourable carbon source. Song *et al.*, (2008) investigated that maximum phosphate solubilization reported by *Burkholderia cepacia* DA23 with glucose followed by sucrose and maltase. Aarab *et al.*, (2015) reported that glucose was the most ideal carbon source for phosphate solubilization by *Pseudomonas* strains. Galactose gave
the very low phosphate solubilization as compared to other carbon sources (Srividya et al., 2009; Pradhan and Sukla 2005).

**Effect of nitrogen sources**

![Dissolved P concentration and Reduced pH](image)

Figure 3.13 Effect of nitrogen sources on phosphate solubilization by *A. awamori*

![Dissolved P concentration and Reduced pH](image)

Figure 3.14 Effect of nitrogen sources on phosphate solubilization by *B. latens*
The phosphate solubilization is also dependent on nitrogen sources. Different inorganic and organic nitrogen sources e.g., (NH$_4$)$_2$SO$_4$, NaNO$_3$, urea and casein are checked for phosphate solubilization of *Aspergillus awamori* and *Burkholderia latens*. *Aspergillus awamori* highest phosphate solubilization (649 ± 6.55 µg mL$^{-1}$) and pH drop was 2.41 with (NH$_4$)$_2$SO$_4$ followed by urea > NaNO$_3$ > casein (Figure 3.13). For *Burkholderia latens* maximum phosphate solubilization was observed in (NH$_4$)$_2$SO$_4$ (596 ± 3.35 µg mL$^{-1}$) and pH drop was 3.35 followed by NaNO$_3$ > urea > casein (Figure 3.14). In the present study, (NH$_4$)$_2$SO$_4$ was found to be a good nitrogen source for *Aspergillus awamori* and *Burkholderia latens* in comparison to other nitrogen sources (NaNO$_3$, urea and casein).

The similar observations have also been made by several researchers. Pradhan and Sukla, (2005) reported that (NH$_4$)$_2$SO$_4$ was the best nitrogen source for maximum phosphate solubilization (411 µg mL$^{-1}$). Illmer and schinner (1992) reported that number of fungi solubilized phosphate only in the presence of ammonium as the nitrogen sources. Asea (1988) investigated that nitrogen sources in form salt seem to be important as it increases phosphate solubilization of rock phosphate. Roos and Luckner (1984) have reported that the presence of NH$_4^+$ in growth medium of *Penicillium cyclopium* resulted in the development of inorganic acid following an operation of NH$_4^+$/H$^+$exchange mechanisms. Scervino et al., (2011) tested different nitrogen sources (urea, ammonium sulphate and l-asparagine) for phosphate solubilization, ammonium sulphate ((NH$_4$)$_2$SO$_4$) was the best nitrogen source. Walpola et al., (2014) reported that (NH$_4$)$_2$SO$_4$ was best nitrogen source for phosphate solubilization of *K. oxytoca*. Karunai and Ravindran (2012) investigated effect of inorganic nitrogen sources for phosphate solubilization by *Bacillus subtilis* with TCP as a phosphate source and maximum activity was found in presence of ammonium sulphate. Pallavi and Gupta (2013) showed that (NH$_4$)$_2$SO$_4$ was best inorganic nitrogen source for phosphate solubilization by *Pseudomonas lurida*. Mardad et al., (2014) studied that highest phosphate solubilization was observed with (NH$_4$)$_2$SO$_4$ in case of *Enterobacter spp.* and *Acinetobacter spp.* Narveer et al., (2014) observed that *Bacillus spp.* solubilized maximum phosphate with (NH$_4$)$_2$SO$_4$ followed by organic nitrogen sources such as tryptone and peptone.
Effect of TCP concentration

Figure 3.15 Effect of concentration of TCP on phosphate solubilization by *A. awamori*

Figure 3.16 Effect of concentration of TCP on phosphate solubilization by *B. latens*

To study the effect of different concentrations of TCP from 0.3 to 0.8% for phosphate solubilization by *Aspergillus awamori* and *Burkholderia latens*. The highest
phosphate solubilization was observed with 0.7% of TCP (714 ± 8.54 µg mL⁻¹) and pH drop was 2.27 for *Aspergillus awamori* (Figure 3.15). Maximum phosphate solubilization was observed with 0.5% of TCP (600.6 ± 9.07 µg mL⁻¹) and pH drop was 3.29 for *Burkholderia latens* (Figure 3.16). In range of 0.3 to 0.8% of TCP concentration, phosphate solubilization was gradually increased upto optimum level then there was decrease in phosphate solubilization with further increase in TCP concentration. There was a no drastic difference observed in phosphate solubilization by various TCP concentrations. Hefnawy *et al.*, (2009) reported that toxic effect of some metal ions like Mn⁺⁺, Ca ++ and Na⁺ which may be released into the culture medium might be affecting the phosphate solubilization. Ivanova *et al.*, (2006) studied effect of different concentrations (0.5, 1 and 2% w/v) of Tunasium phosphorite on phosphate solubilization by *Erwinia spp.* and *Azotobacter spp.* and maximum phosphate solubilization reported at 0.5% w/v of TP. Hefnawy *et al.*, (2002) obtained maximum uranium solubilization at 1% of ore concentration by *A. terrus* and *Penicillium spinulosum*. Hefnawy *et al.*, (2009) studied efficiency of rock phosphate and tri calcium phosphate solubilization at different concentrations ranging from 0.5 to 8% and maximum phosphate solubilization activity was observed at 1% of ore concentration by *A. niger* and *A. fumigates*.

**Effect of glucose concentration**

![Figure 3.17 Effect of different concentrations of glucose on phosphate solubilization by A. awamori](image)

104
Glucose is very important carbon source for phosphate solubilization, so glucose concentration is also important factor for phosphate solubilization. Scervino et al., (2011) reported that the concentration and the nature of the carbon source affected phosphate solubilization. In present study glucose concentration like 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 % were selected for phosphate solubilization. Phosphate solubilization was high at 2.0 % glucose concentration and low at 0.5% glucose (Figure 3.17). For Aspergillus awamori768 ± 6.08 µg mL\(^{-1}\) of phosphate solubilization and pH drop was 2.19 observed at 2.0% glucose concentration. Walpola et al., (2012b) reported that Aspergillus awamori bxq33110 gave the maximum phosphate solubilization with 2% of glucose concentration. Walpola et al., (2014) investigated that maximum phosphate was solubilized with 2% of glucose concentration by Klebsiella oxytoca. Sridevi and Mallaiah (2009) reported that phosphate solubilization increased with increase in glucose concentration up to 2% by Rhizobium strains.

For Burkholderia latens glucose concentrations like 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 % was selected for phosphate solubilization. Phosphate solubilization was very high at 2.5 % of glucose and low at 0.5% glucose by Burkholderia latens (Figure 3.18). Burkholderia latens was solubilized maximum phosphate (632.3 ± 12.58 µg mL\(^{-1}\)) and pH drop was 3.19 at 2.5% of glucose concentration. Park et al., (2010) reported that insoluble phosphate solubilization was enhanced with increasing amounts of
glucose up to 2.5% by *Burkholderia vietnamiensis* M6. Walpola *et al.*, (2012a) investigated that 3% of glucose concentration was ideal for tri calcium phosphate solubilization by *Burkholderia anthina* while other two *Burkholderia* species showed highest tri calcium phosphate solubilization at 2% of glucose concentration. Song *et al.*, (2008) reported that *Burkholderia cepacia* DA23 solubilized maximum phosphate at 3% of glucose concentration.

**CONCLUSION**

*Burkholderia latens* and *Aspergillus awamori* showed 328.7 ± 7.13 µg mL$^{-1}$ and 561 ± 7.47 µg mL$^{-1}$ of phosphate solubilization under unoptimized condition. *Aspergillus awamori* fungal isolates showed maximum phosphate solubilization at different culture conditions like 8 days incubation time, 5% inoculum size, 30°C temperature, pH 6.5, TCP as a phosphate source, (NH$_4$)$_2$SO$_4$ as a nitrogen source, glucose as a carbon source, 0.7% TCP concentration and 2% glucose concentration. Likewise *Burkholderia latens* a bacterial isolates showed highest phosphate solubilization at 3 days incubation time, 3% inoculum size, 32°C temperature, pH 7.5, TCP as phosphate source, (NH$_4$)$_2$SO$_4$ as a nitrogen source, glucose as a carbon source, 0.5% TCP concentration and 2.5% glucose concentration. After completion of optimization study, *Burkholderia latens* and *Aspergillus awamori* showed 632.3 ± 12.58 µg mL$^{-1}$ and 768 ± 6.08µg mL$^{-1}$ of phosphate solubilization respectively. Moreover, this study makes the both strains attractive phosphate solubilizers and might be used as biofertilizers to increase the available phosphorus in soil, to minimize chemical fertilizers and to reduce environment pollution in order to achieve a sustainable agriculture.
REFERENCES


