Full Length Research Paper

Effect of various parameters on the efficiency of zinc phosphate solubilization by indigenous bacterial isolates

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Zinc phosphate solubilization efficiency of ten soil bacteria were studied for various parameters like carbon sources, temperature, pH, variable concentration of sodium chloride and glucose. For majority of the isolates 20°C was appeared to be the optimum temperature for solubilization of zinc phosphate. Glucose was the most favorable carbon source for solubilization while lactose is the least favorable carbon source. pH 7 was the most favorable pH for solubilization while at pH 4 no growth and solubilization was seen. Except CMG859, no isolate solubilized at pH 8 and 9. CMG851 (*Acinetobacter lwoffi*) and CMG852 showed enhanced solubilization in presence of 1% sodium chloride. 1% glucose is required for the solubilization of zinc phosphate and no solubilization was appeared in presence of 0.1% glucose. CMG851 *(A. lwoffi)*, CMG 860 (*pseudomonas aeruginosa*) CMG 857 (*Bacillus thuringiensis*) were found to be the most promising isolates.

Key words: Solubilization, zinc phosphate, carbon sources, *Acinetobacter lwoffi, pseudomonas aeruginosa, Bacillus thuringiensis.*

INTRODUCTION

Interactions between soil microbes and metal play a major role in environmental cycling processes because some processes like solubilization mobilize metals from soil components into forms available for biological up-take. Since phosphorus is one of the most important macronutrient for all living organism, solubilization of insoluble organic phosphate by organic acid production has been the focus of many studies. Solubilizing activity increases the availability of phosphorus to vegetation and improves plant growth (Cuningham and Kuiak, 1992; Babu-khan et al., 1995; Kang, 2002).

Solubilization can be accomplished by a range of mechanisms, which include excretion of metabolites such as organic acids, proton extrusion or production of che-lating agents, (Sayer and Gadd, 1997; Nahas, 1996). The production of gluconic acid seems to be the most fre-quent agent of mineral phosphate solubilization. Several other mechanisms such as production of other inorganic

acids such as sulphuric acid, nitric acid and carbonic acid have also been reported (Rodriguez and Reynaldo, 1999; Seshadre et al., 2002).

The ability to dissolve appreciable amount of zinc phosphate is not common feature amongst the culturable bacteria on the soil surface. (DiSimine et al., 1998). Metal salts solubilization is an important feature as the solu-bilization of metal compounds has application in the recovery of metal from industrial wastes and low grade ores and and on the other hand it also releases anions such as phosphates, sulphates and essential metals such as zinc, calcium etc into biogeochemical cycles. The present paper describes screening of indigenous soil bacteria for solubilization of zinc phosphates. The promising isolates were characterized and solubilizing activity was studied using various parameters such as pH, temperature, etc.

MATERIALS AND METHODS

Isolation, purification and preservation

Soil samples were collected from the various locations of Karachi

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such as auto workshops, nurseries and Karachi University. Bacteria were isolated, purified and screened for the phosphate solubilization activity in tris-minimal media (Fasim et al., 2002) in presence of glucose. Bacterial isolates producing clear haloes on solid medium incorporating zinc phosphate were selected for identification and preservation. They were identified by using API kits and were preserved in 20% glycerol.

Media and chemicals

Tris – minimal medium (Fasim et al., 2002) was employed for solid, liquid culture, containing the carbon source, usually D-glucose10 g l^{-l}(BDH).

Solubilization of metal compounds

Zinc phosphate and Zinc oxide were selected to test for solubilization by various bacterial isolates. These metal compounds were added to the medium to give a final concentration of 5 mM and 14 mM respectively. Inoculation was carried out using a 10 µl drop of bacterial culture, which had been grown in nutrient broth at 37°C for at least 24 h. Centrally inoculated plates in triplicate were incubated at 20°C for 10 days in the incuba-tor and were examined for metal solubilization visualized by the formation of haloes around colonies. For liquid cultures, 250 ml samples of liquid tris- minimal salt medium, unsupplemented (control) or supplemented with 5 mM zinc phosphate or 14 mm zinc oxide, were inoculated with an overnight grown culture of 10 isolates and incubated in shakibutor (100 rpm) at 20°C for 10 days. The diameters of the zones were noted after 10 days and the efficiency of the solubilization was calculated according to the formula (Nguyen, 1992).

Effect of various parameters on the efficiency of solubilization

Carbon sources

Effect of various carbon sources like glucose, fructose, sucrose, and lactose were studied in tris agar plates. The isolates were checked for solubilization activity in tris- minimal agar plates amended with Zinc phosphate. Inoculation was carried out by using 10 µl drop of a bacterial culture that had been grown, in nutrient broth at 37°C for 24 h (Fasim et al., 2002). Centrally inoculated plates in three replicates were incubated at 20°C for 10 days. Growth was observed on second day while clear haloes were observed on third day. Zone diameter was noted and efficiency of solubilization was calculated as mentioned above.

Temperature

To study the effect of various temperatures (20, 25, 30°C) on solubilization efficiency, the isolates were grown in tris- minimal agar plates incorporated with zinc phosphate amended with 1% glucose for 10 day. Growth was observed on second day while clear haloes were observed on third day. Zone diameter was noted and efficiency of solubilization was calculated as described above

pH

To study the effect of pH on the efficiency of zinc phosphate solubilization, all the isolates were grown on tris- minimal agar plates amended with 1% glucose, 20°C for 10 days at ph 4, 5, 6, 7, 8 and 9. Growth was observed on second day while clear haloes were observed on third day. Zone diameter and the diameter of

colony were noted and efficiency of solubilization was calculated as described earlier.

Various concentration of glucose

To study the effect of various concentration of glucose on the efficiency of solubilization isolates were grown on tris-minimal agar plates amended with 0.1, 1, 5, and 10%. Glucose at 20°C for 10 days. Zone diameter and diameter of colony was noted and efficiency of solubilization was calculated as described above

Various concentration of sodium chloride

All the isolates were checked for the solubilization activity on tris-minimal agar plates amended with 1% glucose, at 20°C for 10 days in presence of various concentrations of sodium chloride 0.5, 1, 1.5 and 2%. Efficiency of solubilization was calculated as described above.

RESULT

Isolation, identification and preservation

22 phosphate solubilizing bacteria were isolated and purified from enrichment culture. The isolates were identified through API kits. All the isolates were preserved on 20% glycerol and routinely cultured on nutrient agar and tris-minimal media amended with zinc phosphate. Ten bacterial isolates were selected on the basis of efficient solubilization activity for further studies (Tables 1).

Solubilization of various metal compounds

All the isolates showed solubilization of zinc phosphate in presence of glucose (Tables 1).

Effect of various parameters on   metal solubilization

Carbon source

All the isolates were able to grow and solubilize in presence of glucose. All the isolates except CMG860 were able to solubilize in presence of fructose. CMG 860, CMG 855 and CMG851 were not able to solubilize in presence of lactose while CMG 860 and CMG 852 were not able to solubilize in presence of sucrose (Figure 1).

Temperature

All the isolates were able to grow and solubilize in the range of 20 – 30°C. CMG 851, 852, 853, 854, 857 and 860 showed best solubilization at 20°C while CMG 856, 858 and 859 showed best solubilization at 25°C while all the isolates showed late or week solubilization at 30°C (Figure 2).

Table 1. Identification of isolates and solubilization of zinc phosphate.

Strain code	Source	Identification	Zinc phosphate (5 mM)
CMG851	University of karachi	*A. lwoffii*	+
CMG852	Gulshan nursery	*Pseudomonas* sp.	+
CMG853	Gulshan nursery	*Pseudomonas* sp.	+
CMG854	University of karachi	*B. thuringiensis*	+
CMG855	Gulshan nursery	*Pseudomonas* sp.	+
CMG856	University of karachi	*Pseudomonas* sp.	+
CMG857	University of karachi	*B. thuringiensis*	+
CMG858	Gulshan nursery	*Pseudomonas* sp.	+
CMG859	Auto workshop	*Pseudomonas* sp.	+
CMG860	University of karachi	*Pseudomonas* sp.	+

+ = Clear haloes around the colony.
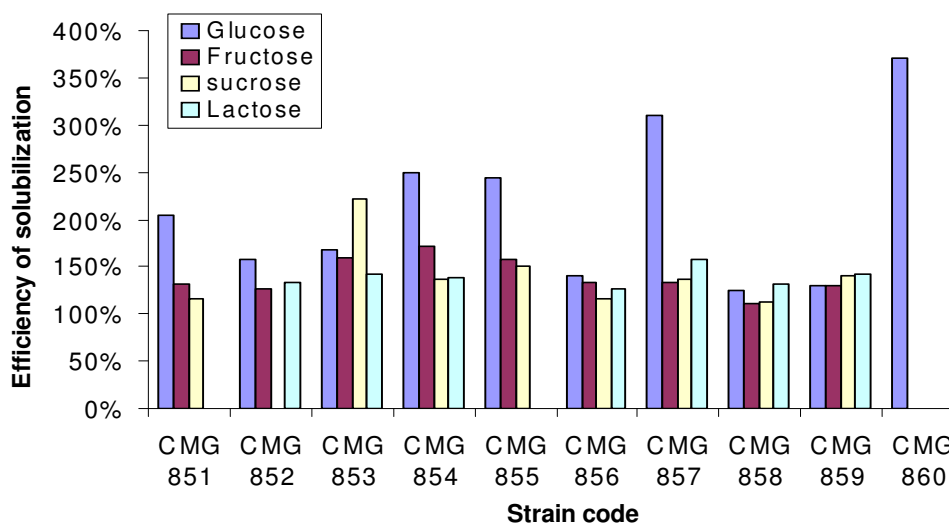
Figure 1. Effect of various carbon sources on the efficiency of solubilization.

pH

All the isolates were able to grow and solubilize at pH 5, 6 and 7. But they showed no solubilization at pH 4 while all the isolates except CMG859 showed no solubilization at pH 8 and 9. (Figure 3).

Various concentration of glucose

All the isolates showed solubilization in presence of 1, 5 and 10% glucose while all isolates showed no solubilization at 0.1% glucose (Figure 4).

Various concentration of sodium chloride

All the isolates were able to grow and solubilize in presence of 0.5% sodium chloride while they lost their activity of solubilization in presence of 2% sodium chloride. CMG 851 and CMG852 showed better solubilization in presence of 1% sodium chloride (Figure 5).

DISCUSSION

Solubilization of metal contaminants provides a route for removal of the metal from soils, sediments and industrial waste by acid leaching or chelation. Different mechanisms of solubilization have been identified including proton excretion, and the production of organic acids and other chelating metabolites (Agnihorti, 1970). Solubilization is of important in nutrient cycling as it releases phosphates as well as metal ions into biogeochemical cycles; the release of this phosphorous is helpful in improving vegetation.

The role of carbon source is important in phosphate solubilization as the production of acid which was com-
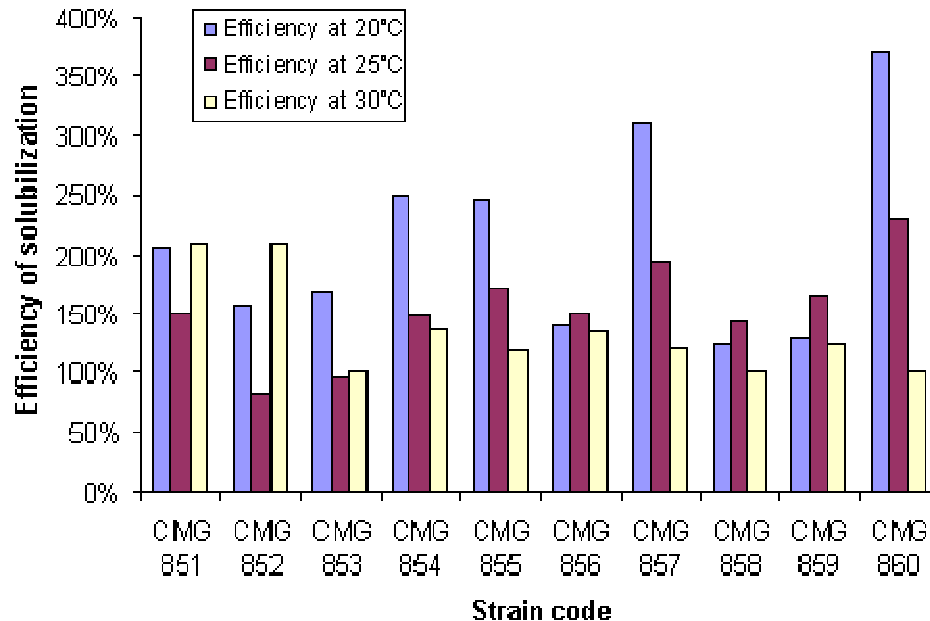
Figure 2. Effect of various temperatures on efficiency of solubilization.
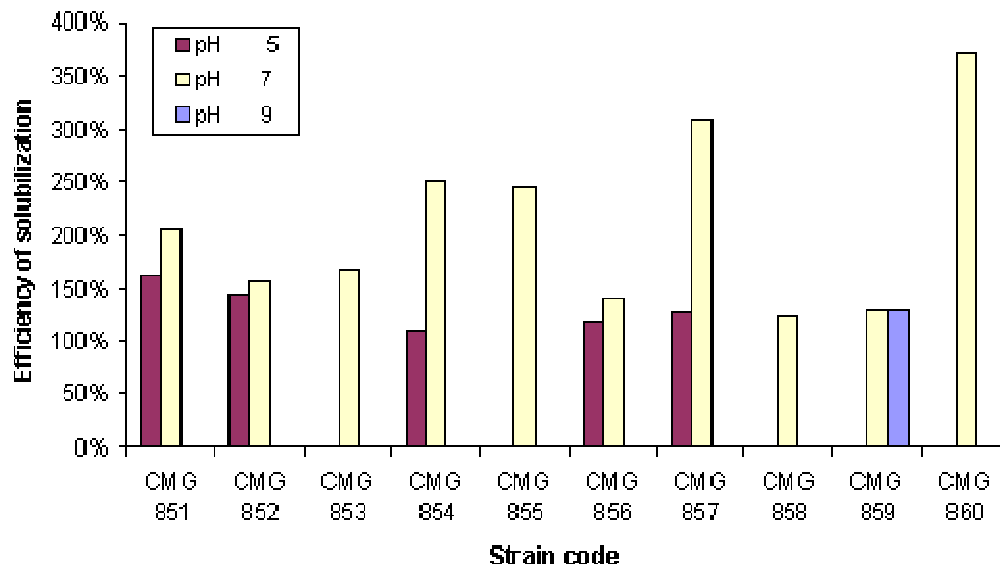

Figure 3. Effect of variable pH on the efficiency of solubilization.

common mechanism of solubilization (Di Simine, 1998) was affected by the carbon source and the nature of acid produced is more important then the quantity of the acid (Agnihorti, 1970). In case of carbon sources all of our isolates were able to grow and solubilize in presence of glucose. Except CMG860 all were able to grow and solubilize in presence of fructose however, except CMG 860, CMG855 and CMG851 all were able to solubilize in

presence of lactose while CMG 860 and CMG852 were not able to solubilize in presence of sucrose.

Fasim et al. (2002) have reported such bacterial iso-lates, which solubilize only in presence of glucose. Simmine, (1998) reported solubilization only in presence of glucose and slight solubilization in presence of man-nose while no solubilization was detected in presence of gluconate, galactose, glycerol, sorbitol and fructose.
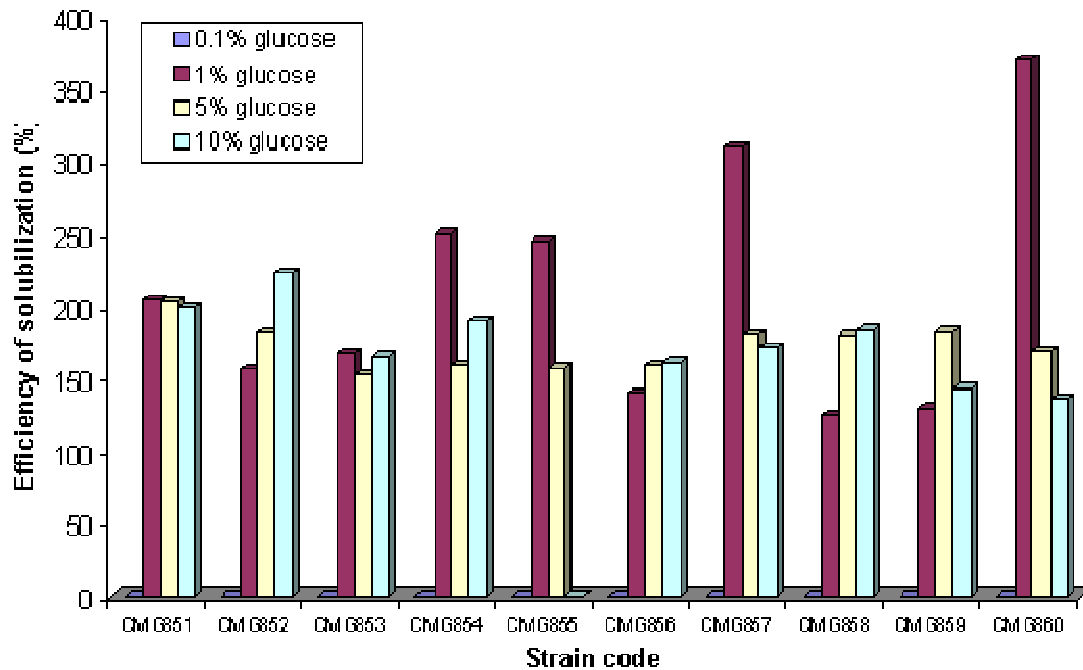
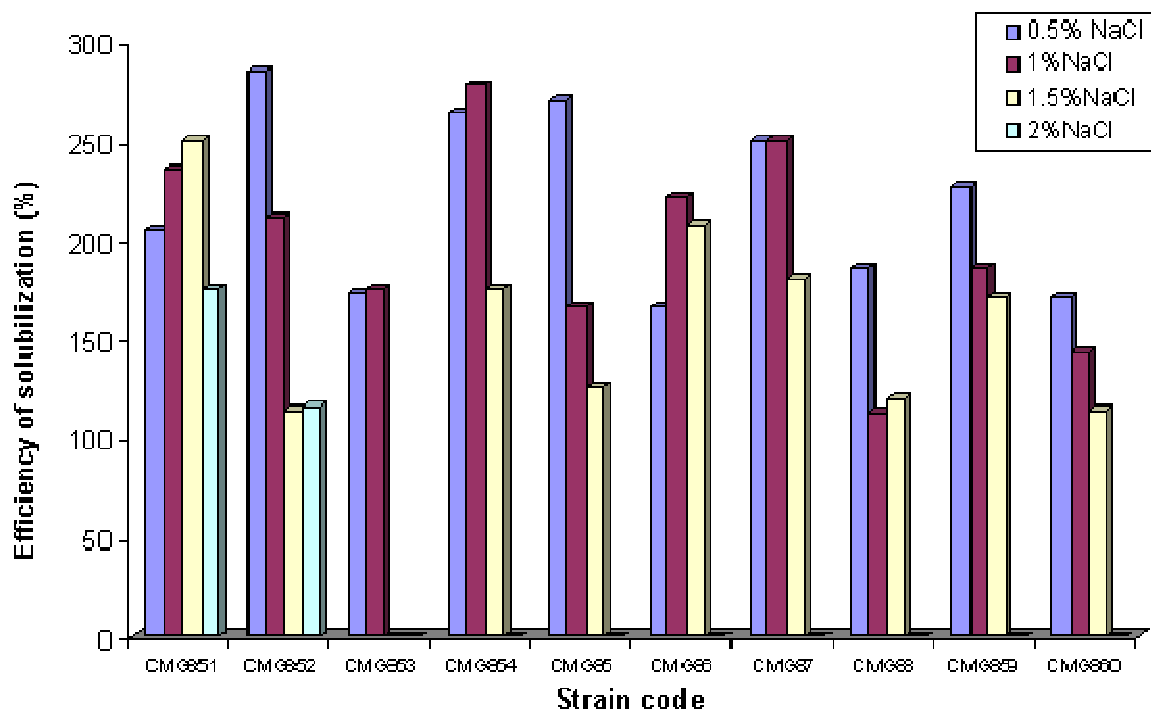**Figure 4.** Effect of various concentration of glucose.

Figure 5. Effect of various concentration of sodium chloride.

While other workers have reported solubilization in presence of a wide range of carbon sources as well. Our results correspond to those of Nautiyal et al., (2000). Narsian, (2000) showed that *A. aculeatus* could use different carbon sources but in relation to highest phosphate solubilizing activity was in the following order: arabinose > glucose > fructose > manitol > xylose > maltose > sorbitol > sucrose > glycerol > galactose >

lactose. However among different carbon sources tested fructose, glucose, xylose sucrose and starch enhanced solubilization more then galactose and maltose (Cerezine et al., 1988).

Our result has shown that for most of the organisms 20°C is the best temperature for solubilization and at 30°C solubilization was weak. But few have shown solubilization at 25°C they include CMG856, CMG858 and CMG 859. Although different temperature have been reported by earlier workers for solubilization, most of the studies have shown 25°C as the optimum temperature (Sayer and Gadd, 1998; Gharieb et al., 1998) few studies have reported 28°C as optimum temperature (Seshadre et al., 2000; Kang, 2002; Varsha, 2002). However Fasim et al. (2002), Johri et al., (1999), Kim et al., (1997) and Rosado et al., (1998) have shown that 30°C is the best temperature for solubilization. Solubilization at extreme temperature has also been reported by few workers such as Nautiyal et al., (2000) and Nahas, (1996) had reported solubilization at 45°C by a desert soil isolate. Johri et al. (1999) has reported solubilization at 10°C. This clearly suggest that bacteria adapt to their indigenous environ-ment, so their metabolic activities are linked to the tem-perature of the environment.

Result of solubilization at different pH has shown that all the isolates solubilized in the range of pH 5 - 7. At pH 4, no growth and solubilization was observed while at pH 8 and 9 all the isolates, except CMG859 were unable to solubilize zinc phosphate although all are growing at pH 8 and 9. pH fall and acidification of media was noted in all the cases. Although other mechanisms also operate in solubilization but acid production has been reported to be a major mechanism involved in solubilization (Fasim et al., 2002; Sayer and Gadd, 1998; Nguyen et al., 1992) and this results in lowering of pH. pH is the vital factor in solubilization. In most of cases phosphate solubilization is the result of organic acid production although other mechanisms such as production of bacterial metabolites and siderophores have also attributes to solubilization however solubilization may occur in alkaline condition as Nautiyal et al. (2000) and Nahas, (1996), have reported solubilization even at pH 12 and suggest other then acid production mechanisms for phosphate solubilization. Nautiyal et al. (2000), Kang (2002), Nahas (1996) and Kim et al. (1997) reported that rock phosphate solu-bilization was associated with sharp decline in pH while this was not the case with other phosphates taken in the study. Nahas, (1996) showed that the solubilization of insoluble phosphates depends upon a multitude of factors including decrease in pH, microorganisms and the insoluble phosphate used.

All the isolates were grew and solubilized in presence of 1% glucose and were not able to solubilize in presence of 0.1% and it was concluded that 1% glucose is the essential requirement for the solubilization to be visua-lized (Figure 4). The result was also in agreement with Dixon et al. (1998) who reported decrease in solubili-

zation activity with the decreasing amount of glucose from 600 to 0.06 mM glucose. Amount of glucose in the media was shown to be an important factor for solubilization as oxalic acid and other acid production was stimulated by the addition of glucose. Increasing sugar concentration can increase the activity of glycolytic enzymes and pyruate carbooxylase which in turn result in increase acid production. Such phenomenon may not occur with all bacteria though oxalic acid production has been found to be promoted under carbon limited conditions in penicilium billai (Cuningham and Kuiak, 1992) and a relatively low sugar concentration was optimal for phosphate solubilization by Penicillium sp. isolated from forest soil. This was explained by the fact that suboptimal growth conditions are often necessary for the production of secondary metabolites (Ilmer and Schineer, 1992).

All the isolates are able to grow and solubilize in pre-sence of 1% sodium chloride and are unable to solubilize in presence of 2% sodium chloride. Although various isolates showed varied efficiency in presence of variable concentration of sodium chloride but for majority of isolates, 0.5% sodium chloride is the optimum concen-tration and majority of isolates showed negative relation with salinity that is as the salinity increased efficiency of solubilization decreased except CMG 851 (Figure 5). Kang (2002) and Kim et al. (1997) reported the enhancement of solubilization in presence of 1% sodum chloride. Jouhari et al. 1999 reported eighteen bacterial isolates out of fifty seven isolates in presence of 5% sodium chloride while two bacterial isolates lost the ability of phosphate solubilization in plate assay in absence of sodium chloride.

Nautiyal (1999) and Rosado et al. (1998) reported solubilization in presence of 10% sodium chloride but there is a general trend of decrease in solubilization activity with the increase of sodium chloride concentrate-ion. This might have two reasons either two stresses at the same time may harm cell growth and proliferation which result in less efficiency of solubilization or it might be possible that too much chloride ions may chelates or neutralize proton ions or acid produced in the media.

Conclusion

It appears that solubilization of phosphate is a multifactor phenomenon and is dependent on the metabolic activities of the organisms. Several factors such as carbon sources, pH, amount of glucose and sodium chloride may affect efficiency of solubilization.

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