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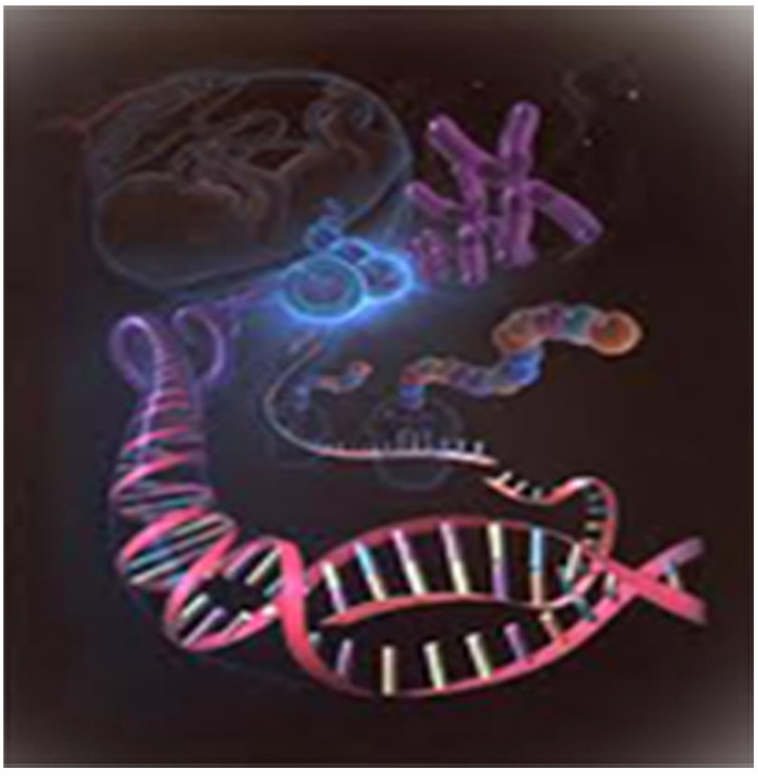

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Research Paper

BACILLUS SUBTILIS: A POTENTIAL SALT TOLERANT PHOSPHATE SOLUBILIZING BACTERIAL AGENT

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Soil salinity is one of the most serious environmental problem. An attempt is made to isolate salt-tolerant phosphate solubilizing bacterium from the soil. The bacterial isolate was identified as *Bacillus subtilis* based on its morphological, cultural and biochemical characteristics. The phosphate solubilizing potential was qualitatively evaluated by the formation of clear zones around the colonies growing on solid medium containing tribasic calcium phosphate as a sole phosphorus source. The results showed that salt tolerant phosphate solubilizing bacterial isolate could be a promising source for the development of saline-alkali soil-based agriculture.

Keywords: Salt tolerant, Phosphate soil salinity solubilization, Calcium phosphate

INTRODUCTION

Phosphorus (P) is one of the major essential macronutrients for plants (Goldstein, 1986). Root development, stalk and stem strength, flower and seed formation, crop maturity and production, nitrogen fixation in legumes, crop quality, and resistance to plant diseases are the attributes associated with phosphorus nutrition.

Phosphorus is applied to soil in the form of phosphatic fertilizers. However, a large portion of applied fertilizer enters in to the immobile pools through precipitation reaction (Gyaneshwar *et al.,* 2002; Hao *et al.*, 2002). Only 0.1% of the total P present is available to the plants, because of its chemical bonding and low solubility (Tilak *et al*., 2005). However, many soil microorganisms have the ability to solubilize and mineralize P from inorganic and organic pools of total soil P, making the element available for plants.

Recently, phosphate solubilizing microorganisms have attracted the attention of agriculturists as soil inoculums to improve the plant growth and yield (Young *et al.*, 1998; Goldstein *et al.*, 1999; Fasim *et al.*, 2002). Bacteria are the predominant microorganisms that can solubilize phosphate compared to fungi and actinomycetes (Goldstein, 1995). Unfortunately, most Phosphate Solubilizing Bacteria (PSB) isolated previously performed relatively low salinity tolerance, being less appropriate for saline-alkali soil-based agriculture. Hence, an attempt is made to isolate and

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characterize halotolerant phosphate solubilizing bacterium from soil.

MATERIALS AND METHODS

Collection of the Soil Sample

Soil sample was collected by random sampling procedures from a cropped field. Approximately 50 g of soil sample was taken from the upper 30 cm of the soil profile aseptically. It was stored at 4° C until use.

Screening of Phosphate Solubilizing Bacteria from Soil Sample

Ten gram of soil sample was aseptically transferred to 250 mL Erlenmeyer flask containing 90 mL distilled water and was shaken on Rotary shaker at 100 rpm for 20 min. The sample was allowed to settle for 10 min. Aliquots of 0.1 mL of the sample was spread plated on a Petri dish containing Pikovskaya medium (Yeast extract-0.5 g, Dextrose-10 g, $Ca_3(PO_4)_2$- 10 g, $(NH_4)_2SO_4$-0.5 g, KCl-0.20 g, $MgSO_4.7H_2O$-0.1 g, $MnSO_4.H_20$-0.0001 g, $FeSO_4.7H_2O$-0.0001 g, Agar-agar-18 g, Distilled water-1000 mL) (Pikovskaya,1948). Plates were incubated at 30°C for 5 days.

Determination of Phosphate Solubilizing Efficiency

Bacterial isolate with the maximum zone of solubilization was selected and again was spot inoculated on Pikovskaya medium and phosphate Solubilizing Efficiency (SE) was calculated by the following formula:

$$SE = \frac{\text{Solubilization diameter}}{\text{Growth diameter}} \times 100$$

Uninoculated plates and *E. coli* inoculated plates served as controls.

Characterization and Identification of PSB Isolate

Cultural, morphological and biochemical characteristics of PSB isolate were studied and with the help of standard literature (Cruickshank *et al*., 1975; Skinner and Lovelock, 1979) the isolate was identified.

Determination of Salt Tolerance

Nutrient agar slants containing different concentration of Sodium chloride (viz., 1%, 1.2%, 1.4%, 1.6%, 1.8% and 2.0%, 2.2% and 2.4%) were inoculated and incubated at 28ºC for 48 h. The growth from different concentrations of NaCl was then compared with the control.

Effect of PSB Isolate on the Growth of *Zea mays* Under Greenhouse Conditions

Soil was subjected for physico-chemical analysis. Three seeds of *Zea mays* were surface sterilized by immersing in 3.25% (v/v) sodium hypochlorite for 1 min, followed by 70% (v/v) ethanol for 1 min and rinsing five times in sterile distilled water. These surface sterilized seeds were then planted into a pot containing sterile saline soil. The culture of PSB isolate grown for 2 days at 28°C in glucose minimum salt medium (OD at 600 nm = 0.9) was added adjacent to the germinated seeds. A thin layer of sterile sand was placed over the soil and watered every day with the 0.3% (w/v) KNO_3 solution. The study was carried put in triplicates. After 4 weeks, plants were harvested. Root and shoot lengths were measured. Roots and shoots were excised, dried in an oven and dry mass of roots and shoots were measured.

RESULTS AND DISCUSSION

Soil sample was subjected for PSB screening using Pikovskaya medium. A bacterial isolate

showing maximum clear zone around colony was selected and spot inoculated on Pikovskaya medium. After incubation, growth diameter and solubilization diameter were measured and SE was calculated and it was found to be 164.

The PSB isolate was confirmed as *Bacillus subtilis* on the basis of morphological, cultural and biochemical characteristics (Figure 1 and Table 1).

Figure 1: Colony of PSB Isolate on NA Plate After Incubation at RT/48 h

Table 1: Morphological, Cultural and Biochemical Characteristics of PSB Isolate

Test	Result
Colony characters on Nutrient agar (incubation at 30°C/24 h)	
Size(in mm)	4
Shape	Circular
Margine	Irregular
Elevation	Flat
Consistancy	Smooth
Opacity	Opaque
Colour	White
Morphological Characteristics	
Gram nature	Gram positive
Morphology	Thick long rods
Motility	Motile
Physiological and Biochemical Characteristics	
Opt pH	7
Opt temp(°C)	30
O_2 reqt	Aerobic
D-Glucose	+
D-Galactose	-
Fructose	+
Sucrose	+
Maltose	+
Oxidase	+
Catalase	+
Gelatinase	+
Urease	+
H_2S production	-
Amylase	+
caseinase,	+
Nitrate reduction	+
Identification	*Bacillus subtilis*

Note: (+) = positive test result and (-) = negative test result.

Salinity test was done for determining salt tolerance capability of PSB isolate. Isolate was able to tolerate upto 2.2% NaCl Conc.

Further the effect of halotolerant PSB isolate was tested on the growth of *Zea mays* under greenhouse conditions. Saline soil was used for the experiment (Table 3). The PSB isolate increased the root and shoot length of *Zea mays* by 11.50 and 9.38% respectively; and root and shoot dry mass by 20.90 and 25.37% respectively

Table 2: Salinity Test of PSB Isolate

PSB Isolate	Salt Conc. (%)							
	1.0	1.2	1.4	1.6	1.8	2.0	2.2	2.4
Bacillus subtilis	++++	++++	+++	++	++	++	+	–

Table 3: Physico-Chemical Properties of the Soil Used for Greenhouse Expt

Parameter	Value
Particle size distribution (%)	Sand=09; Silt=26; Clay=65
pH	8.70
Electrical conductivity (E.C. in mmhos/cm^3)	3.96
Organic carbon(%)	0.35
Available Phosphorus(kg/hactare)	15
Available Potassium(kg/hactare)	309

Figure 2: Effect of PSB Isolate on the Root and Shoot Length of *Zea mays*

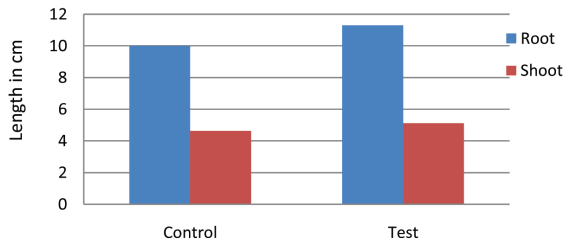
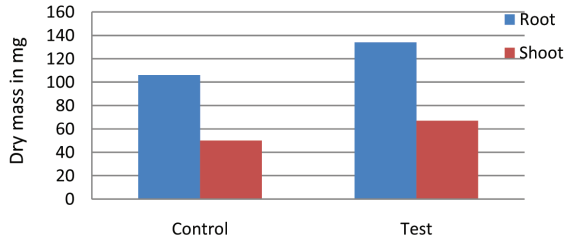


Figure 3: Effect of PSB Isolates on Dry Mass of Root and Shoot of *Zea mays*



as compared to control containing no PSB isolate (Figures 2 and 3).

CONCLUSION

The bacterial isolate have good phosphate solubilization efficiency and is tolerating salt conc upto 2.2%. The associated mechanism of mineral phosphate solubilization could be due to release of low molecular weight organic acids (Kim *et al.*, 1997) or production of siderophores (Chincholkar, 2000). Results of effect on seedling growth due to treatment by the isolated strains of *B. subtilis* are given in Figures 2 and 3. These results suggest that treatment with phosphate solubilizing bacteria is beneficial as a general increase in length and dry mass of test crop plant as compared to control. This enhancement of seedling growth due to seed treatment with phosphate solubilizing bacteria like *B. subtilis* may be due to release of plant growth promoting substances. There is increasing evidence that these bacteria improve plant growth due to biosynthesis of plant growth substances in addition to their action in releasing available phosphorus. The salt tolerance ability of this isolate can help to serve a suitable substrate for

production of bio-fertilizer for saline-alkali soil-based agriculture.

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