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Isolation and characterization of phosphate solubilizing bacteria (*Klebsiella oxytoca*) with enhanced tolerant to environmental stress

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The present investigation was aimed at the isolation and characterization of phosphate solubilizing bacteria with enhanced tolerant to environmental stress. The bacterial strain isolated from a metal-contaminated soil collected from abandoned mines was identified as *Klebsiella oxytoca* according to 16S rRNA analysis. The strain proved the ability to solubilize inorganic phosphate under a wide range of pH (4 to 10), temperature (20 to 40°C) and salt concentrations (0 to 7.5% NaCl). However, the maximum phosphate solubilization (615 µg ml^{-1}) was recorded when the medium contained glucose (2%) and ammonium sulphate (0.1%), respectively as the source of carbon and nitrogen and NaCl (2.5%) with pH adjusted to 7 at 35°C. As revealed by the results of plant growth promoting assays on 1-aminocyclo-propane-1-carboxylic acid (ACC) deaminase activity and production of ammonia, hydrogen cyanide (HCN) and indole acitic acid (IAA), the strain was shown to be good plant growth promoter, which was further confirmed by the enhanced growth of mung bean seedlings inoculated with the strain (31.88 and 45.53% higher shoot and root length compared to un-inoculated control). Based on the results, the strain could be identified as an ideal candidate to be included in developing microbial inoculants suit for stress environments.

Key words: *Klebsiella oxytoca,* phosphate solubilizing bacteria, stress environments, microbial inoculants.

INTRODUCTION

Phosphorous (P) is one of the most important macro-nutrients required by plants. Though soils generally contain high amount of P (0.05%), only very small amount (0.1%) of the total P is available for plant uptake (Chang and Yang, 2009), thus it is considered to be the most limiting nutrient. A large portion of applied inorganic

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Table 1. Composition of different media used for assay phosphate solubilization.

Media	Composition
M1 (PVK; Pikovskaya, 1948)	10 g glucose, 0.5 g $(NH_4)_2SO_4$, 0.1 g $MgSO_4.7H_2O$, 0.5 g yeast extract, 0.2 g KCl, 0.2 g NaCl, 0.002 g $FeSO_4.7H_2O$, 0.002 g $MnSO_4.H_2O$, 5 g $Ca_3(PO_4)_2$ (pH- 7)
M2 (AYG; Halder et al., 1991)	20 g glucose, 1 g $(NH_4)_2SO_4$, 0.5 g $MgSO_4.7H_2O$, 0.2 g yeast extract, trace $FeCl_3$, trace $MnSO_4.H_2O$, 5 g $Ca_3(PO_4)_2$ (pH- 6.8)
M3 (Kim et al., 1997)	10 g glucose, 0.4 g $MgSO_4.7H_2O$, 0.5 g yeast extract, 1 g NaCl, 0.2 g $CaCl_2$, 1.5 g NH_4NO_3, 5 g $Ca_3(PO_4)_2$ (pH- 7)
M4 (Vassilev et al., 1998)	100 g glucose, 0.2 g $MgSO_4.7H_2O$, 0.5 g NH_4NO_3, 0.004 g $ZnSO_4$, 5 g $Ca_3(PO_4)_2$ (pH- 5)
M5 (NBRIY; Nautiyal, 1999)	10 g glucose, 0.5 g $(NH_4)_2SO_4$, 0.1 g $MgSO_4.7H_2O$, 0.2 g KCl, 0.2 g NaCl, 0.002 g $FeSO_4.7H_2O$, 0.002 g $MnSO_4.H_2O$, 5 g $Ca_3(PO_4)_2$ (pH- 7)
M6 (NBRIP; Nautiyal, 1999)	10 g glucose, 0.1 g $(NH_4)_2SO_4$, 0.25 g $MgSO_4.7H_2O$, 0.2 g KCl, 5 g $MgCl_2.6H_2O$, 5 g $Ca_3(PO_4)_2$ (pH-7)

soluble phosphate fertilizer to soil is rapidly becoming unavailable to plants, which leads to excess application. Recently, emphasis has been paid to utilize unavailable P forms through the action of phosphate solubilizing microorganisms (PSMs).

PSMs are naturally found in the soils; however, their growth and phosphate solubilization highly vary with environmental conditions such as temperature, pH and salt concentration of the soils. Though, there are some information regarding the stress tolerant PSMs such as *Rhodotorula* sp., PS4 is highly tolerant to temperature, pH and salt variations (Mundra et al., 2011); *Kushneria* sp. YCWA18 is tolerant to salt conditions (Zhu et al., 2011); *Bacillus megatherium* is tolerant to salt conditions (Xiang et al., 2011); *Burkholderia vietnamiensis* M6 is tolerant to temperature, pH and salt conditions (Park et al., 2010), and *Pantoea agglomerans* R-42 is tolerant to temperature, pH and salt conditions (Sharan et al., 2008; Son et al., 2006); most of the previously isolated PSMs are reported to perform poorly under stress conditions making them less appropriate for such environments. In this context, PSMs with the genetic potential for increasing tolerance to high temperature, pH and high salt concentration are still considered to be important for the establishment, multiplication and production of environmentally friendly bio-inoculants. The present study was aimed at the isolation and characterization of plant growth promoting, temperature, pH and salt tolerant phosphate solubilizing bacteria, *Klebsiella oxytoca*.

MATERIALS AND METHODS

Isolation of phosphate solubilizing bacterial strains

The strain was isolated from heavy metal contaminated soil collected from abandoned mines at Boryeong area in South Korea. 10 g of field moist soil from each soil samples was weighed and transferred to 250 ml Erlenmeyer flask containing sterilized 90 ml of 0.85% NaCl solution. The mixture was then shaken for 30 min at approximately 150 rpm. Immediately after shaking, a series of tenfold dilutions of the suspension was made by pipetting 1 ml aliquots into sterilized 9 ml of 0.85% NaCl solution. Aliquots of 0.1 ml of the sample from each of these dilutions were spread onto a Petri dish on National Botanical Research Institute Phosphate (NBRIP) medium containing 10 g glucose, 5 g $Ca_3(PO_4)_2$, 5 g $MgCl_2.6H_2O$, 0.25 g $MgSO_4.7H_2O$, 0.2 g KCl, 0.1 g $(NH_4)_2SO_4$ in 1 L distilled water (Nautiyal, 1999). The pH of the media was adjusted to 7. The Petri plates were incubated for 7 days at 30°C and examined for colonies developing clear zone. Colonies with conspicuous clear zones around them were picked up and further purified by repeated sub-culturing. The isolated strain (PSB-23) showed marked phosphate solubilizing activity on NBRIP agar plates as visualized by the clear zone.

Strain identification

The partial sequencing of 16S rRNA for the bacterial strains was done with the help of DNA sequencing service, SOLGENT, Daejeon, South Korea using universal primers, 27F (5'-AGAGTTTGATCCTGGCTCAG -3') and 1492R (5'-GGTTACCTTGTTACGACTT -3'). The online program BLAST was used in identifying the related sequences with known taxonomic information available at the databank of NCBI (http://www.ncbi.nlm.nih.gov/BLAST). A Phylogenetic tree was constructed using CLUSTAL X program (Thompson et al., 1997), which involved sequence alignment by neighbor joining method (Saitou and Nei, 1987) and maximum parsimony using the MEGA4 program (Kumar et al., 2001). Grouping of sequences was based on confidence values obtained by bootstrap analysis of 1,000 replicates. Gaps were edited in the BioEdit program and evolutionary distances were calculated using Kimura two parameter model. Reference sequences were retrieved from GenBank under the accession numbers indicated in the trees.

Assay of inorganic phosphate solubilization

The optimal medium for the phosphate solubilization was assayed using different types of liquid media (Table 1) as described by

Srividya et al. (2009). Bacterial strain was grown in sterilized liquid NBRIP medium (20 ml) at 30°C for 2 days with continuous shaking at 150 rpm/min. Aliquots of culture (1 ml) was then transferred to a 500 ml flask (n=3 per strain) containing sterilized liquid medium (250 ml) and incubated with continuous shaking at 30°C. Sterilized uninoculated medium served as a control. A sample (10 ml) of each cultured and control were taken and centrifuged at 8000 rpm for 15 min. The clear supernatant was used in determining the pH and amount of phosphorous released into the medium.

The isolate was also tested for the capacity to solubilize phosphates under different carbon and nitrogen sources. Effect of carbon source on phosphate solubilization was tested by adding fructose, galactose, sorbitol, mannitol, xylose, sucrose, maltose and lactose instead of glucose in the NBRIP medium. To test the effect of nitrogen source, $(NH_4)_2SO_4$ in the NBRIP medium was replaced by NH_4Cl, NH_4NO_3, KNO_3, $NaNO_3$, $Ca(NO_3)_2$ and yeast.

Phosphate solubilization under stress conditions

Phosphate solubilizing capacity of the strain was assayed under high pH, temperature and high salt concentration in the medium. The effect of salt on phosphate solubilization was tested by growing the strain on NBRIP containing different concentrations (0, 2.5, 5and 10%) of NaCl, KCl and $CaCl_2$. Further the pH of NBRIP medium was adjusted (from 4 to 12) using HCl or NaOH to assess the effect of pH on phosphate solubilization. For estimation of high temperature induced phosphate solubilization, NBRIP medium inoculated strain was incubated at different temperature conditions (20-45°C). In all cases, phosphate solubilization, microbial growth and pH of the culture medium were recorded after 2 days of incubation.

The phosphorus solubilization was determined using phosphomolybdate blue color method (Murphy and Riley, 1962). Samples from cultures grown in NBRIP medium were diluted 1:1 (v/v) using 1 N HCl to dissolve the residual insoluble phosphate and absorbance was recorded at 660 nm against a blank to measure the growth of the strain (Rodriguez et al., 2000).

Assay of plant growth promoting characteristics

Indole acetic acid (IAA) production was determined using the method described by Gutierrez et al. (2009). The strain grown in sterilized liquid NBRIP medium (100 ml) containing 1 ml of 0.2% tryptopan was incubated for 72 h with continuous shaking at 30°C. The centrifuged clear supernatant of 1 ml was mixed with 4 ml of the Salkowski's reagent (50 ml of 35% perchloric acid and 1 ml of 0.05 M $FeCl_3$ solution). Development of pink color indicated the IAA production, which was then quantified with optical density measurements taken at 530 nm using UV spectrophotometer (Shimadzu UV-VIS).

1-aminocyclopropane-1-carboxylic acid (ACC) deaminase activity was assayed according to Penrose and Glick (2003). Solid DF minimal medium containing ACC was inoculated with 10 µl of starter culture (grown overnight at 30°C). Plates were then incubated at 30°C in dark and colony emergence was checked daily for consecutive 3 days.

The ammonia production was tested using peptone water. Fresh cultures were inoculated into 10 ml peptone water and incubated for 48 h at 30°C. Nessler's reagent (0.5 ml) was added to each tube. Development of brown to yellow colour indicated the production of ammonia (Cappucino and Sherman, 1992).

HCN production was assessed by growing the bacteria in 10% tryptic soy agar (TSA) supplemented with glycine (4.4 g/L). Filter paper soaked in picric acid and Na_2CO_3 (0.5 and 2% respectively) was fixed to the underside of the lids of plates and incubated for 5 days at 30°C. A change in filter paper color from yellow to orange-brown was considered to be the indication of HCN production (Donate-Correa et al., 2005).

Plant growth promotion bioassay with mung bean (*Vigna radiata*)

Plant growth promotion ability of the strain was determined with pot culture assay for 4 weeks. Mung bean seeds were soaked in bacterial suspension at the concentration of 10^8 cells/ml about 30 min prior to plant. At the end of 4 weeks, seedlings were uprooted and washed with running water to measure the root and shoot length.

Statistical analysis

The data were subjected to analysis of variance (ANOVA) using SAS package (SAS, 1999). The Duncan's Multiple Range Test (DMRT) was applied to test the significance of treatment means at $P \leq 0.05$.

RESULTS

Isolation and identification of phosphate solubilizing bacterial strain

Selected bacterial strain had a marked solubilizing ability of insoluble phosphate as visualized by the clear zone developed around the colony after 3 days of incubation. According to 16S rRNA sequence analysis, the strain showed close proximity with *K. oxytoca* JCM1665. Phylogenetic tree (Figure 1) shows the position of isolated phosphate solubilizing bacterial strain with respect to related species.

Assay of inorganic phosphate solubilizing ability

Different culture mediums with different compositions have been previously employed to assay inorganic phosphate solubilization and to select optimal medium for solubilization. Our isolated strain showed significantly different ($P \leq 0.05$) phosphate solubilization with each assayed medium. As depicted in Figure 2, maximum phosphate solubilization was observed with the medium 6 (NBRIP) (Nautiyal, 1999) followed by medium 2 (AYG) (Halder et al., 1991). Solubilization was recorded as 463 and 440 µg/ml with the pH drop from 7.00 to 3.35 and from 6.80 to 3.85, respectively for the medium 6 and 2 after 3 days of incubation. Based on the amount of glucose and other ingredients used in the medium, NBRIP was recognized to be the most cost effective medium for the present strain without much compromising the phosphate solubilization.

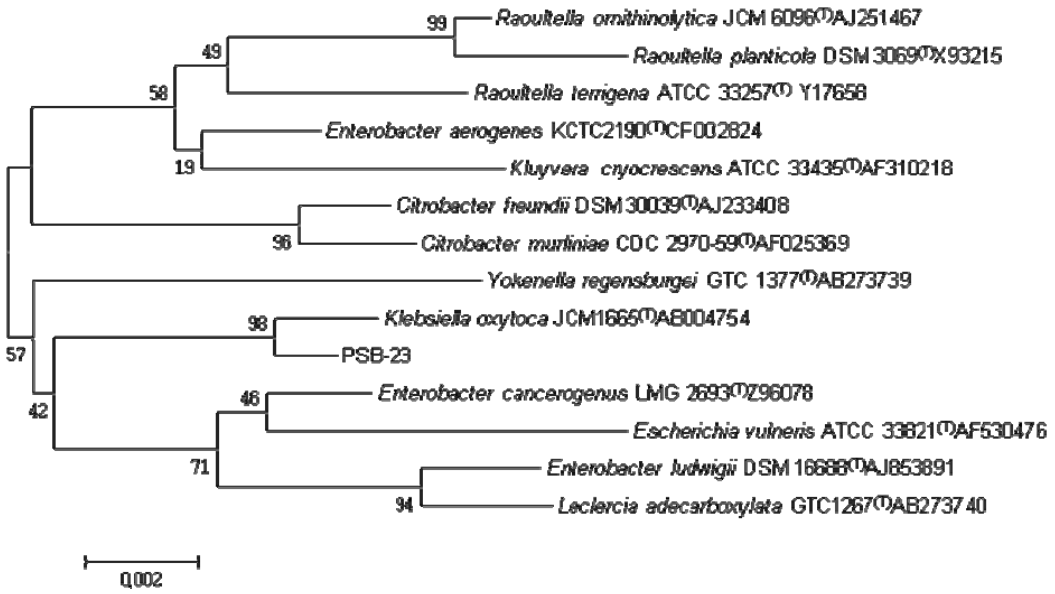

Figure 1. Phylogenetic tree based on 16S rDNA gene sequences, showing the position of isolated efficient phosphate solubilizing bacterial strain (PSB-23) with respect to related species. The scale bar indicates 0.002 substitutions per nucleotide position and accession numbers are given in parenthesis.

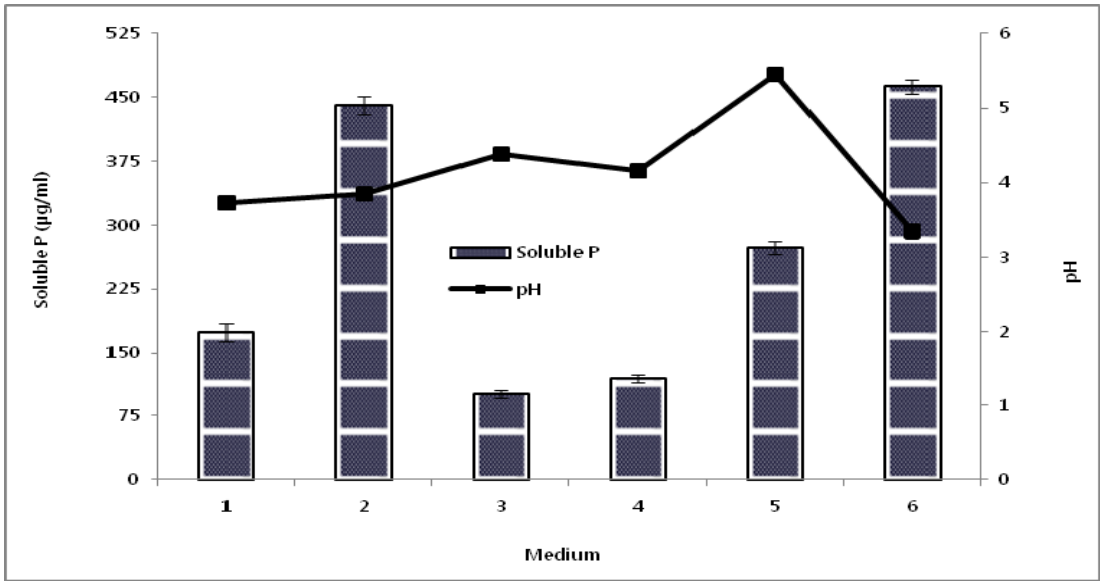

Figure 2. Effect of different media on insoluble phosphate solubilization by *Klebsiella oxytoca.* Values are mean ± SD of three replicates. 1, PVK (Pikovskaya, 1948); 2, AYG (Halder et al., 1991); 3: (Kim et al., 1997), 4: (Vassilev et al., 1998); 5, NBRIY (Nautiyal, 1999); 6, NBRIP (Nautiyal, 1999).

Inorganic phosphate solubilizing capacity of the strain was assessed in the presence of eight different carbon sources and five different nitrogen sources by replacing glucose and $(NH_4)_2SO_4$ respectively in NBRIP medium (Table 2). Strain showed diverse levels of phosphate solubilizing activity in the presence of various carbon and nitrogen sources. As can be seen in Table 2, the highest phosphate solubilization was recorded with glucose (460 µg/ml) followed by fructose (444 µg/ml) and galactose (435 µg/ml) in the medium. The strain exhibited very poor

Table 2. Effect of various carbon and nitrogen sources on phosphate solubilization. Values are means ±SD for triplicates.

Parameter	Soluble P (µg ml^{-1})	Growth (A_{660})	Final pH
Carbon source			
Glucose	460.06^{a}±7.11	0.423^{a}±0.015	3.61^{e}±0.18
Fructose	444.02^{b}±0.54	0.416^{a}±0.017	3.63^{e}±0.15
Galactose	435.22^{b}±5.19	0.412^{a}±0.015	3.76^{de}±0.04
Sorbitol	234.59^{f}±5.68	0.213^{d}±0.012	4.46^{b}±0.05
Mannitol	325.78^{d}±4.83	0.315^{c}±0.012	3.76^{de}±0.07
Xylose	312.26^{e}±6.61	0.303^{c}±0.006	3.96^{c}±0.08
Sucrose	398.42^{c}±7.86	0.380^{b}±0.015	3.68^{e}±0.02
Maltose	391.51^{c}±4.99	0.379^{b}±0.014	3.86^{dc}±0.05
Lactose	141.51^{g}±6.53	0.176^{e}±0.008	5.58^{a}±0.08
Nitrogen source			
$(NH_4)_2SO_4$	460.06^{a}±17.12	0.423^{a}±0.015	3.40^{d}±0.02
NH_4Cl	287.42^{c}±19.79	0.356^{c}±0.017	3.69^{b}±0.06
NH_4NO_3	339.68^{b}±4.85	0.386^{b}±0.007	3.58^{c}±0.04
KNO_3	314.47^{cb}±6.94	0.326^{d}±0.020	3.65^{cb}±0.02
$NaNO_3$	299.69^{c}±27.73	0.369^{c}±0.006	3.68^{b}±0.02
Yeast extract	214.78^{d}±8.02	0.184^{e}±0.003	3.84^{a}±0.01

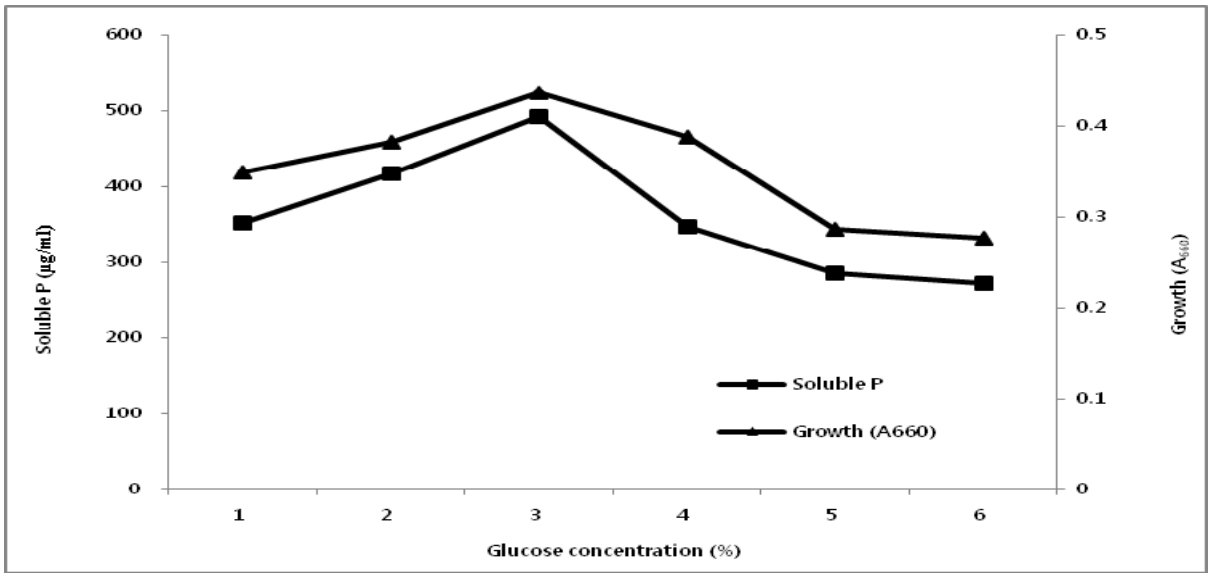

Figure 3. Effect of glucose concentration on phosphate solubilization by *Klebsiella oxytoca.*. Values are mean ± SD of three replicates.

phosphate solubilization with lactose (141 µg/ml). Similarly, the growth of the strain was also shown to be high with glucose while poor with lactose. The highest pH reduction was also recorded with glucose (3.61) in the medium. As depicted in Figure 3, phosphate solubilization was enhanced by increased amount of glucose (up to 2%), followed by reduction in both phosphate solubilization and growth. The results of the effect of nitrogen source on phosphate solubilization are presented in Table 2. Among the different sources, $(NH_4)_2SO_4$ was identified as the best nitrogen source (460 µg/ml) for growth and phosphate solubilization of *K. oxytoca*; whereas yeast resulted in poor growth and phosphate solubilization (215 µg/ml). However, the pH reduction in the medium was

Table 3. Effect of different temperature (20-45°C), pH (4-10) on phosphate solubilization. Values are means ±SD for triplicates.

Parameter	Soluble P (µg ml^{-1})	Growth (A_{660})	Final pH
Temperature (°C)			
20	175.15^{d}±1.44	0.217^{d}±0.008	4.27^{b}±0.06
25	283.65^{c}±11.78	0.260^{c}±0.007	3.99^{c}±0.03
30	352.52^{b}±7.21	0.348^{b}±0.006	3.68^{d}±0.05
35	545.60^{a}±27.81	0.452^{a}±0.012	3.21^{f}±0.09
40	332.71^{b}±13.61	0.338^{b}±0.013	3.56^{e}±0.07
45	59.66^{e}±5.14	0.063^{e}±0.008	5.75^{a}±0.05
pH			
4	328.30^{d}±6.60	0.394^{b}±0.011	2.92^{e}±0.01
5	359.74^{b}±7.85	0.411^{b}±0.005	2.99^{d}±0.05
6	370.12^{ba}±2.17	0.454^{a}±0.022	3.54^{c}±0.00
7	379.24^{a}±7.48	0.453^{a}±0.008	3.52^{c}±0.00
8	344.65^{c}±10.61	0.411^{b}±0.010	3.56^{c}±0.01
9	309.74^{e}±8.02	0.339^{c}±0.004	3.72^{b}±0.03
10	277.67^{f}±6.13	0.315^{d}±0.007	3.87^{a}±0.05

found to be less affected by the source of nitrogen.

Phosphate solubilization under stress conditions

Effects of different temperature conditions and pH on phosphate solubilization of *K. oxytoca* are shown in Table 3. As revealed by the results, the strain was able to grow and solubilize phosphate in the temperature range of 20 to 40°C. The maximum phosphate solubilization (546 µg/ml) and growth was observed at 35°C followed by gradual reduction as temperature further increased.

Initial pH in the medium also had significant effect on phosphate solubilization and growth of *K. oxytoca.* As depicted in Table 3, the strain was able to grow and solubilize phosphate in the pH range of 4 to 10. The highest phosphate solubilization (379 µg/ml) was recorded at initial pH 7 followed by pH 6 (370 µg/ml). However, phosphate solubilization and growth at the initial pH 6 and 7 were not significantly different ($P \leq 0.05$).

Table 4 presents the effect of different salts (NaCl, KCl and $CaCl_2$) on phosphate solubilization and growth of *K. oxytoca.* NaCl and KCl at the concentrations up to 2.5% enhanced phosphate solubilization and growth of the strain followed by concentration-depended reductions in both parameters. Contrary to this, phosphate solubilization and growth were decreased with increasing $CaCl_2$ concentrations. The maximum phosphate solubilization (615 µg/ml) was recorded when the medium contained glucose (2%) and $(NH_4)_2SO_4$ (0.1%) respectively as the source of carbon and nitrogen and NaCl (2.5%) with pH adjusted to 7 at temperature 35°C.

Assay of other plant growth promoting characteristics

As the other plant growth promoting characteristics of the strain, ACC deaminase activity, ammonia production and HCN production was measured qualitatively and IAA production was measured quantitatively (Table 5). The strain showed positive responses for all the tested plant growth promotion traits. The highest IAA production (21.5 µg/ml) was recorded within the first 24 h followed by reduction (15.2 µg/ml after 48 h) as time progressed.

Plant growth promotion bioassay

K. oxytoca inoculated mung bean seedlings showed significantly higher shoot and root growth when compared with un-inoculated seeds. As shown in Table 5, inoculated seedlings recorded 31.88 and 45.53% higher shoot and root lengths respectively compared to un-inoculated control.

DISCUSSION

Phosphate solubilization is recognized to be a complex process with many factors especially nutritional, physiological and growth conditions of the cultures it is involved in. In the present study, we isolated *K. oxytoca*, a phosphate solubilizing bacteria tolerant to temperature, pH and salt variations. This could be the first report of such *Klebsiella* spp., although previous reports described some *Klebsiella* spp. as phosphate solubilizers (Ghosh et al., 2012; Islama et al., 2007; Chung et al., 2005).

Nutritional condition of the culture medium is detrimental on microbial growth as well as phosphate solubilization (Jain et al., 2012). Out of the six tested mediums, the strain exhibited the maximum phosphate solubilization and growth in NBRIP which is recognized to be the most cost effective medium also. Phosphate solubilizing microorganisms can utilize a variety of carbon sources to meet their energy requirement; however, their growth and phosphate solubilization may vary with the carbon source. Carbon source is considered to be an important factor for active proliferation of organisms and production of organic acids. The nature of available carbon sources is thus decisive in determining the type and concentration of organic acid produced by the phosphate solubilizing microorganisms which in turn controls the amount of phosphate solubilization by lowering the pH (Patel et al., 2008). Glucose (2%) resulted in significantly higher phosphate solubilization with maximum growth of *K. oxytoca*. Based on phosphate solubilization and growth,

Table 4. Effect of different concentrations (0, 2.5, 5 and 10%) of NaCl, KCl and $CaCl_2$ on phosphate solubilization. Values are means ±SD for triplicates.

Parameter	Soluble P (µg ml^{-1})	Growth (A_{660})	Final pH
NaCl			
0	352.52^{b}±7.20	0.348^{a}±0.006	3.68^{d}±0.05
2.5	525.16^{a}±28.4	0.361^{a}±0.012	3.48^{e}±0.04
5.0	227.67^{c}±6.13	0.295^{b}±0.007	4.17^{c}±0.04
7.5	97.17^{d}±6.80	0.103^{c}±0.007	6.05^{b}±0.04
10.0	0.00^{e}±0.00	0.000^{d}±0.000	6.49^{a}±0.02
KCl			
0	316.98^{c}±9.00	0.289^{c} ±0.006	3.72^{d}±0.01
2.5	599.69^{a}±20.75	0.438^{a} ±0.002	3.68^{d}±0.02
5.0	551.89^{b}±3.40	0.359^{b} ±0.002	3.85^{c}±0.01
7.5	234.28^{d}±20.78	0.116^{d}±0.005	4.59^{b}±0.03
10.0	0.00^{e}±0.00	0.000^{e}±0.000	6.49^{a}±0.02
$CaCl_2$			
0	316.98^{a}±9.00	0.438^{a}±0.006	3.72^{a}±0.01
2.5	298.74^{b}±6.27	0.080^{b}±0.005	3.15^{b}±0.00
5.0	69.49^{c}±3.57	0.072^{c}±0.005	3.52^{c}±0.01
7.5	0.00^{d}±0.00	0.023^{d}±0.002	3.99^{d}±0.01
10.0	0.00^{d}±0.00	0.000^{e}±0.000	6.49^{e}±0.02

Table 5. Plant growth promoting characteristics and results of the pot experiment. Values are means ±SD for triplicates.

Parameter	Results	
Plant growth promoting characteristics		
IAA production	21.5 µg ml^{-1}	
Ammonia production	Positive	
HCN production	Positive	
ACC deaminase activity	Positive	
Pot experiment with mung bean seedlings		
	Inoculated	Un-inoculated
Shoot length (cm)	23.12	15.34
Root length (cm)	17.52	10.64

the different carbon sources can be listed in the following order: glucose > fructose > galactose > sucrose > maltose > mannitol > xylose > sorbitol > lactose. Dave and Patel (2003) have reported that the monosaccharides are superior to disaccharides, polysaccharides and alcohols for phosphate solubilization, which is in agreement with our results with monosaccharides such as glucose, fructose and galactose. However, based on the results with *Citrobacter* sp. DHRSS, Patel et al. (2008) listed the carbon source in the order of maltose > glucose > sucrose > fructose and stated that phosphate solubilization depends on the nature of the sugar. In the case of nitrogen source, the highest and the lowest phosphate solubilization was recorded with $(NH_4)_2SO_4$ and yeast respectively. In the presence of ammonium ions, inorganic acids production through proton exchange mechanisms accelerates the phosphate solubilization (Ahuja et al., 2007; Nautiyal et al., 2000), which is in line with the present results of significantly high phosphate solubilization recorded with nitrogen sources such as $(NH_4)_2SO_4$, NH_4NO_3, and NH_4Cl.

Ability to withstand against various stress conditions is an important factor which determines the growth and survival of microorganisms in soil. Though the present strain recorded the highest phosphate solubilization in NBRIP medium at pH 7, temperature 35°C and 2.5% NaCl, it showed the ability to solubilize phosphate under a wide range of pH (4 to 10), temperature (20 to 40°C) and salt concentrations (0 to 7.5% NaCl). Therefore, *K. oxytoca* could be considered as thermo, acid, alkali and

salt tolerant phosphate solubilizing bacterium with a high potential to be used in a wide range of soils. Among the other strains, only *Burkholderia vietnamiensis* M6 has so far been reported to perform well over wide range of soils (Park et al., 2010). Therefore, *K. oxytoca* is highly impressed to be applied in maintaining phosphorus level in extreme environment conditions, where the other microbes showed reduced phosphate solubilizing capacity.

In addition to the phosphate solubilizing activity under extreme conditions, the strain also showed several plant growth promoting characteristics, which may promote plant growth directly or indirectly or synergistically. The IAA production by the strain was well over the production range (3.0 to 20.3 µg/ml) reported by Banerjee et al. (2010) with *Arthrobacter* sp. and *Bacillus* sp. However, Chaiharn and Lumyong (2011) reported extremely high IAA production (291.97 g/ml) and phosphate solubilization (334 g/ml) with strain *Klebsiella* SN 1.1 isolated from rice rhizospheric soils in Northern Thailand. It has been reported that microbial IAA promotes root growth either directly by stimulating plant cell elongation or cell division or indirectly by its influence on the ACC deaminase activity (Patten and Glick, 2002). The increased shoot and root length of mung bean could be associated with cell elongation and multiplication induced by greater absorption of nutrients, particularly P as well as other plant growth promoting activities of the strain. Therefore, in addition to providing available phosphorus to plants, the isolated strain can enhance the growth of plant through several different mechanisms when inoculated in soil. However, as field conditions are much more complex than *in vitro*, further studies under field based trials would be ideal in confirming the present results.

Conclusion

Our results clearly indicate that isolated *K. oxytoca* is a potent thermo, acid, alkali and salt tolerant phosphate solubilizing bacteria which retains its phosphate solu-bilizing capacity over a wide range of pH, tempera-ture and salt concentrations. Furthermore, the strain is capable of producing plant growth promoting traits such as IAA production, ACC deaminase activity, ammonia and HCN production implying that the strain is an ideal candidate to be included in developing microbial inoculants suit for stress environments also.

Conflict of Interests

The authors have not declared any conflict of interests.

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