CHAPTER 3

Characterization of MPS abilities of *E. asburiae* PSI3 containing Gluconate dehydrogenase operon (*gad*) from *Pseudomonas putida* KT2440.
Chapter 3 Characterization of MPS abilities of E. asburiae PSI3 containing Gluconate dehydrogenase operon (gad) from Pseudomonas putida KT2440.

3.1: INTRODUCTION

Phosphorus is a major macronutrient after nitrogen required for plant growth (Theodorou et al., 1991). P is abundant in the soil in the form of organic and inorganic metal complexes (Ca, Al, Fe) depending on nature of soil but not available to plants. Soluble P forms, $H_2PO_4^-$ and $HPO_4^{2-}$, are used by plants (Ae et al., 1991). Application of chemical fertilizers in the field is not very efficient due to high P refixing capacity of the soil (Goldstein et al., 1995). Rhizobacteria promote plant growth by different mechanism and one of them is mineral phosphate solubilization (Kucey et al., 1989). A large number of bacteria belong to different genera and many fungi possess mineral phosphate solubilization ability which has been attributed to secretion of organic acid viz, D- gluconic acid (GA), citric acid, oxalic acid and 2-keto-D-gluconic acid (2-KG) etc. (Goldstein 1995; Khan et al., 2006; Archana et al., 2011).

Solubilization of mineral phosphate has been extensively studied in Gram negative bacteria using glucose as a carbon source (Goldstein 1995; Gyaneshwar et al., 1999; 2002). GA is prominent organic acid produced via direct oxidation pathway by membrane bound quinoprotein dependent glucose dehydrogenase (GDH). Pyrroloquinoline quinone (PQQ) is the cofactor for GDH enzyme and its biosynthesis requires many genes which vary in bacteria (Choi et al., 2008). Genes conferring mineral phosphate solubilization ability were cloned from phosphate solubilizing microorganisms (PSMs) like Erwinia herbicola, Pseudomonas cepacia, Enterobacter intermedium, Serratia marcescens, Rahnella aquatilis and non-PSMs Synechocystis PCC 6803 ( Gyaneshwar et al., 1998a; Zaidi et al., 2009). Incorporation of pqqE gene from these PSMs has enabled to develop mineral phosphate solubilization ability in

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*Escherichia coli*, *Burkholderia cepacia* IS-16, *Pseudomonas* sp. PSS and *Azospirillum* sp. Field studies of plant inoculations with PSMs had inconsistent effect on plant growth and crop yields. This has been attributed to variations in soil, crop and environmental factors influencing the survival and colonization of the rhizosphere. Buffering capacity of Alkaline vertisol, refixation of acidic alfisols, availability of carbon sources, nature of nitrogen source, nature of mineral phosphates and catabolite repression of organic acid secretion have been demonstrated to determine the efficacy of PSMs in field conditions (Kucey et al., 1989; Gyaneshwar et al., 1998b; Srivastava et al., 2007; Patel et al., 2011).

*Enterobacter asburiae* PSI3, an isolate of pigeon pea (*Cajanus cajan*) rhizosphere, solubilizes RP in buffered conditions by secretion of GA mediated by phosphate starvation inducible GDH (Gyaneshwar et al., 1999). In addition to glucose, *E. asburiae* PSI3 can utilize various mono- and di-saccharides for solubilizing RP due to the broad substrate specificity of GDH and it requires a mixture of seven aldosugars at 15mM concentrations for solubilizing RP in buffered medium (Sharma et al., 2005). 2-ketogluconic acid (2-KG) is much stronger than GA and also efficiently chelates calcium in soils (Moghimi et al., 1978; Moghimi and Tate 1978). Many bacteria are known to secrete 2-KG (Misenheimer et al., 1965; Webley and Duff 1965; Yum et al., 1997; Hwangbo et al., 2003; Saichana et al., 2009; Vyas and Gulati, 2009; Gulati et al., 2010; Park et al., 2010). GA gets converted to 2-KG in the periplasm by gluconate dehydrogenase (GAD) enzyme encoded within *gad* operon (Yum et al., 1997; Toyama et al., 2007; Saichana et al., 2009). GAD has been characterized and purified from *Pseudomonas*, *Klebsiella pneumoniae*, *Serratia*
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marcescens, acetic acid bacteria (Gluconobacter sp.) and Erwinia cypripedii ATCC 29267. gad operon encodes for three subunits namely, FAD dependent gluconate dehydrogenase, cytochrome c and smallest third subunit of unknown function (Matsushita et al., 1982; Yum et al., 1997; Toyama et al., 2007; Saichana et al., 2009). In present study, Pseudomonas putida KT 2440 gad operon was expressed in E. asburiae PSI3 which led to secretion of 2-KG and solubilized rock phosphate more efficiently in buffered condition.
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3.2 Material and Methods

3.2.1 Bacterial strains, plasmids and media

The bacterial strains and plasmid used in this study are listed in Table 3.1. Routine DNA manipulation was done in *E. coli* DH10B (Invitrogen) using standard molecular biology protocols (Sambrook and Russel, 2001). pTTQ18 and pGM160 were generously gifted by Dr. Michael J.R. Stark (Leicester Biocentre, University of Leicester) and Dr. Günther Muth (University of Beilfeld, Germany). *E. coli* DH10B, *E. asburiae* PSI3 (*Cajanu cajan* rhizosphere isolate) and its plasmid derivatives were grown on Luria-Bertanni (LB) medium (Hi Media, India) containing 20 µg/ml streptomycin, 50 µg/ml ampicillin, erythromycin (20µg/ml) and 10 µg/ml gentamycin as and when required.
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Table 3.1 Bacterial strains and plasmids used in this study

<table>
<thead>
<tr>
<th>Plasmid/Strains</th>
<th>Relevant characteristics</th>
<th>Source/Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Plasmids</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pTTQ18</td>
<td>Cloning vector-ColE1 origin, $P_{tac}$, Ap&lt;sup&gt;†&lt;/sup&gt;</td>
<td>Stark, 1987</td>
</tr>
<tr>
<td>pGM160</td>
<td>Cloning vector, Gm&lt;sup&gt;†&lt;/sup&gt;</td>
<td>Muth <em>et al.</em>, 1989</td>
</tr>
<tr>
<td>pTTQGm&lt;sup&gt;†&lt;/sup&gt;</td>
<td>pTTQ18 with acc, Ap&lt;sup&gt;†&lt;/sup&gt; Gm&lt;sup&gt;†&lt;/sup&gt;</td>
<td>This study</td>
</tr>
<tr>
<td>pJET 2.1</td>
<td>Blunt end PCR cloning vector</td>
<td>Fermantas</td>
</tr>
<tr>
<td>pCNK12</td>
<td>pJET2.1 with gad operon</td>
<td>This study</td>
</tr>
<tr>
<td>pCNK14</td>
<td>pTTQGm&lt;sup&gt;†&lt;/sup&gt; with acc gene, gad operon, Ap&lt;sup&gt;†&lt;/sup&gt; Gm&lt;sup&gt;†&lt;/sup&gt;</td>
<td>This study</td>
</tr>
<tr>
<td><strong>Bacterial Strains</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>E. coli</em> DH10B</td>
<td>Host strain for routine DNA manipulation experiments and plasmid maintenance</td>
<td>Invitrogen</td>
</tr>
<tr>
<td><em>E. asburiae</em> PSI3</td>
<td><em>Cajanus cajan</em> rhizosphere isolate</td>
<td>Gyaneshwar <em>et al.</em>, 1998b</td>
</tr>
<tr>
<td><em>E. a.</em> PSI3(pCNK14)</td>
<td><em>E. asburiae</em> PSI3 containing pCNK14</td>
<td>This study</td>
</tr>
<tr>
<td><em>E. a.</em> PSI3(pTTQGm&lt;sup&gt;†&lt;/sup&gt;)</td>
<td><em>E. asburiae</em> PSI3 containing pTTQGm&lt;sup&gt;†&lt;/sup&gt;</td>
<td>This study</td>
</tr>
</tbody>
</table>

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3.2.2: Cloning of gluconate dehydrogenase (gad) operon in blunt end cloning vector pJET2.1

Genomic DNA isolation from Pseudomonas putida KT2440 was done as described by Sambrook and Russel (2001). Sequence of gad operon was obtained from P. putida KT2440 genome database (Accession No. AE015451). Gene specific primer pair 5′CGGATCCCGAGGGAATGTCATGCCTGAGCATGCCCC3′ and ‘GCTCTAGAGCTCAGCGAAGCGACTTTACATC 3‘ were used for obtaining gad amplicon. Underline sequence showed BamHI and XbaI restriction enzyme site used for cloning. Italic sequence indicates RBS. PCR amplification was done using XT-20 polymerase (Banglore Genei, India) from 10 ng template DNA of Pseudomonas putida KT2440. Amplified PCR product was ligated into blunt end PCR cloning vector pJET2.1 (MBI Fermantas, India) according to manufacturer instruction. Positive clones were confirmed by restriction digestion and designated as pCNK12.

3.2.3: Cloning of aacC3 gene confer for gentamycin in pTTQ18

E. asburiae PSI3 is naturally resistant to ampicillin. Hence, aminoglycoside-(3)-N-acetyltransferase III (aacC3) gene from pGM160 (1.6kb HindIII fragment) was cloned in HindIII site of pTTQ18 to obtain pTTQGm′. Recombinant plasmid was confirmed by growing them on gentamycin containing LA plates and restriction digestion. Plasmid was designated to pTTQGm′.
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3.2.4: Construction of pCNK14 containing gluconate dehydrogenase (gad) operon of *Pseudomonas putida* KT2440 under control of *tac* promoter

pCNK12 was digested with *BamHI* and *XbaI* which gives 3.8 kb release of GAD operon. 3.8 kb fragment was gel eluted and subcloned in gel purified pTTQGm′ digested with *BamHI* and *XbaI* to obtained pCNK14. Clone was confirmed by restriction digestion. Functionality of the operon was confirmed by GAD enzyme assay.

3.2.5: Physiological experiments

Bacterial cells were prepared by growing the cells overnight at 37 °C in LB. Batch culture studies were performed under aerobic conditions in Orbitek rotary shaker with temperature maintained at 37°C and agitation speed was kept constant at 200 rpm. The composition of minimal medium used for the physiological studies is as follows: TRP minimal medium- 100mM Tris-Cl (pH 8.0), (45mM, 50 mM and 60mM) glucose, 10mM NH₄Cl, 10mM KCl, 2mM MgSO₄, 0.1mM CaCl₂ and micronutrient cocktail (Gyaneshwar et al., 1998b) with 1mg per ml Senegal Rock phosphate (RP) as sole phosphorus source. Erythromycin and gentamycin was added to a final concentration of 7.5 g ml⁻¹ and 2.5 g ml⁻¹, respectively.

3.2.6: Monitoring of MPS ability of recombinant *E. asburiae* PSI3

MPS ability of recombinant *E. asburiae* PSI3 was determined on TRP minimal medium plates (100mM tris buffer pH-8.0, RP 1mg/ml, methyl red, 1.8% agar and varying concentration of glucose *viz* 60 mM, 50mM and 45 mM). Same combination was used for shake flask experiments without agar. Absorbance at 600
nm was used for cell growth and pH drop was used as acidification of medium. Supernatant of medium was used for Pi estimation using KH$_2$PO$_4$ as standard (Ames, 1964).

3.2.7: Measurement of GDH and GAD activities

Wild type and recombinant *E. asburiae* PSI3 were grown on TRP containing minimal medium containing 75mM glucose as carbon source. After pH dropped below 5.5, cells were harvested (10000 g for 5 min) and washed with sterile saline and resuspended in 50mM Tris buffer pH 8.75. Whole cells were used for GDH and GAD enzyme assay and the assay was performed according to Matsushita and Ameyama (1982) and Matsushita et al (1982). GDH (EC 1.1.5.2) was determined spectrophotometrically by following the coupled reduction of DCIP at 600nm. The molar absorbance of DCIP was taken as 15.1 mM$^{-1}$cm$^{-1}$ at pH 8.75. The reaction mixture included: Tris-Cl buffer (pH 8.75), 16mM; DCIP, sodium salt, 0.05mM; PMS, 0.66mM; sodium azide, 4mM; D-glucose, 66mM; whole cells and water to 3.0 ml. For GAD activity, the protocol was similar to GDH assay except the 0.01M potassium phosphate buffer pH 6.0 containing 66mM D-gluconate. The molar absorbance of DCIP was taken as 10.0 mM$^{-1}$cm$^{-1}$ at pH 6.0.

Total whole-cell protein was estimated using a modified Lowry’s method (Peterson, 1979). Units of activity of GDH and GAD are defined as nmol of 2, 6-dichlorophenolindo-phenol (DPIP) reduced per minute using glucose or gluconate as substrates, respectively. Specific activity is defined as units per mg total protein.
3.2.8: Analytical methods

All the physiological experiments used an initial cell density of ~0.025 OD at 600nm as monitored spectrophotometrically (path length of 1cm; Shimadzu UV-1700 spectrophotometer). Growth was monitored as increase in absorbance at 600nm and pH of the medium was monitored at 12h time intervals. When pH of the medium reached below 5 sample (2 ml) were taken from flask were centrifuged at 9,200 g for 10 min at 4 °C and the culture supernatants were used to estimate organic acid and P release in the medium. For HPLC analysis, the culture supernatant was passed through 0.2µm nylon membranes (Pall Life Sciences, India) and the secreted organic acids were quantified using Luna C-18 column (Phenomenex, India). The column was operated at room temperature using mobile phase of 5mM H₂SO₄ at a flow rate of 1.0 ml min⁻¹ and the column effluents were monitored using a UV detector at 210 nm. Standards of organic acids were prepared in double distilled water, filtered using 0.2µm nylon membranes and were subjected to chromatography under similar conditions for determining the individual retention time. Comparison of peak area with external standards was used for quantification. The statistical analysis of all the parameters has been done using Graph Pad Prism (version 5.0) software.

3.2.9: Plant inoculation study

For plant inoculation study was carried out in pots of sterile local alkaline vertisol. Mung bean (Vigna radiata) seeds were surface sterilized with 0.1% HgCl₂ solution, followed by 70% ethanol washed thoroughly and germinated in sterile distilled water. Overnight 50 ml LB grown wild type and genetic transformants of E. asburiae PSI3 were washed thrice with normal saline and dissolved in 5 ml saline.
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Mung bean seeds were soaked in bacterial culture for 30 min. Uninoculated control seeds were treated with saline. 15 to 20 grown seeds were inoculated in a single pot. Three sets of 5-6 pots each of uninoculated control, E. asburiae PSI3 (pTTQGm) and E. asburiae PSI3 (pCNK14) were grown under natural light at ambient temperature for 15 days. Uprooted plant shoot and root were separated and dried at 65°C for constant dry weight measurement. Dried plant shoots (n=6) were ashed in muffle furnace at 550°C for 15 h and ashes were dissolved in 0.9M H₂SO₄ at 10 mg dry weight per ml acid (Richardson et al., 2001). Subsequently P contents of H₂SO₄ extract was estimated by molybdate- blue method (Murphy and Reilay, 1962).

3.2.10: Data analysis

Physiological experiments were done in three independent triplicates. Data are expressed in mean and standard deviation. In plant experiments, three independent duplicate studies were performed. Differences in mean values were determined using general analysis of variance (ANOVA) and linear regression analysis was done using GraphPad Prism5.0.
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3.3 Results

3.3.1 Cloning and heterologous overexpression of gad operon of P. putida KT 2440 in E. asburiae PSI3.

Cloning strategy of gad operon schematically represented in Figure 2. gad operon was cloned from genomic DNA of P. putida KT 2440 in blunt end cloning vector pJET 2.1. Clone was confirmed by RE digestion with BamHI/XbaI, BglIII and BamHI/HindIII. BamHI/XbaI digestion gives release of 3.8kb gad operon and 2.6kb vector. BglIII present in both side of MCS so digestion will give 3.8 kb gad operon release and vector backbone. BamHI/HindIII digestion gives 4.2kb due to presence of HindIII in vector, 400bp downstream of MCS. 3.8 kb gad operon was subcloned in BamHI/XbaI RE sites of expression vector pTTQGm© under the control of tac promoter to obtain pCNK14. pCNK14 was confirmed by RE digestion with BamHI/HindIII. BamHI /HindIII digestion give 3.8 kb gad operon, 1.6kb gentamycin and 4.5 kb vector backbone.

pTTQGm and pCNK14 were transformed into E. asburiae PSI3. E. asburiae PSI3 gad operon transformant showed similar GAD activity in the presence and absence of IPTG indicative of constitutive expression. In LB, GAD enzyme activity was found to be 985±44 which was enhanced by ~1.7 fold in presence of 2% D-gluconate. In TRP medium containing 75mM glucose, GAD and GDH specific activities of the E. asburiae PSI3 (pCNK14) were found to be 438.3± 0.113 U and 884.2±0.182 U, respectively. There was no significant difference in GDH activity was observed as compared to wild type and plasmid control (Table 3.2).
Chapter 3  Characterization of MPS abilities of E. asburiae PSI3 containing Gluconate dehydrogenase operon (gad) from Pseudomonas putida KT2440.

![Image 1](image1.png)

**Fig. 3.1** Restriction digestion pattern of pCNK11

![Image 2](image2.png)

**Fig. 3.2** Restriction digestion pattern of pCNK14

3.3.2 Effect of *gad* overexpression on mineral phosphate solubilization ability of *E. asburiae* PSI3

*E. asburiae* PSI3 (pCNK14) showed acidification and mineral phosphate solubilization ability on TRP plates containing 45mM glucose while *E. asburiae* PSI3 and *E. asburiae* PSI3 (pTTQGm³) did not show acidification and mineral phosphate solubilization ability when the glucose concentration was less than 75 mM (**Fig. 3.3**).
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In liquid TRP medium, *E. asburiae* PSI3 (pCNK14) grew up to 0.3 O.D. and acidified the medium to pH 3.9 in 96 h (Fig.2). Pi release was found to be 0.84mM. *E. asburiae* PSI3 secreted ~ 50 mM GA in presence of 75 mM glucose in TRP medium whereas *gad* transformant secreted 11.5 mM 2-KGA and 21.6 mM GA when grown in TRP medium containing 45mM glucose as a carbon source (Table 3.3). Thus, the efficiency of GA conversion to 2KGA by *E. asburiae* PSI3 (pCNK14) is ~30%.

![Fig.3.3 MPS ability of *E. asburiae* PSI3 (pTTQGm) and *E. asburiae* PSI3 (pCNK14) in TRP agar medium with glucose as carbon source.](image_url)

**Table 3.3** MPS ability of *E. asburiae* PSI3 (pTTQGm) and *E. asburiae* PSI3 (pCNK14) in TRP agar medium with glucose as carbon source.
Characterization of MPS abilities of *E. asburiae* PSI3 containing Gluconate dehydrogenase operon (gad) from *Pseudomonas putida* KT2440.

**Fig. 3.4:** Growth and pH profile of *E. asburiae* PSI3 (pTTQGm) and *E. asburiae* PSI3 (pCNK14) in TRP medium containing 45 mM glucose.

**Table 3.2** Activities of GAD and GDH enzymes.

<table>
<thead>
<tr>
<th>Strain</th>
<th>GAD activity (U)</th>
<th>GDH activity (U)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. putida</em> KT2440</td>
<td>328±0.048</td>
<td>ND</td>
</tr>
<tr>
<td><em>E. asburiae</em> PSI3 (pTTQGm)</td>
<td>UD</td>
<td>991±0.13</td>
</tr>
<tr>
<td><em>E. asburiae</em> PSI3 (pCNK14)</td>
<td>438.3±0.032</td>
<td>884±0.052</td>
</tr>
</tbody>
</table>

*E. asburiae* PSI3 was grown in 75 mM glucose and 100 mM Tris buffered medium. *P. putida* KT2440 was grown in 75 mM glucose and 50 mM Tris buffered condition. UD- Undetected, ND- Not Done
Characterization of MPS abilities of *E. asburiae* PSI3 containing Gluconate dehydrogenase operon (gad) from *Pseudomonas putida* KT2440.

**Table 3.3** Amount of Organic acid secretion and P release by *E. asburiae* PSI3 (pTTQGm) and *E. asburiae* PSI3 (pCNK14) in TRP medium containing 45 mM glucose.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Initial O.D</th>
<th>Final O.D</th>
<th>Gluconic acid</th>
<th>2-ketogluconic acid</th>
<th>Pi (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. asburiae</em> PSI3</td>
<td>0.02±0.002</td>
<td>0.21±0.01</td>
<td>33.74±1.78</td>
<td>UD</td>
<td>UD</td>
</tr>
<tr>
<td>(pTTQGm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>E. asburiae</em> PSI3</td>
<td>0.03±0.01</td>
<td>0.31±0.03</td>
<td>21.65±0.94</td>
<td>11.63±0.93</td>
<td>0.84±0.04</td>
</tr>
<tr>
<td>(pCNK14)</td>
<td></td>
<td></td>
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</tbody>
</table>

UD- Undetected

### 3.3.3 Effect of gad overexpression *E. asburiae* PSI3 transformant on *Vigna radiata*.

Mung bean (*Vigna radiata*) growth was monitored in pot experiments in sterile alkaline vertisol soil. Dry shoot weight, dry shoot/root weight and shoot P content were significantly improved in *E. asburiae* PSI3 pCNK14 as compared to vector control as as well as uninoculated control while dry shoot weight and dry shoot/root weight were improved in vector control compared to uninoculated control but no significant P content was observed (**Table 3.4**).
Table 3.4 Effect of *E. asburiae* PSI3 (pCNK14) inoculation on the mung bean (*Vigna radiata*) dry shoot weight, dry shoot/root weight ratio and shoot P content after 15 days.

<table>
<thead>
<tr>
<th>Bacterial strain</th>
<th>Dry shoot weight (mg)</th>
<th>Dry shoot/root weight ratio of plant</th>
<th>Shoot P concentration of plants (mg/g dry weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uninoculated control</td>
<td>22.65</td>
<td>3.654</td>
<td>5.98</td>
</tr>
<tr>
<td><em>E. asburiae</em> PSI3</td>
<td>pTTQGm</td>
<td>pCNK14</td>
<td>pTTQGm</td>
</tr>
<tr>
<td>E</td>
<td>25.49*</td>
<td>29.39**†</td>
<td>5.02*</td>
</tr>
</tbody>
</table>

Plants were grown (12 plants, 5 replicates) for 15 days and data are means of individual experiments (for dry weight and shoot/root ratio of plants, n=12, and for shoot P content, n=6). ns: comparison with uninoculated control (Fisher LSD test, p<0.05); *: significance in comparison with uninoculated control; †: significance in comparison with the corresponding vector control (single sign: p<0.05; double sign: p<0.001).

Fig. 3.5: Effect of *E. asburiae* PSI3 (pCNK14) on the growth of mung bean (*Vigna radiata*) after 15 days in alkaline vertisol soil. UC- Uninoculated control, VC- *E. asburiae* PSI3 (pTTQGm),
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3.4 Discussion

Nature and amount of organic acid determine the efficacy of phosphate solubilization by rhizobacteria. Most of the PSMs secrete GA but not 2KGA which is much stronger acid. Hence, incorporation of gad in GA producing PSM could significantly improve their plant growth ability. In the present study, incorporation of P. putida KT 2440 gad operon in E. asburiae PSI3 showed ~ 438 U and 985 U of enzyme activity in TRP and LB media respectively. Earlier reports of incorporation of the gad operon from E. cypripedi ATCC 29267 with ~ 1360 U enzyme activity in E. coli resulted in ~410 U GAD activity in LB medium which was increased to 2150 U in presence of gluconate (Yum et al., 1997). Similar increase in GAD activity (1738 U) was found in E. asburiae PSI3 gad transformant in presence of gluconate. E. asburiae PSI3 requires 75mM glucose to solubilize rock phosphate in TRP medium. However, gad transformant could solubilize rock phosphate in presence of 45 mM glucose due to secretion of ~21 mM GA and ~11mM 2KGA. In contrast, E. coli containing E. cypripedi ATCC 29267 gad operon could secrete ~ 13mM 2KGA in LB containing 100mM glucose (Yum et al., 1997).

Recent plant growth studies with PSMs have demonstrated improvement in P status upon supplementation with tricalcium phosphate (Vyas and Gulati, 2009; Gulati et al., 2010). Acinetobacter rhizosphaerae strain, which produced very high (upto ~ 87mM) GA, enhanced maize plant growth upon supplementation with tricalcium phosphate as compared to the plants supplemented with super phosphate (Gulati et al., 2010). On the other hand, phosphate solubilizing fluorescent pseudomonads strains improved maize plant growth as well as P content on