Isolation and characterization of Endophytic-Rhizospheric phosphate solubilizing bacteria of cumin and their evaluation in vitro

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DOI: https://doi.org/10.22271/chemi.2020.v8.i5v.10530

Abstract
The present investigation was carried out during the year 2017-18 and 2018-19 in Laboratory of Microbiology, Devison of crop production, ICAR-National Research Centre for Seed spices Ajmer, Rajasthan (India). The maximum P solubilization zone was 19 mm followed by 17 mm recorded in isolate DCU-251 which was characterized as Pseudomonas aeruginosa (Accession no. MN192165) and DCU-262 (Kosakonia oryzendophytica strain NRCSSDCU262 Accession no. MN192166), respectively. The least solubilization zone was 11 mm due to isolate DCU-551. Phosphate solubilization index ranged 1.6-3.1. Highest SI was 3.1 followed by 2.9 and 2.8 associated with isolate DCU-251(Pseudomonas aeruginosa strain NRCSSDCU251 Accession no. MN192165) and DCU-262 (Kosakonia oryzendophytica strain NRCSSDCU262 Accession no. MN192166) and DCU-22 (Bacillus paramycoides strain NRCSSDCU22 Accession no. MN192162), respectively. The Lowest value of SI was 1.5 due to isolate DCU-551. The highest soluble P (326 µg ml⁻¹) in pikovskaya’s broth was recorded in bacterial isolate DCU-251(Pseudomonas aeruginosa strain NRCSSDCU251 Accession no. MN192165) and least soluble P was 152 µg ml⁻¹ recorded in isolate DCU-553.

Keywords: Cumin, P solubilizing rhizobacteria

Introduction
Cumin (Cuminum cyminum) is a member of Umbelliferae (Apiaceae) family and an annual plant, which is widely cultivated in arid and semi-arid regions. Cumin has along history of use as food flavours, perfumes and medicine. In addition to its common use as spice in our daily life, recent studies have indicated its pharmaceutical and medicinal importance. Total area, total production and yield of cumin in India is 966170 hactare, 688660 tonnes and 713 kg per hectare, respectively, (Directorate of arecanut and spices development, Calicut, 2017-18). Among seed spices, cumin is an important crop of western Rajasthan and is mainly grown in the districts of Jaisalmer, Jalore, Pali, Barmer, Ajmer, Nagaur, Tonk and Jodhpur. In fact, the extreme susceptibility to disease like wilt, powdery mildew and blight and also to aphids and lack of knowledge of suitable agricultural practices are the reasons of poor productivity in this crop. There is no doubt that this crop has tremendous scope and the availability of suitable improved practices will result in increase in area as well as production by solving the above constraints.

Nitrogen fixing and P solubilizing bacteria act as PGPR for secretion of phytohormones, siderophore production, HCN production which help in the increase growth and yield of plant life (Kumar et al., 2014) [5]. Biofertilizers have emerged as a brand new idea of plant growth promoting rhizobacteria, manner the product containing carrier based dwelling microorganisms which can be agriculturally useful in terms of N fixation, P solubilization and nutrient mobilization, to increasing crop production and to maintain the fertility of soil (Zahir et al., 2012) [15].

Some researchers mentioned that certain bacteria had been more energetic than other type of microorganisms, within the conversion of P (Igual et al., 2001; Sadia et al., 2002; Thakuria et al., 2004) [4, 10, 14]. Phosphate solubilizing bacteria constitute 1-50 percent. In particular, soil microorganisms specially phosphate solubilizing bacteria (PSB) are powerful in liberating P

P-ISSN: 2349-8528
E-ISSN: 2321-4902
www.chemijournal.com
IJCS 2020; 8(5): 1583-1593
© 2020 IJCS
Accepted: 21-08-2020
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from inorganic and natural pools of total soil P by solubilization and mineralization (Chen et al., 2006) [3]. Keeping this in view, the present study was planned to isolate PGPR from cumin roots and rhizospheric soil, to elucidate different mechanisms involved in plant growth so that they can be exploited as potential bioinoculants for cumin.

**Methods**

The present investigation was carried out in Microbiology laboratory Devison of Crop production, ICAR-NRCSS, Tabijji, Ajmer for isolation and characterization of Phosphate solubilizing bacteria from cumin rhizosphere and roots.

**Collection of samples:** A total of 155 soil and plant samples were collected from cumin grown agricultural fields of Ajmer, Barmer, Jalore, Nagaur, Jaisalmer and Jodhpur Districts of Rajasthan (India). All rhizospheric soil samples were collected from 0-15 cm depth by carefully uprooting the plants and for endophytes healthy cumin roots were collected. The samples were properly tagged, sealed and stored. Collected soil samples were preserved in a polythene bag for physico-chemical properties and microbial analysis.

**Isolation of rhizobacteria:** The isolation of rhizobacteria from cumin roots as well as rhizosphere soil of cumin was done using six different media viz. Nutrient agar (NA) and Pikovskaya’s agar media for P solubilizing bacteria. All the media were prepared and autoclaved at 15 psi and 121 °C for 20 minutes.

**Isolation of rhizobacteria from rhizospheric soil:** Ten grams of the fresh soil was transferred to Erlenmeyer flask (150 ml) containing 90 ml sterile distilled water (10^-1) and was shaken at 120 rpm for 15 min. Then, 1.0 ml of this suspension was transferred into a 9 ml blank (10^-2). This serial dilution was continued up to 10^-10, followed by pour plating on Nutrient agar (NA) and Pikovskaya’s agar media. The petri plates were inoculated and incubated for 24-48 hrs at 28 °C. Colonies which appeared to be morphologically different were isolated and sub cultured.

**Isolation of bacteria from cumin roots:** The collected plant material used for the isolation was first surface sterilized following the method of Santos et al. (2003) with few modifications. Plant material was first cleaned by washing several times under running tap water then Surface sterilization was performed by sequentially rinsing the plant material with 70% ethanol (C2H5OH) for 30 seconds, then with 0.01% mercuric chloride (HgCl2) for 5 minutes followed by 0.5% sodium hypochlorite (NaOCl) for 2-3 minutes and finally with sterile distilled water for 2-3 times. Plant roots was then dried in between the folds of sterile filter papers. After proper drying, the surface sterilized roots were cut vertically into small segments each segment was placed on different types of medium. All the plates were incubated at 28°C to promote the growth of endophytes and were regularly monitored for any microbial growth. On observing the microbial growth, sub culturing was done. Each endophytic culture was checked for purity and transferred to freshly prepared medium plate. Appropriate controls were also set up in which no plant tissues were inoculated.

**Screening for P solubilizing bacterial isolates:** All the bacterial isolates were tested for their ability to solubilize the insoluble tri calcium phosphate [Ca3(PO4)2] on Pikovskaya’s agar media for isolate P solubilizing bacteria.

**Biochemical characterization of rhizobacteria:** Biochemical characterization of bacterial isolates was done on the basis of catalase production, nitrate reduction, starch hydrolysis and methyl red test. These were conducted as per the standard methods (Cappuccino and Sherman 1992) [2].

- **Catalase production:** A drop of 3% H2O2 was taken on a glass slide and small amount of bacterial culture was mixed with platinum inoculation loop. Rapid and sustained production of gas bubbles or effervescence constituted positive test.

- **Nitrate reduction test:** 5ml nitrate broth was inoculated with pure culture of the test organism. It was incubated at 28 °C for 48 hours. Equal volume (0.5 ml) of both the reagents A(Sulfanilic acid 8g + Acetic acid 1000 ml) and B (5 g Alpha naphthylamine + 5N Acetic acid 1000 ml) were added. The development of red color within 30 seconds indicated the positive test.

- **Hydrolysis of starch:** Sterilized starch agar medium was poured onto petriplates. The log phase cultures were spotted on the plates and incubated at 28 °C for 48 hrs. After full growth of cultures, the petriplates were flooded with Gram’s iodine. The hydrolysis of starch was observed as a colorless zone surrounding the colonies against purple background. A blue or purple zone indicated that starch was not hydrolyzed.

- **Methyl red (MR) test:** Dye was dissolved in alcohol followed by addition of water to make 100 ml volume. It was stored at room temperature. A tube of GPPW (5 ml) was inoculated with pure culture of the test organism. It was incubated at 28 °C for 48 hours. At the end of this, 5 drops of the MR reagent was added directly to the broth. The development of a stable red color indicated positive test.

**Biochemical Test:** HiAssorted™ Biochemical Test kit is a standardized colorimetric identification system utilizing seven conventional biochemical tests and five carbohydrate utilization tests . The tests are based on the principle of pH change and substrate utilization. On incubation organisms undergo metabolic changes which are indicated by a colour change in the media that can be either interpreted visually or after addition of the reagent.

- **Qualitative assay for P solubilization:** One loop full of overnight matured cultures of P solubilizing bacterial isolates were spotted on Pikovskaya’s agar media to observe the zone of clearance by the isolates. The plates were incubated at 28 °C for 48 h and the zone of clearance was observed and expressed in mm. SI was measured using the following formula (Premenon et al., 1996) [9].

\[
SI = \frac{(Colony \ diameter + Halo \ zone \ formation)}{(Colony \ diameter)}
\]

- **Quantitative assay for P solubilization:** The PSB isolates that showed halo zone formation were further tested for their ability to release soluble P from insoluble tri calcium phosphate using UV Spectrometer. The PSB were grown in 50.0 ml Pikovskaya’s broth containing 0.5 % tri calcium phosphate (Pikovskaya, 1948) at
28 °C for different interval of days (5th, 10th and 15th day) with three replicates in incubator cum shaker at 120 rpm. At 5th, 10th and 15th days after incubation, cultures were withdrawn and transferred aseptically to centrifuge tubes. They were centrifuged at 8,000 rpm for 15 min at 4 °C. The supernatant was collected in test tubes. Then 1.0 ml aliquot from the supernatant was transferred to 50.0 ml standard flask and the volume was made up to 50.0 ml using distilled water. This was followed by addition of 10 ml Vanadomolybdate solution, which was added along the sides of the flask. The contents of the flasks were diluted with distilled water and the volume was made up to 50.0 ml. The intensity of the yellow color of the solution was measured in a colorimeter using blue filter. The soluble ‘P’ was estimated from standard curve by plotting colorimeter readings drawn from standard solution against µg of P taken.

Determination of pH
The pH of the PSB culture filtrates and the uninoculated samples was determined at 5, 10 and 15 days after inoculation. The culture was filtered using Whatman No.1 filter paper. The pH was estimated using Elico pH meter.

Molecular characterization of rhizobacteria
The genomic DNA of the promising bacterial isolates was isolated using standard protocol by Sambrook and David (2000) [11]. Molecular identification of bacteria was done using 16S rDNA sequence analysis. The 16S rDNA was amplified using 27F and 1492R primers (Woese 1987; Stackebrandt and Goebel 1994) in 25 µL of reaction mixture containing 1x buffer (10 mM Tris pH 9, 50 mM KCl, 0.01% gelatin), 100 µm dNTP mix, 3 mM MgCl2, 10 µg BSA, 5pm each primer, 0.5 units of Taq DNA polymerase and 100 ng template DNA. Thermo cycling condition consisted of an initial denaturation at 94 °C for 1 min 10 s, 48 °C for 30 s, 72 °C for 2 min 10 s and a final polymerization step of 72 °C for 6 min with eppendorf master thermal cycler. The final PCR product was resolved in 0.8% agarose gel in Tris acetate EDTA buffer. The sequencing was performed with Eurofins Genomics India Pvt Ltd. Bengaluru (India). Sequence was subjected to BLAST analysis and compared with registered sequences in the Gen Bank database using NCBI Blast server (http://www.ncbi.nlm.nih.gov).

Statistical Analysis: Statistical analysis of data was carried out using online statistical analysis pack-age (OPSTAT, Computer section, CCS HAU Hisar, Haryana) for calculation of ANOVA.

Results & Discussion
In first stage of screening 32 bacterial isolates were obtained those had capacity to solubilizing P in vitro. Out of these 32, eight isolates viz., DCU-22 (Bacillus paramycoides strain NRCSDDCU22 Accession no. MN192162), DCU-112 (unidentified), DCU-184(Pseudomonas aeruginosa strain NRCSDDCU184 Accession no. MN192163), DCU-188 (Pseudomonas aeruginosa strain NRCSDDCU188 Accession no. MN192164), DCU-251(Pseudomonas aeruginosa strain NRCSDDCU251 Accession no. MN192165), DCU-451(Pseudomonas aeruginosa strain NRCSDDCU451 Accession no. MN192169) and DCU-651 (Bacillus pacificus strain NRCSDDCU651 Accession no. MN192170) also had able to solubilize another mineral like Zn or K or both Zn and K, those are depicted in Table 1.

Those 32 bacterial isolates were subjected to detailed cultural, morphological and biochemical characterization according to Bergey's Manual of Systematic Bacteriology. The cultural and morphological characteristic of rhizobacterial isolates is presented in Table 2 (Plate 1). Out of 32 bacterial isolates, 9 were gram positive and 23 were gram negative. All were found rods. Out of 32, Pseudomonas (15), Bacillus (9), Azotobacter (7) and Kosakonia (1). The biochemical characteristic of bacterial isolates is presented in Table 3 (Plate 2). Out of 32 bacterial isolates, 19 (starch hydrolysis), 19 (catalase production), 35 (methylene red), 17 (Citrate utilization), 9 (Lysine utilization), 12 (Ornithine utilization), 13 (Urease), 11 (Phenylalanine deaminase), 8 (Nitrate reductase), 10 (H₂S Production), 5 (Glucose), 2 (Adonitol), 7 (Lactose), 10 (Arabinose) and 7 (Sorbitol) were positive.

Qualitative assay for phosphate solubilization
Phosphate solubilizing microorganisms play an important role in utilization of unavailable native phosphates as well as added phosphates. 32 bacterial isolates were screened for phosphate solubilization ability, those could solubilize tricalcium phosphate, however, the P-solubilizing potential varied amongst these isolates as evidenced by the size of halo zone on Pikovskaya’s agar plates. Phosphate solubilization zone and solubilization index of rhizobacterial isolates were presented in Table 4 (Fig. 1, Plate 3). The maximum P solubilization zone was 19 mm followed by 17 mm recorded in isolate DCU-251 (Pseudomonas aeruginosa strain NRCSDDCU251 Accession no. MN192165) and DCU-262 (Kosakonia oryzendophytica strain NRCSDDCU262 Accession no. MN192166), respectively. The least P solubilization zone was 11 mm recorded in isolate DCU-551. Phosphate solubilization index ranged 1.6-3.1. Highest SI was 3.1 followed by 2.9, 2.8 and 2.7 associated with isolate DCU-251 (Pseudomonas aeruginosa strain NRCSDDCU251 Accession no. MN192165), DCU-262 (Kosakonia oryzendophytica strain NRCSDDCU262 Accession no. MN192166), DCU-22 (Bacillus paramycoides strain NRCSDDCU22 Accession no. MN192162) and DCU-4, respectively. The Lowest value of SI was 1.5 due to isolate DCU-551. The results are in harmony with the findings of Mishra et al. (2015) [7] in isolates from fennel crop, Atekan et al. (2014) [1], Malboobi et al. (2009) [6].

Quantitative assay for Phosphate solubilization
The amount of P which solubilized by bacterial isolates at interval was presented in Table 5 (Fig. 2). The minimum solubilized P in broth medium containing tricalcium phosphate (0.5%) were recorded on day 5, afterwards the solubilized P increased up to day 15 of incubation. At 5 days highest amount of soluble P was 103 followed by 101, 99 and 98 µg ml⁻¹ due to isolate DCU-251(Pseudomonas aeruginosa strain NRCSDDCU251 Accession no. MN192165), DCU-262 (Kosakonia oryzendophytica strain NRCSDDCU262 Accession no. MN192166), DCU-184 (Pseudomonas aeruginosa strain NRCSDDCU184 Accession no. MN192163) and DCU-551, respectively, and least soluble P was 75 µg ml⁻¹ recorded in isolate DCU-101. At 10 days highest amount of soluble P was found 248 followed by 233, 227 and 207 µg ml⁻¹ due to isolate DCU-251(Pseudomonas aeruginosa strain NRCSDDCU251 Accession no. MN192165), DCU-184 (Pseudomonas
Pseudomonas aeruginosa strain NRCSSDCU184 Accession no. MN192163), DCU-112 and DCU-262 (Kosakonia oryzendophytica strain NRCSSDCU262 Accession no. MN192166), respectively, and least was 122 µg ml⁻¹ associated with isolate DCU-553. At 15 days of inoculation highest amount of released P was recorded 326 followed by 305, 298 and 282 µg ml⁻¹ associated with DCU-251 (Pseudomonas aeruginosa strain NRCSSDCU251 Accession no. MN192165), DCU-262 (Kosakonia oryzendophytica strain NRCSSDCU262 Accession no. MN192166), DCU-22 (Bacillus paramycoides strain NRCSSDCU22 Accession no. MN192162), and DCU-4, respectively, and least was 152 µg ml⁻¹ recorded in DCU-553. However the superior P solubilizing bacterial isolate was DCU-251, showing the strong ability to solubilize tri calcium phosphate in vitro. Variation of isolate for reduction in pH was observed in most of the cultures over incubation time. From 5th days after incubation to 15th days of inoculation pH value were drop which indicate that if soluble P were increased then pH were reduced (Table 5). These results are in close conformity with the findings of Teng et al. (2018) [13] due to the phosphate solubilizing bacterial species of Enterobacter asburiae, Acinetobacter sp., Bacillus cereus, Paul and Narayan (2017) [8] due to PSB identified as Pseudomonas aeruginosa strain KUPSB12.

Molecular characterization of promising bacterial isolates
After evaluation of in vitro experiments viz. Qualitatively P solubilization and Quantitatively P solubilization 8 bacterial isolates were identified using molecular marker 16S rRNA. The 16S rRNA gene sequence is an essential part of the description of a novel organism.
The data obtained from the partial 16S rRNA sequencing of PCR-amplified product was subjected to BLAST analysis and compared with registered sequences in the Gen Bank database using NCBI Blast server (http://www.ncbi.nlm.nih.gov). The query sequence revealed out of Eight, 5 isolates were strains of Pseudomonas aeruginosa, 1 isolate was Bacillus paramycoides, 1 isolate was Bacillus pacificus and 1 isolate was Kosakonia oryzendophytica. The sequence data of all 8 isolates were deposited in Gen Bank and generated accession number. Details of molecular identified bacterial with their accession number are depicted in Table 6 (Fig. 3).

Table 1: Screened bacterial isolates on the basis solubilization ability

| S.N. | Isolates | Microbe               | P Solubilization | K Solubilization | Zn Solubilization |
|------|----------|-----------------------|------------------|------------------|-------------------|
| 1    | DCU-4    | Bacteria              | +                | -                | -                 |
| 2    | DCU-22   | Bacteria              | +                | +                | -                 |
| 3    | DCU-101  | Bacteria              | +                | -                | -                 |
| 4    | DCU-104  | Bacteria              | +                | -                | -                 |
| 5    | DCU-107  | Bacteria              | +                | -                | -                 |
| 6    | DCU-112  | Bacteria              | +                | -                | +                 |
| 7    | DCU-116  | Bacteria              | +                | -                | -                 |
| 8    | DCU-121  | Bacteria              | +                | +                | +                 |
| 9    | DCU-184  | Bacteria              | +                | -                | +                 |
| 10   | DCU-188  | Bacteria              | +                | -                | +                 |
| 11   | DCU-251  | Bacteria              | +                | +                | +                 |
| 12   | DCU-252  | Bacteria              | +                | -                | -                 |
| 13   | DCU-253  | Bacteria              | +                | -                | -                 |
| 14   | DCU-261  | Bacteria              | +                | -                | -                 |
| 15   | DCU-262  | Bacteria              | +                | -                | -                 |
| 16   | DCU-264  | Bacteria              | +                | -                | -                 |
| 17   | DCU-451  | Bacteria              | +                | +                | +                 |
| 18   | DCU-453  | Bacteria              | +                | +                | +                 |
| 19   | DCU-551  | Bacteria              | +                | -                | -                 |
| 20   | DCU-553  | Bacteria              | +                | -                | -                 |
| 21   | DCU-555  | Bacteria              | +                | -                | -                 |
| 22   | DCU-563  | Bacteria              | +                | -                | -                 |
| 23   | DCU-567  | Bacteria              | +                | -                | -                 |
| 24   | DCU-568  | Bacteria              | +                | -                | -                 |
| 25   | DCU-569  | Bacteria              | +                | -                | -                 |
| 26   | DCU-570  | Bacteria              | +                | -                | -                 |
| 27   | DCU-651  | Bacteria              | +                | +                | +                 |
### Table 2: Cultural and morphological characteristics of cumin rhizobacterial isolates

| Isolate | Margin   | Texture | Elevation | Consistency | Optical feature | Pigment Colour | Gram’s reaction | Shape        | Probable genera   |
|---------|----------|---------|-----------|-------------|----------------|----------------|----------------|--------------|-------------------|
| DCU-4   | Undulate | Smooth  | Flat      | Viscous     | Opaque         | yellow         | -              | Rod          | Pseudomonas       |
| DCU-22  | Undulate | Rough   | Flat      | Dry         | Opaque         | -              | +              | Rod          | Bacillus         |
| DCU-101 | Entire   | Smooth  | Crateriform | Butyrous   | Translucent    | -              | +              | Rod          | Bacillus         |
| DCU-104 | Circular | Smooth  | Flat      | Dry         | Opaque         | -              | -              | Rod          | Azotobacter       |
| DCU-107 | Circular | Smooth  | Flat      | Viscous     | Translucent    | -              | -              | Rod          | Pseudomonas       |
| DCU-112 | Undulate | Smooth  | Flat      | Viscous     | Opaque         | Light brown    | +              | Rod          | Bacillus         |
| DCU-116 | Irregular| Smooth  | Flat      | Butyrous    | Translucent    | -              | -              | Rod          | Azotobacter       |
| DCU-121 | Entire   | Smooth  | Raised    | Butyrous    | Opaque         | Orange         | -              | Rod          | Pseudomonas       |
| DCU-184 | Undulate | Smooth  | Raised    | Viscous     | Opaque         | Yellowish dark green | -            | Rod          | Pseudomonas       |
| DCU-188 | Entire   | Smooth  | Flat      | Viscous     | Opaque         | Yellowish green | -              | Rod          | Pseudomonas       |
| DCU-251 | Entire   | Smooth  | Flat      | Moist       | Opaque         | Yellow         | -              | Rod          | Pseudomonas       |
| DCU-252 | Undulate | Smooth  | Flat      | Viscous     | Translucent    | -              | +              | Rod          | Bacillus         |
| DCU-253 | Undulate | Smooth  | Flat      | Viscous     | Opaque         | Yellowish green | -              | Rod          | Pseudomonas       |
| DCU-451 | Entire   | Smooth  | Flat      | Viscous     | Opaque         | Yellowish green | -              | Rod          | Pseudomonas       |
| DCU-453 | Irregular| Smooth  | Flat      | Viscous     | Opaque         | yellow         | -              | Rod          | Pseudomonas       |
| DCU-551 | Undulate | Smooth  | Flat      | Viscous     | Opaque         | Light brown    | -              | Rod          | Bacillus         |
| DCU-553 | Undulate | Smooth  | Flat      | Viscous     | Opaque         | Light brown    | -              | Rod          | Azotobacter       |
| DCU-555 | Undulate | Smooth  | Flat      | Viscous     | Opaque         | Light brown    | -              | Rod          | Pseudomonas       |
| DCU-563 | Entire   | Smooth  | Flat      | Moist       | Translucent    | -              | -              | Rod          | Pseudomonas       |
| DCU-567 | Undulate | Smooth  | Flat      | Viscous     | Opaque         | -              | -              | Rod          | Azotobacter       |
| DCU-568 | Undulate | Smooth  | Flat      | Viscous     | Opaque         | -              | -              | Rod          | Azotobacter       |
| DCU-569 | Irregular| Smooth  | Flat      | Viscous     | Opaque         | -              | -              | Rod          | Pseudomonas       |
| DCU-570 | Circular | Smooth  | Flat      | Viscous     | Translucent    | -              | -              | Rod          | Pseudomonas       |
| DCU-651 | Undulate | Smooth  | Flat      | Viscous     | Opaque         | yellow pigment | -              | Rod          | Pseudomonas       |

### Table 3: Biochemical tests of cumin rhizobacterial isolates

| S.N. | Isolates | Starch hydrolysis | Catalase production | Methyl Red test | Hi Assorted Biochemical test kit |
|------|----------|-------------------|---------------------|-----------------|----------------------------------|
|      |          |                   |                     |                 |                                  |
| 1.   | DCU-4    | -                 | +                   | +               | 1 2 3 4 5 6 7 8 9 10 11 12     |
| 2.   | DCU-22   | +                 | +                   | -               |                                  |
| 3.   | DCU-101  | +                 | -                   | +               |                                  |
| 4.   | DCU-104  | -                 | +                   | -               |                                  |
| 5.   | DCU-107  | +                 | -                   | -               |                                  |
| 6.   | DCU-112  | +                 | +                   | +               |                                  |
| 7.   | DCU-116  | -                 | -                   | +               |                                  |
| 8.   | DCU-121  | +                 | +                   | -               |                                  |
| 9.   | DCU-184  | +                 | +                   | +               |                                  |
| 10.  | DCU-188  | -                 | +                   | -               |                                  |
| 11.  | DCU-251  | +                 | -                   | +               |                                  |
| 12.  | DCU-252  | +                 | +                   | +               |                                  |
| 13.  | DCU-253  | +                 | +                   | -               |                                  |
| 14.  | DCU-254  | -                 | +                   | -               |                                  |
| 15.  | DCU-256  | -                 | +                   | -               |                                  |
| 16.  | DCU-258  | +                 | +                   | +               |                                  |
| 17.  | DCU-259  | -                 | +                   | +               |                                  |
| 18.  | DCU-260  | -                 | +                   | +               |                                  |
| 19.  | DCU-261  | +                 | -                   | +               |                                  |
| 20.  | DCU-262  | -                 | +                   | -               |                                  |
| 21.  | DCU-264  | -                 | -                   | -               |                                  |
| 22.  | DCU-451  | +                 | +                   | +               |                                  |
| 23.  | DCU-453  | +                 | +                   | +               |                                  |
| 24.  | DCU-551  | +                 | +                   | -               |                                  |
| 25.  | DCU-553  | +                 | +                   | -               |                                  |
| 26.  | DCU-555  | +                 | +                   | +               |                                  |
| 27.  | DCU-563  | +                 | +                   | +               |                                  |
| 28.  | DCU-567  | +                 | -                   | +               |                                  |
| 29.  | DCU-568  | +                 | +                   | +               |                                  |

`1587`
Table 4: Qualitative assay for P Solubilization by rhizobacterial isolates

| S.N. | Isolates | Microbes | Solubilization zone (mm) | Solubilization Index |
|------|----------|----------|--------------------------|----------------------|
| 1.   | DCU-22   | Bacteria | 16                       | 2.8                  |
| 2.   | DCU-4    | Bacteria | 15                       | 2.7                  |
| 3.   | DCU-101  | Bacteria | 12                       | 1.6                  |
| 4.   | DCU-184  | Bacteria | 13                       | 2.3                  |
| 5.   | DCU-188  | Bacteria | 16                       | 2.5                  |
| 6.   | DCU-104  | Bacteria | 15                       | 2.4                  |
| 7.   | DCU-107  | Bacteria | 12                       | 1.8                  |
| 8.   | DCU-251  | Bacteria | 19                       | 3.1                  |
| 9.   | DCU-252  | Bacteria | 14                       | 2.2                  |
| 10.  | DCU-253  | Bacteria | 13                       | 2.1                  |
| 11.  | DCU-254  | Bacteria | 14                       | 2.4                  |
| 12.  | DCU-258  | Bacteria | 15                       | 2.5                  |
| 13.  | DCU-259  | Bacteria | 14                       | 2.3                  |
| 14.  | DCU-260  | Bacteria | 13                       | 2.5                  |
| 15.  | DCU-261  | Bacteria | 15                       | 2.2                  |
| 16.  | DCU-262  | Bacteria | 17                       | 2.9                  |
| 17.  | DCU-112  | Bacteria | 14                       | 2.6                  |
| 18.  | DCU-116  | Bacteria | 13                       | 2.1                  |
| 19.  | DCU-121  | Bacteria | 14                       | 2.5                  |
| 20.  | DCU-551  | Bacteria | 11                       | 1.5                  |
| 21.  | DCU-553  | Bacteria | 14                       | 2.4                  |
| 22.  | DCU-555  | Bacteria | 13                       | 2.2                  |
| 23.  | DCU-451  | Bacteria | 14                       | 2.5                  |
| 24.  | DCU-256  | Bacteria | 13                       | 2.3                  |
| 25.  | DCU-453  | Bacteria | 15                       | 2.5                  |
| 26.  | DCU-563  | Bacteria | 13                       | 2.3                  |
| 27.  | DCU-567  | Bacteria | 12                       | 2.1                  |
| 28.  | DCU-568  | Bacteria | 14                       | 2.5                  |
| 29.  | DCU-569  | Bacteria | 12                       | 2.4                  |
| 30.  | DCU-570  | Bacteria | 13                       | 2.5                  |
| 31.  | DCU-651  | Bacteria | 15                       | 2.6                  |
| 32.  | DCU-264  | Bacteria | 14                       | 2.3                  |
|      |          |          | SEm                      | 0.681                |
|      |          |          | CV                       | 0.166                |
|      |          |          | CD(5%)                   | 1.456               |
|      |          |          |                          | 0.388               |
|      |          |          | CV                       | 5.152               |
|      |          |          |                          | 4.134               |

Table 5: Quantitative estimation of soluble P in broth containing tri calcium phosphate by rhizobacterial isolates

| S.N. | Isolates | Microbes | 5 Days Soluble P (µg/ml) | pH | 10 Days Soluble P (µg/ml) | pH | 15 Days Soluble P (µg/ml) | pH |
|------|----------|----------|--------------------------|----|--------------------------|----|--------------------------|----|
| 1.   | DCU-22   | Bacteria | 95                       | 6.11 | 190                       | 6.68 | 298                       | 5.72 |
| 2.   | DCU-4    | Bacteria | 92                       | 6.83 | 185                       | 6.67 | 282                       | 5.69 |
| 3.   | DCU-101  | Bacteria | 75                       | 6.11 | 160                       | 6.68 | 268                       | 5.72 |
| 4.   | DCU-184  | Bacteria | 99                       | 6.34 | 233                       | 6.23 | 279                       | 5.84 |
| 5.   | DCU-188  | Bacteria | 94                       | 6.43 | 180                       | 6.23 | 270                       | 6.12 |
| 6.   | DCU-104  | Bacteria | 93                       | 6.47 | 136                       | 6.52 | 171                       | 5.86 |
| 7.   | DCU-107  | Bacteria | 95                       | 5.12 | 141                       | 5.0  | 253                       | 4.23 |
| 8.   | DCU-251  | Bacteria | 103                      | 6.69 | 248                       | 6.41 | 326                       | 5.39 |
| 9.   | DCU-252  | Bacteria | 88                       | 6.87 | 184                       | 5.88 | 245                       | 5.23 |
| 10.  | DCU-253  | Bacteria | 82                       | 7.11 | 138                       | 6.49 | 261                       | 5.41 |
| 11.  | DCU-254  | Bacteria | 92                       | 7.34 | 180                       | 6.47 | 219                       | 5.84 |
| 12.  | DCU-258  | Bacteria | 97                       | 7.34 | 195                       | 6.35 | 270                       | 5.66 |
| 13.  | DCU-259  | Bacteria | 84                       | 6.21 | 180                       | 6.68 | 276                       | 5.72 |
| 14.  | DCU-260  | Bacteria | 88                       | 6.45 | 154                       | 6.12 | 297                       | 5.23 |
| 15.  | DCU-261  | Bacteria | 86                       | 6.67 | 178                       | 5.97 | 246                       | 5.34 |
| 16.  | DCU-262  | Bacteria | 101                      | 6.95 | 207                       | 6.81 | 305                       | 5.91 |
| 17.  | DCU-112  | Bacteria | 86                       | 7.23 | 227                       | 6.64 | 253                       | 5.63 |
| 18.  | DCU-116  | Bacteria | 82                       | 6.98 | 131                       | 7.15 | 195                       | 6.23 |
| 19.  | DCU-121  | Bacteria | 87                       | 6.22 | 159                       | 5.88 | 188                       | 5.56 |
| 20.  | DCU-551  | Bacteria | 88                       | 6.77 | 126                       | 6.39 | 193                       | 5.73 |
Table 6: Molecular identification of potential rhizobacterial isolates

| S. N. | Isolates | Name of bacteria | strain | Isolation source | Matching with NCBI database including accession number | % Similarity | Accession number |
|-------|----------|------------------|--------|------------------|-----------------------------------------------------|-------------|-----------------|
| 1.    | DCU-22   | *Bacillus* paramycoides | NRCSSDCU22 | cumin rhizospheric soil | *Bacillus paramycoides* strain MCCC 1A04098 16S ribosomal RNA, partial sequence [NR_157734.1] | 100 % | MN192162 |
| 2.    | DCU-184  | *Pseudomonas* aeruginosa | NRCSSDCU184 | cumin root | *Pseudomonas aeruginosa* strain ATCC 10145 16S ribosomal RNA, partial sequence [NR_114471.1] | 99.44% | MN192163 |
| 3.    | DCU-188  | *Pseudomonas* aeruginosa | NRCSSDCU188 | cumin rhizospheric soil | *Pseudomonas aeruginosa* strain DSM 50071 16S ribosomal RNA, partial sequence [NR_117678.1] | 99.60% | MN192164 |
| 4.    | DCU-251  | *Pseudomonas* aeruginosa | NRCSSDCU251 | cumin rhizospheric soil | *Pseudomonas aeruginosa* strain NBRC 12689 16S ribosomal RNA, partial sequence [NR_113599.1] | 99.40% | MN192165 |
| 5.    | DCU-262  | *Kosakonia* oryzendophytica | NRCSSDCU262 | cumin root | *Kosakonia oryzendophytica* strain REICA_082 16S ribosomal RNA, partial sequence [NR_125586.1] | 99.03% | MN192166 |
| 6.    | DCU-451  | *Pseudomonas* aeruginosa | NRCSSDCU451 | rhizospheric soil | *Pseudomonas aeruginosa* strain DSM 50071 16S ribosomal RNA, partial sequence [NR_117678.1] | 99.74% | MN192168 |
| 7.    | DCU-453  | *Pseudomonas* aeruginosa | NRCSSDCU453 | rhizospheric soil | *Pseudomonas aeruginosa* strain NBRC 12689 16S ribosomal RNA, partial sequence [NR_113599.1] | 100% | MN192169 |
| 8.    | DCU-651  | *Bacillus* pacificus | NRCSSDCU651 | cumin root | *Bacillus pacificus* strain MCCC 1A06182 16S ribosomal RNA, partial sequence [NR_157733.1] | 99.08% | MN192170 |

Fig 1: Qualitative assay for P solubilization by rhizobacterial isolates.
Fig 2: Quantitative estimation of soluble P in broth containing tri calcium phosphate by rhizobacterial isolates

**Phylogenetic Tree:**

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Fig 2. Molecular Phylogenetic analysis by Maximum Likelihood method
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Fig 2. Molecular Phylogenetic analysis by Maximum Likelihood method
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Fig 2. Molecular Phylogenetic analysis by Maximum Likelihood method
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Fig 2. Molecular Phylogenetic analysis by Maximum Likelihood method
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Fig 3: Phylogenetic tree based on analysis of partial 16S rDNA nucleotide sequences

Plate 1: Morphological and cultural characteristics of PSB isolates
Catalase activity

Plate 2: Biochemical tests of rhizobacterial PSB isolates
Conclusion
Keeping in view of experimental findings it can be concluded that the isolate DCU-251 (Pseudomonas aeruginosa strain NRCCSDCU251 Accession no. MN192165) is the most potent phosphate solubilizers followed by isolate DCU-262 (Kosakonia oryzendophytica strain NRCCSDCU262 Accession no. MN192166). However, these isolates can be used in arid and semi arid region of country especially Rajasthan for phosphate solubilization to maintain the soil fertility.

Acknowledgement
I am greatful to the Devision of crop production, ICAR-National Research Centre on Seed Spices, Tabiji, Ajmer (Rajasthan) for providing facilities for this research.

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