Describing *Paenibacillus mucilaginosus* strain N3 as an efficient plant growth promoting rhizobacteria (PGPR)

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**Abstract:** Bacterium *Paenibacillus mucilaginosus* strain N3 was isolated from agricultural farm soil (located at Boriavi village, Gujarat, India). Isolate showed an evidence of non-symbiotic nitrogen fixation, when grown in nitrogen-free bromothymol blue growth medium. It was tested positive for direct plant-growth-promoting traits like Indole-3-acetic acid production, solubilization of Tri-calcium-phosphate, and ammonia production. Further, N3 isolate was tested positive for siderophore production of catecholate type and catalase production as an indirect plant-growth-promoting trait. Biochemical tests along with 16s rRNA gene sequence analysis confirmed the strain N3 to be *P. mucilaginosus*. To determine its efficacy as a plant-growth-promoting rhizobacteria (PGPR), its talc-based biofertilizer was prepared and tested on the growth of green gram (*Vigna radiata*). Seeds treated with this biofertilizer showed an increase in overall dry biomass by 17% and sapling length by 28% (as compared to non-treated controls) after 10 days of sowing in pots. Thus, multiple plant-growth-promoting traits of *P. mucilaginosus* N3 determined in vitro along with its ability to promote growth in green gram in vivo we characterize this strain as an efficient PGPR.

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**PUBLIC INTEREST STATEMENT**

Commercially available microbial biofertilizers contain strains of bacteria which associate with the roots of the plant and imparts beneficial effect on plant. Such microbial strains possess symbiotic relation with plants. Most widely available biofertilizers contain nitrogen-fixing microbes like *Rhizobium*, *Azotobacter*, *Azospirillum*, *Blue green Algae*, or contain phosphate solubilizing strains of *Pseudomonas* and *Bacillus*. To improvise microbial biofertilizers, there is a need to isolate and characterize new microbial strains which are more efficient or possess more than one trait which aids growth promotion in plants. Thus, with this article, we describe isolation and characterization of new microbial strain possessing ability to enhance growth of the plant, such research will introduce new and efficient strains which can be used to develop more efficient biofertilizers.
1. Introduction

*Paenibacillus* genus was separated from *Bacillus* before 20 years by Ash, Priest, and Collins (1993). They claimed that strains of *Paenibacillus* have dissimilarity in the consensus region of 16s rRNA as compared to strains of *Bacillus*. To our interest, species of *Paenibacillus* strains provides them superiority. Characteristics that distinguish the genus *Paenibacillus* from other members of *Bacillus* includes, rod-shaped bacterial cells with a flagellum; producing ellipsoidal spores with swollen sporangia; positive for catalase; negative for H₂S production; variable for oxidase; G + C content of their DNA ranging between 45% and 54 mol%, with anteiso-C₁₅: 0 as the major cellular fatty acid, meso-diaminopimelic acid as the diagnostic diamino acid, and more than 89.6% intra-genus similarity in 16s rRNA gene sequences (Ash et al., 1993).

*Paenibacillus polymyxa* (formerly *Bacillus polymyxa*) is most widely studied strain from this genus; it has ability to fix nitrogen and capable of producing antibiotic named polymyxin, and it is widely used as biocontrol agent for control of phytopathogens in agriculture (Eastman, Weselowski, Nathoo, & Yuan, 2014). Microbes are considered as a major resource for agricultural applications (Patel, Jha, Tank, & Saraf, 2011); however, strains other than *P. polymyxa* belonging to this genus are poorly studied for their role in agriculture.

Importance of microbes in agriculture become popular since late 1970s and such plant beneficial bacteria are termed as PGPR (Kloepper & Schroth, 1978). But genus *Paenibacillus* was proposed much later and described in 2nd edition of Bergey’s manual of systematic bacteriology which was published in 2004 (Naing et al., 2014). Since then there are limited reports describing strains of *Paenibacillus* as PGPR. Strains of *Paenibacillus* have not received comparable importance as efficient PGPR as strains of *Acinetobacter*, *Agrobacterium*, *Arthrobacter*, *Azotobacter*, *Sinorhizobium*, *Azospirillum*, *Burkholderia*, *Bradyrhizobium*, *Rhizobium*, *Frankia*, *Serratia*, *Thiobacillus*, *Pseudomonads*, and *Bacillus* have received (Jacobson, Pasternak, & Glick, 1994; Sunar, Dey, Chakraborty, & Chakraborty, 2013; Vessey, 2003). However, over the period of last 10 years, few strains of *Paenibacillus* were rediscovered as nitrogen fixers which helped them to prove superiority over other extensively studied PGPR. Few nitrogen-fixing *Paenibacillus* strains include *P. polymyxa*, *P. macerans*, *P. durus*, *P. peoriae*, *P. borealis*, *P. brasilensis*, *P. graminis*, *P. odorifer*, *P. wynnii*, *P. massiliensis*, and *P. sabinae* (Beneduzi et al., 2010; Xie et al., 2014).

Thus, there is a huge scope in isolating new stains of *Paenibacillus* and studying them for their plant-growth-promoting traits which can help them to characterize as cogent PGPR. Such study will help to introduce new and efficient strains for agricultural applications.

To portray any new strain as PGPR, it is necessary to know the mechanics employed by PGPR to enhance plant growth. The augmentative effect of PGPR occurs through various mechanisms which are primarily distinguished in the direct and indirect mechanisms. Direct mechanisms include production of plant hormones such as indole acetic acid (IAA), gibberellins, and cytokinins (Dey, Pal, Bhatt, & Chauhan, 2004; Patten & Glick, 1996) along with asymbiotic nitrogen fixation (Kennedy et al., 1997); and solubilization of phosphates (Bonerjee & Yasmin, 2002; de Freitas, Banerjee, & Germida, 1997; Richardson, 2001). On the other hand, indirect mechanisms are the production of iron chelators, siderophores, and cyanides (Ahmad, Ahmad, & Khan, 2008; Cattelan, Hartel, & Fuhrmann, 1999; Goswami, Dhandhukia, Patel, & Thakker, 2014) since they act as antagonists to plant pathogens.

Under present study one such strain N3, capable of growing in nitrogen-free medium belonging to *Paenibacillus* genus was isolated from fertile agricultural soil. Strain was identified using 16s rRNA gene sequencing which showed high similarity with *Paenibacillus mucilaginosus*. The aim of the research was to portray this strain capable of growing in nitrogen-free growth medium, isolated from...
fertile rhizospheric soil as a PGPR. *In-vitro* biochemical analysis was performed to assess its multiple plant–growth-promoting traits which included ammonia production, phosphate solubilization, nitrogen fixation, IAA production, siderophore production, hydrogen cyanide (HCN) production, and catalase production. Plant-growth-promoting ability of strain N3 was *in vivo* determined by preparing talc-based biofertilizer and checking its effect on the seedling growth of green gram (*Vigna radiata*).

2. Materials and methods

2.1. Isolation of bacterial strain

The rhizospheric soil samples were collected from the agricultural field 15 cm below the soil surface, located at Boriavi village, Anand, Gujarat, India (22°61′ N, 72°93′ E). Soil sample was aseptically suspended in sterile distilled water and after allowing it to stand for an hour, supernatant was spread on nitrogen-free medium (Glucose: 50 g l⁻¹, K₂HPO₄: 0.2 g l⁻¹, NaCl: 0.2 g l⁻¹, K₂SO₄: 0.1 g l⁻¹, CaCO₃: 5.0 g l⁻¹, Agar: 25 g l⁻¹, pH 6.8, prepared in distilled water) with appropriate dilution and the plates were incubated at 27°C for 48 h.

2.2. Identification of the organism

Morphological analyses were performed by using a standardized method (Murray, Doetsch, & Robinow, 1994). Biochemical tests described in Bergy’s Manual of Systematic Bacteriology were performed as per the methods described by Shi, Takano, and Liu (2012). All the media and reagents required to perform biochemical tests were obtained from HiMedia.

For identification of bacterial isolate, 16s rRNA gene sequence was determined and analyzed. Genomic DNA from strain N3 was extracted and 16s RNA gene was amplified using polymerase chain reaction using universal forward primer (5′-AGAGTTTGATCCTGGCTCAG-3′) and reverse primer (5′-AAGGAGGTGATCCAGCCGCA-3′) which were procured from 1st BASE (Agile Life Science Technologies India Pvt. Ltd) (Goswami, Dhandhukia, et al., 2014; Goswami, Pithwa, Dhandhukia, & Thakker, 2014). The reaction was carried out in a 50 μl mixture containing 1.5 mM MgCl₂, 0.2 mM each dNTP, 25 pmol of forward and reverse primers, 50 ng DNA template, and 5 U Taq DNA polymerase with its reaction buffer. Reaction was performed for 34 cycles using thermocycler at 94°C for 45 s, 58°C for 45 s, and 72°C for 105 s followed by a final extension of 10 min at 72°C. Amplified gene product of (1.6 Kb) was sequenced at 1st BASE (Agile Life Science Technologies India Pvt. Ltd). The BLASTn search program (http://www.ncbi.nlm.nih.gov) was used for sequence homology analysis. The gene sequences were also submitted to GenBank and accession numbers were assigned. The sequence obtained was then aligned by ClustalW using MEGA 4.0 software (Tamura, Dudley, Nei, & Kumar, 2007) and a neighbor-joining (NJ) tree with bootstrap value 1,000 was generated using the software.

2.3. Qualitative and quantitative estimation of phosphate solubilization

Qualitative determination of phosphate solubilization was performed on Pikovskaya’s agar plate. Isolate was spot inoculated and incubated at 27 ± 2°C, and the size of the halo corresponding to phosphate solubilization was measured after 7 days of incubation. Quantitative estimation of tri-calcium phosphate solubilization was performed by growing the strain in Pikovskaya’s broth. The concentration of the soluble phosphate was determined from the culture supernatant at 10th, 15th, and 20th day after inoculation by stannous chloride method (King, 1932). Organic acid produced due to glucose metabolism (in Pikovskaya’s broth) was determined by monitoring the change in the pH of the medium.

2.4. IAA production

Indole-3-acetic acid (IAA) production was estimated using the method described by Bric, Bostock, and Silverstone (1991). About 10% of exponentially grown culture of strain N3 was inoculated in 100 ml nitrogen-free medium with varying concentration of L-tryptophan which ranged from 0 to 500 μg ml⁻¹ in different flasks. Bacterial cell-free supernatant was taken at 24, 48, and 72 h for performing quantitative estimation of IAA. Briefly, 1 ml of cell-free supernatant was mixed vigorously with 1 ml of Salkowsky’s reagent (1 ml of 0.5 M FeCl₃ in 50 ml of 35% HClO₄) followed by the addition of two drops of orthophosphoric acid and assay system was kept at 27 ± 2°C in dark for 20 min till
pink color developed. Optical density of this colored solution was measured spectrophotometrically at 535 nm. The concentration of IAA in each sample was determined from the standard curve of IAA ranging from 10 to 100 μg ml⁻¹.

2.5. Siderophore estimation
Siderophore production was determined in deferrated MM9 medium supplemented with 1% (w/v) Glucose, using CAS-shuttle assay (Payne, 1994). Strain N3 was grown in deferrated MM9 medium with 1% (w/v) glucose. About 10 ml sample was withdrawn and centrifuged at 2,700 g for 15 min; 0.5 CAS assay solution was added to 0.5 ml of culture supernatant and mixed. This mixture was allowed to stand for 20 min. Siderophore (if present in the culture supernatant) will remove the iron from the dye complex, resulting in a loss of blue color of the solution. Decrease in the intensity of blue color was determined by measuring absorbance at 630 nm. Siderophore produced was calculated using the formula: 

\[ \frac{(A_r - A_s)}{A_r} \times 100 = \% \text{siderophore units.} \]

where, \( A_r \) is the absorbance of reference (minimal media + CAS assay solution) and \( A_s \) is the absorbance of sample (culture supernatant + CAS assay solution).

For qualitative estimation of catecholate type of siderophores, 1 ml of culture supernatant was mixed with 1 ml of 0.5 N HCl, followed by the addition of 1 ml of sodium molybdate reagent (10 g of sodium nitrite and 10 g of sodium molybdate in 100 ml of distilled water), and allowed to stand for 15 min. To this mixture, 1 ml of 1 N NaOH was added. Pink color, if develops in this solution, indicates the presence of catecholate type of siderophores (Carson, Holliday, Glenn, & Dilworth, 1992).

2.6. Non-symbiotic nitrogen fixing ability and ammonia production
Nitrogen-fixing ability was qualitatively studied by allowing the strain N3 to grow in nitrogen-free bromothymol blue (NfB) medium (Sucrose: 10 g l⁻¹, K₂HPO₄: 0.6 g l⁻¹, MgSO₄: 0.20 g l⁻¹, NaCl: 0.2 g l⁻¹, K₂SO₄: 0.1 g l⁻¹, CaCO₃: 2.0 g l⁻¹, pH 6.8, 2.0 ml of 0.5% Bromothymol blue solution). Here, the strain possessing the ability to fix nitrogen will increase the pH of the medium which is determined by the color change of the medium from green to blue (Jha et al., 2010).

Ammonia production by the strain N3 was determined in peptone water broth. Briefly, strain N3 was inoculated into the medium and was allowed to grow at 27 ± 2°C, where the assay for ammonia production was performed for 4 days at an interval of every 24 h. Broth was collected, centrifuged, and the amount of ammonia in the supernatant was estimated by means of Nesslerization reaction where, 1 ml Nessler’s reagent was added to 1 ml of supernatant and volume of this mixture was made up to 10 ml by addition of ammonia-free distilled water. Development of brown to yellow color indicated positive result for ammonia production and its optical density was measured by spectrophotometer at 450 nm (Cappucino & Sherman, 1992). The concentration of ammonia was estimated based on a standard curve of ammonium sulfate ranging from 0.1 to 1 μmol ml⁻¹.

2.7. Catalase test
Presence of catalase was checked qualitatively using the method described by Goswami, Patel, et al. (2014). Solution of 6% H₂O₂ was added on the colonies grown on nitrogen-free agar plates; effervescences of O₂ released from the bacterial colonies indicate the presence of catalase activity.

2.8. Preparation of talc-based biofertilizer and seed bacterization
The talc-based formulations of N3 isolate was prepared by the method described by Goswami, Vaghela, Parmar, Dhandhukia, and Thakker (2013). Briefly, talc powder was taken in a sterilized metal tray and its pH was adjusted to neutral by addition of 15 g of CaCO₃ per kg of talc. To this, 10 g of CMC was added and the mixture was autoclaved. About 400 ml of 48 h grown bacterial suspension was mixed with carrier-cellulose mixture under aseptic conditions. After drying (approximately 35% moisture content) overnight under sterile conditions, it was used for seed bacterization. Slurry of talc-based biofertilizer was prepared and surface-sterilized seeds (seeds dipped in 70% ethanol
for 3 min and rinsed with sterile distilled water) were soaked overnight for the biofertilizer to get coated on seeds. Efficacy of biofertilizer was tested on wild-type seeds of green gram (V. radiata). These seeds treated with biofertilizer were sown in the pots sized (10 × 6 cm) and the growth parameters of seedlings were analyzed. All the pot experiments were carried out during the month of May where the average temperature was 37.0 ± 2.0°C and average humidity was 45%. Further, the soil used for the growth for chick pea and green gram was tested for its physical and chemical properties. Physical attributed Sieve analysis and Atterberg limit (Plastic limit, Liquid Limit and Plasticity Index; Dhandhukia, Goswami, Thakor, & Thakker, 2013). For chemical properties, total nitrogen, conductivity, TDS, pH, phosphates, sodium, potassium, calcium and magnesium were estimated from the soil sample (Ibekwe, Poss, Grattan, Grieve, & Suarez, 2010).

2.9. Statistical analysis
After the germination of test plants, the difference resulted by the biofertilizer treatment on the seedling growth was analyzed using statistical analysis. For this, analysis of variance (ANOVA) was carried out using triplicate value to identify significant difference in each vegetative parameter between treated and non-treated seeds. Mean values of triplicates were compared at significance levels of 5 and 1%.

3. Results

3.1. Isolation of bacterial strain
From the soil sample used to isolate bacterial strains on nitrogen-free medium, total three types of colonies were obtained. The most dominant strain growing on the minimal medium was isolated and purified. Strain was designated as N3, where “N” stands for ability of the strain to grow in absence of nitrogen. Colonies were big, round, entire, transparent, colorless, sticky, and odorless. Image of colonies of strain N3 growing on nitrogen-free agar medium is shown in Figure 1.

3.2. Identification of the organism
Microscopic observation of strain N3 showed that it was gram negative and produced large capsules. Strain showed optimum growth at 30°C, with constant agitation at neutral pH of the growth medium. Biochemical tests showed that strain N3 was positive for oxidase, catalase, and nitrite reduction test; however, strain was found negative for amylase, gelatinase, urease, indole production, methyl-red test, and Vogus–Proskauer (VP) test. Strain showed acid production when grown with glucose, galactose, glycerol, sucrose, and mannitol; whereas, fructose, lactose, starch, sorbitol, maltose, citrate were not utilized by the strain (Table 1).

Phylogenetic analysis was done by comparing 16s rRNA gene sequence of N3 and the sequences of other type strains of genus Paenibacillus from GenBank to identify the isolated strain. Accession numbers of Paenibacillus type strains were obtained from “Bergey’s Manual of Systematic Bacteriology, Second Edition, Volume Three: The Firmicutes” to construct phylogenetic tree. Phylogenetic analysis
showed that 16s rRNA gene sequence of strain N3 matched best with gene sequence of type strain P. mucilaginosus AS1.231T under GenBank ID: DQ898308 (Figure 2). Based on biochemical characterization and phylogenetic analysis, strain N3 was designated as P. mucilaginosus strain N3 and its 16s rRNA gene sequence was submitted to nucleotide sequence database under GenBank ID: JX154206.

### 3.3. Estimation of phosphate solubilization

It was observed that strain N3 could solubilize phosphate on Pikovaskya's agar where the zone diameter of phosphate solubilization was 20 mm and diameter of spot inoculant was 12 mm after its growth for 7 days. Quantitative estimation of phosphate solubilization was carried out in liquid Pikovaskya's medium which contained glucose as a carbon source. Here, quantitative estimation of phosphate solubilized was determined at 10th, 15th, and 20th day of its growth. Strain N3 solubilized maximum of 11 μg ml⁻¹ of tri calcium phosphate after 15 days of incubation, though on 20th day the determined phosphate solubilized was less than 11 μg ml⁻¹ which may be due to utilization of solubilized phosphate by the strain. Analysis of pH altered in the growth medium by the bacterial metabolism suggested that the pH decreases from neutral to acidic due to organic acid production as a product of glucose metabolism (Figure 3).

### 3.4. IAA production

It was observed that strain N3 could produce IAA only when L-tryptophan was supplemented in the medium. IAA production by N3 isolate was determined at an interval of every 24 h after incubation (Figure 4). Maximum IAA produced was 13 μg ml⁻¹ after 72 h of incubation when L-tryptophan concentration in the medium was maximum (500 μg ml⁻¹). In the growth medium with absence of L-tryptophan, IAA was not detected even after 72 h. This shows that there is a direct correlation in IAA production and supplemented L-tryptophan in the medium.

| Table 1. Biochemical characterization of P. mucilaginosus strain N3 |
|---------------------------------------------------------------|
| **Biochemical test** | **Reaction** |
| Gram reaction | − |
| Capsule formation | + |
| Carbohydrate utilization |  |
| Fructose, lactose, starch, sorbitol, maltose, citrate | − |
| Glucose, galactose, glycerol, sucrose, mannitol | + |
| Oxidase | + |
| Catalase | + |
| Indole production | − |
| Nitrate reduction | + |
| Methyl red test | − |
| Vagés–Proskauer | − |
| Gelatin hydrolysis | − |
| Casein hydrolysis | − |
| Urease production | − |
| H₂S production | − |
| 2.0% NaCl tolerance | − |
| Growth at various temperatures (°C) |  |
| 25 | + |
| 37 | + |
| 40 | − |

Notes: “+” indicates positive and “−” indicates negative.
Figure 2. The phylogenetic analysis based on 16s rRNA gene sequences available from European Molecular Biology Laboratory (EMBL) library constructed after multiple alignments of data by ClustalX.

Notes: Figure shows the relationship between *P. mucilaginosus* strain N3 and related members of the genus *Paenibacillus*. Distances and clustering with the neighbor-joining method was performed using MEGA 4.0 software package. Bootstrap values based on 1,000 replications listed as per percentages at the branching points.

Figure 3. The phosphate solubilization by *P. mucilaginosus* strain N3 in liquid Pikovskaya’s medium that contains glucose as a carbon source.

Note: Organic acids produced in the growth medium is attributed by drop in the pH of the medium.
3.5. Siderophore and catalase production

Strain N3 showed production of siderophore in the iron-free MM9 minimal medium supplemented with 1% glucose which was detected by CAS-Shuttle assay. Maximum siderophore produced was 32% units (Figure 5). The culture supernatant when mixed with CAS solution, its inherent blue color was gradually lost which indicated presence of siderophore (Figure 6(a)). Further, it was found that strain N3 could produce catecholate type of siderophores which was determined by the reaction of culture supernatant with sodium molybdate reagent under standard reaction condition produced pink color in the solution (Figure 6(b)). Strain N3 was also found to produce catalase as strong effervescences of \( \text{O}_2 \) evolved when 6% \( \text{H}_2\text{O}_2 \) solution was flooded on the colonies grown on nitrogen-free agar.
3.6. Non-symbiotic nitrogen fixation and ammonia production

Ammonia production by strain N3 was determined in peptone water broth by Nesslerization reaction (Figure 6(d)). Maximum ammonia produced by the strain was 3.6 μmol ml⁻¹ after 96 h of growth (Figure 5). There are two evidences that prove strain N3 can fix nitrogen. First, the strain can easily grow in absence of any nitrogen source, indicating the nitrogen required for its growth must be available from the environment. Second, NFB medium which is nitrogen-free medium containing limiting amount of carbon source shows an increase in the pH when strain N3 is grown in it. The pH increase in the medium is visualized by the color change of the medium from green to blue, as the NFB contains pH sensitive dye bromothymol blue (Figure 6(c)). The increase in the pH caused by the metabolic activity of strain N3 in NFB medium is due to nitrogen fixation. Thus, these experiments are suggestive that the strain can fix atmospheric nitrogen.

3.7. Effect of bio-fertilizer on seedling growth of green gram

P. mucilaginosus strain N3 showed multiple traits of PGPR including production that included IAA, ammonia, siderophore, nitrogen fixation, and solubilization of phosphate; its efficacy as a PGPR was tested in vivo on the growth of green gram. Chemical properties of soil used for pot study are shown in Table 2. Green gram seeds were treated with biofertilizer prepared from P. mucilaginosus strain N3 and seeded in pots. After 15 days, the germinated seedlings were uprooted and their vegetative parameters including stem and root mass (Fresh mass and Dry mass), total length, root length, and stem length were measured. It was observed that biofertilizer-treated seedlings showed 29.0% increase in total length and 26.9% increase in the total fresh mass as compared to non-treated control (Figure 7). ANOVA analysis showed significant effect of biofertilizer treatment on vegetative parameters of the plant.

4. Discussion

Large soil microbial diversity is yet to be explored (Glick, 2014). Rhizosphere possess 100 folds higher bacterial density than in bulk soil as 5–21% of plant photosynthetic product is secreted by roots in form of different sugars which is in turn utilized by microbial populations (Govindasamy et al., 2011; Jha, Patel, Rajendran, & Saraf, 2010). Thus, the rhizospheric soil was selected for present study to isolate efficient strain (P. mucilaginosus strain N3) that can be categorized as efficient PGPR.

Over the period of last 10 years, new Paenibacillus strains are identified as nitrogen fixers (Xie et al., 2014). Lu, Xue, Cao, Yang, and Hu (2014) reported various strains of P. mucilaginosus could fix nitrogen and suggested its potential application in agriculture. Under present study, nitrogen

| Physical properties of soil |  |
|----------------------------|---|
| Gravel (+4.75 mm)          | 0% |
| Sand (0.075–4.75 mm)       | 31%|
| Silt + Clay (<0.075 mm)    | 69%|
| Liquid limit               | 26%|
| Plastic limit              | 24%|
| Plasticity index           | 02%|

| Chemical properties of soil |  |
|-----------------------------|---|
| pH                          | 6.8 |
| Electrical conductivity     | 0.21 mS cm⁻¹ |
| Salinity equivalent to NaCl | 32 mmol |
| Sodium% (as Na⁺)            | 0.038 |

Notes: Physical properties of soil were examined at Birla VishvakarmaMahavidyodaya Engineering College, Material Testing Department, VallabhVidhyanagar, Gujarat and chemical properties of soil were examined at Gujarat Laboratory, Ahmedabad.
fixing ability detected by *P. mucilaginosus* strain N3 supports the findings of Lu et al. (2014). Despite nitrogen fixing ability possessed by *Paenibacillus* strains they are poorly characterized for other traits aiding plant growth promotion. This lack of research has opened a new horizon to portray on the *Paenibacillus* strains as PGPR for development of sustainable agriculture. To overcome this constrain, present study is focused to demonstrate *P. mucilaginosus* strain N3 as nitrogen fixing bacteria along with possessing other plant-growth-promoting traits like IAA production, phosphate solubilization, siderophore production, ammonia, and catalase production.

Nitrogen and phosphorus are the most essential nutrient required for plant growth where, phosphorus is the second-most limiting nutrient for plants. Plants can absorb only mono and dibasic phosphate which are the soluble forms of phosphate (Jha, Patel, & Saraf, 2012). Phosphate-solubilizing microbes mineralize organic phosphorous in soil by solubilizing complex insoluble phosphates (Tri-calcium phosphate, Rock phosphate, Aluminum phosphate, etc.) and aid the phosphate availability to plants. Under present study, *P. mucilaginosus* strain N3 could efficiently solubilize tri-calcium phosphate in Pikovaskya’s medium. It was also observed that strain could produce organic acids which increases acidity of the medium. Here, phosphate solubilized by the strain is ascribed by acid produced; as under acidic environment complex form of phosphates brakes in to simple form, which is considered primary mechanism for phosphate solubilization by microbes (Goswami, Pithwa, et al., 2014). Stains of *Paenibacillus* that are known to solubilize phos-phate include *P. telluris* PS38, *P. polymyxa* B1-4, *P. brasilensis* PB177, and *P. kribensis* CX-7 (Ai-min, Gang-yong, Shuang-feng, Rui-ying, & Bao-cheng, 2013; Arthurson, Hjort, Muleta, Jäderlund, & Granhall, 2011; Lee, Kim & Yoon, 2011).

PGPRs are known to produce several phytohormones such as gibberellins, cytokinins, auxins, etc. IAA—an auxin is an efficient molecule known to enhance plant growth by stimulating apical dominance and root growth (Bal, Das, Dangar, & Adhya, 2013). Majority of PGPR produce IAA form L-tryptophan as a primary precursor, whereas some does not require L-tryptophan (Idris, Iglesias, Talon, & Borriss, 2007). *P. mucilaginosus* strain N3 showed IAA production only when L-tryptophan was supplemented in the growth medium indicating the pathway used to produce IAA was L-tryptophan dependent.

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**Figure 7. The growth analysis of green gram when treated with N3-based talc biofertilizer with non-treated control.**

Notes: “ns” suggests (p value greater than 0.05) non-significant as compared to control (ANOVA); “*” suggests (p value between 0.05 and 0.01) significant at 5% (ANOVA); and “**” suggests (p value between 0.01 and 0.001) significant at 1% as compared to control (ANOVA).
The most widely studied strain for IAA production among the genus Paenibacillus is *P. polymyxa*. Similar study was also performed by Lebuhn, Heulin, and Hartmann (1997) where they showed IAA production by *P. polymyxa* was directly dependent on supplementation of L-tryptophan. They also claimed that indole-3-ethanol, indole-3-lactic acid, and indole-3-carboxylic acid were also produced by *P. polymyxa* in addition to IAA on L-tryptophan supplementation.

*P. mucilaginosus* strain N3 also showed production of siderophores. Siderophores are produced in the rhizosphere by PGPRs to quench iron. Iron-deficient condition in the rhizosphere caused by siderophore-producing PGPR inhibits the growth of unwanted organisms. Thus, siderophore-producing microbes antagonize the growth of plant pathogens in the rhizosphere eventually helps plant to remain healthy (Haas & Défago, 2005; Jan, Azam, Ali, & Haq, 2011).

To portray any rhizobacterial strain as PGPR, it should be recognized to IAA production, phosphate solubilization, ammonia production, nitrogen fixation, and siderophore production, further, it is inevitable for strain to show plant growth promotion *in vivo* under pot trials (George, Gupta, Gopal, Thomas, & Thomas, 2013; Goswami et al., 2013). Our previous reports suggest that *Pseudomonas* spp. strain OG which showed several traits of PGPR also enhanced growth of green gram (*V. radiata*). Similarly, Mehta, Walia, Kulshrestha, Chauhan, and Shirkot (2014) described phosphate solubilizing, *Bacillus circulans* CB7 can enhance the growth of tomato (*Solanum lycopersicum* L.) and described the strain as an efficient PGPR. Urease-producing *Pseudomonas aeruginosa* strain BG isolated from marine water was reported to show several traits of PGPR-enhanced growth of chick pea (*Cicer arietinum* L.; Goswami, Patel, et al., 2014). Under present study, we can categorize *P. mucilaginosus* strain N3 as an efficient PGPR as it possesses important PGPR traits and also shows growth promotion in green gram can be considered as an efficient PGPR.

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