Environment and Ecology 37 (1B) : 363—372, January—March 2019
Website: environmentandecology.com ISSN 0970-0420

# Response of Nanogypsum on the Performance of Plant Growth Promotory Bacteria Recovered from Nanocompound Infested Agriculture Field

**Parul Chaudhary, Anita Sharma**

Received 26 September 2018 ; Accepted 1 November 2018 ; Published on 22 November 2018

**Abstract** In the present study, 20 bacterial isolates were recovered from a nanogypsum infested agricultural field of the University Campus, under wheat cultivation in the year 2016-2017. Recovered bacterial isolates had PGPR properties like solubilization of phosphate, zinc and production of siderophore, indole acetic acid, ammonia and hydrogen cyanide. Two bacterial isolates (PC1 and PC4), selected on the basis of best plant growth promotory properties had 98% homology with *Pseudomonas taiwanensis* and *Pantoea agglomerans* based on 16S rDNA sequence analysis and showed enhanced growth in the presence of 50 ppm nanogypsum. Results suggest that the application of nanogypsum supports bacterial growth in liquid medium hence can be used as bioformulation to enhance the shelf life of bacterial cultures, plant growth and productivity in agricultural field.

**Keywords** Nanogypsum, Nanoparticles, PGPR, Rhizobacteria, Infested agriculture field.

## Introduction

Plant growth promoting rhizobacteria (PGPR) play important role in the development of sustainable agriculture. These bacteria use a variety of (direct and indirect) mechanisms to stimulate plant growth (Arif et al. 2017). Direct mechanisms include mechanisms like solubilization of insoluble phosphate, Zn compounds and other nutrients (Johri et al. 1999), fixation of atmospheric nitrogen (Boddey and Dobereiner 1995) and hormone production such as gibberellic acid, auxins and cytokinins (Glick1995). Indirect mechanisms reveal the role of PGPR in control of pathogens through production of antibiotics, chitinase, Hydrogen cyanide and siderophores which chelate iron and make it available to the plants (Goswami et al. 2013). Fungi have been extensively studied for solubilization of insoluble zinc and phosphate compounds. Phosphorus is the second important key element after nitrogen required to enhance plant health. Although phosphate is abundantly present in soils in organic and inorganic forms, but its availability is restricted as it occurs mostly in insoluble forms. Role of P-solubilizing microorganisms (PSM) in providing phosphorus for enhanced growth of different cropping systems has been reported by many authors. Several bacterial (*pseudomonads* and *bacilli*) and fungal strains (*Aspergilli* and *Penicillium*) have been identified as PSM (Sharma et al. 2013). HCN, a common secondary metabolite and volatile in nature plays a significant role in antagonism of bacteria against phytopathogens and affects growth and development of plants and microorganisms (Siddiqui 2006). Ammonia production is most important traits of PGPR which is beneficial to various crops. Joseph et al. (2012) reported ammonia production in *Bacillus* sps. (95%), *Pseudomonas* (94.2%), *Rhizobium* (74.2%) and *Azotobacter* (45%). According to Mishra et al. (2010), *B. subtilis* strain MA-2 and *Pseudomonas fluorescens* strain MA-4 were good ammonia producer and increased biomass of *Geranium,* a medicinal aromatic plant. Interaction

Parul Chaudhary*, Anita Sharma
Department of Microbiology, College of Basic Science and Humanities, G. B. Pant University of Agriculture and Technology, Pantnagar, Uttarakhand, India
e-mail: parulchaudhary1423@gmail.com
*Corresponding author

between soil microbes and minerals play a major role in nutrient recycling processes, which leads to the mobilization of nutrients from soil components into available forms for biological uptake and enhance plant growth and yield.

At present nanotechnology is extensively used in different sectors including agriculture to promote concept of precision agriculture. Nanocompounds having one or more dimensions in the order of 100 nm or less show extra ordinary physical properties and may find application in plant protection and nutrition management because of their size, high surface to volume ratio and unique optical properties (Auffan et al. 2009, Ghormade et al. 2011). Nanoparticles with unique properties can be easily synthesized from different biological sources and applied in agriculture. Gypsum is a soluble source of calcium and sulfur which improves plant growth. Amendments of gypsum can also improve physical properties of some soils. It promotes soil aggregation which prevents dispersion of soil particles, reduces surface crust formation, promotes seedling emergence and increases water infiltration rates and movement through the soil profile (Dontsova et al. 2005).

The main emphasis of this research is to screen the best PGPRs from nanocompound infested soil and their growth pattern in presence of nanogypsum @ 50 mg/L concentration.

## Materials and Methods

### Soil sample collection

Soil samples were collected from the agriculture field where nanogypsum was applied through soil on wheat crop. The experimental site was located at Norman E. Borlogue Crop Research Center of G. B. Pant University of Agriculture and Technology, Pantnagar. This site is situated at an altitude of 243.84 above mean sea level, 29$^{o}$N latitude and 79.3$^{o}$E longitudes.

### Isolation of bacteria

A composite soil sample was serially diluted up to $10^{6}$ dilution and plated in nutrient agar 100 µl of the diluted sample was poured in 25 ml of nutrient agar. After mixing properly inoculated plates were incubated at 27±1ºC for 24 h. Twenty bacterial colonies were selected on the basis of their morphological characteristics.

### Screening of bacterial isolates with plant growth promotory properties

All the recovered bacterial isolates were screened qualitatively and quantitatively for P and zinc solubilization and production of siderophore, indole acetic acid, hydrogen cyanide and ammonia.

### Qualitative estimation of PGPRs properties

#### *Zinc solubilization*

Screening of Zn solubilizing bacteria (ZSB) was done on basal agar medium supplemented with 0.1% ZnO according to Saravanan et al. (2003). Recovered bacteria were tested for their Zn solubilizing potential based on halo zone formation around the bacterial colonies.

#### Phosphate solubilization

Bacterial cultures were spot inoculated on Pikovskaya medium (HI media) and incubated for 4 to 6 days at 28ºC. Formation of halo zone around bacterial colony indicates phosphate solubilization by the bacteria (Pikovskaya 1948).

#### Siderophore production

Siderophore production in selected bacterial isolates was detected by using universal method. Bacterial cultures were spot inoculated on CAS agar and then incubated at 30ºC for 60 to 72 h. Formation of orange or yellow halo zone around the bacterial colony indicates a positive test for siderophore production. CAS agar plates were prepared by mixing Chrome Azurol'S (60.5 mg) in distilled water (50 ml), to which 10 ml 1 mM $FeCl_3$. $6H_2O$ in 10 mM HCL was added gradually. The mixture was then added to HDTMA solution (72.9 mg in 40 ml distilled water). Dark blue color solution obtained was autoclaved at 15 lb psi for 20 min. Sterilized nutrient agar (300 ml)

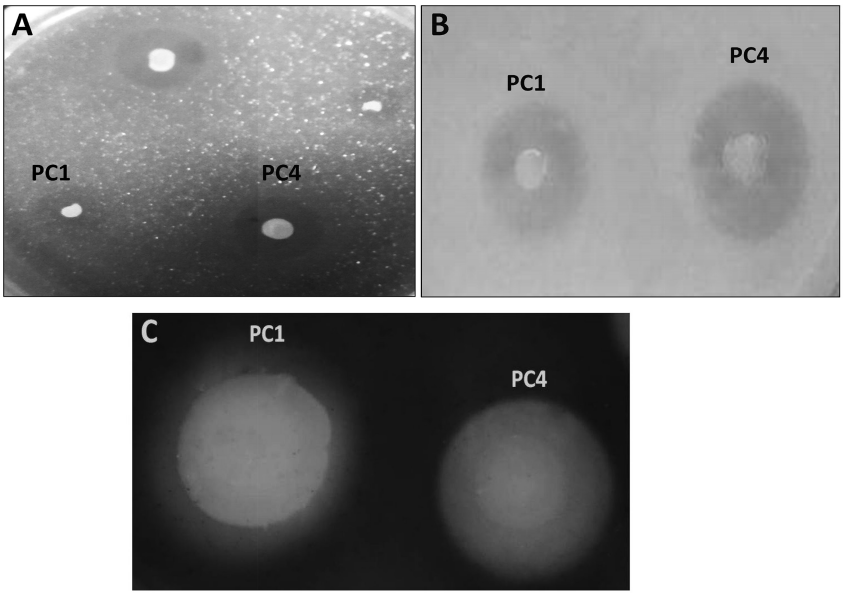


**Fig. 1.** PGPR properties (A) Zinc solubilization (B) Phosphate solubilization (C) Siderophore production of bacterial isolates PC1 and PC4.

was mixed with CAS solution in the ratio of 1 : 10 (CAS solution : media) (Yeole et al. 2001).

Quantitative estimation of indole acetic acid production (Gorden and Webber 1951)

Actively growing bacterial culture was inoculated in 5 ml of sodium succinate broth, supplemented with 100 µg / ml tryptophan. After incubation at 28±1ºC for 48 h, broth was centrifuged for 10 min at 10,000 rpm. After centrifugation, one ml culture supernatant was mixed with Salkovaski reagent (2 ml) and incubated at 30ºC for 25 min in dark to observe color change. Development of pink color indicates a positive test for IAA production by the test bacteria. Optical density of the colored mixture was recorded at 530 nm by using UV/visible spectrophotometer.

Ammonia production

Actively growing bacterial cultures were inoculated in 10 ml Peptone water and incubated for 72 h at 27±2°C in a rotatory shaker at 100 rpm. Production of ammonia was tested by adding Nessler's reagent (1 ml) to the bacterial culture after 4 days of incubation (Cappuccino and Sherman 1992). Presence of yellow to brown color indicates production of ammonia.

Qualitative estimation of HCN production

Test bacterial isolates were streaked on King's B medium amended with glycine (4.4 g/1). Sterile filter paper saturated with picric acid solution (2.5 g of picric acid ; 12.5 g of $Na_2CO_3$, 1000 ml of distilled water) was placed in the upper lid of the petri plate. Petri plates were sealed with parafilm and incubated at 28°C for 48 h. Change in color of the filter paper from yellow to light brown, brown or reddish-brown was recorded (Bakker and Schipper 1987).

Effect of nanogypsum on the growth of bacterial isolates

Effect of nanogypsum (50 ppm) was observed on the growth pattern of PC1 and PC4. Different treatments used are as follows. Blank (No culture and no nanogypsum, PC1, PC4, PC1+nanogypsum (50 ppm), PC4+nanogypsum (50 ppm).

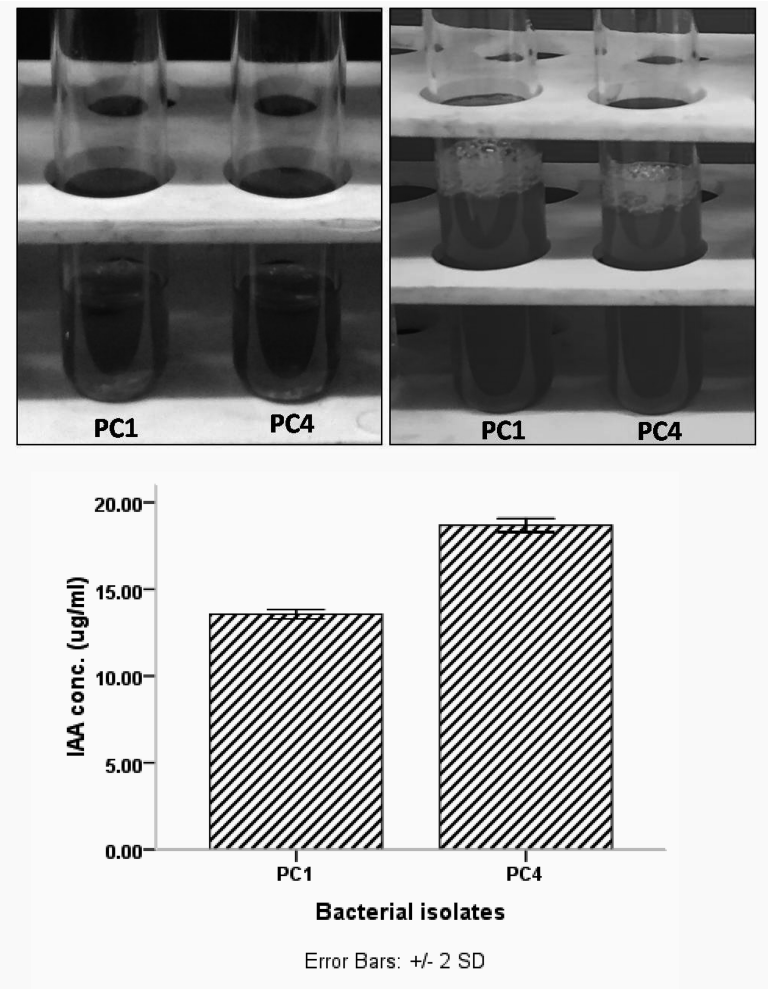


**Fig. 2.** IAA and ammonia production by bacterial isolates PC1 and PC4.

Stock solution of nanogypsum (2000 ppm) was sonicated at 20 KHz for 5–10 min. Aliquot of 1.25 ml from the stock solution of nanogypsum was added to nutrient broth (50 ml). Broth was autoclaved at 15 lb psi for 20 min. 50 µl of the active bacterial culture was inoculated into 50 ml of sterile nutrient broth with or without nanogypsum. Aliquots of 4 ml were regularly with drawn at an interval of 0, 24, 36, 48 and 72 h. 3 ml aliquot was used for taking the absorbance at 600 nm under visible spectrophotometer and 1 ml of the aliquot was used for pour plating after dilution. One ml from $10^5$ dilution was used for pour plating using nutrient agar. Plates were incubated at 27°C for 28 h and observed for bacterial counts.

Molecular characterization of bacterial isolates

Genomic DNA of 2 bacterial isolates (PC1 and PC4) was extracted by using HiPureA$^{TM}$ Bacterial Genomic DNA Purification Kit and 16S rDNA region was amplified using universal primer (27F: 5′-AGAGTTTGATCMTGGCTCAG-3′ and 1492R 5′-TACCTTGTTACGACTT-3′). Amplified genomic products of both the isolates were fully sequenced by Genei laboratories at Bangalore.

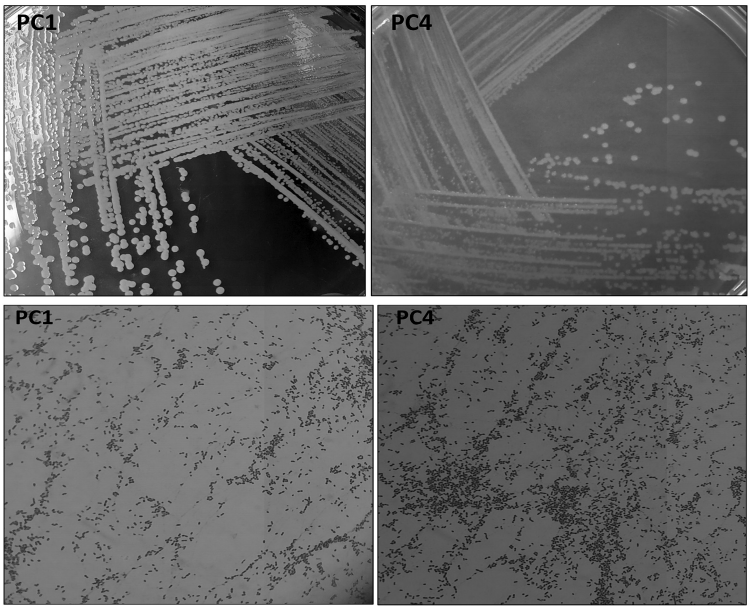


**Fig. 3.** Culture morphology and Gram staining of PC1 and PC4.

*Insilico analysis of sequence data*

16S rDNA sequences of test bacterial isolates were analyzed for homology with known 16S rDNA sequences available in NCBI (National Center) for Biotechnology Information) database using BLAST (Basic Local Alignment Search Tool) (Altschuh et al. 1990). Sequences were aligned by multiple sequence alignment using Clustal W algorithm program. To deduce the evolutionary relatedness of the aligned sequences, dendrogram was constructed using UPGMA (Unweighted Pair Group Method with Arithmetic Mean) with MEGA 6.0 (molecular evolutionary genetic analysis) software (Tamura et al. 2013). Neighbour joining method generates phylogenetic tree on the basis of distance matrix calculated from sequence data.

Statistical analysis

Analysis of variance (ANOVA) was done with statistical software SPSS statistics (version 19.0). All the experiments were conducted in triplicate and the data are shown as mean values ± standard deviation (SD). Results are considered statistically significant at 95% confidence interval ($p < 0.05$).

## Results

Zinc solubilization

Out of 20 bacterial isolates, 6 isolates did not show zone of clearance on basal medium supplemented with ZnO. Maximum zinc was solubilized by PC1 and PC4 (Fig. 1A).

Phosphate solubilization

Sixteen bacterial isolates solubilized phosphate on Pikovaskaya medium. PC1 and PC4 showed maximum P solubilization. Seven isolates showed moderate, 6 isolates showed least solubilization

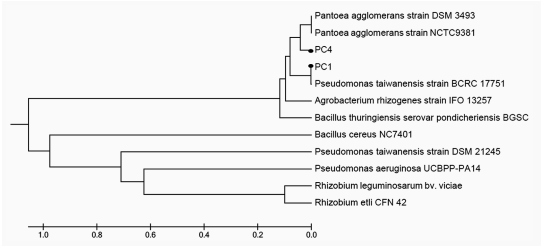


**Fig. 4.** Phylogenetic tree of isolated bacterial cultures constructed using MEGA 6.

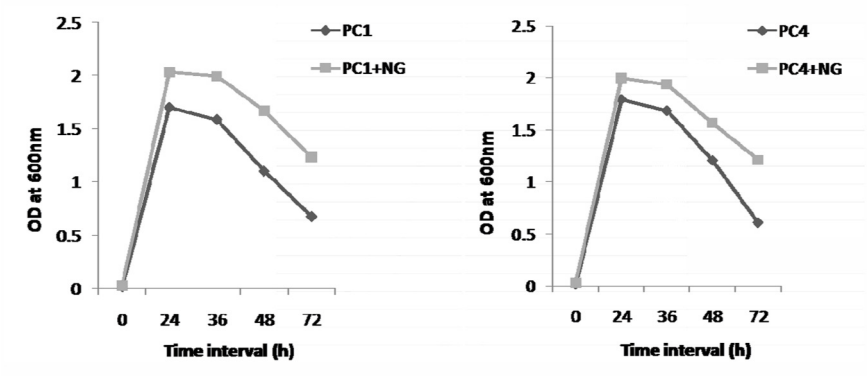


**Fig. 5.** Effect of nanogypsum on the growth of PC1 and PC4.

and 4 bacterial isolates did not solubilize phosphate compounds (Fig. 1B).

Siderophore production

Out of 20 bacterial isolates, PC1 and PC4 showed maximum siderophore production. Five isolates showed moderate production range, 9 isolates showed minimum production. Four bacterial cultures did not produce siderophore on CAS medium (Fig. 1C).

IAA production

All the 20 bacterial cultures showed IAA production. Four isolates produced maximum indole acetic acid. Nine isolates gave intermediate results while 7 bacterial isolates showed very low level of IAA production (Fig. 2).

Ammonia production

Out of 20 bacterial isolates 19 showed positive response for ammonia production. PC1 and PC4 gave best results for ammonia production and 11 isolates showed intermediate range of production. Three isolates showed minimum production of ammonia (Fig. 2).

HCN production

HCN is produced by many rhizobacteria. It is postulated to play a major role in biological control of pathogens. Two isolates PC6 and PC8 produced HCN.

Culture identification

On the basis of best PGPR properties PC1 and PC4 were selected for further studies. Both the isolates were gram negative and had short rods (Fig. 3).

*Molecular characterization*

Bacterial isolates (PC1 and PC4) were identified as *Pseudomonas taiwanensis* and *Pantoea agglomerans* respectively on the basis of BLAST match (Fig. 4).

Effect of nanogypsum on the growth of bacterial isolates

Overall growth of PC4 was higher than PC1. Both the bacterial cultures showed enhanced growth in the presence of nanogypsum (50 mg/L). CFU counts of bacterial cultures are related to optical density taken at 660 nm and supported positive response of nanogypsum on bacterial growth (Figs. 5 and 6).

**Discussion**

PGPRs play an important role in agriculture praces to improve plant and soil health. Agriculture scientists can take advantage of symbiotic relation hips between plants and microbes to enhance plant productivity and crops by manipulating the composition of microbial population of the soil (Asei et al. 2015). Different bacterial strains have the ability to induce

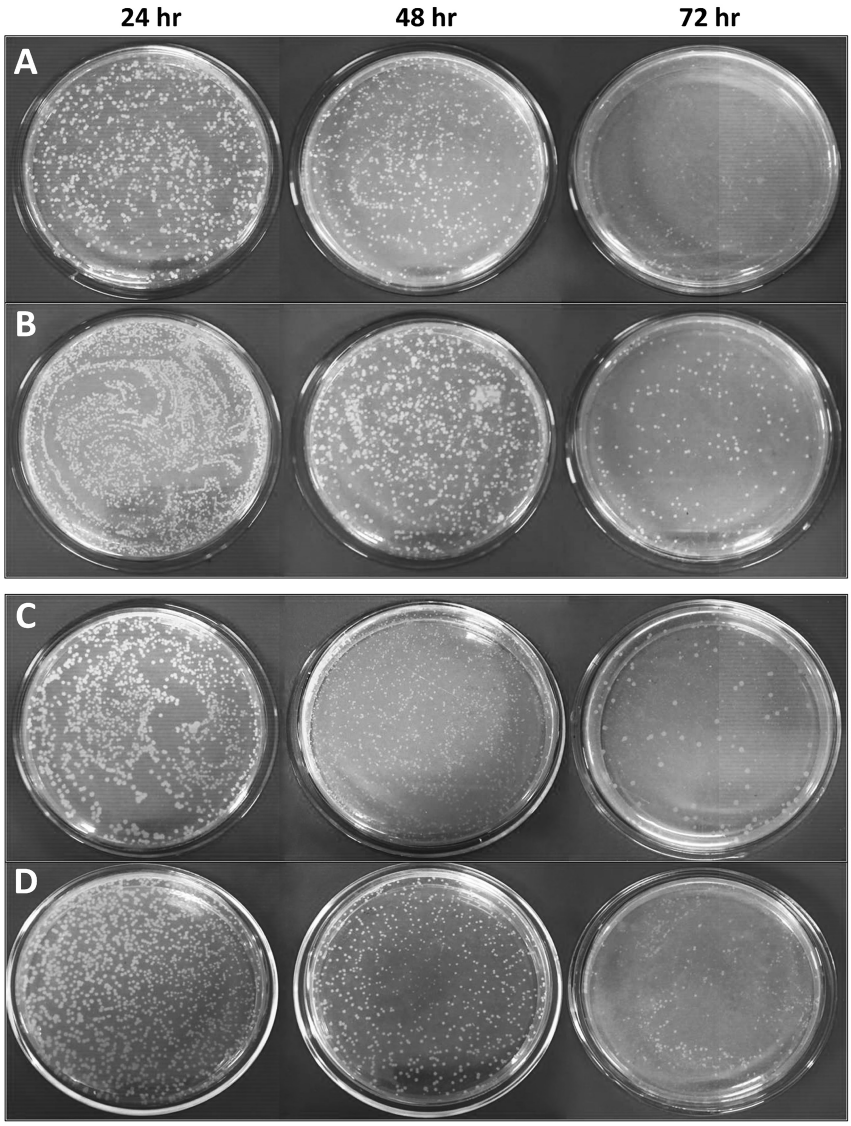


**Fig. 6.** CFU count (A) PC1 only (B) PC1 with nanogypsum (C) PC4 only (D) PC4 with nanogypsum.

growth promotion on diverse crop production (Nieto-Jacobo et al. 2017). In the present study 2 bacterial isolates PC1 and PC4, recovered from nanocompound infested wheat field showed best PGPR properties.

Zinc is among the essential micronutrients and required for optimum plant growth. In general inorganic zinc compounds are present in the soil but in unavailable to the plants. However, zinc solubilizing bacteria are potential alternatives for providing zinc. These microbes convert applied inorganic zinc to available forms (Kamran et al. 2017). Number of Zn solubilizing bacterial species like *Pseudomonas* sp., *Gluconacetobacter* sp., *Thiobacillus* sp., *Bacillus* sp., *Acinetobacter* sp. have been reported by various scientists (Vidyashree et al. 2016).

Phosphorus is the second most important key element next to nitrogen in the soil. It plays very important role in all the major metabolic pathways

in plants including photosynthesis, energy transfer, signal transduction, macromolecular biosynthesis and respiration (Khan et al. 2010). The main P solubilization mechanisms employed by soil microorganisms include: release of complexing or mineral dissolving compounds e.g. organic acid anions, liberation of extracellular enzymes like acid phosphatase and the release of P during substrate degradation (biological P mineralization) (McGill and Cole 1981). The use of efficient PSM (phosphate-solubilizing microorganisms), opens up a new horizon for better crop productivity besides maintaining soil health. Therefore, there is a need for extensive and consistent efforts to identify and characterize more PSM with greater efficiency for their ultimate application under field conditions. *Pseudomonas*, *Bacillus* and *Rhizobium* are some known P solubilizers.

Iron is an important micronutrient required by most of the living systems including microbes. Iron is highly insoluble in soil and often acts as a limiting factor in the rhizosphere. Some bacteria promote plant growth by producing low density iron chelator biomolecules called siderophore which act as chelating agents for iron. Iron binding ligands (siderophores) for iron acquisition have a competitive advantage over other microorganisms. Siderophores bind to ferric iron of the soil or the root zone and then taken up by outer membrane receptors. Affinity for ferric iron in siderophores depends on their structure, which are of 2 types hydroxymate- and phenolate / catecholate-type. The ability of bacterial siderophores to suppress phytopathogens through siderophores could be of significant agronomic importance (Beneduzi et al. 2013). In the present study, out of 20 bacterial cultures 16 isolates were found positive for siderophore production.

Indole acetic acid (IAA) is one of the most physiologically active auxins. It is a common product of L-tryptophan metabolism produced by several microorganisms including Plant Growth Promoting Rhizobacteria (PGPR) (Lynch 1985). Level of IAA production by bacteria may vary among different species and strains and it is also influenced by culture condition, growth stage and substrate availability (Mutluru and Konada 2007). The use of Salkowski reagen for the detection of IAA has found great significance. IAA positively influences root growth and development, thereby enhancing nutrient uptake (Khalid et al. 2004, Kisiel et al. 2016, Vandana et al. 2018). Auxins play a pivotal role in root growth and development (Overvoorde et al. 2010). Among all the isolates PC1 and PC 4 showed higher IAA production 13.56 and 18.67 µg/ml respectively.

Inorganic compounds such as HCN , $H_2S$, nitric oxide are recently referred as bacterial volatile compounds (BVC). HCN producing isolates have shown antagonistic activity against pathogenic fungi. It inhibits metalloenzyme cytochrome oxidase activity and plays a key role in biocontrol activity (Audrain et al. 2015). HCN producing *Pseudomonas* spp. have been well documented in the literature with reference to their biocontrol activity and their correlation with Phl (phenazine 1-carboxylic acid) (Siddiqui 2006). In our study, HCN production was found in lesser extent only 2 isolates exhibited HCN production.

Nitrogen (N) is the most important mineral nutrient required by plants. Ammonia oxidizing bacteria (AOB) and ammonia-oxidizing archaea (AOA) are the key drivers that are responsible for the conversion of N into usable forms. Ammonia is ubiquitous in nature and acts as a substrate to produce nitrate (Amoo and Babalola 2017). Ammonia occurs naturally in the environment and can be found in the soil, where it is an important source of nitrogen for plants. Ammonium does not typically accumulate in soils because it is quickly converted to nitrate by soil microbes (Daebeler et al. 2014). Ammonia producing bacteria convert organic nitrogen to ammonical nitrogen which leads to increase in soil nitrogen content. *Paracoccus denitrificans*, *Pseudomonas putida*, *Thiosphaera pantotropha* and *Alcaligenes faecalis* are some heterotrophic nitrifying bacteria. Out of 20 bacterial isolates, 19 were found positive for $NH_3$ production. Three isolates had fair and 11 isolates had good and 5 isolates had excellent $NH_3$ activity.

Bacterial cultures PC1 and PC4 showed enhanced growth in the presence nanogypsum when added @ 50 ppm in nutrient broth. The observation shows an interaction between nanoparticles and bacteria, which strongly supports the viability of PGPR isolates. Similar results were also reported

by Palmqvist et al. (2015) who investigated that *B. amyloliquefaciens* 5113, cultured with nanotitania showed higher OD at 600 nm. The study supports that TNs could be used to support beneficial rhizobacteria in the colonization of plant roots. They observed the nanotitania not only supported the growth of PGPR strains but also enhanced their colonization on plant roots. In contrary to this, several nanocompounds act as antimicrobial agent and retard the microbial growth and hence can also be harmful for the PGPR strains. Fullerenes have been found to inhibit the growth of commonly occurring soil and water bacteria (Oberdorster et al. 2004). Application of nanosilica can enhance microbial population from $4 \times 10^5$ CFU (control) to $8 \times 10^5$ CFU per gram of soil (Karunakaran et al. 2013). It was concluded that the nanogypsum supports the growth of bacterial isolates and does not have any toxic effect on bacterial cultures. Once the nutrients in the medium are exhausted the microorganisms enter stationary and then death phase. Nanoparticles enhance nutrient use efficiency and allow slow release of nutrient and help the isolates to survive for a longer period of time. Same pattern was found in the plate count assay in both the bacterial isolates.

## Conclusion

In the present study, 2 indigenous bacterial isolates recovered from nanocompound infested agriculture field had best PGPRs properties and also showed enhanced CFU in the presence of nanogypsum. It can also be concluded that application of nanogypsum at 50 ppm concentrations does not cause any harmful effect on bacterial growth. Hence these bacterial isolates can be used as bioinoculants in different crops for enhanced plant/soil health and productivity.

## Acknowledgements

The authors are grateful to the Department of Microbiology, G. B. Pant University of Agriculture and Technology, Pantnagar, India for providing necessary research facilities.

### References

Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ (1990) Basic local alignment search tool. J Mol Biol 215 : 403—410.

Amoo AE, Babalola OO (2017) Ammonia-oxidizing microorganisms: Key players in the promotion of plant growth. J Soil Sci Pl Nutr 17 : 935—947.

Arif MS, Riaz M, Shahzad SM, Yasmeen T, Ali S, Akhtar MJ (2017) Phosphorus-mobilizing rhizobacterial strain *Bacillus cereus* GS6 improves symbiotic efficiency of soybean on an aridisol amended with phophorus-enriched compost. Eur J Soil Biol 57 : 27.

Asei R, Ewusi-Mensah N, Abaidoo RC (2015) Response of soybean (*Glycine max* L.) to rhizobia inoculation and molybdenum application in the Northern savannah zones of Ghana. J Pl Sci 3 : 64—70.

Audrain B, Létoffé S, Ghigo JM (2015) Airborne bacterial interactions: Functions out of thin air ? Front Microbiol 6 : 1476.

Auffan M, Rose J, Bottero YJ, Lowry GV, Jolivet JP, Wiesner MR (2009) Towards a definition of inorganic nanoparticles from an environmental, health and safety perspective. Nat Nanotechnol 4 : 634—664.

Bakker AW, Schipper B (1987) Microbial Cyanide production in the rhizosphere in relation to potato yield reduction and *pseudomonas* spp.-M plant growth-stimulation Soil. Etol Biothem 19 : 457.

Beneduzi A, Ambrosini A, Luciane, Passaglia MP (2013) Plant growth-promoting rhizobacteria (PGPR): Their potential as antagonists and biocontrol agents. Genet Mol Biol 35 : 1044—1051.

Boddey RM, Dobereiner J (1995) Nitrogen fixation associated with grasses and cereals : Recent progress and perspectives for the future. Fort Res 42 : 241.

Cappuccino JC, Sherman N (1992) In : Microbiology : A laboratory Manual , New 16 : 125—179.

Daebeler A, Bodelier PL, Yan Z, Hefting MM, Jia Z, Laanbroek HJ (2014) Interactions between Thaumarchaea, Nitrospira and methanotrophs modulate autotrophic nitrification in volcanic grassland soil. The ISME J 8 : 2397—2410.

Dontsova K, Lee YB, Slater BK, Bigham MJ (2005) Ohio State University Extension Fact Sheet ANR-20-05. Gypsum for Agricultural Use in Ohio—Sources and Quality of Available Products. http://ohioline.osu.edu/anrfact/0020.html.

Ghormade V, Deshpande MV, Paknikar KM (2011) Perspectives for nano-biotechnology enabled protection and nutrition of plants. Biotechnol Adv 29 : 792—803.

Glick BR (1995) The enhancement of plant growth by free-living bacteria. Can J Microbiol 41 : 109.

Gorden SA, Webber RP (1951) Colorimetric estimation of indole acetic acid. Pl Physiol 26 : 192—195.

Goswami D, Vaghela H, Parmar S, Dhandhukia P, Thakker J (2013) Plant growth promoting potentials of *Pseudomonas* spp. strain OG isolated from marine water. J Pl Interact 8 : 281.

Johri JK, Surange S, Nautiyal CS (1999) Occurrence of salt, pH and temperature–tolerant, phosphate–solubilizing bacteria in alkaline soils. Curr Microbiol 39 : 89.

Joseph B, Ranjan RP, Lawrence R (2012) Characterization of plant growth promoting rhizobacteria associated with chickpea (*Cicer arientinum* L.). Int J Pl Prod 1 (2) : 141—152.

Kamran S, Shahid I, Baig ND, Rizwan M, Malik AK, Mehnaz

S (2017) Contribution of zinc solubilizing bacteria in growth promotion and zinc content of wheat. Frontiers in Microbiol 8 : In press.

Karunakaran G, Suriyaprabha R, Manivasakan P, Yuvakkumar R, Kannan PN (2013) Effect of nanosilica and silicon sources on plant growth promoting rhizobacteria, soil nutrients and maize seed germination. IET Nanobiotechnol 7 (3) : 70—78.

Khalid A, Arshad M, Zahir ZA (2004) Screening plant growth promoting rhizobacteria for improving growth and yield of wheat. J Appl Microbiol 96 : 473—480.

Khan MS, Zaidi A, Ahemad M, Oves M, Wani PA (2010) Plant growth promotion by phosphate solubilizing fungi-current perspective. Arch Agron Soil Sci 56 : 73—98.

Kisiel A, Kępczyńska E, Gaertn TM (2016) As a model for understanding the mechanism of growth promotion by bacteria from rhizosphere and nodules of alfalfa. Planta 243 : 1169—1189.

Lynch JM (1985) Origin, Nature and Biological Activity of Aliphatic Substances and Growth Hormones Found in Soil. Soil Organic Matter and Biol Activity, pp 151—174.

McGill WB, Cole CV (1981) Comparative aspects of cycling of organic C, N, S and P through soil organic matter. Geoderma 26 : 267—268.

Mishra RK, Prakash O, Alam M, Dikshit A (2010) Influence of plant growth promoting rhizobacteria (PGPR) on the productivity of *Pelargonium Graveolens* I. herit, Recent res. In Sci And Technol 2 (5) : 2010.

Mutluru S, Konada VM (2007) Bioproduction of indole acetic acid by *Rhizobium* strains isolated from root nodules of green manure crop, *Sesbania sesban* (L.) Merr. Iranian J Biotechnol 5 (3) : 178—182.

Nieto–Jacobo MF, Steyaert JM, Salazar-Badillo FB, Nguyen DV, Rostás M, Braithwaite M, De Souza JT, Jimenez-Bremont JF, Ohkura M, Stweart A (2017) Environmental growth conditions of *Trichoderma* spp. affects indole acetic adic derivates, volatile organic compounds and plant growth promotion. Front Pl Sci 8 : 102.

Oberdörster G, Sharp Z, Atudorei V, Elder A, Gele in R, Kreyling W (2004) Translocation of inhaled ultrafine particles to the brain. Inhal Toxicol 16 : 437—445.

Overvoorde P, Fukaki H, Beeckman T (2010) Auxin control of root development. Cold Spring. Harb Perspect Biol 2 : a001537.

Palmqvist NGM, Bejai S, Meijer J, Seisenbaeva GA, Kessler VG (2015) Nanotitania aided clustering and adhesion of beneficial bacteria to plant roots to enhance crop growth and stress management. Sci Rep 5 : 10146.

Pikovskaya RI (1948) Mobilization of phosphorus in soil connection with the vital activity of some microbial species. Microbiologiya 17 : 362—370.

Saravanan VS, Subramonium SR, Raj SA (2003) Assessing *in vitro* solubilization potential of different zinc solubilizing bacterial (zsb) isolates. Brazillian J Microbiol 34 : 121—125.

Sharma SB, Sayyed RZ, Trived MH, Gobi TA (2013) Phosphate solubilizing microbes : Sustainable approach for managing phosphorus deficiency in agricultural soils. Springer Plus 2 : 587.

Siddiqui ZA (2006) PGPR : Prospective biocontrol agents of plant pathogens. PGPR : Biocontrol and Biofertilization. Springer, Berlin.

Tamura K, Stecher G, Peterson D, Filipski, Alan, Kumar S (2013) MEGA 6 : Molecular evolutionary genetics analysis (MEGA) software version 6.0. Mol Biol and Evolut 10 : 1093.

Vandana UK, Chopra A, Choudhury A, Adapa D, Mazumder PB (2018) Genetic diversity and antagonistic activity of plant growth promoting bacteria, isolated from tea-rhizosphere: A culture dependent study. Biomed Res 29 : 853—864.

Vidyashree DN, Muthuraju R, Panneerselvam P, Saritha B, Ganeshamurthy AN (2016) Isolation and characterization of zinc solubilizing bacteria from stone quarry dust powder. Int J Agric Sci 8 : 3078—3081.

Yeole RD, Dave BP, Dube HC (2001) Siderophore production by *fluroscent Pseudomonas* colonizing roots of certain crop plants. Ind J Experim Biol 39 : 464—468.