Environment and Ecology 37 (2) : 521—527, April—June 2019
Website: environmentandecology.com ISSN 0970-0420

Influence of Nanozeolite on Plant Growth Promotory Bacterial Isolates Recovered from Nanocompound Infested Agriculture Field

Priyanka Khati, Parul Chaudhary, Saurabh Gangola, Anita Sharma

Received 19 November 2018; Accepted 24 December 2018; Published on 14 January 2019

Abstract The objective of the study was to assess the impact of nanozeolite (a nanocompound used in agriculture field) on plant growth promotory rhizobacteria isolated from nanocompound infested soil under wheat cultivation. Fifteen bacterial strains were isolated from the wheat field treated with nanoparticles for 4 to 5 years (Crop Research Center Pantnagar). Screening on the basis of plant growth promotory properties like phosphate solubilization, siderophore, indole acetic acid, ammonia and hydrogen cyanide production was done. The effect of nanoparticle on the growth pattern and total protein concentration of bacterial isolates was studied. There was a slight improvement in the growth pattern after the nanozeolite amendment, as observed in plate assay. The concentration of protein in bacterial isolates after nanozeolite treatment was significantly enhanced (>0.05) in comparison to controlled one. Two bacterial isolates with best plant growth promotory traits (PS2 and PS10) were selected for further observations. The results suggest that the application of nanozeolite had positive impact on bacterial growth and protein expression.

Keywords Nanozeolite, Nanocompound, PGPR, Protein, Rhizobacteria.

Priyanka Khati*, Parul Chaudhary, Saurabh Gangola, Anita Sharma
Department of Microbiology, College of Basic Science and Humanities, GBPUA & T Pantnagar, US Nagar 263145, Uttarakhand, India
e-mail: priyankakhati712@gmail.com
*Corresponding author

Introduction

Plant growth promotory rhizobacteria (PGPR) are known to support plant growth through various mechanism especially by p-solubilizing, nitrogenfixation, siderophore, hormone production and they are also involved in developing induced systemic resistance (ISR) and systemic acquired resistance (SAR). PGPR are free-living soil-borne bacteria from the rhizosphere and are known to enhance the growth of plant when applied to seeds or crops (Kloepper et al.1980). Plant growth promoting rhizobacteria (PGPR) are amongst the most complex and important assemblages in the biosphere found in the vicinity of plant rhizosphere (Khan 2005). They are considered as a group of beneficial free-living soil bacteria used for sustainable agriculture (Babalola 2010). PGPR like *P. aeruginosa, P. putida, P. fluorescens, B. subtilis* and soil nitrogen cycle bacteria (nitrifying bacteria and

denitrifying bacteria) have shown varying degree of inhibition when exposed to nanoparticles (Mishra and Kumar 2009). Nanotechnology is a rapidly growing industry due to its wide applicability and demand in agriculture sector. Nanoparticles being small (under 100 nm) in size, are very reactive identities. Eco toxicoligical properties and the risks of these nanoparticles have not yet been fully characterized. Many nanoparticles have already been reported to have anti-microbial properties and thus directly affect microorganisms. Iron and copper based nanoparticles are presumed to react with peroxides present in the environment and generate free radicals that are highly toxic to microorganisms like *P. aeruginosa.* A sub-lethal dose of CuO nanoparticle impaired pyoverdine (PVD) function in a gram-negative bacterium. Some nontoxic and biodegradable nanoparticles have shown to be supportive for the microbial population in soil after application. Silica nanoparticles were observed to significantly enhance (<0.05) the microbial population and total biomass content (Suriyaprabha et al. 2014). Although most of the reports point out negative effect of nanoparticles on PGPRs but the effect of nanozeolite on PGPRs was never worked out before and nanocompounds like it are natural and thus least toxic and biodegradable. Nanozeolites due to water retentive and mineral chelating properties are supposed to support the growth of PGPR by enhancing nutrient use efficiency. Present study was planned to observe the effect of nanozeolite on PGPR isolates through systematic investigation of growth parameters and protein profiling which ultimately reflects the state of bacterial isolates.

Materials and Methods

Soil sample collection

Soil samples used for isolation of plant growth promoting bacterial isolates were collected from a wheat field experiment where the nanoparticles were applied by spray method @ 0.03 g each in 1.5 liter. The site is located at Norman E. Borlogue Crop Research Center of G.B. Pant University of Agriculture and Technology, Pantnagar, Dist. Udham Singh Nagar (Uttarakhand). The soil without nanoparticle treatment was considered as control.

Isolation of bacteria and screening for plant growth promotory properties

The soil sample was serially diluted up to 10^7 dilution and plated in nutrient agar supplemented with nystatin (antifungal) to check the fungal growth. Fifteen bacterial colonies were selected on the basis of morphological and physiological characteristics and screened qualitatively or quantitatively for p-solubilization (Nautiyal 1999, Gupta et al. 2007), siderophore production (Schwyn and Neilands 1987), indole acetic acid production (Gordon and Weber 1951), hydrogen cyanide production (Bakker and Schipper 1987) and ammonia production (Cappuccino and Sherman 1992).

Effect of nanozeolite on bacterial isolates

Four isolates i.e. PS2, PS7, PS9 and PS10 were selected to study the effect of nanozeolite on their growth. Duplicate treatments were made accordingly. The treatments were : PS2 only, PS2+nanozeolite, PS7, PS7+nanozeolite, PS9, PS9+nanozeolite, PS10, PS10+nanozeolite and control (No nanoparticles + no bacteria). Overnight active culture was made by inoculating a single colony in nutrient broth. 2000 ppm concentration (stock) of different nanoparticles were ultrasonicated at 20 kHz for 30 min, for the proper dispersion of nanoparticles in DW and added in the broth at the concentration of 50 ppm. Hundred µl of the active culture was inoculated into 50 ml of sterile nutrient broth with and without different nanoparticles. Aliquots of 3 ml + 1 ml were regularly withdrawn at the interval of 0, 24, 48, 60 and 70 h, 3 ml aliquot was used for recording the absorbance at 600 nm under visible spectrophotometer (Perkin Elmer) and 1 ml of aliquot was used for pour plating. Serial dilution (upto 10^{-4}) of 1 ml aliquot was done for pour plating to reduce the bacterial population to countable range. One ml of 10^4 dilution was used for pout plating with nutrient agar and incubated at 27^0C for 48 h. Colony forming unit (CFU) was calculated by the formula:

CFU = No. of colonies × dilution factor/volume of sample taken (ml).

Table 1. Qualitative plant growth promotory properties by bacterial isolates. a: Phosphate solubilization (diameter of zone of clearance); (-) : Very low, (+) : Medium ,(++) : high. b: Siderophore production (diameter yellow halo zone); (-): Very low, (+) : Medium, (++) : High. c: Indole acetic acid production (intensity of pink color) : (-) : Very low, (+) : Medium, (++): High. d : HCN production (color change from yellow to brown); (-) :Very low, (+) : Medium, (++): High. e : Ammonia production (orange color intensity); (-) : Very low, (+) : Medium, (++) : High.

Strains	P solubilization [a]			Siderophore production [b]			Indole acetic acid production [c]			HCN production [d]			Ammonia production [e]		
	-	+	++	-	+	++	-	+	++	-	+	++	-	+	++
PS1			■		■			■		■				■	
PS2		■				■			■	■			■		
PS3	■				■		■			■			■		
PS4		■			■			■		■			■		
PS5	■				■			■		■			■		
PS6	■				■				■	■				■	
PS7		■			■				■	■			■		
PS8	■				■			■		■			■		
PS9		■			■			■		■				■	
PS10			■			■		■		■				■	
PS11		■				■		■		■			■		
PS12	■				■		■			■			■		
PS13	■				■			■		■			■		
PS14		■				■		■		■			■		
PS15	■				■					■			■		

Protein extraction and quantification

Protein extraction was done according to modified method of Giard et al. (2000). Pellet was washed thrice in cold 0.1M TrisCl pH (6.8) and resuspended in 200 µl Tris buffer (0.1M, pH 6.8). After centrifugation, pellet was suspended in 200 µl extraction buffer and kept in boiling water bath for 20- 30 minute. Protein samples were centrifuged and supernatant was stored at -20^{0}C for further studies. Quantification of protein was done according to Bradford (1976). Absorbance was taken at 595 nm with Bovine serum albumin as control.

Molecular characterization of PGPR isolates

The genomic DNA of 2 bacterial isolates (PS2 and PS10) was extracted using the modified method by Bazzicalupo and Fani (1995) and 16SrDNA region was amplified using universal primer (27F and 1492 R). Amplified sequences were partially sequenced by Central Instrumentation Facilities, South campus Delhi. Sequences obtained were analyzed by basic local alignment search tool (BLAST).

Statistical analysis

The data were statistically treated using general linear model procedure (SPSS, Ver 16.0) to revel significant effect of nanozeolite on bacterial total protein. Duncan's test was applied for testing difference between individual events.

Results and Discussion

PGPR properties

PGPR properties of bacterial isolates are listed in Table 1. Siderophore production by biocontrol agents (BGA) and plant growth promoting microbes (PGPM) is one of the important mechanisms for plant growth promotion (Kloepper et al. 1980) and disease suppression (Husen 2003). Among the 15 bacterial isolates (PS1 to PS15) siderophore production was given by all isolates and best results was shown by PS2, PS10, PS11 and PS14. Typically, microbial siderophore are classified as catecholates, hydroxamates and α -carboxylates, depending on chemical nature of their coordination sites with iron (Heymann et al. 2002), phenolates (Haag et al. 1993) and mixed (both hydroxamate and catecholate functional groups) (Meyer and Abdallah 1978). Phosphate solubilizing microorganisms (PSM) through various mechanisms of mineralization are able to solubilize and convert inorganic soil p (which are insoluble form) to plant accessible forms (Kumari et al. (2009) PS1 and

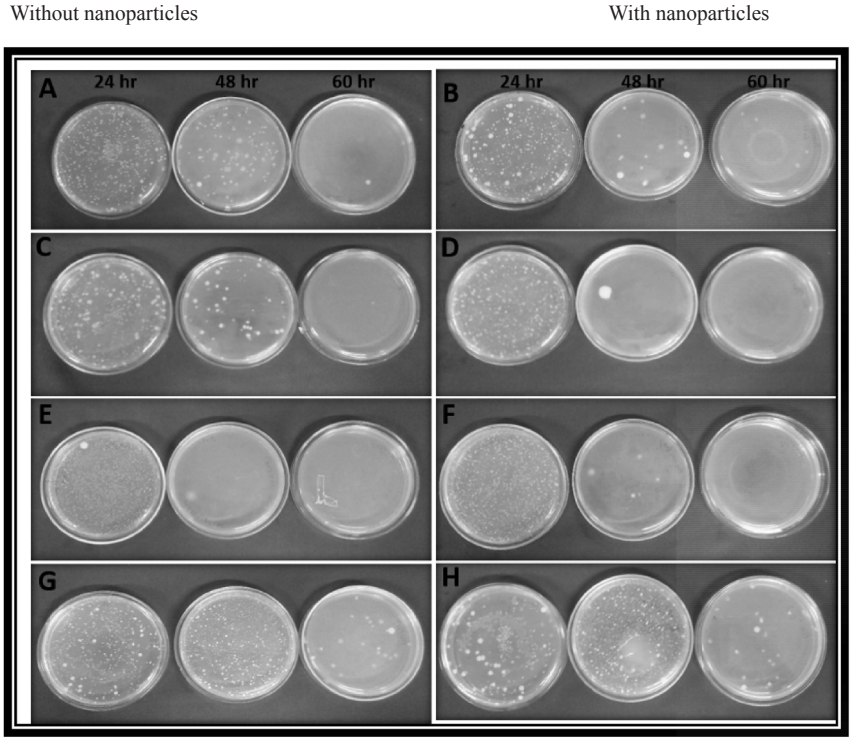

Fig. 1. Enumeration of bacterial population in different time interval for (A) PS2, (B) PS2 + Nanozeolite, (C) PS7, (D) PS7 + Nanozeolite, (E) PS9, (F) PS9 + Nanozeolite, (G) PS10, (H) PS10 + Nanozeolite.

PS10 solubilized maximum phosphate followed by PS2, PS4, PS7, PS9, PS11 and PS14 with medium p. solubilization and the rest (PS3, PS5, PS6, PS8, PS12, PS13 and PS15) did not produced any zone of clearance. Both bacterial and fungal strains exhibiting p-solubilizing activity are detected by the formation of clear halo (a sign of solubilization) around their colonies (Sharma et al. 2013).

IAA production is another PGPR property which was maximally produced by PS2 and PS7 followed by PS1, PS4, PS5, PS6, PS8, PS9, PS10, PS11, PS13, PS14 and PS15, which gave intermediate results while PS1 and PS12 showed least IAA. The results were in support to previous study by Ghosh and Basu (2002). It has been reported that IAA production by bacteria can vary among different species and strains and it is also influenced by culture condition, growth stage and substrate availability (Sridevi and Mallaiah 2007). HCN production was negative for all the 15 isolates. Ammonia production marked by color change from yellow to orange was positive for only PS1, PS6, PS9 and PS10. Four isolates on the basis of best PGPR properties were selected for further studies (i.e. PS2, PS7, PS9 and PS10).

Effect of nanozeolite on the growth of bacterial isolates

Effect of nanozeolite on the growth pattern of 4 PGPR isolates is evidenced in Figs. 1 and 2. A gentle decline in growth after the treatment of nanozeolite with PS2 was observed in comparison to steep decline in control (Fig. 2). The CFU counts obtained also correlates with optical density (OD at 660 nm) results (Fig. 1). Similarly the gowth rate of nanozeolite amended PS7 was higher than PS7, but the plate count assay do not follow the same. The growth was suppressed by nanozeolite for PS9 but on the other hand for PS10,

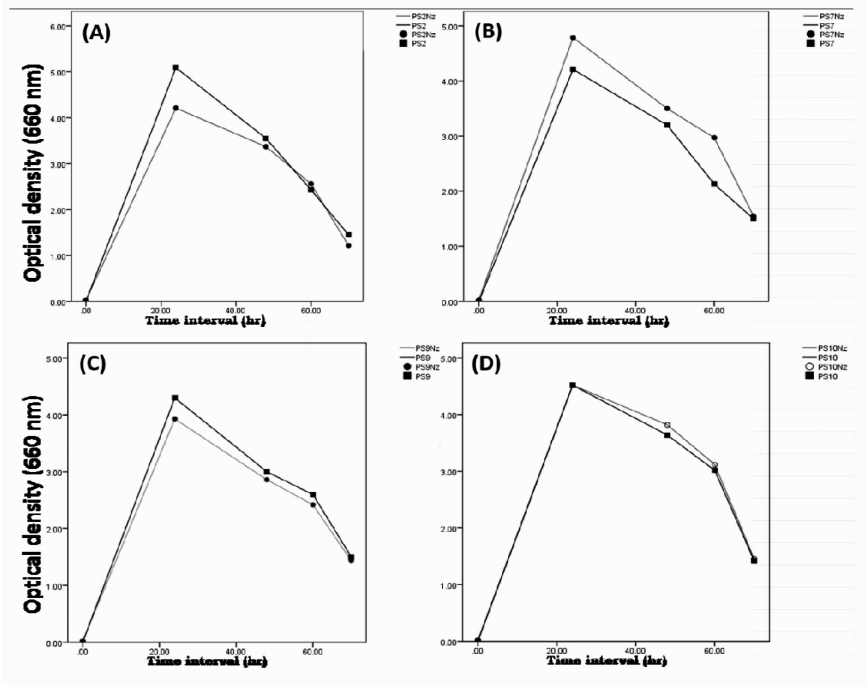

Fig. 2. Growth curve pattern of different isolates with (bold square) and without nanozeolite (bold circle).

although the maximum growth obtained was similar in both the cases (PS10 alone and PS10 + nanozeolite) but the decline in case of PS10 alone was much steeper than nanozeolite amended PS10. There was a gradual decline after stationery phase in case of nanozeolite amended isolates which also correlates with the plate assay and the best result were obtained for PS2 and PS10 (Figs. 1 and 2). This means that there is an interaction,between nanoparticles and bacteria, which strongly increases the viability of bacterial isolates by extending the death phase. Once the nutrients in the medium are exhausted the microorganisms enters stationary and then death phase.

The nanoparticle help enhance nutrient use efficiency and allow slow release of nutrient which allow the isolates to survive for a longer period of time. Similar pattern of results were also inferred by Palmqvist et al. (2015) who worked on effect of nanotitania on PGPR strains and their clustering pattern on plant roots which is an important trait for PGPR strains.They observed the nanotitania not only supported the growth of PGPR strains but also enhanced their colonization on plant roots.

Nanosilica was found to double the colony forming unit from 4×10^5 CFU (control) to 8×10^5 CFU per gram of soil (Karunakaran et al. 2013). In contrary to it several manoparticles are reported as antimicrobial agent and retard the growth of bacteria and hence can also be harmful for the PGPR strains. Effect of iron oxide and gold nanoparticles on *E. coli* was studied by Chatterjee et al. (2011) and preliminary growth analysis data revealed the inhibitory effect of iron oxide on bacterial whereas gold nanoparticle did not show any inhibitory effect. An evident increase in bacterial population shows enhanced bacterial division by gold nanoparticle. Tong et al. (2007) recently reported that introduction of fullerene nanoparticles in the soil had no influence on the soil bacterial diversity but on the other side. Fullerenes have been found to inhibit the growth of commonly occurring soil and water bacteria.

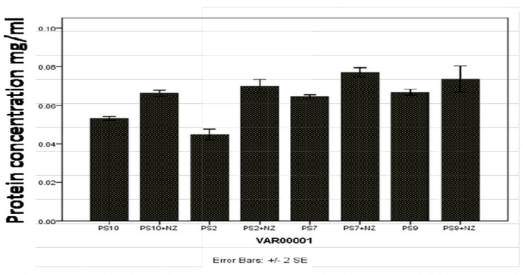

Fig. 3. Protein concentration for different treatments, with bars representing +/-2SE.

Molecular characterization

Two bacterial isolates PS2 and PS10 were identified as *Bacillus* sp. on the basis of BLAST match and were allotted with Accession number as KX650178 and KX650179 respectively by NCBI.

Protein quantification

The level of total protein enhanced in all the bacterial isolates after treatment with nanozeolite (Fig. 3). This enhanced protein expression suggest the nanoparticle induce bacterial isolates for growth and more protein production which help in better sustenance.

The mechanism involved is still not worked out but the possible way is the better water entrapment and enhanced nutrient use efficiency in the presence of nanozeolite, which supports the microbial growth. Similarly, Karunakaran et al. (2013) also observed increase in protein concentration in broth after nanosilica treatment.

Conclusion

A positive impact of nanozeolite on PGPRs indicates tremendous applicability of nanozeolite in agricultural fields for enhancing crop productivity while maintaining soil health. It can also be concluded that application of nanozeolite at certain concentrations in bacterial formulations as organic manure or directly in agricultural fields may come forward as a boon for the agricultural productivity.

References

Babalola OO (2010) Beneficial bacteria of agricultural importance. Biotechnol L 32 : 1559—1570.

Bakker AW, Schipper BB (1987) Microbial cyanide production in the rhizosphere in relation to potato yield reduction and *Pseudomonas* SPP-mediated plant growth-stimulation. Soil Biol and Biochem 19 (4) : 451—457.

Bazzicalupo M, Fani R (1995) The use of RAPD for generating specific DNA probes for microorganisms. In : Clapp JP (ed). Methods in Molecular Biology. Humana, Totowa, NJ, pp 155—175.

Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytic Biochem 72 : 248—254.

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

Chatterjee S, Bandyopadhyay A, Sarkar K (2011) Effect of iron oxide and gold nanoparticles on bacterial growth leading towards biological application. J Nanobiotechnol 9 : 34.

Ghosh AC, Basu PS (2002) Growth behavior and bioproduction of indole acetic acid by a Rhizobium species isolated from root nodules of a leguminous tree *Dalbergia lanceolarea.* Ind J Exp Biol 40 : 796—801.

Giard JC, Rince A, Capiaux H, Auffray Y, Hartke A (2000) Inactivation of the stress and starvation-inducible gls24 operon has a pleiotropic effect on cell morphology, stress sensitivity and gene expression in *Enterococcus faecalis*. J Bacteriol 182 : 4512—4520.

Gordon SA, Weber RP (1951) Colorimetric estimation of Indoleacetic acid. Pl Physiol 26 : 192—195.

Gupta N, Sabat J, Parida R, Kerkatta D (2007) Solubilization of tricalcium phosphate and rock phosphate by microbes isolated from chromite, iron and manganese mines. Acta Bot Croat 66 (2) : 197—204.

Haag H, Hantke K, Drechsel H, Stojiljkovic I, Jung G, Zahner H (1993) Purification of Yersinia bactin : A siderophore and possible virulence factor of *Yersinia enterocolitica.* J General Microbiol 139 : 2159—2165.

Heymann P, Gerads M, Schaller M, Dromer F, Winkelmann G, Ernst JF (2002) The siderophore iron transporter of *Candida albicans* (Sit 1p/Arn 1p) mediates uptake of ferrichrome-type siderophores and is required for epithelial invasion. Infect Immun 70 (9) : 5246—5255.

Husen E (2003) Sereening of soil bacteria for plant growth promotion activities *in vitro.* Indon J Agric Sci 4 : 27—31.

Karunakaran G, Suriyaprabha R, Manivasakan P, Yuvakkumar R, Rajendran V, Prabu P, Kannan N (2013) Effect of nanosilica and silicon sources on plant growth promoting rhizobacteria, soil nutrients and maize seed germination. IET Nanobiotechnol 7 (3) 70—77.

Khan AG (2005) Role of soil microbes in the rhizospheres of plants growing on trace metal contaminated soils in phytoremediation. J Trace Elements Med Biol 18 : 355—364.

Kloepper JW, Leong J, Teintze M, Schroth MN (1980) Enhanced plant growth by siderophores produced by plant growth promoting rhizobacteria. Nature 286 : 885—886.

Kumari M, Vasu D, Ul-Hasan Z, Dhurwe UK (2009) Effects of PSB (phosphate solubilizing bacteria) morphological on ch-

aracters of *Lens culinaris*. Med Med Biol Forum Int J 1 : 5—7.

Meyer JM, Abdallah MA (1978) The fluorescent pigment of *Pseudomonas fluorescens:* Biosynthesis, purification and physico-chemical properties. J General Microbiol 107 (3) : 319—328.

Mishra VK, Kumar A (2009) Impact of metal nanoparticles on the plant growth promoting rhizobacteria. Digest J Nanomat and Biostruc 4 (3) : 587—592.

Nautiyal CS (1999) An efficient microbiological growth medium for screening phosphate solubilizing microorganisms. FEMS Microbiol L 170 (1) :265—270.

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

Schwyn, Neilands (1987) Universal chemical assay for the detection and determination of siderophores. Anal Biochem 160 (1) : 47—56.

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

Sridevi MM, Mallaiah KV (2007) Bioproduction of indole acetic acid by rhizobium strains isolated from root nodules of green manure crop, Sesbaniasesban (L.) Merr. Tran J Biotechnol 5 (3) : 178—182.

Suriyaprabha R, Karunakaran G, Yuvakkumar R, Prabu P, Rajendran V, Kannan N (2014) Effect of silica nanoparticles on microbial biomass and silica availability in maize rhizosphere. Biotechnol and Appl Biochem 61 (6) : 1—8.

Tong Z, Bischoff M, Nies L, Applegate B, Turco R (2007) Impact of fullerene (C60) on a soil microbial community. Environm Sci and Technol 41 (8) : 2985—2991.