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ORIGINAL PAPER

Potential plant growth-promoting activity of *Serratia nematodiphila* NII-0928 on black pepper (*Piper nigrum* L.)

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Abstract A potential bacterial strain designated as NII-0928 isolated from Western *ghat* forest soil with multiple plant growth promoting attributes, and it has been identified and characterized. Plant growth promoting traits were analyzed by determining the P-solubilization efficiency, Indole acetic acid production, HCN, siderophore production and growth in nitrogen free medium. It was able to solubilize phosphate (76.6 μg ml^{-1}), and produce indole acetic acid (58.9 μg ml^{-1}) at 28 ± 2°C. Qualitative detection of siderophore production and HCN were also observed. At 5°C it was found to express all the plant growth promotion attributes except HCN production. The ability to colonize roots is a sine qua non condition for a rhizobacteria to be considered a true plant growth-promoting rhizobacteria (PGPR). 16S rRNA gene sequencing reveals the identity of the isolate as *Serratia nematodiphila* with which it shares highest sequence similarity (99.4%). Seed bacterization with black pepper cuttings in greenhouse trials using Sand: Soil: FYM with three individual experimental sets with their respective control showed clearly the growth promoting activity. Hence, *Serratia nematodiphila* NII-0928 is a promising plant growth promoting isolate showing multiple PGPR attributes that can significantly influence black pepper cuttings. The result of this study provides a strong basis for further development of this strain as a bioinoculants to attain the desired plant growth promoting activity in black pepper growing fields.

Keywords Plant growth promotion · Black pepper · *Serratia nematodiphila*

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Introduction

Black pepper *(Piper nigrum L.)* famous as "Black Gold" and also known as "King of Spices" is one of the important agricultural commodities of commerce and trade in India since pre-historic period. The crop is the major source of income and employment for rural households in the predominantly pepper growing State of Kerala where more than 2.5 million farm families are involved in pepper cultivation. Black Pepper is a plant of humid tropics requiring adequate rainfall and humidity. The hot and humid climate of sub-mountainous tracts of Western *ghat* is ideal for its cultivation. It grows successfully between 20° North and South latitude and from sea level up to 1500 meters above sea level. The crop tolerates temperature between 10° and 40°C. A well distributed annual rainfall of 125–200 cm is ideal for the crop. Ideal pH ranges from 4.5 to 6.5. Nitrogen and phosphorus are essential nutrients for plant growth and development. Intensive agriculture entails the risk of excessive fertilization.

Microorganisms are important in agriculture in order to promote the circulation of plant nutrients and reduce the need for chemical fertilizers as much as possible. Plant growth-promoting rhizobacteria (PGPR) are able to exert a beneficial effect upon plant growth. Beneficial plant–microbe interactions in the rhizosphere are the determinants of plant health and soil fertility (Jeffries et al. 2003). In the era of sustainable agricultural production, the

interactions in the rhizosphere play a pivotal role in transformation, mobilization, solubilization etc., from a limited nutrient pool in the soil and subsequent uptake of essential plant nutrients by the crop plants to realize full genetic potential of the crop. Soil microorganisms are very important in the biogeochemical cycles of both inorganic and organic nutrients in the soil and in the maintenance of soil health and quality (Lucy et al. 2004; Diby and Sarma 2006). Thus, the need of the hour is to enhance the efficiency of the meager amount of external inputs by employing the best combinations of beneficial microbes for sustainable agricultural production. In the present study we are reporting a potential isolate of Serratia sp isolated from Western *ghat* dense forest with their effective plant growth promoting activity in black pepper.

Materials and methods

Isolation and characterization of Rhizobacteria

The soil used for bacterial isolation was collected from a root-free soil of Silent valley of Western *ghat* [GPS coordinates for the sample site are 74° 52′E, 8°18′N] located in the Nilgiri Hills, Palakkad district, Kerala, in South India. The rich forests of the Western *ghat* harbor a large portion of India's biological diversity and include most of the endemic species (http://en.wikipedia.org/wiki/Western_Ghats). The wide variation of rainfall patterns in the Western *ghats*, coupled with the region's complex geography, produces a great variety of vegetation types. These include scrub forests in the low-lying rain shadow areas and the plains, deciduous and tropical rainforests up to about 1,500 meters, and a unique mosaic of montane forests and rolling grasslands above 1,500 meters with no obvious soil management, the soil characteristics were pH 6.0, available N (1231 kg ha^{-1}), available P (14.6 kg ha^{-1}), available K (189 kg ha^{-1}), organic carbon (18290 kg ha^{-1}). The processed soil sample was serially diluted, spread plated on full strength nutrient agar and incubated at 28°C for 48 h. A total of 520 different colonies were isolated on nutrient agar (NA) and were purified with repeated culturing and maintained in 20% glycerol at −80°C. A potential isolate was screened and selected on the basis of halo zone produced in Pikovskaya agar. Strains were assessed for morphology, physiology and Gram reaction and other characterization.

Characterization, identification and phylogenetic analysis

Preliminary biochemical characterization was carried out as per standard methodologies (Collins and Lyne 1980). The temperature range for growth was checked on NA from 4 to 50°C at an interval of 5°C, by inoculating 1 ml of an exponential culture (10^8 CFU ml^{-1}) in 100 ml of nutrient broth (initial population log 7.0 CFU ml^{-1}), and estimating the cell population at 2-h intervals. The numerical values were log transformed and plotted against time. Growth at different pH values ranging from 4.0 to 12.0 (at a 0.5 intervals) were studied at 28°C. The identity of the isolate was revealed on the basis of Biolog carbon source utilization, and further validated by the 16S rRNA gene sequencing performed using an ABI PRISM BigDye Terminator cycle sequencing kit (as recommended by the manufacturer). Bacterial suspensions were inoculated into Biolog GN2 Microplates as described in instruction manuals and incubated at 30°C for 24 h. The results were interpreted with Biolog Micro Log 34.20.04 software (Biolog, Hayward, CA). The universal primer 27F and 1492R was used for the partial sequencing of the 16S rRNA gene (1081 nucleotides). Sequence was deposited in GenBank under FJ897465 accession. Sequence analysis was done at the RDP-II (Ribosomal Database Project, Michigan State University, MI, USA) using Seqmatch version 3 (Cole et al. 2007). Similarity scores were obtained by the similarity rank analysis function at RDP data version 9.50. Nucleotide sequences were aligned using the Clustal X 1.81 algorithm (Thompson et al. 1997). Phylogenetic and molecular evolutionary analyses were conducted using mega version 4.0 (Kumar et al. 2004). The phylogenetic tree were constructed by the neighbor- joining method (Saitou and Nei 1987) using the distance matrix from the alignment. Distances were calculated using the Kimura method (1980).

Quantitative estimation of phosphate solubilization

Initial qualitative estimation of the P-solubilizing activity of the isolate was carried out on Pikovskaya agar (1948) and followed the quantitative estimation of P solubilization as per standard methodology (Mehta and Nautiyal 2001), by inoculating 1 ml of bacterial suspension (3×10^7 cells ml^{-1}) in 50 ml of National Botanical Research Institute Phosphate (Mehta and Nautiyal 2001) broth in Erlenmeyer flasks (250 ml), and incubating the flasks for 15 days. Every 2 days of the incubation period the cell suspension was centrifuged at 10,000 rev min^{-1} for 10 min and the P content in the supernatant was spectrophotometrically estimated by the ascorbic acid method (Murphy and Riley 1962). All the studies were repeated on three independent dates to confirm the results. In order to observe the effect of cultural conditions for insoluble phosphate solubilization, the bacterial cells were cultured at three different temperature (5, 20 and 30°C) and pH (pH 4–12).

Quantification of bacterial IAA

Strains were grown in L-broth supplemented with a filter-sterilized solution of 1 g L-tryptophan. The liquid medium was inoculated by bacterial culture adjusted to optical density 0.5 measured at 600 nm by spectrophotometer. Inoculated tubes were incubated at 30°C for 24–48 h. After incubation cells were removed from the culture medium by centrifugation (5,000 rev/min for 15 min). Auxin was detected in 1 ml of supernatant using Salkowski reagent (Gordon and Weber 1951; Sarwar et al. 1992). A standard curve was drawn for comparison to determine auxin production by isolates. The presence of IAA was further confirmed by HPLC. Filter-sterilized supernatant was analysed by using a Shimadzu HPLC equipped with a Hypersil-Keyston ODS column (5 lm; 4.6 × 250 mm). The mobile phase was methanol/water/acetic acid (36:64:1) at a flow rate of 1 ml/min. Eluates were detected at 220 nm and IAA was quantified by integrating the areas under the peaks. Authentic IAA (Sigma) was used as standard.

Qualitative estimation for siderophore and HCN production

Isolates, subcultured on quarter-strength King's B agar for 48 h, were initially screened qualitatively for production of cyanide by using picrate/Na_2CO_3 saturated filter paper fixed to the underside of Petri dish lids (Bakker and Schipper 1987), which were sealed with parafilm before incubation at 28 ± 2°C. Color change of the filter paper from yellow to light brown, brown, or reddish brown was recorded at 4, 24, and 48 h as an indication of weak, moderate, or strong cyanogenic potential, respectively. Reactions from inoculated plates were visually compared with corresponding control plates containing no culture.

CAS assay was used to detect siderophores produced by rhizobacteria. Siderophore production was tested on Petri dishes contained CAS-agar. The composition of CAS blue solution for this assay was prepared according to Schwyn and Neilands (1987). Pure isolates were stabbed on CAS agar plates using sterile toothpicks and incubated at 28°C for 2 weeks in the dark. The colonies with orange zones were considered as siderophore–producing strains. Assay in solid media were carried out in triplicate. The control plates of CAS-agar (uninoculated) were incubated under the same conditions as described above and no color change in the CAS-blue agar was observed, after incubation periods of 1–14 days.

Black pepper bioassays

For the bioassays, stem cuttings (*c*. 15 cm in height) of the black pepper cultivar 'Karimunda' were obtained from runner shoots of plants from a healthy black pepper orchard. To study the plant growth-promoting activities of the selected bacterial isolate, a natural soil was collected from a garden field in NIIST institute campus, black pepper was not grown in this soil before. This soil was mixed with sand and farm yard manure (FYM) in the ratio 1: 1: 1 (v/v) and sterilized for 30 min at 121°C for three consecutive days and then transferred to plastic pots. For the bioassays, the disease-free cuttings (15 cm height) were surface-sterilized with ethanol (70%) for 5 min followed by several washings with ample amounts of sterile water. Excess water adhering to the cuttings was removed using sterile facial tissues. Fresh cuts were made at the ends of the cuttings to yield sterile end tissues. The lower half of the stem cuttings, including the first node, was dipped in water (control) and bacterial suspensions (10^9 cfu ml^{-1}) for 30 min prior to transplanting to the pots. The bioassays were conducted in the greenhouse without temperature and humidity control. The population dynamics of the bacterial isolates on the roots of black pepper seedlings was assessed after 60 days of plant growth in the greenhouse. Population densities of the introduced bacterial strains were assessed by dilution plating of rhizosphere suspensions on nutrient agar medium. The height of the new black pepper shoots was scored after 60 days of plant growth. Additionally, the number of roots per cutting and the length of the roots were determined.

Statistical analysis

Data were statistically analyzed by analysis of variance using the general linear model developed by the SAS Institute (version 9.1; Cary, NC), and means were compared using the least significant difference (LSD) method; $P \leq 0.05$ was considered significant.

Results

Isolation and characterization of the bacterial isolate

A potential bacterial strains producing more than 20 mm zone of phosphate solubilization after 5 days incubation on Pikovskaya agar and morphologically different were subjected further for phenotypic and physiological characteristics. The bacterial colonies were circular, smooth, convex, and entire and Gram negative. Isolate NII-0928 was able to grow over a wide range of temperature 5–40°C, with optimum at 28 ± 2°C and pH tolerance over the range of 6–12, with optimum 7.0 ± 0.2 and could tolerate up to 7% of NaCl concentration (w/v). Phenotypic and biochemical characterization were done using Biolog based carbon source utilization and Hi25 Kit (HiMedia, Mumbai). Strain

NII-0928 was positive for nitrate reduction, oxidase, catalase, H_2S production, citrate utilization, esculin hydrolysis, casein hydrolysis, gelatinase and methyl red. Strain found negative for voges proskaures test, starch hydrolysis, indole production and urease. Strain NII-0928 utilize adonitol, saccharose, glucose, lysine, malonate, trehalose and does not utilize arabinose, xylose, rhamnose, cellobiose, melibiose, raffinose and. Molecular analysis based on 16S rRNA gene sequencing reveals that isolates NII-0928 showed highest similarity to *Serratia nematodiphila* KCTC 22130(99.4%) available in the public domain. The phylogenetic tree (Fig. 3) constructed using 16S rRNA gene sequences reveals the position and placing of potential isolate to their respective genus.

P-solubilization activity and IAA production

Isolate was found to solubilize phosphate significantly at 28 ± 2°C. Although the zone of solubilization around the bacterial colony on Pikovskaya agar after 72 h of incubation varies from 5°C to 30°. Quantitative estimation of phosphate solubilization, estimated after incubation for 15 days (at 3, 5, 7, 9, 11, 13 and 15 day intervals), maximum solubilization was observed at 28 ± 2°C (Fig. 1). At this temperature, maximum solubilization of 76.6 ± 0.6 µg/ml was recorded after 9th day of incubation, after which the solubilization continued to decline with increased incubation. The pH of the broth was found to decline, in each case, due to bacterial activity; lowering of pH coincided with increase in the efficiency of phosphate-solubilizing activity. The pH was found to decline from 7.0 (control) to <4.0–3.0. And maximum IAA production from 20.2 to 58.9 µg ml^{-1} in tryptophan amended media with different isolates at 30°C after 48 h of incubation. The

Table 1 Effect of inoculation plant growth promoting *Serratia nematodiphila* NII-0928 on plant growth promoting parameters and nutrient uptake of black pepper cuttings

Treatments	Control	NII-0928
Shoot length (cm)	3.2 ± 0.3	5.1 ± 0.4
Number of roots	17.0 ± 1.0	62.0 ± 3.0
Length of roots (cm)	18.0 ± 1.0	32.0 ± 2.0
Leaf area (cm^2)		
Nutrient uptake	4.4 × 3.0	6.2 × 3.25
N (mg g^{-1} tissue)	15.60 ± 1.2	21.3 ± 1.0
P (mg g^{-1} tissue)	1.62 ± 0.03	3.2 ± 0.08
K (mg g^{-1} tissue)	3.5 ± 0.08	5.0 ± 0.06

Results obtained were of mean of triplicates. Results obtained were of mean of triplicates. Data were analysed using one-way analysis of variance and treatment means were compared ($P \leq 0.05\%$). 20 ml of culture filtrate (10^8 CFU) was given to sprouts and growth promotion was observed in black pepper after 60 days. Distilled water (DW) was used as control

bacterial strain was able to grow at all the temperature tested, and grew especially well at 28 ± 2°C. The survival of isolate under different temperatures was of significant (Fig. 1). Although more research work is needed to evaluate their efficiency under temperature stress conditions but it was well documented that the relative effectiveness of plant inoculation was higher under extreme conditions of soil temperature in different experiments. The influence of initial pH on the insoluble phosphate solubilization was investigated in the range of pH 4.0 to 12.0. The cell growth and insoluble phosphate solubilization were varies for all pH values tested. However, pH range 4 and above pH 9 resulted less cell growth of bacterial biomass (0.04 OD_{600}), were as soluble P-solubilization in pH 4.0 is very high, this

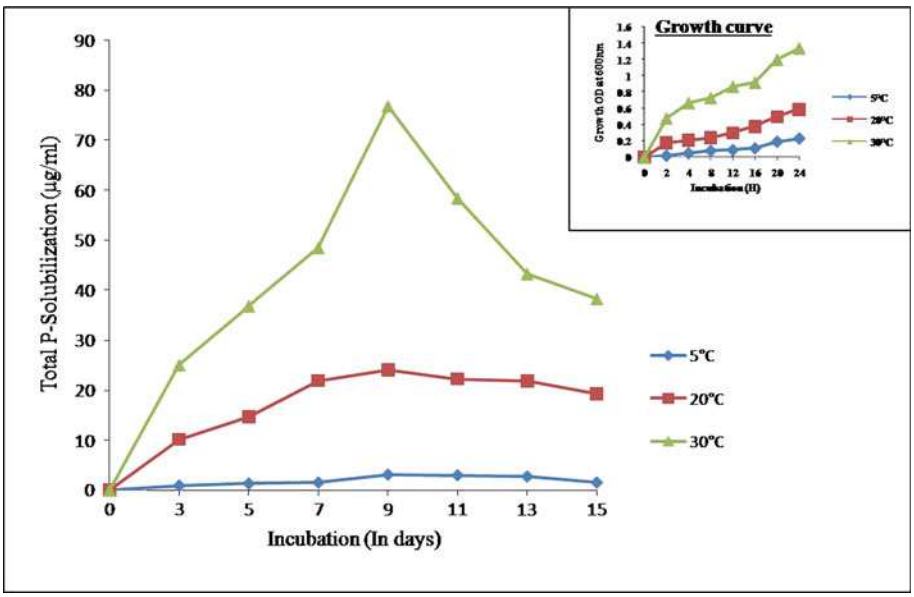

Fig. 1 Total P-solubilization by *Serratia nematodiphila* NII-0928 at different temperature for up to 15 days of incubation. Inner figure shows the growth curves of the isolate at three different temperature

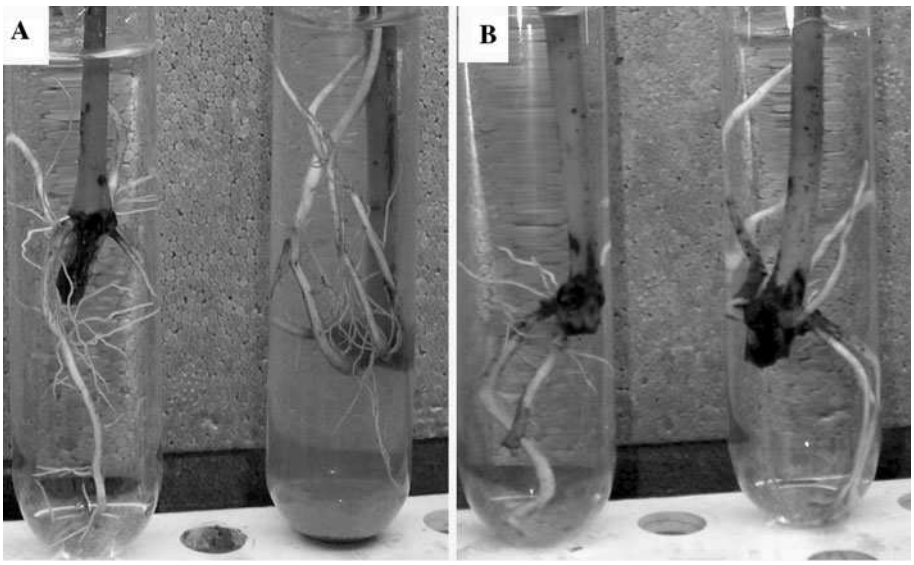

Fig. 2 Effect of inoculation of PGPR isolates on black pepper cutting over uninoculated after 60 days. **a** Cuttings treated with strain NII-0928 **b** cutting treated with sterile distilled water (control)

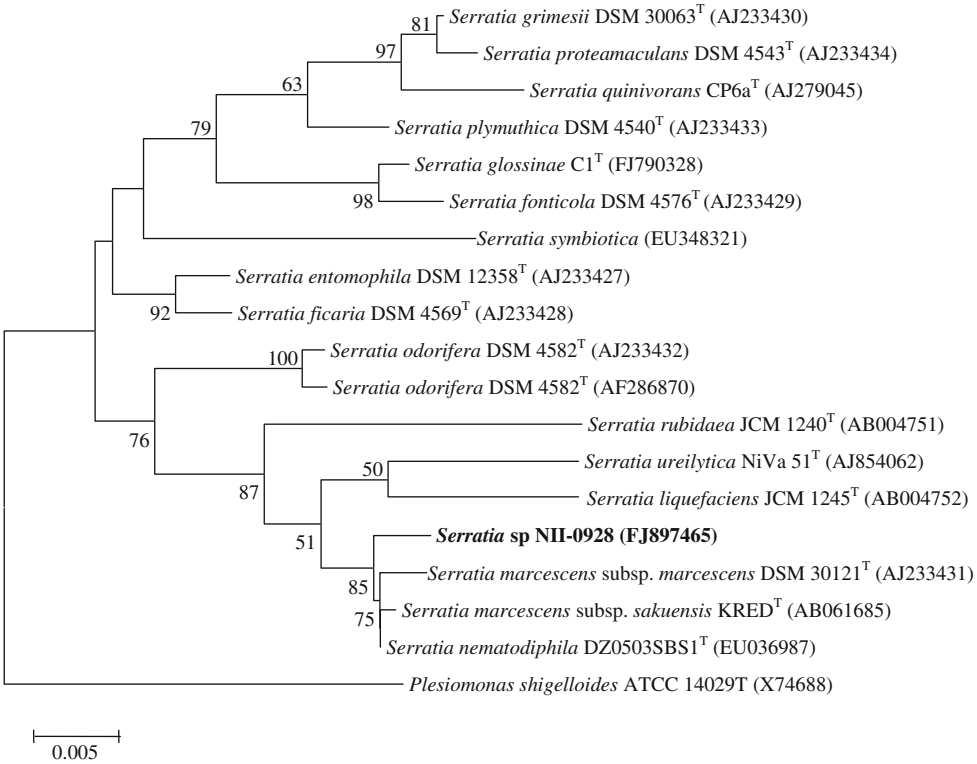

Fig. 3 Neighbour-joining phylogenetic dendrogram based on 16S rRNA sequences showing relationships between isolates and their related taxa. Only the bootstrap percentages higher than 50% are shown at branching points. Bar 0.005 substitutions per nucleotide position. *Plesiomonas shigelloides* ATCC 14029 was used as the outgroup to root the tree. Accession numbers are given in parentheses

result strongly support, as decline pH will increase the P-solubilization. These results indicated that isolates of Western *ghat* are acid- and alkali-tolerant bacterium which can be applied to the acidic as well as alkaline soil.

Plant yield parameters

The activity of isolate NII-0928 exerted a considerable influence on black pepper and recorded with 59% higher root length, 77.7% high shoot lengths and 3.6 times higher in root numbers when compared with untreated control (Table 1; Fig. 2). Seed bacterization resulted in greater enhancement of the root growth, as compared with the shoot growth, as well as increase in number roots was observed in the bacterized treatment over the uninoculated controls (Fig. 3).

Discussion

Bacterial plant growth promotion is a well-established and complex phenomenon, and is often achieved by the

activities of more than one plant growth-promoting traits exhibited by the associated bacterium (Lifshitz et al. 1987). It is well established fact that improved phosphorous nutrition influences overall plant growth and root development (Jones and Darrah 1994). Siderophore production by the isolate assumes significance for iron nutrition of plants grown under iron deficient conditions (Pieterse et al. 2001). HCN production by rhizospheric bacterium has been variably viewed, while it is considered effective from the biocontrol point of view. In this study, a potential *Serratia* isolate NII-0928 isolated from Western *ghat* forest possessed multiple plant growth traits, like P-solubilization, IAA and Siderophore production. P-Solubilization activity in *Serratia* sp NII-0928 was in accordance with previous studies showing that members of the *Serratia* genus display high P-solubilization activities (Chen et al. 2006; Hameeda et al. 2006; Selvakumar et al. 2007; Pérez et al. 2007). Strain NII-0928 significantly increases the total root biomass of the black pepper plants. The number of roots have been significantly increased apart from increasing the root length and their by root area (Table 1). Strain NII-0928 was able to solubilize the complex forms of phosphorous to plant available form as evidenced in the In vitro study conducted. Microorganisms are critical for the transfer of phosphorous from poorly available forms and are important for maintain phosphorous in readily available pools. The present studies proved that, potential isolate of PGPR isolated from Western *ghat* mediated P-solubilization and thereby enhanced uptake by the plants, which resulted in increased root proliferation. The microorganism used in this study was found to produce indole acetic acid and siderophore, which may chelating metal ions associated with the bound phosphorous and release phosphorous from complex form. These beneficiary attributes can be corroborated with the hormonal and nutritional factors by which the rhizobacteria influence the plant. Plant growth regulators viz. IAA produced by this strain, as detected by HPLC, these factors not only stimulated the root for higher absorption of nutrients and minerals but also improved root health. Most root-promoting bacteria synthesize IAA and this has been clearly demonstrated in many cropping systems. While low levels of IAA stimulate root elongation, high levels of bacterial IAA, whether from IAA over producing mutants or strains that naturally secrete high levels or from high-density inocula, stimulate the formation of lateral and adventitious roots (Khalid et al. 2004; Berleth and Sachs 2001).

In conclusion, worldwide there is a profound need to explore varied agro-ecological niches for the presence of native beneficial micro-organisms. Many studies have been undertaken to understand the nature and properties of these unique microbes which harbor potential plant growth promoting traits. With increasing awareness about the chemical-fertilizers-based agricultural practices (Ahmad et al. 2008), it is important to search for region-specific microbial strains which can be used as a potential plant growth promoter to achieve desired product. In this study, isolate NII-0928 stimulated the growth of black pepper seedlings under pot culture conditions. The increased nutrient uptake parameters could be attributed to the enhancement of the root growth and development. Although other parameters could have positively influenced the growth of black pepper seedlings, auxin production by the isolates is proposed as a major means of attaining growth promotion. Future studies are required to prove the nature of these isolates and to harness their potential as bio-inoculants in agriculture.

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References

Ahmad F, Ahmad I, Khan MS (2008) Screening of free-living rhizospheric bacteria for their multiple plant growth-promoting activities. Microbiol Res 163:173–181

Bakker AW, Schipper B (1987) Microbial cyanide production in the rhizosphere in relation to potato yield reduction and Pseudomonas spp. mediated plant growth stimulation. Soil Biol Biochem 19:451–457

Berleth T, Sachs T (2001) Plant morphogenesis: long distance coordination and local patterning. Curr Opin Plant Biol 4:57–62

Chen YP, Rekha PD, Arun AB, Shen FT, Lai WA, Young CC (2006) Phosphate solubilizing bacteria from subtropical soil and their tricalcium phosphate solubilizing abilities. Appl Soil Ecol 34:33–41

Cole JR, Chai B, Farris RJ, Wang Q, Kulam-Syed-Mohideen AS, McGarrell DM, Bandela AM, Cardenas E (2007) The ribosomal database project (RDP-II): introducing myRDP space and quality controlled public data. Nucleic Acid Res 35:D169–D172

Collins CH, Lyne PM (1980) Microbiological methods. Butterworth and Co. (Publishers) Ltd., London

Diby P, Sarma YR (2006) Antagonistic effects of metabolites of strains on the different growth phases of Phytophthora capsici, foot rot pathogen of black *Pseudomonas fluorescens* pepper (Piper nigrum L.). Arch Phytopathol Plant Protect 39:113–118

Gordon AS, Weber RP (1951) Colorimetric estimation of indole acetic acid. Plant Physiol 26:192–195

Hameeda B, Reddy Y, Rupela OP, Kumar GN, Reddy G (2006) Effect of carbon substrates on rock phosphate solubilization by bacteria from composts and macrofauna. Curr Microbiol 53:298–302

Jeffries P, Gianinazzi S, Perotto S, Turnau K, Barea JJM (2003) The contribution of arbuscular mycorrhizal fungi in sustainable maintenance of plant health and soil fertility. Biolog Fertil Soils 37:1–16

Jones DL, Darrah PR (1994) Role of root derived organic acids in the mobilization of nutrients from the rhizosphere. Plant Soil 166:247–257

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

Kimura M (1980) A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. J Mol Evol 16:111–120

Kumar S, Tamura K, Nei M (2004) MEGA3: integrated software for molecular evolutionary genetics analysis and sequence alignment. Brief Bioinform 5:150–163

Lifshitz R, Kloepper JW, Kozlowski M, Simonson C, Carlson J, Tipping EM, Zaleska I (1987) Growth promotion of canola (rapeseed) seedlings by a strain of *Pseudomonas putida* under gnotobiotic conditions. Can J Microbiol 8:102–106

Lucy M, Reed ER, Glick B (2004) Application of free living plant growth promoting rhizobacteria. Antonie Van Leeuwenhoek 86(1):1–25

Mehta S, Nautiyal CS (2001) An efficient method for qualitative screening of phosphate solubilizing bacteria. Curr Microbiol 43:51–56

Murphy JP, Riley JP (1962) A modified single solution method for the determination of the phosphate in natural waters. Anal Chem Acta 27:31–36

Pérez E, Sulbaran M, Ball M, Yarzabal LA (2007) Isolation and characterization of mineral phosphate-solubilizing bacteria naturally colonizing a limonitic crust in the south-eastern Venezuelan region. Soil Biol Biochem 39:2905–2914

Pieterse CMJ, Van Pelt JA, Van Wees SCM, Ton J, Leon-Kloosterziel KM, Keurentjes JJB, Verhagen BMW (2001) Rhizobacteria mediated induced systemic resistance: triggering, signaling and expression. Eur J Plant Pathol 107:51–61

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

Saitou N, Nei M (1987) The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol 4:406–425

Sarwar M, Arshad M, Martens DA, Frankenberger WT Jr (1992) Tryptophan- dependent biosynthesis of auxins in soil. Plant Soil 147:207–215

Schwyn B, Neilands JB (1987) Universal chemical assay for the detection and determination of siderophore. Anal Biochem 160:47–56

Selvakumar G, Mohan M, Kundu S, Gupta AD, Joshi P, Nazim S, Gupta HS (2007) Cold tolerance and plant growth promotion potential of *Serratia marcescens* strain SRM (MTCC 8708) isolated from flowers of summer squash (*Cucurbita pepo*). Lett Appl Microbiol 46:171–175

Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG (1997) The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res 25:4876–4882