Growth promotion of wheat seedlings by *Exiguobacterium acetylicum* 1P (MTCC 8707) a cold tolerant bacterial strain from the Uttarakhand Himalayas

G. Selvakumar · S. Kundu · Piyush Joshi · Sehar Nazim · A. D. Gupta · H. S. Gupta

Received: 18 June 2007 / Accepted: 31 March 2008

Abstract *Exiguobacterium acetylicum* strain 1P (MTCC 8707) is a gram-positive, rod-shaped, yellow pigmented bacterium isolated from soil on nutrient agar plates at 4°C. The identity of the bacterium was arrived on the basis of the biochemical characterization, BIOLOG sugar utilization pattern and sequencing of the 16S rRNA gene. It grew at temperatures ranging from 4 to 42°C, with temperature optima at 30°C. It expressed multiple plant growth promotion attributes such as phosphate solubilization, indole acetic acid (IAA), siderophore and hydrogen cyanide (HCN) production, differentially at suboptimal growth temperatures (15 and 4°C). At 15°C it solubilized phosphate (21.1 µg of P ml⁻¹ day⁻¹), and produced IAA (14.9 µg ml⁻¹ day⁻¹) in tryptophan amended media. Qualitative detection of siderophore production and HCN were possible at 15°C. At 4°C it retained all the plant growth promotion attributes. Seed bacterization with the isolate, positively influenced the growth and nutrient uptake parameters of wheat seedlings in glass house studies at suboptimal cold growing temperatures.

Keywords Cold tolerance · *Exiguobacterium acetylicum* · Plant growth promotion

Introduction

The current trends in agriculture which focus on the reduction of chemical pesticides and inorganic fertilizers have prompted the search for alternatives that sustain agricultural outputs and environmental quality [1]. An important means of achieving this is through the use of microbial inoculants that have a bearing on soil health and plant growth promotion. Bacteria that have a beneficial effect on plant growth and development are referred to as plant growth promoting bacteria (PGPB) [2]. Such strains usually stimulate plant growth either by nitrogen fixation [3], solubilization of nutrients [4], production of growth hormones, or through biological control mechanisms such as siderophore production, chitinase activity, antibiotics and cyanide production [5–8]. Though the beneficial effects of inoculation of such PGPB have been well documented at several locations, there is an urgent need to evolve location specific strains that are able to positively influence plant growth in prevailing soil and edaphic conditions.

In recent years, growing attention in research has been devoted to cold-adapted microorganisms. These microbes successfully colonize cold habitats, which compose more than 80% of the earth's biosphere, and play a major role in the processes of nutrient turnover at low temperatures [9]. Among the different cold tolerant bacterial genera that have been explored, the genus *Exiguobacterium* is emerging as a bacterium with an impressive diversity in its geographic distribution. In addition to the diverse origins of the type strain, *Exiguobacterium* has been isolated from the Siberian permafrost environment [10], a glacial ice core sample in Greenland [11], the stem of potato in Austria [12], and hot springs in Yellowstone National Park [13]. The

G. Selvakumar (✉) · S. Kundu · P. Joshi · S. Nazim ·
A. D. Gupta · H. S. Gupta
Vivekananda Institute of Hill Agriculture,
Indian Council of Agricultural Research,
Almora - 263 601, India

E-mail: gselva74@rediffmail.com

species *Exiguobacterium oxidotolerans* has been described as a novel alkaliphile with high catalase activity [14].

In the state of Uttarakhand in the N.W. Indian Himalayas, the mean minimum temperature during the winter months varies from 3.5°C to subzero levels depending on the geographical location. Snowfall is quite common in the upper region and the ground remains frozen for varying periods. Due to this the mean soil temperature ranges from 2 to 10°C. Moreover the soils of hilly regions of Uttarakhand state are generally acidic in reaction, with low native P and high phosphorus fixing capacity, leading to low productivity of major crops [15]. Considering the unique ecological and edaphic features of the hill and mountain agro-ecosystem, it is most evident that the benefits of microbial inoculation would be realized only when locally adapted strains are used as bioinoculants. In this context, we report the isolation characterization and phylogenetic analysis of a cold tolerant *Exiguobacterium acetylicum* strain 1P, from a high altitude soil in the Uttarakhand Himalayas, and its effect on the growth of wheat seedling under cold temperature conditions.

Materials and methods

Strain isolation and maintenance

The rhizospheric soil used for isolation was collected from an altitude of 2200 m above mean sea level in Almora district (29°36'N and 79°40'E), of Uttarakhand state, in the N.W. Indian Himalayan Region. The vegetation of the location was predominantly of apple (*Malus domestica*), with no obvious soil management, the soil characteristics were pH 6.0, available N (369.9 kg ha⁻¹), available P (14.6 kg ha⁻¹), available K (189 kg ha⁻¹), organic carbon (0.28 %). The processed soil sample was serially diluted (10,000 fold), spread plated on full strength nutrient agar and incubated at 4°C for 48 h. At the end of the incubation period, two bacterial colonies with distinct colony morphologies were recovered on the incubation plates. The isolated colonies were purified by streaking on nutrient agar. Gram staining revealed that one culture was gram-positive while the other culture was gram-negative. The cultures were initially screened for their plant growth promotion traits (phosphate solubilization, siderophore and indole acetic acid (IAA) production at 15°C). Based on the preliminary results, the gram-positive culture was chosen for intensive study.

Growth, identification and characterization of the bacterial isolate

Preliminary morphological and physiological characterization was carried out as per standard methodologies [16].

The observation that the isolate grew at temperatures ranging from 4 to 42°C, prompted us to determine its plant growth promoting abilities at cold temperatures. A growth curve of the isolate was drawn at three temperatures viz., 4, 15 and 30°C, by inoculating 1ml of the exponential culture (3 × 10⁸ cells ml⁻¹) in 100 ml of nutrient broth (initial population log 6 cells ml⁻¹), and determining the cell population at 2 h intervals. The population values were log transformed and plotted against time. Sequencing of the 16S ribosomal RNA gene and determination of the BIOLOG sugar utilization pattern were performed at Institute of Microbial Technology (IMTECH), Chandigarh. The universal eubacterial primer 27f (5'-GAGAGTTTGATCCTGGCTCAG-3'), was used for partial sequencing (448 bp fragment) of the 16S rRNA gene. The gene sequence was subjected to a similarity search using the BLAST algorithm [17]. Nucleotide sequences were aligned using the CLUSTAL X 1.81 algorithm [18]. Phylogenetic and molecular evolutionary analyses were conducted using MEGA version 3.1 [19]. The phylogenetic tree was constructed by the neighbor-joining method [20] using the distance matrix from the alignment. Distances were calculated using the Kimura method [21]. The GenBank accession number of the isolate is EF596775. The cell culture of the isolate has been deposited in the Microbial Type Culture Collection (MTCC), Chandigarh, India.

Quantitative estimation of phosphate solubilization and IAA production

Quantitative estimation of tricalcium phosphate (TCP) solubilization and IAA production were carried out at three different incubation temperatures viz., 4, 15 and 30°C. Quantitative estimation of phosphate solubilization was determined in NBRIP broth [22], by inoculating 1ml of bacterial suspension (3 × 10⁷ cells ml⁻¹) in 50 ml of NBRIP broth in Erlenmeyer flasks (150 ml), and incubating the flasks for 7d. At the end of the incubation period, the cell suspension was centrifuged at 10000 rpm for 10 min. The P content in the supernatant was spectrophotometrically estimated by the ascorbic acid method [23]. Estimation of IAA was done by inoculation of 200 µl of bacterial suspension (3 × 10⁷ cells ml⁻¹) in 10 ml Luria Bertani (LB) broth amended with L-tryptophan (100 µg ml⁻¹), and incubating it for a period of 48 h. The IAA content in the culture suspension was estimated by the standard colorimetric procedure [24].

Qualitative estimation for siderophore production and HCN production

Siderophore and hydrogen cyanide (HCN) production by the isolate were estimated qualitatively, at three different

incubation temperatures viz., 4, 15 and 30°C. Siderophore production was detected by the Chrome Azurol-S (CAS) assay [25], and the diameter of the clearing zone was rated visually. HCN production was inferred by the qualitative method [26]. The change in the color of the filter paper dipped in 0.05% picric acid prepared in 2% sodium carbonate, from yellow to dark brown was rated visually depending on the intensity of the color change.

Plant growth promotion assay

The plant growth promotion ability of the isolate was assessed using wheat seedlings (cv. VL 802) in non-sterile soil under glass house conditions. Seed bacterization was performed as per standard procedure [27], by soaking surface sterilized seeds in a culture suspension of the bacterium (10^8 cfu ml⁻¹) for 15 min and coating with a charcoal based inoculant of the bacterium (10^6 cfu g⁻¹). Control seeds were soaked in sterile uninoculated medium and coated with sterile charcoal. Control seeds were treated with sterile uninoculated medium and coated with sterile charcoal. Seeds were sown in plastic pots (15 cm diameter) containing 2 kg of non-sterile soil, and placed in a temperature controlled growth chamber at $22 \pm 1^\circ\text{C}$ for 42 d. The mean soil temperature recorded during the experimental period was $18 \pm 0.5^\circ\text{C}$. Thinning of seedlings was done 7 d after sowing and two seedlings per pot were maintained throughout the experimental period. The soil was moistened to 60% of its water holding capacity. The treatments were arranged in a completely randomized design with ten replications. At the end of the experimental period, plants were uprooted, washed under running water and root and shoot lengths were measured. The plant biomass was dried at a constant temperature of 65°C and dry weights were recorded. The oven dried samples were ground to pass through a 0.2 mm sieve for estimation of N content in a CHN analyzer (CHN-O – Rapid Foss Heareus). The P content was estimated by the ammonium molybdate method [28] and the K

content by flame photometry [29]. The nutrient uptake was calculated from the respective nutrient contents. Statistical analysis was performed with the SPSS package; version 2.2 and treatment means were compared at 5% level of significance [30].

Results and discussion

The isolate was gram-positive, and appeared as small rods with scattered arrangement. Raised, circular, white colonies (1–5 mm in diameter) appeared on nutrient agar. The colonies turned yellow and later orange on aging. The biochemical properties of the isolate are presented in Table 1. The bacterium was able to tolerate salt concentrations upto 8% and grew at temperatures ranging from 4 to 42°C . Positive BIOLOG sources were β -cyclodextrin, dextrin, glycogen, tween-40, N-acetyl-D glucosamine, amygdalin, arbutin, D-cellobiose, D-fructose, L-fructose, D-galactose, D-galacturonic acid, gentiobiose, α -D-glucose, maltose, maltotriose, D-mannitol, D-melibiose, α -methyl-D-galactoside, 3-methyl-D-glucose, α -methyl-D-glucoside, β -methyl-D-glucoside, palatinose, D-psicose, D-ribose, salicin, sedoheptulosan, D-sorbitol, stachyose, sucrose, D-trehalose, D-xylose, succinic acid mono-methyl ester, pyruvic acid, L-alanyl-glycine, L-glutamic acid, glycyl-L-glutamic acid, 2,3-butanediol, glycerol, adenosine, 2'-deoxy adenosine, inosine, thymidine and uridine.

The growth pattern of the isolate recorded at three different incubation temperatures is graphically represented in (Fig. 1). The growth curves clearly showed that the isolate grew at three different incubation temperatures and attained exponential growth at 15 and 4°C when incubated for 24 h. The 16S rRNA gene sequence had 98% similarity with sequences of *Exiguobacterium acetylicum* available in the public database. The phylogenetic analysis (Fig. 2) revealed that it formed a close cluster with the type strain *Exiguobacterium acetylicum* DSM 20416, where as it was distant

Table 1 Morphological, biochemical and physiological characteristics of *Exiguobacterium acetylicum* 1P

Morphological characteristics

Colony characteristics: Medium sized circular, slightly raised, yellow pigmented, smooth colonies of 4–6 mm diameter on nutrient agar at 15°C after 3–4 days incubation

Cell features: Gram-positive, short rods with scattered arrangement of cells

Biochemical properties

Positive for: Methyl red test, citrate utilization, caesin utilization, gelatin hydrolysis, arginine decarboxylation, catalase, oxidase, utilization of maltose, fructose, dextrose, trehalose, sucrose, mannose and mannitol as sole carbon sources

Negative for: Indole test, VP reaction, starch utilization, ornithine decarboxylation, nitrate reduction, lecithinase, DNase. Utilization of raffinose, mellibiose, L-arabinose, lactose, xylose, galactose, rhamnose and sorbitol as sole carbon sources

Physiological characteristics

Temperature tolerance: 4 to 42°C , optimal growth temperature 30°C , pH tolerance 4 to 10, NaCl tolerance 2 to 8%

from other gram-positive bacteria such as *Brevibacterium mariniaquaticum*, *Brevibacterium otidis* and *Flavobacterium aquatile*.

The different plant growth promotion attributes of the isolate were estimated at three different incubation temperatures (Table 2). The higher values of all plant growth promotion traits were recorded at 30°C, but all the traits were retained at 15 and 4°C. At 15°C it solubilized phosphate (21.1 µg of P ml⁻¹ day⁻¹), and produced IAA (14.9 µg ml⁻¹ day⁻¹) in tryptophan amended media. Qualitative detection of siderophore production and HCN were possible at 15°C. At 4°C, the lower temperature extreme for its growth, the isolate solubilized phosphate (1.5 µg of P ml⁻¹ day⁻¹), and produced IAA (10.4 µg ml⁻¹ day⁻¹), besides producing siderophore and HCN.

The plant growth promotion ability of the cold tolerant *Exiguobacterium acetylicum* strain 1P was determined under non-sterile soil conditions at cold temperatures. Bacterized wheat seedlings recorded 16.54 and 8.16% higher root and shoot lengths respectively, compared to uninoculated control (Table 3). A corresponding increase in the root and shoot biomass was also observed in bacterized seedlings. Seed bacterization resulted in greater enhancement of the root growth, as compared to the shoot growth. An additional manifestation of the plant growth promotion was the increased nutrient uptake observed in the bacterized plants. An increase of 51.9, 38.5 and 45.4% in the uptake of N, P

and K respectively was recorded in the bacterized plants over the uninoculated controls.

The alpine and subalpine regions of the N.W. Indian Himalayan region are known to be a rich source of microbial diversity, and several novel cold tolerant bacterial species have been isolated from this region [31]. Though *Exiguobacterium acetylicum* has been widely studied for its extremophilic nature and enzyme production abilities, its plant growth promotion potential, especially under sub-optimal temperatures was not known earlier. Considering the fact that this strain was isolated at 4°C, and grew at temperatures ranging from 4 to 42°C, it would be appropriate to call it as a cold tolerant plant growth promoting strain.

Bacterial plant growth promotion is a well established and complex phenomenon that is often achieved by the activities of more than one plant growth promoting trait exhibited by the associated bacterium [32]. Most studies have quantified bacterial plant growth promotion as an incremental increase in plant biomass over the uninoculated controls [33]. Alternately, an increase in nutrient uptake as a result of bacterization has been demonstrated in this study. Though our strain possessed multiple plant growth promotion traits, we would attribute the increased nutrient uptake by bacterized seedlings mainly to auxin production by the bacterium, which is known to stimulate root growth, thereby facilitating an increased uptake of nutrients from the soil. An earlier observation by Khalid et al. [34], who observed a linear positive correlation between *in vitro* auxin production and increase in early growth parameters of inoculated wheat seeds, is in support of the present results. Another possible factor is the enhanced availability of soluble P in the rhizospheric region, as a result of the P solubilization by the bacterium. The role of the siderophores produced by this bacterium and their role in disease suppression and iron nutrition of wheat seedlings is a researchable issue.

HCN production by rhizospheric bacteria has been variably viewed, while it is considered beneficial from the biocontrol point of view, it is considered detrimental due to its interference with the cytochrome P₄₅₀ system, of certain plants [35]. In this study, seed bacterization did not have a detrimental effect on plant growth and development. HCN resistance in certain plants has been attributed

Fig. 1 Growth curve of cold tolerant *Exiguobacterium acetylicum* 1P, at three different incubation temperatures

Table 2 Plant growth promotion attributes of *Exiguobacterium acetylicum* 1P as influenced by temperature of incubation

Temperature of incubation (°C)	IAA production (µg ml ⁻¹ day ⁻¹)	P solubilization (µg of P ml ⁻¹ day ⁻¹)	HCN production	Siderophore production
4	10.4 ± 0.04	1.5 ± 0.01	+	+
15	14.9 ± 0.06	21.1 ± 1.18	+	+
30	17.1 ± 0.02	22.6 ± 2.16	+++	++

*Values are mean ± SE of three independent observations

+ Low; ++ medium; +++ high

Fig. 2 Neighbor joining tree showing phylogenetic relationship between *Exiguobacterium acetylicum* 1P and various representative gram-positive eubacterial genera (GenBank accession numbers within parentheses). The bar represents 0.02 substitutions per site. Values displayed at the nodes ($n=1000$) indicate bootstrap values. Type strain of *Exiguobacterium acetylicum* DSM 20416^T

Table 3 Effect of inoculation of the *Exiguobacterium acetylicum* 1P on the nutrient uptake parameters of wheat (cultivar VL 802)

Treatment	Root length (cm)	Shoot length (cm)	Dry root biomass (gm)	Dry shoot biomass (gm)	N uptake (mg pot ⁻¹)	P uptake (mg pot ⁻¹)	K uptake (mg pot ⁻¹)
Control	27.8	19.6	0.9	0.8	10.2	29.0	18.5
<i>E. acetylicum</i> 1P	32.4	21.2	1.1	1.0	15.5	40.2	26.9
LSD (p=0.05)	3.4	1.2	0.1	0.1	0.4	0.7	0.6

Values are the mean of ten replicates. Data were analyzed using one way analysis of variance and treatment means were compared at 5% level of significance. Each pot contained two kg of non-sterile soil and two 42 d old wheat seedlings.

to the presence of a cyanide resistant respiratory pathway [36]. Cyanogenesis by rhizospheric bacteria has been proposed as a major means of control of the take all disease of wheat caused by the fungal pathogen *Gaeumannomyces graminis* var *tritici*, while simultaneously promoting wheat root growth [37]. In a separate study we observed that this isolate was antagonistic to the growth and development of several plant pathogenic fungi under in vitro conditions (data not shown). The biological control potential of this isolate as a result of cyanogenesis is to be explored further.

The North Western Indian Himalayas present a virgin opportunity for exploration and characterization of cold tolerant microorganisms, due to the unique ecological niche of the region that includes glaciers, alpine and subalpine regions. We had earlier reported the occurrence of cold tolerant plant growth promoting strains of *Pantoea dispersa* and *Serratia marcescens* from this region [38, 39]. With increasing awareness about the perils of chemical fertilizer based

agricultural practices, it is imperative to scout such pristine environments for beneficial microbial strains. These strains would ideally retain their plant growth promotion traits at cold temperature conditions. In this context we propose that this cold adapted strain of *Exiguobacterium acetylicum*, needs to be explored further for its plant growth promotion ability under field conditions.

Acknowledgments This research work was supported by ICAR, under the project “Application of Microorganisms in Agriculture and Allied Sectors.” The technical assistance of Sanjay Kumar is gratefully acknowledged.

References

1. Ahmed S (1995) Agriculture–fertilization interface in Asia issue of growth and sustainability, Oxford and IBH publishing Co, New Delhi

2. Andrews JH and Harris RF (2003) The ecology and biogeography of microorganisms on plant surfaces. *Annu Rev Phytopathol* 38:145–180
3. Han J, Sun L, Dong X, Cai Z, Yang H, Wang Y and Song W (2005) Characterization of a novel plant growth-promoting bacteria strain *Delftia tsuruhatensis* HR4 both as a diazotroph and a potential biocontrol agent against various pathogens. *Syst Appl Microbiol* 28:66–76
4. Rodriguez H and Fraga R (1999) Phosphate solubilizing bacteria and their role in plant growth promotion. *Biotechnol Adv* 17:319–339
5. Correa JD, Barrios ML and Galdona RP (2004) Screening for plant growth-promoting rhizobacteria in *Chamaecytisus proliferus* (tagasaste), a forage tree-shrub legume endemic to the Canary Islands. *Plant Soil* 266:75–84
6. Haggag WM (2002) Sustainable agricultural management of plant diseases. *J Biol Sci* 2:280–284
7. Renwick AR, Campbell A and Coe S (1991) Assessment of in vivo screening systems for potential biocontrol agents of *Gaeumannomyces graminis*. *Plant Physiol* 40: 524–532
8. Pal KK, Tilak KVBR, Saxena AK, Dey R and Singh CS (2001) Suppression of maize root diseases caused by *Macrophomina phaseolina*, *Fusarium moniliforme* and *Fusarium graminearum* by plant growth promoting rhizobacteria. *Microbiol Res* 156:209–223
9. Kottmeier ST and Sullivan CW (1990) Bacterial biomass and production in pack ice of Antarctica marginal ice age zones. *Deep Sea Res* 37:1311–1330
10. Rodrigues DF, Goris J, Vishnivetskaya T, Gilichinsky D, Thomashow MF and Tiedje JM (2006) Characterization of *Exiguobacterium* isolates from the Siberian permafrost. Description of *Exiguobacterium sibiricum* sp. nov. *Extremophiles* 10:285–294
11. Miteva VI, Sheridan PP and Brenchley JE (2004) Phylogenetic and physiological diversity of microorganisms isolated from the deep Greenland glacier ice core. *Appl Environ Microbiol* 70:202–213
12. Reiter B, Pfeifer U, Schwab H and Sessitsch A (2002) Response of endophytic bacterial communities in potato plant to infection with *Erwinia carotovora* subsp. *atroseptica*. *Appl Environ Microbiol* 68:2261–2268
13. Knudston KE, Haas EJ, Iwen PC, Ramaley WC and Ramaley RF (2001) Characterization of gram-positive, non-spore-forming *Exiguobacterium* like organism isolated from a West Colorado (USA) hot spring. *Abstr Ann Meet Am Soc Microbiol* I-92, p 30
14. Yumoto I, Narisawa MH, Hirota K, Shingyo T, Takebe F, Nodasaka Y, Matsuyama H and Hara I (2004) *Exiguobacterium oxidotolerans* sp. nov., a novel alkaliphile exhibiting high catalase activity. *Int J Syst Evol Microbiol* 54:2013–2017
15. Pal SS (2000) Management of soil microbial population and crop yield with indigenous phosphate solubilizing bacterial culture in Garhwal Himalaya. *J Indian Soc of Soil Sci* 48: 184–188
16. Collins CH and Lyne PM (1980) Microbiological methods. Butterworth and Co. (Publishers) Ltd. London
17. Altschul SF, Gish W, Miller W, Myers EW and Lipman DJ (1990) Basic local alignment search tool. *J Mol Biol* 215: 403–410
18. Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F and Higgins DG (1997) The Clustal X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res* 24:4876–4882
19. Kumar S, Tamura K and Nei M (2004) MEGA3: Integrated Software for Molecular Evolutionary Genetics Analysis and Sequence Alignment. *Brief Bioinform* 5:150–163
20. Saitou N and Nei M (1987) The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* 4:406–425
21. Kimura M (1980) A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. *J Mol Evol* 16:111–120
22. Mehta S and Nautiyal CS (2001) An efficient method for qualitative screening of phosphate-solubilizing bacteria. *Curr Microbiol* 43:51–56
23. Murphy JP and Riley JP (1962) A modified single solution method for the determination of the phosphate in natural waters. *Anal Chem Acta* 27:31–36
24. Gordon AS and Weber RP (1951) Colorimetric estimation of indole acetic acid. *Plant Physiol* 26:192–195
25. Schwyn B and Neilands JB (1987) Universal chemical assay for the detection and determination of siderophore. *Anal Biochem* 160:47–56
26. Bakker AW and 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
27. Elliot LF and Lynch JM (1984) *Pseudomonas* as a factor in the growth of winter wheat (*Triticum aestivum* L.). *Soil Biol Biochem* 16:69–71
28. Olsen SR and Sommers LE (1982) Phosphorus, in soil chemical and microbiological properties, part 2. (Page AL, Miuller RH, Keeney DR eds). *Methods of soil analysis*, Amer Soc Agron, Madison, Wisconsin, USA. 403–430
29. Jackson ML (1973) Soil chemical analysis. Prentice Hall of India Pvt Ltd., New Delhi, pp. 25–214
30. SAS Institute Inc (2001) SAS user's guide, version 8.2, SAS Institute Inc, Cary, North Carolina, USA
31. Shivaji S, Chaturvedi P, Reddy GSN and Suresh K (2005) *Pedobacter himalayensis* sp. nov., from Hamta glacier located in the Himalayan mountain range in India. *Int J Syst Evol Microbiol* 55:10830–1088
32. Lifshitz R, Kloepper JW, Kozłowski M, Simonson C, Carlson J, Tipping EN and Zaleska I (1987) Growth promotion of canola (rape-seed) seedlings by a strain of *Pseudomonas putida* under gnotobiotic conditions. *Can J Microbiol* 8: 102–106
33. Hameeda B, Rupela OP and Reddy G (2006) Application of plant growth-promoting bacteria associated with composts and macrofauna for growth promotion of pearl millet (*Pennisetum glaucum* L.). *Biol Fertil Soils* 43:221–227
34. Khalid A, Arshad M and Zahir ZA (2004) Screening plant growth promoting rhizobacteria for improving growth and yield of wheat. *J Appl Microbiol* 96:473
35. Kremer RJ and Souissi T (2001) Cyanide production by rhizobacteria and potential for suppression of wheat seedling growth. *Curr Microbiol* 43:182–186
36. Lambers H (1982) Cyanide-resistant respiration: A non phosphorylating electron transport pathway act as an energy overflow. *Physiol Plant* 55:478–485

37. Sarniguet A, Lucas P, Lucas M and Samson R (1992) Soil conduciveness to take all of wheat: Influence of the nitrogen fertilizers on the structure of populations of fluorescent pseudomonads. *Plant Soil* 145:29–36
38. Selvakumar G, Kundu S, Joshi Piyush, Nazim Sehar, Gupta AD, Mishra PK and Gupta HS (2008) Characterization of a cold tolerant plant growth promoting bacterium *Pantoea dispersa* 1A, isolated from a subalpine soil in the North Western Indian Himalaya. *World J Microbiol Biotechnol* 24:955–960
39. Selvakumar G, Mohan M, Kundu S, Gupta AD, Joshi P, Nazim S and 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