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ORIGINAL PAPER

Drought-tolerant endophytic actinobacteria promote growth of wheat (*Triticum aestivum*) under water stress conditions

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Abstract Drought tolerant endophytic actinobacteria *Streptomyces coelicolor* DE07, *S. olivaceus* DE10 and *Streptomyces geysiriensis* DE27 were isolated from cultivated plants of arid and drought affected regions of Rajasthan, India. These isolates exhibited plant growth promotion traits and intrinsic water stress tolerance from −0.05 to −0.73 MPa. Maximum auxin production was observed in majority of actinobacterial cultures in the logarithmic to stationary phase of growth. Significant enhancement of wheat seedling vigour was recorded by the inoculation of these endophytic actinobacteria. *S. olivaceus* DE10 recorded maximum accumulation of indole 3-acetic acid (84.34 μg mg^{-1} protein). Culture and cell-free extract of the endophytes was applied on to wheat seeds to assess the effect on growth in water-stressed soil. Maximum yield was recorded with the inoculation of *S. olivaceus* DE10 culture (492.77 kg ha^{-1}) and cell-free extract (262.31 kg ha^{-1}). Co-inoculation of *S. olivaceus* DE10 + *S. geysiriensis* DE27 recorded highest yield of 550.09 kg ha^{-1} while their cell-free extract yielded 524.92 kg ha^{-1}. Overall, wheat seeds treated with cultures showed better plant growth and yield in comparison to control. Direct coating of cultures on seeds yielded better performance than cell-free extract coated on seeds and co-inoculation of cultures or cell-free extract proved better than single culture inoculations. Production of phytohormones, plant growth promotion traits combined with water stress tolerance potential in these endophytic actinobacteria played a cumulative synergistic role that supported enhanced plant growth promotion of wheat in the stressed soil.

Keywords Indole 3-acetic acid · Drought-tolerance · Endophyte · Plant growth promotion · *Streptomyces*

Introduction

Water deficit is among the most common environmental stresses to affect plant growth and influence crop quality and productivity in the arid and semiarid regions (Jones 2009). Plants growing under detrimental environmental conditions, such as those occurring in arid and semiarid soils, undergo water limitation and nutrient deficiencies. Rhizosphere microorganisms are adapted to adverse conditions and may compensate for such detrimental conditions (Ruiz-Lozano et al. 1996). Inoculation with native beneficial microorganisms may increase drought tolerance of plants growing in arid or semiarid areas (Marulanda et al. 2007). Endophytic microorganisms reside within living plant tissues at some phases of their life cycle without causing apparent damage to them (Petrini 1991). They can be extracted from inner plant parts or isolated from surface-disinfested plant tissues (Hallmann et al. 1997). Endophytic bacteria and filamentous fungi have been widely reported from seeds, roots, stems, leaves, needles, twigs and barks of various plant species and the role of endophytic microbial community in endophyte-plant associations has been intensively discussed (Seena and Sridhar 2004; Reinhold-Hurek and Hurek 2011). Root-colonizing non-pathogenic endophytic bacteria can

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increase plant resistance to biotic and abiotic stress factors (Dimkpa et al. 2009).

Plant-associated microorganisms are involved in symbiotic and associative microbial activities that help in plants to establish in their environment (Morrissey et al. 2004). These are economical and safer source of nutrition for increasing agricultural production and improving soil fertility. Suggested mechanisms for microbe associated plant growth include production of cytokinins, gibberellins, indole-3-acetic acid (IAA, an auxin), increased mineralization and nitrogen availability in the soil (Coombs and Franco 2003). Indole-3-acetic acid is quantitatively the most abundant phytohormone secreted by most plant-associated bacteria and play positive role in plant growth. IAA biosynthesis is related to environmental stress, including acidic pH, osmotic and matrix stress, and carbon limitation (Spaepen et al. 2007).

Microbial endophytes include both commensal microorganisms that have no direct effect on plants and beneficial microorganisms that could be used for the benefits of plants (Procopio et al. 2009; Shi et al. 2010). The role of endophytic microorganisms in the promotion of plant growth has received increasing attention in recent years as the introduction and/or manipulation of endophytic microbial population may provide a consistent and effective enhancement in the productivity of crops (Morrissey et al. 2004; Shi et al. 2010).

Actinobacteria are constantly found in the phytosphere (Shirokikh et al. 2006; Norovsuren et al. 2007; Merzaeva and Shirokikh 2006). They are the exciting novel sources of bioactive compounds and have been reported from several hosts such as tomato, banana, wheat, and maize with promising anti-microbial activity against pathogenic strains (Coombs and Franco 2003; Cao et al. 2004; Castillo et al. 2007) and root endophytic actinobacteria play an important role in plant development (Verma et al. 2009). Their involvement in the phytohormonal regulation of plant growth (Khamna et al. 2010; Shi et al. 2010) has received little attention in relation to crop plants under environmental stresses. The relevant literature contains just sparse references to the ability of the representatives of the genera *Streptomyces*, *Micromonospora, Corynebacterium, Frankia, Mycobacterium* (Khamna et al. 2010), and *Rhodococcus* (Tsavkelova et al. 2005) in plant growth promotion. Their presence as endophytic symbiont with plant and ability to help plants in coping with the detrimental soil conditions like drought has not gained much attention although actinobacterial existence with the plants is an established phenomenon (Khamna et al. 2010). This versatility of endophytic actinobacteria encouraged us to explore these organisms from arid and drought affected regions for their utilization in alleviating drought stress in wheat (*Triticum aestivum*) crop.

Materials and methods

Site description and sample collection

The plant samples were collected from arid and drought-affected fields of Bikaner and Jaisalmer, Rajasthan, India. The roots from five plant species Bui (*Aerva tomentosa,* Amaranthaceae), Keeker (*Acacia nilotica,* Mimosaceae), Kheep (*Leptadenia pyrotechnica,* Asclepiadaceae), Phog (*Calligonum polygonides,* Polygonaceae) and Bajra (*Pennisetum glaucum,* Poaceae) were collected from Beechwal (28°04′N,73°20′E), Arjanser (28°56′N,73°52′E), Napaser (27°57′N,73°33′E) in Bikaner and Sam (26°49′N,70°30′E) village in Jaisalmer, India. The samples were kept in sterile polythene bags and transported to laboratory in Styrofoam boxes maintained at 4 °C.

Isolation and characterization of endophytic actinobacteria

Plants roots were thoroughly washed by a sonication step to dislodge soil and organic matter, dipped in Tween 20 for 5 min and sterilized by sequential immersion in 70 % ethanol for 5 min and in a solution of sodium hypochlorite (0.9 % available chlorine) for 20 min (Coombs and Franco 2003). Samples were washed (3×) in sterile water and soaked in 10 % $NaHCO_3$ solution for 10 min to disrupt plant tissues and inhibit the growth of endophytic fungi (Baker 1990). After washing for three times for 15 min in sterile distilled water, roots were homogenized with necessary dilutions (10^{-2}), plated on petriplates containing three different media (Actinomycetes isolation agar, Starch casein agar, Humic acid-Vitamin agar) with 50 μg ml^{-1} each of cycloheximide and nalidixic acid and incubated at 32 °C for 7–15 days. Actinobacterial colonies appearing on isolation plates were picked on the basis of colony morphology and diffusible pigments and characterized for plant growth promoting (PGP) attributes like ammonia (Cappuccino and Sherman 1992), siderophore (Schwyn and Neilands 1987) and IAA production (Brick et al. 1991). In addition to PGP attributes, lysozyme sensitivity, urea and casein hydrolysis, gelatin degradation and hydrogen sulphide production were also tested according to Cappuccino and Sherman (1992). To study the drought tolerance ability of actinomycetes at various water stress levels, Actinomycetes isolation agar (AIA) plates with different water potentials (−0.05, −0.15, −0.30, −0.49, and −0.73 MPa) were prepared by adding filter sterilized 5–25 % polyethylene glycol (PEG6000) (Michel and Kaufmann 1973) and was allowed to dry overnight in laminar airflow chamber. The plates were inoculated with actinobacterial cultures and incubated at 32 °C for 1–2 weeks under continuous observation. Growth of isolates at various stress levels was

recorded. All experiments were repeated three times and pooled data was analyzed.

Scanning electron microscopy

Scanning electron microscopy was carried out to study the mycelial morphology and root colonization. Germinated seedlings with mycelia were taken (after 10 days of sowing) and washed in 0.1 M sodium cacodylate buffer (pH 7.4). Mycelia were fixed in 2.5 % glutaraldehyde in 0.1 M sodium cacodylate buffer for 4 h at 4 °C followed by post-fixation with 1 % OsO_4 in 0.1 M sodium cacodylate buffer (pH 7.4) and dried in a critical point dryer (EMITECH model K850, Hitachi). The preparations were mounted onto aluminium holders, sputter-coated with 10 nm Au and observed by Scanning electron microscope (Hitachi model S3400 at 15–30 kV, 2–5.00 μm).

Identification of endophytic actinobacteria

Genomic DNA of actinobacterial isolates was extracted by the modified protocol of Boudjella et al. (2006). 16S rRNA gene was PCR amplified using two universal primer pairs fD1 (5′-GAGTTTGATCCTGGCTCA-3′) and Rp2 (5′-CG GCTACCTTGTTACGACTT-3′) according to the method of Malviya et al. (2011). The forward and reverse rDNA sequences were joined into contiguous sequence using CAP3 Sequence Assembly Program (http://pbil.univ-lyon1.fr/cap3.php). The 16S rRNA sequences were identified by comparing the partial 16S rRNA sequences of the isolated strains with those available in the GenBank database using BLASTn program. A total of 3 sequences of 16S rRNA gene were deposited in public databases (GenBank, NCBI) under the accession numbers JN204723, JN204724 and JN204725, and the cultures were deposited under the accession number B-1006, B-1007 and B-1008 at the National Agriculturally Important Microbial Culture Collection, National Bureau of Agriculturally Important Microorganisms, Maunath Bhanjan, Uttar Pradesh, India.

Effect of water stress on growth kinetics

Effect of water stress on the growth kinetics and IAA production by endophytic actinobacteria was investigated using Bioscreen-C Micro Biology Reader (Labsystems). Actively growing culture ($\sim 10^6$ CFU ml^{-1}) was inoculated in −0.73 MPa concentration of polyethylene glycol (PEG6000) in liquid media according to manufacturer's instructions and subjected to growth kinetics analysis (absorbance 600 nm) at 24 h interval. The liquid media without PEG6000 served as control for each culture. The experiment was repeated thrice and pooled data was used for analysis.

Extraction of IAA

Cultures were tested for IAA production with same inoculum size using the procedure of Brick et al. (1991). The content of IAA produced from each isolate at different phase of growth was determined using high performance liquid chromatography (HPLC). Seven selected isolates were grown in 10 ml of GYMA broth (gl^{-1}: 4.0 g glucose; 4.0 g yeast extract; 10.0 g malt extract) in 3 replicates and allowed to grow in incubator shaker at 32 °C for 15 days. Supernatant was collected by centrifugation at $10{,}000 \times g$ for 10 min at every 24 h from each replicate, added with equal volume of ethyl acetate in screw cap tubes, rigorously shaken for 2 h on a gyratory shaker (50 rpm) and the content was allowed to settle for 1 h at 37 °C. The ethyl acetate extract was finally air dried and re-dissolved in methanol (HPLC grade) prior to HPLC analysis.

HPLC analysis

HPLC analysis was performed on HPLC system (Waters, USA) equipped with binary 515 reciprocating pumps, variable photodiode array (PDA) detector (Waters 2996), and system controller equipped with Waters® Empower™ software for data integration and analysis. Reverse phase analysis was carried out in isocratic conditions on C-18 column (250 × 4.6 mm i.d., 5 μm particle size) at 25 ± 1 °C and detection at 254 and 280 nm. IAA was analyzed at a flow rate of 1 ml min^{-1} of methanol: 1 % aqueous acetic acid (60:40, v/v). Samples were filtered through a 0.45 μm membrane filter prior to injection in the sample loop (injection volume 10 μl). HPLC grade solvents and chemicals (EMerck and HiMedia, India) were used throughout the analysis. Qualitative characterization of the compounds in the sample was done by comparing retention time (R*t*) and co-injection while quantitative analysis was performed by comparing peak areas of the standard compounds obtained from HiMedia, India.

Plant growth under drought stress

Seedling vigour assay

Wheat seeds (cultivar WR-544) of late variety sensitive to drought stress obtained from Directorate of Seed Research, Maunath Bhanjan, Uttar Pradesh, India were used for field experiments including seedling vigour assays. Seeds were surface sterilized with a mixture of 3 % hydrogen peroxide and 96 % ethanol (1:1), coated with actinobacterial culture ($\sim 10^8$ cells ml^{-1}) and cell-free extract using 1 % carboxymethyl cellulose (CMC) as binder. Coated seeds were dried overnight and sown in a plastic pot filled with

sterilized soil in a greenhouse. The plants were uprooted at 24 h intervals after 2 days of germination for measurement of root and shoot length. The seedling vigour indices were calculated after 10 days using the formula: Seedling vigour index = (mean root length + mean shoot length) × germination (%). The experiment was repeated three times and the data was pooled before statistical analysis.

Field experiments

Field experiment was conducted in drought affected field of Directorate of Seed Research, Maunath Bhanjan, Uttar Pradesh, India. The air-dried and sieved (<2 mm) field soil possessed following physico-chemical parameters: sand 71 %, silt 3 %, and clay 26 % with bulk density 1.60 mg m^{-3}, total porosity 38.9 %, and water holding capacity 36.9 %; pH 8.8, EC 4.6 dS m^{-1}, organic C 0.41 %, total N 0.14 %, and Olsen P 101 mg per 100 g soil. Surface sterilized, actinobacteria and cell-free extract coated wheat seeds were sown in the field in randomized complete block design (RCBD). Surface-sterilized non-inoculated seeds served as control. Two independent RCBD experimental fields were maintained for culture and cell-free extract coated seeds. Fertilizer application and other package of practices were carried out till harvest of the crop. Experimental field with wheat crop received irrigation only at the germination stage to maintain the stress level. After harvesting, shoot and root length, fresh shoot and root weight of the plants along with their dry weight, number of tillers and panicles and yield were recorded. The field experiment was repeated three times and pooled data was used for analysis.

Statistical analysis

One-way ANOVA was applied to determine the significance between different treatments using SPSS 16.0 statistical software (SPSS, Inc., Chicago, IL, USA). Critical difference ($P \leq 0.05$) and standard error of means (*SEM*) were tabulated. Mean separations were calculated based on the mean rankings at $P \leq 0.05$ using Duncan's Multiple Range Test.

Results

Isolation and screening of drought tolerant endophytic actinobacteria

Fourty six morphotypes of endophytic actinobacteria were isolated from drought tolerant plant roots. Most of the endophytes possessed dry colonies with varying colour of aerial and substrate mycelium. Only 19 morphotypes were able to show growth under −0.73 MPa (25 % PEG) water stress condition. Growth kinetics of water stress tolerant isolates indicated normal growth curve with typical life cycle of 6–8 days. All isolated actinobacteria showed different biochemical attributes like lysozyme sensitivity, urea and casein hydrolysis, gelatin degradation and hydrogen sulphide production. Among the endophytes 7 isolates DE07, DE10, DE20, DE27, DE39, DE46 and DE52 that showed significant growth and IAA production at −0.73 MPa of PEG6000 were chosen for seedling vigour assay and colonization studies (Supplementary table).

Seedling vigour assay and colonization

Among the seven actinobacterial isolates, DE07, DE10 and DE27 showed higher seedling vigour with cell treatment as compared to cell-free extracts (Fig. 1). Maximum seedling vigour was recorded with isolate DE10, followed by DE07 and DE27 and showed significant vigour over the control. Out of seven actinobacterial cell-free extracts, only DE10 cell-free extracts showed significant vigour over control. Isolate DE20 showed decrease in seedling vigour in comparison to culture extract. Over all the cultures DE07, DE10 and DE27 increased the seedling vigour ($P \leq 0.05$) significantly in comparison to other isolates with cell culture. Scanning electron microscope studies also showed actinobacterial colonization on the root surface by the isolate DE07, DE10 and DE27, where actinobacteria were connected together by an extracellular polymeric matrix colony formation under water stress conditions (Fig. 2).

Identification of endophytic actinobacteria

BLASTn homology search of NCBI for 16S rRNA gene sequences of all the three strains were confirmed as *Streptomyces* sp. The strain DE10 had 100 % similarity with *Streptomyces olivaceus* whereas strains DE07 and DE27 showed 99 % similarity with *Streptomyces coelicolor* and *S. geysiriensis*. The gene sequences of DE07, DE10 and DE27 were submitted to GenBank (NCBI) with accession numbers JN204723, JN204724 and JN204725, respectively (Table 1). The molecular identification was further confirmed on the basis of morphological characteristics using scanning electron microscopy. The scanning electron microscopic observations ascertained that spores of all the three actinobacteria were spirals and loops forms (Fig. 2) with characteristic aerial mycelium, type I cell wall (LL-DAP and without characteristics sugars) indicating that they belonged to the *Streptomyces* genus.

Actinobacterial IAA synthesis in water stress condition

Quantitative IAA estimation at different time intervals resulted through growth kinetics and IAA synthesis in liquid culture demonstrated that actinobacterial isolates

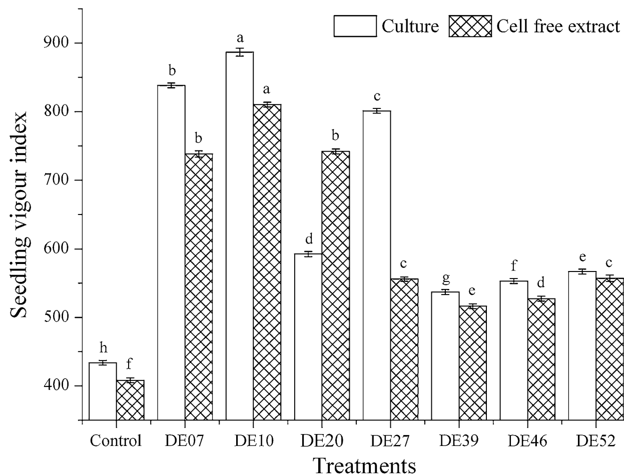

Fig. 1 Seedling vigour indices of culture and cell free extracts of actinobacteria treated wheat seeds at 10 days after inoculation. Control: seedlings without treatment of culture or cell free extracts of actinobacteria, DE07: *Streptomyces coelicolor* DE07, DE10: *S. olivaceus* DE10, DE27: *S. geysiriensis* DE27, DE20: *Streptomyces* sp. DE20, DE39: *Streptomyces* sp. DE39, DE46: *Streptomyces* sp. DE46, DE52: *Streptomyces* sp. DE52. Experiment was repeated three times and mean with different letters on the top of error bars indicate statistically different values at $P \leq 0.05$ using Duncan's Multiple Range Test (DMRT). Seedling vigour index = (mean root length + mean shoot length) × germination (%)

grew well in normal conditions and synthesized IAA significantly as compared to water stress conditions (Fig. 3). Isolate DE07 and DE10 synthesized and accumulated IAA in the logarithmic phase whereas isolate DE27 produced IAA after stationary phase of growth till to death phase under normal conditions. However, isolate DE07 and DE27 produced highest IAA under water stress condition till end of logarithmic phase of growth, whereas DE10 produced highest IAA in the midst of logarithmic phase. In general cultures showed increased IAA production under normal conditions, and decreased IAA production under water stress conditions after peak growth phase. DE27 showed minimum accumulation in normal and water stress condition in comparison to other isolates.

Plant growth under drought stress

All the treatments with actinobacterial inoculation (single and combined) were significant ($P \leq 0.05$) in comparison to control for all the studied growth and yield parameters. Among all the treatments, *S. geysiriensis* DE10 culture treated plants recorded maximum shoot length, number of tillers and panicles, fresh shoot and root weight, dry shoot and root weight, and yield. In combined inoculations, *S. coelicolor* DE07 + *S. geysiriensis* DE27 resulted highest plant growth through root length, number of tillers and panicles, fresh shoot and root weight and dry shoot weight. Highest yield was recorded with combined inoculation of *S. olivaceus* DE10 + *S. geysiriensis* DE27 cultures and lowest yield with *S. coelicolor* DE07 + *S. geysiriensis* DE27 in comparison to control (242 kg ha^{-1}). Combined inoculations were better in yield as compared to single inoculations and cell culture treatments were better than cell-free extract treated seeds (Table 2).

Discussion

Beneficial plant–microbe interactions, impact of microbial inoculation on plant growth and differential mechanisms underlying growth promotion under stress conditions are documented (Yang et al. 2008; Meena et al. 2010; Jha et al. 2011). Bacteria isolated from different stressed habitats possess stress tolerance along with the plant growth-promoting traits and therefore are potential candidates for seed treatment (Tiwari et al. 2011). Impact assessment of drought tolerant actinobacteria on plant growth promotion and underlying biochemical mechanisms is scarcely documented, and therefore, need for special attention because of the potential intrinsic properties of actinobacteria isolated from drought soil ecosystems.

Water stress tolerant morphotypes of endophytic actinobacteria isolated from roots of five plant species produced dry powdery colonies on plates and possessed morphological characteristics of *Streptomyces* genus that has been the predominant genera of endophytes in the majority of hosts (Sardi et al. 1992; Coombs and Franco 2003). Among 46 morphotypes, nineteen were capable of producing IAA in the growth medium (Supplementary table). Majority of actinobacteria and coryneform bacteria associated with plants have been reported to produce auxins (Merzaeva and Shirokikh 2006). Seven endophytes producing relatively high levels of IAA enhanced root and shoot growth in wheat seedlings and out of seven three actinobacteria (DE07, DE10 and DE27) enhanced maximum seedling vigour and produced maximum auxin. Molecular characterization of these potent isolates confirmed their identification as *S. coelicolor* DE07, *S. olivaceus* DE10 and *S. geysiriensis* DE27 with 99, 100 and 99 % similarity (Table 1), which was further validated by scanning electron microscopic studies (Fig. 2). Cell-free extracts showed relatively more enhanced seedling vigour over culture as in case of DE20 (Fig. 1). Scanning electron microscopy of isolate DE07, DE10 and DE27 revealed successful actinobacterial colonization on the root surface by an extracellular polymeric matrix colony formation under water stress conditions (Fig. 2). Possible reasons for decreased vigour of DE20 culture coated seedlings over the extracts might be attributed to relatively slow colonization of actinobacteria on the roots. Similarly, root exudates have definite selective and promoting effects on specific

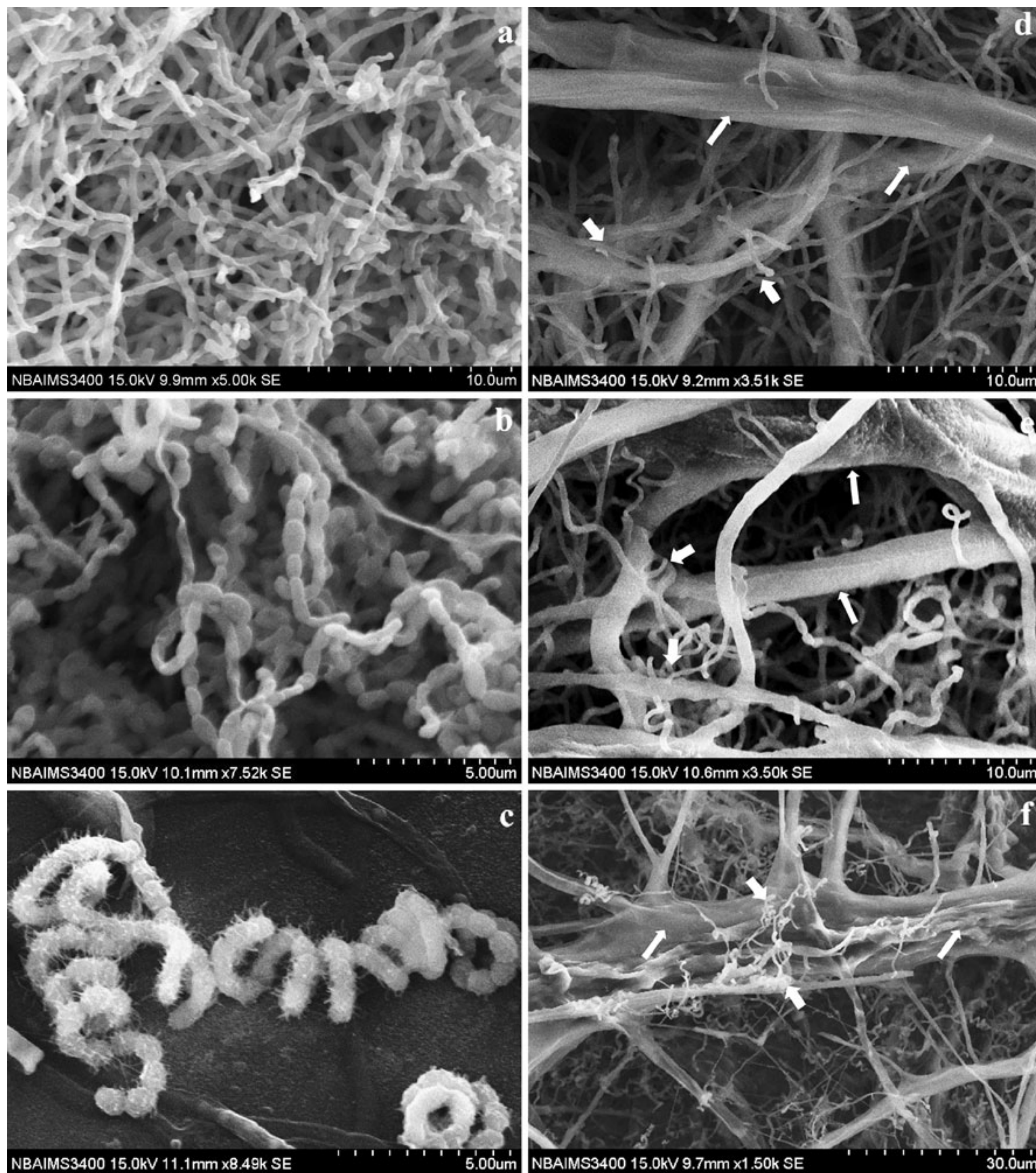

Fig. 2 Scanning electron microscopy (SEM) of actinobacteria isolates, showing variations in spore chain morphology of **a** *Streptomyces coelicolor* DE07, **b** *S. olivaceus* DE10, **c** *S. geysiriensis* DE27 and wheat root colonization with **d** *Streptomyces coelicolor* DE07, **e** *S. olivaceus* DE10 and **f** *S. geysiriensis* after 10 days of inoculation. *Thin arrows* represent the wheat roots and *thick arrows* represent endophytic actinobacterial colonization on roots

microbial population (Hartmann et al. 2009) and actinobacteria, being a slow grower in the soil, may colonize roots successfully but slowly. Since, *Streptomyces* species isolated from unexplored and underexplored habitats are reported to be a rich source of bioactive compounds (Bull et al. 2005; Fiedler et al. 2005), the coating of cell-free extracts of actinobacteria to plant seeds may serve the purpose of potent actinobacterial inoculation in a more pronounced manner.

Comparison of biomass and IAA concentration in liquid culture revealed that maximum auxin accumulation observed in the logarithmic phase to stationary growth phase (Fig. 3), which corresponded well with other reports on the dependence of auxin synthesis on the growth phase of cultures (Muronets et al. 1997; Tsavkelova et al. 2005).

Microbial population as natural bio-inoculants is advantageous, not only from the economical but also from

Table 1 Organisms and their habitats used in the study, accession numbers, PGP traits and water stress tolerance properties

Organisms	*Streptomyces coelicolor* DE07	*Streptomyces olivaceus* DE10	*Streptomyces geysiriensis* DE27
Habitat	Bikaner, Rajasthan, India (28.01°N,73.17°E)	Bikaner, Rajasthan, India (28.01°N,73.17°E)	Jaisalmer, Rajasthan, India (26.55°N,70.55°E)
GenBank accession number	JN204723	JN204724	JN204725
similarity (%)	99	100	99
PGP traits			
IAA (μg mg^{-1} protein)[a]	79.53	84.34	82.48
Siderophore production	ND	+	+
Ammonia production	+	+	+
Biochemical characterization			
Lysozyme sensitivity	+	+	+
Gelatin liquefaction	ND	ND	+
Hydrogen sulphide production	+	ND	ND
Casein hydrolysis	ND	+	ND
Urea hydrolysis	+	+	+
Water stress tolerance potential			
Water stress tolerance limit (MPa)[b]	−0.05 to −0.73	−0.05 to −0.73	−0.05 to −0.73

ND not detected, + positive

[a] IAA production estimated using HPLC

[b] Polyethylene glycol (PEG6000) range on which the organisms were grown successfully

the ecological point of view (Diby et al. 2005). Some microbes live inside plants for at least a part of their life cycle as endophytes conferring on the host benefit such as stress reduction, increased root growth and nutrient availability (Hardoim et al. 2008). Microbial inoculations significantly alter the rhizospheric changes particularly in the small molecules like sugars, amino acids, fatty acids, flavonoids and strigolactones, as well as various classes of proteins leading to interesting modification in the plant (Badri and Vivanco 2009). In the rhizosphere, plant–microbe interactions are interdependent as plants exude a wide array of organic and inorganic compounds (Lugtenberg and Kamilova 2009). In turn, microbes inhabiting the rhizosphere release phytohormones, small molecules, or volatile compounds which regulate plant growth and root morphogenesis (Castro-Sowinski et al. 2007). Although growth and development of plants is reduced in stress conditions due to damaged biochemical and physiological mechanisms, such stresses may be relieved to some extent by the application of microbial inoculants which evoke various natural mechanisms to help plants sustain their growth under stress conditions (Yang et al. 2008). In the present study, single as well as combined inoculation of actinobacteria performed better plant growth promotion in comparison to control; combined inoculation of microbes has always been proved to be more effective than single inoculation in plant growth promotion as is also reported in this study (Meena et al. 2010; Jha et al. 2011). It is interesting to note that wheat seeds coated with cell-free extracts yielded reduced plant growth parameters in comparison to culture inoculation (Table 2) except in case of DE20. Long-term effect of actinobacterial live cultures colonized on roots served as a continuous source of phytohormones throughout different plant growth stages rather than one time initial application of cell-free extracts (Shakirova et al. 2003). Endophytic actinobacteria *S. geysiriensis* DE27, *S. coelicolor* DE07 and *S. olivaceus* DE10 showed increase in shoot and root length, number of tillers and panicles, biomass, and yield of wheat crop under field conditions (Table 2). These observations are in accordance with the role of endophytic actinobacteria to produce auxins and enhance drought tolerance in plants (Hasegawa et al. 2004; Meguro et al. 2006). Our results support the hypothesis that microbial colonization induce different physical and biochemical changes in plants with enhanced plant growth under stressed soil conditions. Endophytic actinobacteria have been implied in the production of metabolites that affect plant's life directly by affecting physiology of the host plants (Hasegawa et al. 2004; Meguro et al. 2006) which is correlated with the results of current investigation. The plant–microbe and microbe–microbe interactions are complex and dependent on

Fig. 3 Comparison of growth and indole 3-acetic acid production of actinobacteria *Streptomyces coelicolor* DE07, *S. olivaceus* DE10 and *S. geysiriensis* DE27. Experiment repeated three times and mean with standard errors were used

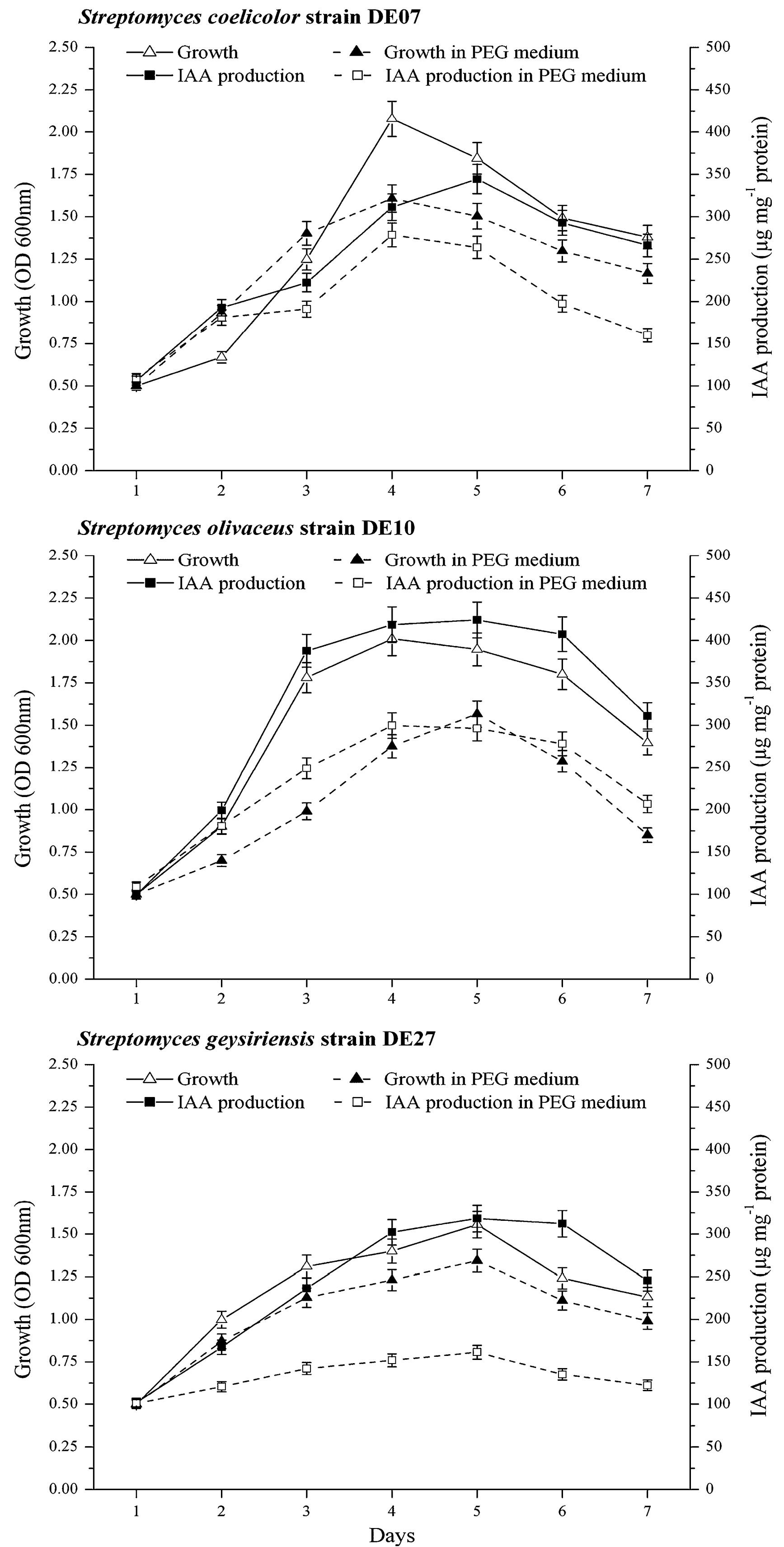

Table 2 Effect of actinomycetes cultures and cell free extracts on growth of wheat plants at the time of harvest in single and co-inoculated formulations

Treatment	Application mode	Shoot length (cm)	Root length (cm)	Number of tillers	Number of panicles	Fresh shoot weight (g)	Fresh root weight (g)	Dry shoot weight (g)	Dry root weight (g)	Yield (kg ha^{-1})
Streptomyces coelicolor DE07	Culture	60.31ef	10.25g	6.83c	14.03e	44.33g	7.68f	17.54h	4.14f	335.98g
	Extract	55.57h	8.12i	4.59g	9.56j	41.01h	4.65i	22.15f	3.61g	143.68k
Streptomyces olivaceus DE10	Culture	67.20b	15.53b	7.37b	17.04b	79.31b	12.77b	27.80c	6.59c	492.77c
	Extract	55.91h	14.54c	6.01d	14.79d	61.78d	9.37d	25.89d	4.09f	262.31h
Streptomyces geysiriensis DE27	Culture	65.83c	12.49e	7.32b	16.04c	77.56b	12.74b	25.43d	6.13d	442.74d
	Extract	54.29i	10.94f	4.94f	10.74h	54.31f	6.84g	24.13e	3.83fg	233.88i
DE07 + DE10	Culture	63.00d	13.17d	6.12d	14.03e	58.70e	10.55c	26.09d	6.27cd	406.69e
	Extract	57.82g	8.84h	4.59g	12.72f	56.40f	8.02f	23.51e	4.12f	252.65hi
DE07 + DE27	Culture	60.85e	12.50e	7.24b	14.03e	69.80c	8.65e	25.54d	5.37e	381.52f
	Extract	54.77hi	8.66h	4.59g	11.68g	54.97f	5.79h	20.65g	3.58g	242.93hi
DE10 + DE27	Culture	69.85a	17.22a	7.67a	18.04a	85.48a	14.32a	33.91a	10.34a	550.09a
	Extract	59.23f	13.45d	6.72c	14.86d	62.91d	12.92b	31.35b	7.50b	524.92b
Control[a]	–	44.63j	6.02j	5.60e	10.02i	40.16hi	4.39i	22.12f	2.37h	194.49j
	–	41.60k	5.24k	5.44e	9.55j	38.57i	3.42j	17.69h	2.32h	116.96l
SEM±		0.41	0.18	0.06	0.15	0.77	0.19	0.24	0.11	7.74
CD ($P = 0.05$)		1.18	0.52	0.17	0.42	2.24	0.54	0.69	0.33	22.51
CV (%)		1.20	2.70	1.70	1.90	2.30	3.70	1.70	3.90	4.10

Cell free extracts coated with wheat seeds using CMC (1 %)

SEM standard error of means, *CD* critical difference at significance level $P \leq 0.05$, *CV* coefficient of variance

[a] Surface-sterilized non-inoculated seeds

multiple traits. The strong promotion of growth and yield of wheat plants due to combined inoculation may serve as a new finding to improve crop productivity, particularly for dry soils, using stress-tolerant actinobacteria.

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