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ORIGINAL ARTICLE

Plant growth promoting potential and soil enzyme production of the most abundant *Streptomyces* spp. from wheat rhizosphere

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Keywords
Actinomycetes, plant growth promoting rhizobacteria, rhizosphere, soil enzymes, solid state fermentation, wheat growth.

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Abstract

Aim: To evaluate the plant growth promotion (PGP) potential and soil enzyme production under solid state fermentation (SSF) by most abundant *Streptomyces* spp. isolated from the wheat rhizosphere and to evaluate their effect on plant growth parameters.

Methods and Results: Actinomycetes were isolated from wheat rhizosphere and screened for PGP activities. Three actinomycete isolates having significantly higher PGP activities (*Streptomyces rochei* IDWR19, *Streptomyces carpinensis* IDWR53, *Streptomyces thermolilacinus* IDWR81) were selected. The soil enzymes production potential of these isolates using soil extract and wheat straw under ssf was assessed. Utilization of soil extract as a fermentation medium for soil enzyme production by Actinomycetes has been reported first time in this study. Maximum chitinase (*S. rochei* IDWR19 12·2 U mg^{-1} protein) and phytase activity (*S. carpinensis* IDWR53 5·2 U mg^{-1} protein) was produced on 7th day of incubation, whereas maximum alkaline protease (*S. rochei* IDWR19 3·2 U mg^{-1} protein) was produced on 6th day of incubation. For cellulase (*S. rochei* IDWR19 7·4 U mg^{-1} protein) and invertase (*S. carpinensis* IDWR53 451 U mg^{-1} protein) maximum activity was observed on 4th as well as 5th day of incubation. On the basis of PGP activity and enzyme production, two actinomycete isolates (*S. rochei* IDWR19 and *S. thermolilacinus* IDRWR81) were selected for plant growth experiment. An increase of 12·2 and 24·5% in shoot length of plants inoculated with *S. rochei* IDWR19 and *S. thermolilacinus* IDWR81 was observed, respectively. A similar increase in biomass of 1·8- and 2·3-fold was also recorded for the two isolates, respectively.

Conclusions: It could be concluded that *Streptomyces* sp. with high PGP activities and soil enzyme production capability significantly improved growth and development of wheat cv.

Significance and Impact of the Study: The abundant Actinomycetes obtained in this study (*S. rochei* IDWR19 and *S. thermolilacinus* IDWR81) are rhizosphere competent and effective strains.

Introduction

Current status of agriculture at the global scenario demands the researchers to come up with superior technologies for increasing the yield and quality of food grains and crop yield. The conventional methods for enhancing crop yield by indiscriminate use of chemical fertilizers and pesticides have largely affected

humans, environment and the food quality. Public awareness to these problems has shifted the approach towards alternative strategies (Shaxson 2006; Javaid and Shah 2010). It is well known that a considerable number of bacterial species, mostly those associated with the plant rhizosphere are able to exert beneficial effect upon plant growth. Therefore, their use as biofertilizers or control agents for agriculture improvement has been a focus of researchers for a number of years. This important group of bacteria has been termed as 'Plant Growth Promoting Rhizobacteria' (PGPR) (Glick 1995). PGPR strains are broadly distributed amongst many taxa including Actinobacteria, Bacteroidetes, Cyanobacteria, Firmicutes and Proteobacteria (Tilak *et al.* 2005).

Actinomycetes are gram positive, aerobic and mycelia-forming bacteria, known for nutrient cycling, production of secondary metabolites and plant growth promotion (PGP) ability. Actinomycetes are found in various habitats including sea water, fresh water, soil, marsh area etc.; however, they are dominant in dry, humic and calcareous type of soil. Actinomycetes also play an important role in the rhizosphere by secreting a wide range of antimicrobial products thus preventing growth of common root pathogens. However, apart from their biocontrol potential, other PGP traits of Actinomycetes are scarcely reported. There are few reports regarding their ability of phosphate solubilization, organic acid production, siderophore production and secretion of large number of enzymes, which directly or indirectly help plant growth (Doumbou *et al.* 2001; Al-Aksar 2012; Sadeghi *et al.* 2012).

Soil enzymes like cellulase, phytase, chitinase, protease, phosphatase play a critical role in maintaining soil ecology, fertility and health (Sinsabaugh *et al.* 1991). The enzyme levels in soil system are influenced by organic matter content, composition and inhabitant macro- and micro-organisms of soil (Kiss *et al.* 1978). These enzymes can be produced by Actinomycetes under submerged as well as solid state fermentation (SSF) (Suneetha and Zaved 2011). SSF is a process where in insoluble substrate is fermented with sufficient moisture but without free water (Chahal 1985). The substrates generally used are low cost agro residues such a wheat straw, wheat bran, baggase, soya hulls, etc (Mohana *et al.* 2008). Enzyme production under SSF by Actinomycetes is very sparsely reported (Basha *et al.* 2009; El-Dein *et al.* 2010) and needs a lot more attention.

In the present study, we have extended PGP potential [phosphate solubilization, siderophore and indole acetic acid (IAA) production] of the most abundant Actinomycete of wheat rhizosphere to soil enzyme production and explored their potential as a bioinoculant in plant growth experiment.

Materials and methods

Rhizosphere: sample collection and characterization

Soil samples were collected from the rhizosphere of 3-month-old wheat plants grown in Idar region of Gujarat, India (lat 23° 50′ E and long N 73° 02′) by composite sampling. Three samples each from five plants of ten fields were taken. The soil loosely adhered to roots was removed, whilst rhizospheric soil was collected and mixed in sterile polyvinyl bags. The iron, phosphorous and magnesium contents of soil were determined by AES-ICP (Model: Optima-3300RL; Perkin–Elmer, Waltham, MA, USA). The carbon and nitrogen content of soil was determined by C-H-N analyzer (Model: 2400 Series II; Perkin–Elmer).

Actinomycete isolation

The rhizosphere soil samples were enriched with SDS (0·1%) and yeast extract (5%) and incubated for 15 min at room temperature to cultivate most abundant Actinomycetes. Appropriate dilutions (100 μl) of soil suspension were spread onto Humic acid Vitamin agar (pH 7·2) (gl^{-1}: Humic acid 1·2, Na_2HPO_4 0·6, KCl 2·052, $MgSO_4{\cdot}7H_2O$ 0·6, $FeSO_4{\cdot}7H_2O$ 0·012, $CaCO_3$ 0·024, agar 21·6) supplemented with Nalidixic acid (10 mg l^{-1}), Trimethoprim (20 mg l^{-1}) and Cycloheximide (20 mg l^{-1}) and incubated at 30°C for 2-3 weeks (Hayakawa and Nonomura 1989). All the media and antibiotics were supplied by Hi-media, India.

Screening for PGP activities

Actinomycete isolates obtained were screened for PGP activities on buffered (100 mmol l^{-1}) tri calcium phosphate (TCP) agar, crome azurol sulphonate agar and tryptone soy agar (containing 5 mmol l^{-1} tryptophan) for phosphate solubilization, siderophore production and IAA production, respectively (Schwyn and Neilands 1987; Bric *et al.* 1991; Stephen *et al.* 2009). The isolates that tested positive on media plates were quantified for phosphate solubilization by free phosphate estimation, using phosphomolybdate method at 820 nm (Ames 1964), IAA estimation by Salkouski method at 535 nm (Gordon and Weber 1951) and hydroxamate-type siderophore at 400 nm (Mayer and Abdallah 1978). The best three isolates thus identified were selected for further studies.

Morphological, chemotaxonomic and molecular identification

The selected Actinomycetes were characterized on tryptone yeast medium (ISP1), yeast malt-extract medium

(ISP2), oat meal medium (ISP3), inorganic salt starch medium (ISP4), glycerol asparagine medium (ISP5), peptone yeast iron medium (ISP6) and tyrosine medium (ISP7) for morphological characterization including substrate aerial mycelia colour, sporulation, colonial characters, pigmentation and microscopic characteristics (Shirling and Gottlieb 1966). Isomers of diaminopimelic acid (DAP) in cell wall hydrolysates of Actinomycetes were determined by thin layer chromatography following the standard method of Boone and Pine (1968). The cultures were identified to the genus level by comparing the characteristics of isolated strain to the known species described in Bergey's manual of systemic bacteriology (Cross 1989).

The molecular identification of the isolates was carried out as under:

The genomic DNA of the selected PGP Actinomycete isolates was extracted as described by Kieser *et al.* (2000). The 16S ribosomal DNA (rDNA) gene was amplified with Actinomycete specific primer pair, 243f (5′-GGATGAGCCCGCGGCCTA-3′) and 1378r (5′-CGGTGTGTACAAGGCCCGGGAACG-3′ (Heuer *et al.* 1997) using Mastercycler Personal (Eppendorf, Germany). For the specific amplification of 16S rDNA fragments of Actinomycetes, the reaction mixture contained, 2 μl of template DNA (*ca.* 80–100 ng), 100 mol l^{-1} F243 and R1378 100 mol l^{-1}, 5 μl 5X GC-rich Buffer A (Invitrogen, Carlsbad, CA) and 0·5–1 U GC-rich DNA polymerase (Invitrogen). PCR amplification was carried out as follows: initial denaturation step of 3 min at 94°C followed by 30 cycles of denaturation at 94°C for 30 s, annealing at 63°C for 30 s, extension at 72°C for 1 min and final extension at 72°C for 10 min. The PCR products were purified using the QIAquick PCR purification kit (Qiagen) and sequenced using automatic ABI 310 DNA sequencer (Big Dye Terminator cycle sequencing, ready reaction kit; Perkin–Elmer). The 16S rDNA sequences determined were manually aligned with the published sequences of validly described species available from the EMBL/GenBank/DDBJ databases. The evolutionary history was inferred using maximum likelihood method based on the Kimura 2-parameter model (Kimura 1980). Topography of the constructed tree was evaluated by bootstrap analysis with 1000 replicates (Felsenstein 1985). The evolutionary analyses including phylogenetic tree construction were conducted in MEGA5 using 50 nucleotide sequence (Tamura *et al.* 2011).

Plant sugar utilization profile

The plant sugar utilization was determined by growing the isolates on minimal medium (gl^{-1}: $(NH_4)_2SO_4$ 0·2, $MgSO_4{\cdot}7H_2O$ 0·06, PEG 6000 50) supplemented with sugars (2 gl^{-1}) (Arabinose, Fructose, Glucose, Mannose, Sucrose, Xlyose) added separately in each tube, 1 ml of trace salts solution (gl^{-1}: $ZnSO_4{\cdot}7H_20$ 1·0, $MnCl_2{\cdot}4H_2O$ 1·0, $CaCl_2$ 1·0) (Hopwood *et al.* 1985) and bromocresol purple to monitor drop in pH. Minimal medium without any carbon source served as control. The sugar utilization profile was determined by observing Actinomycete growth after 1 week incubation at 30°C.

Solid state fermentation

The potential of Actinomycete isolates to produce soil enzymes like cellulase, invertase, chitinase, alkaline protease and phytase was determined in solid state fermentation.

Fermentation medium

Enzyme production was carried out using soil extract medium containing rhizosphere soil extract (pH 7·2) (gl^{-1}: total carbon 26, total nitrogen 54, phosphorous 0·21, iron 4·6, magnesium 0·9). The fermentation media were supplemented with enzyme inducers (5 gl^{-1}) viz. carboxymethyl cellulose, sucrose, colloidal chitin, casein and sodium phytate for cellulase, invertase, chitinase, protease and phytase production, respectively.

Inoculum preparation

Inoculum of $1{\cdot}6 \times 10^8$ spores ml^{-1} was prepared by harvesting spores from a week-old inorganic salt starch agar (ISP-4) slants of selected isolates by adding 10-ml-distilled water containing small amount of Tween 80. One millilitre inoculum was added to fermentation medium.

Enzyme production

The enzyme production was carried out in Erlenmeyer flask (250 ml) containing 3 g wheat straw as substrate matrix and soil extract (12 ml) as the fermentation medium sterilized separately by autoclaving at 121°C for 15 min. Fermentation medium containing one millilitre inoculum was added to substrate matrix and mixed thoroughly and incubated at 30°C for 7 days under stationary conditions. The crude enzyme from each flask was extracted using minimum volume of 0·25 mol l^{-1} Acetate buffer (pH 5·5) (for cellulase, invertase, chitinase, phytase) or 0·25 mol l^{-1} Phosphate buffer (pH 7·4) (for alkaline protease) and filtered through a wet muslin cloth by squeezing. The filtrate was centrifuged at 6000 ***g*** for 15 min and the clear supernatant thus obtained was used for further assays.

Enzyme assay

The filter paper assay was used to estimate total cellulase activity from crude enzyme extract (Mandel *et al.* 1975). Chitinase and Invertase were estimated according to the method as described by Reissig *et al.* (1955), Sumner and

Howell (1935), respectively. The reducing equivalents of the enzyme hydrolysate were estimated by 3, 5 dinitrosalicylic acid (DNS) method (Miller 1959). The alkaline protease and phytase assay were performed according to the method as described by Hagihara *et al.* (1958), Harland and Harland (1980), respectively. Protein estimation was carried out by Lowry method (Lowry *et al.* 1951), and specific activity of enzyme was reported as units per mg of protein.

One unit of enzyme was defined as the amount of enzyme required to release one μmol of product per minute per ml of enzyme under standard assay conditions. Two isolates which showed significantly high enzyme production, and PGP activities were selected for pot experimentation.

Plant growth experiment

The rhizosphere soil sample was air-dried, sieved (2 mm mesh) and sterilized by repeated autoclaving before filling the pots. *Streptomyces rochei* IDWR19, and *Streptomyces thermolilacinus* IDWR81 were selected for pot experiment. Wheat (var. Lokwan) seeds were surface sterilized by 95% ethanol and 0·2% $HgCl_2$ solution for 3 min followed by several washings with sterile distilled water. The surface sterilization was checked by rolling seeds on nutrient agar plates. For each treatment, a set of three pots were individually sown with eight to ten seeds inoculated with 1 ml (10^8 spores) of the selected Actinomycete culture. After germination, five plants were maintained in each pot. The uninoculated pots with sterile seeds served as control. The pots were kept under natural sunlight and temperature. At the end of 28 days, plants were uprooted and measured for root length, shoot length and biomass.

Statistical analysis

The data were analysed by SIGMA PLOT (Windows ver. 11.0; Systat Software Inc., Richmond, CA, USA). A one-way analysis of variance (ANOVA) was used to determine the statistical significance, which was assumed to be different when the comparison showed a significance level of $P \leq 0{\cdot}05$. Results were reported as mean ± SE.

Results

Actinomycete isolation and PGP screening

The composite soil samples from wheat rhizosphere of Idar region had the total content ($g\,l^{-1}$) of Carbon, 26; Nitrogen, 54; Phosphorous, 0·21; Iron, 4·6; Magnesium, 0·9; and pH of 7·2. A total of 15 morphologically distinct Actinomycetes, abundantly distributed in test soil samples were obtained and screened for various PGP activities. Most of the isolates produced significant IAA on tryptophan supplemented tryptone soy agar, whilst failing to solubilize phosphate on tris-buffered tricalcium phosphate agar. Amongst 15 isolates tested, 78% (12 cultures) produced auxins (ranging from 2·6 to 19·22 mg l^{-1} IAA equivalents), 60% (10 cultures) produced siderophores (hydorxymate type) (ranging from 1·3 to 34·17 mg l^{-1}) and only five solubilized phosphate on buffered tricalcium phosphate agar. *Streptomyces thermolilacinus* IDWR81 showed highest phosphate solubilization (911·6 mg l^{-1}) and also produced a red halo around the colony indicating drop in pH (8–5·5) (data not shown) on tris-buffered tricalcium phosphate agar. Three Actinomycete isolates with maximum PGP activities were selected for further studies (Table 1).

Identification of Actinomycetes

The three best PGP Actinomycete isolates obtained belonged to the genus *Streptomyces* as they were gram positive, filamentous, sporulating Actinomycetes with L-DAP isomer in the cell wall (Table 2).

The 16S rDNA amplicon sequences (~700 bp) were aligned to databases and the isolates were identified as *S. rochei* (IDWR19), *Streptomyces carpinensis* (IDWR53) and *S. thermolilacinus* (IDWR81) as they exhibited 97, 98 and 98% homology with *S. rochei* P42 (accession JN967802), *S. carpinensis* NBRC 14214 (accession NR041157) and *S. thermolilacinus* (accession AB184585), respectively. The amplicon sequences of the isolates have been deposited in GeneBank under the accession numbers JQ432712, JQ432711 and JQ432713 respectively. The taxanomic distances of the isolates in the *Streptomyces* tree are presented (Fig. 1).

Utilization of plant sugar

The plant sugar utilization profile of Actinomycete isolates is presented (Table 3). Amongst the three strains, *S. rochei* IDWR19 recorded maximum growth on all the C sources; however, the other two isolates also utilized the tested plant sugars.

Soil enzymes

In the present study, we have explored the potential of Actinomycetes to produce extracellular soil enzymes using soil extract medium under solid state fermentation. The enzyme activity of the isolates is shown (Fig. 2).

Maximum chitinase (*S. rochei* IDWR19 12·2 U mg^{-1} protein) and phytase activity (*S. carpinensis* IDWR53

Table 1 PGP potential of actinomycete isolates

Strain	Siderophore production*† (mg l^{-1})	Phosphate solubilization*† (mg l^{-1})	IAA production*† (mg l^{-1})
IDWR17	1·34 ± 0·08	NA	19·22 ± 1·9
IDWR19	34·17 ± 0·07	95·40 ± 5·1	17·81 ± 2·1
IDWR22	12·2 ± 0·02	NA	14·23 ± 1·3
IDWR30	32·12 ± 0·09	NA	NA
IDWR32	NA	123 ± 4·2	NA
IDWR39	10·1 ± 0·12	NA	2·63 ± 1·2
IDWR48	21·12 ± 0·04	NA	18·23 ± 0·9
IDWR53	27·63 ± 0·08	405·17 ± 6·2	13·36 ± 2·3
IDWR55	NA	NA	7·1 ± 1·3
IDWR56	25·12 ± 0·1	NA	14·2 ± 1·2
IDWR62	NA	NA	3·2 ± 0·9
IDWR64	NA	NA	4·1 ± 1·1
IDWR65	NA	100·21 ± 2·2	8·2 ± 0·8
IDWR81	26·9 ± 0·07	911·6 ± 5·3	11·5 ± 2·1
IDWR82	22·7 ± 0·03	NA	NA

NA, no significant activity; IAA, indole acetic acid; PGP, plant growth promotion.
*Data are mean ± SE for three determinations ($n = 3$).
†The values represent mean which were significantly different ($P \leq 0·05$).

Table 2 Morphological and chemotaxonomical characteristics of actinomycete isolates

Characteristic	IDWR19	IDWR53	IDWR81
Aerial spore mass	Grey	White	Pink
Aerial mycelium	White	Grey	White
Soluble pigment	None	Dark brown	None
DAP isomer	L-DAP	L-DAP	L-DAP
Gram staining	Positive	Positive	Positive

DAP, diaminopimelic acid.

5·2 U mg^{-1} protein) was produced on 7th day of incubation, whereas maximum alkaline protease (*S. rochei* IDWR19 3·2 U mg^{-1} protein) was produced on 6th day of incubation. For cellulase (*S. rochei* IDWR19 7·4 U mg^{-1} protein) and invertase (*S. carpinensis* IDWR53 451 U mg^{-1} protein) maximum activity was observed on 4th as well as 5th day of incubation, respectively.

Plant growth experiment

The most potent PGP and soil enzyme producers (*S. rochei* IDWR19 and *S. thermolilacinus* IDWR81) were selected for plant growth studies in a pot experiment. Inoculation of wheat with Actinomycete isolates significantly improved plant growth as compared to uninoculated control group after 4 weeks (Table 4, Fig. 3).

The wheat plants inoculated with Actinomycete isolates had high number of root branches; however, significant change in root length was not observed. Inoculated plants also showed higher number of branches and significantly higher biomass (2 and 2·5 times for *S. rochei* IDWR19 and *S. thermolilacinus* 81, respectively) as compared to uninoculated control. An increase of 12·2 and 24·5% in shoot length of plants inoculated with *S. rochei* IDWR19 and 81 was observed, respectively. A similar increase in biomass of 1·8- and 2·3-fold was also recorded with the two isolates (Table 4).

Discussion

In the present study, we explored the potential of PGP Actinomycete from wheat rhizosphere to produce soil enzymes under SSF. Three abundant Actinomycetes with ability to promote plant growth significantly were isolated in this study. The morphological and chemotaxonomical characterization of the isolates identified indicated that they belonged to *Streptomyces* genera (Table 2). Further 16S rDNA amplication confirmed them to be *S. rochei* IDWR19, *S. carpinensis* IDWR53 and *Streptomyces thermolilacinus* IDWR81 (Fig. 1). In soil, *Streptomyces* species constitute more than 90% of total Actinomycete (Jiang and Xu 1990; Mokni *et al.* 2009). Three Actinomycetes reported here have not been evaluated for PGP potential except biocontrol activity. To the best of our knowledge, we are the first to explore the PGP potential of these *Streptomyces* species. The three isolates had significantly higher IAA and siderophore production potential. In *Streptomyces,* IAA biosynthesis occurs by Indoleacetamide (IAM) pathway, wherein tryptophan supplemented in the medium acts as precursor. It is hypothesized that IAA acts a common regulating agent for sporulation and

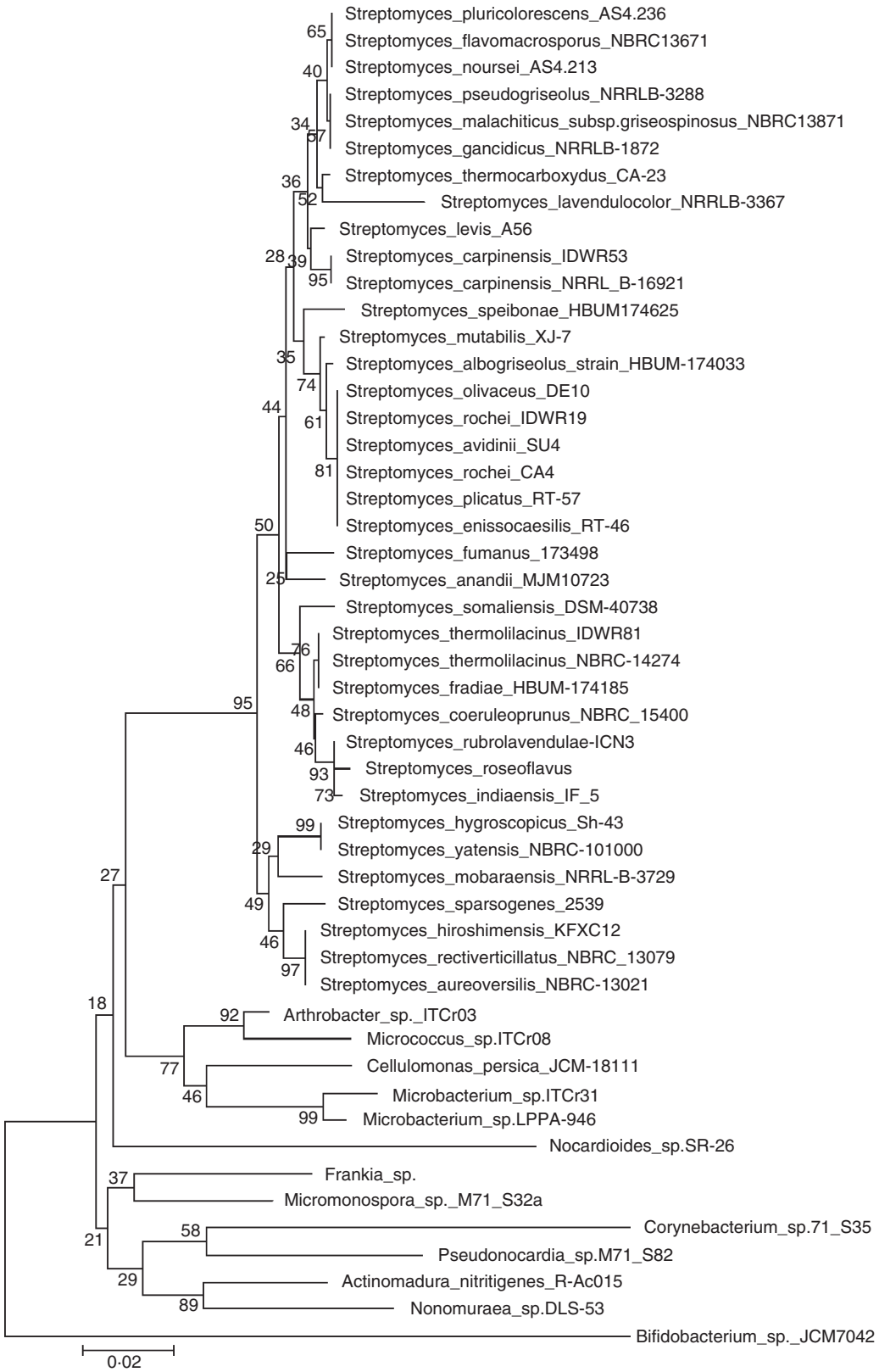

Figure 1 Maximum likelihood tree based on 16S rDNA sequences, showing relationships amongst strain IDWR19, IDWR53, IDWR81 and other representatives of *Streptomyces* family Bar 0·02 nucleotides substitution per site. Numbers on branches indicate confidence limits estimated from bootstrap analysis of 1000 replicates.

Table 3 Plant sugar utilization profile of actinomycete isolates

	Plant sugars (MM+ 2%) added*					
Strain	Glucose	Fructose	Mannose	Sucrose	Arabinose	Xylose
IDWR19	++	++	++	++	++	++
IDWR53	+	+	+	+†	+	+
IDWR81	+	+	+	+	+	+

+, presence of growth; ++, significantly high growth; MM, minimal medium.
*Control with only MM showed no growth.
†Broth colour changed to yellow indicating sugar oxidation.

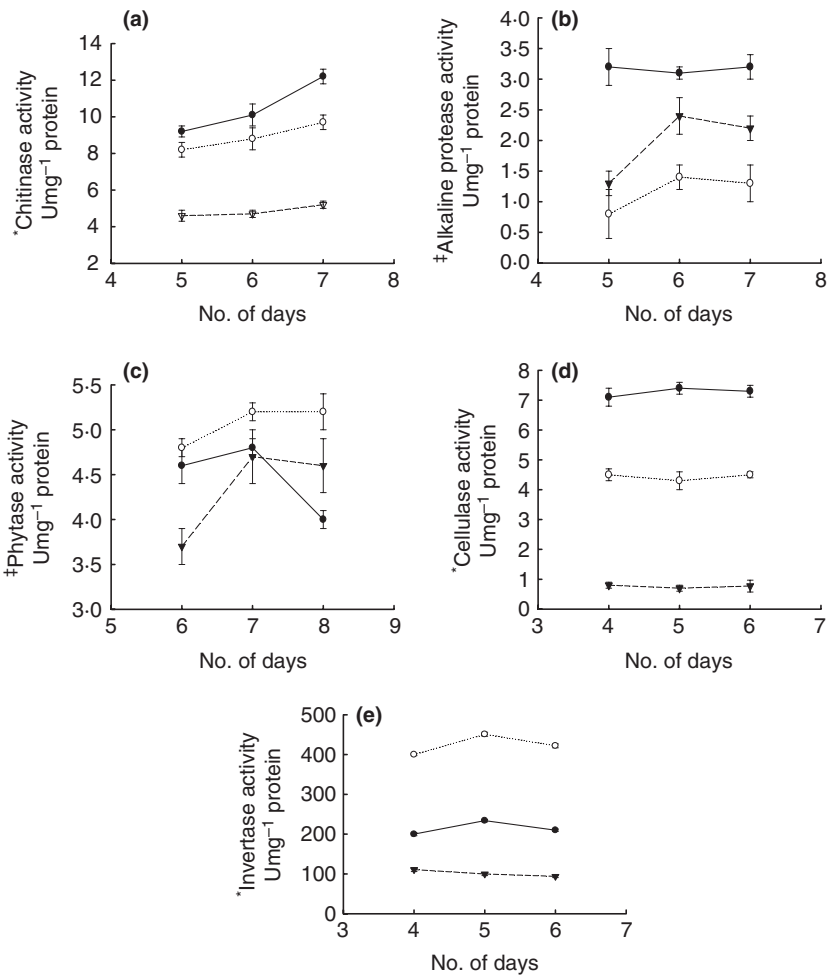

Figure 2 Soil enzyme production by three selected *Streptomyces* spp. (a) Chitinase, (b) Alkaline protease, (c) Phytase, (d) Cellulase, (e) Invertase under ssf using soil extract medium and wheat straw. The number in the legend indicates specific strain under study. Data are mean ± SE for three determinations ($n = 3$). Enzyme production was extremely significant for chitinase*, cellulase* and invertase* ($P \leq 0{\cdot}001$); least significant ($P \leq 0{\cdot}05$) for alkaline protease† whilst non significant for phytase‡ ($P \geq 0{\cdot}05$). ●, *S. rochi* IDWR 19; ○, *Streptomyces carpinensis* IDWR 53; ▼, *S. thermolilacinus* IDWR81.

secondary metabolite production in Actinomycetes (Matsukawa *et al.* 2007). In our study, maximum IAA production observed for *S. rochei* IDWR19 (17·8 mg l^{-1}) was higher to that reported for *S. albidoflavus* (7·1 mg l^{-1}) (Table 1) (Narayana *et al.* 2009) and comparable to other Actinomycetes (0·22 to 15·14 mg l^{-1}) (Nimnoi *et al.*

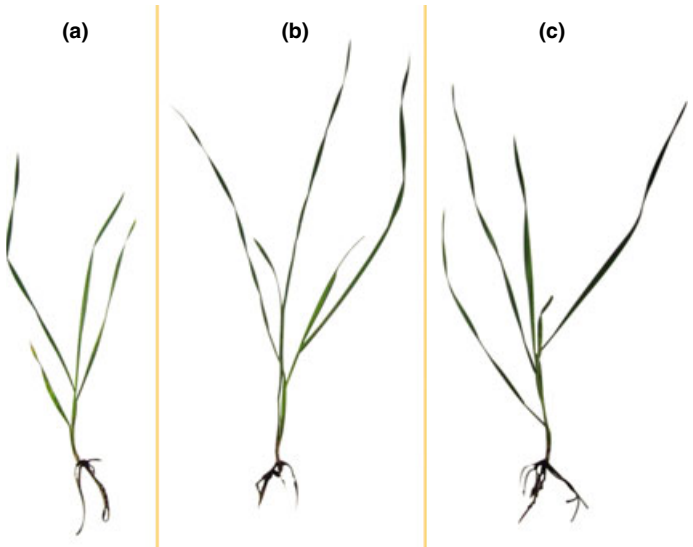

Figure 3 Effect of Actinomycete inoculation on wheat plants (a) Control (uninoculated), (b) Inoculated with *Streptomyces thermolilacinus* IDWR81, (c) Inoculated with *Streptomyces rochei* IDWR19.

Table 4 Effect of actinomycete inoculation on plant growth parameters

Strain	Root length (cm)*†‡	No. of root branches*†	Shoot length (cm)*†	No. of shoot branches*†	Biomass (g)*†
Control	6·0 ± 0·2	05	28·5 ± 1·2	04	0·195 ± 0·02
IDWR19	6·5 ± 0·3	07	32 ± 1·0	05	0·370 ± 0·01
IDWR81	6·0 ± 0·2	07	35·5 ± 0·4	05	0·450 ± 0·02

*Data are mean ± SE for three determinations (n = 3).
†Means in data are significantly different as compared to control ($P \leq 0{\cdot}05$).
‡Values do not significantly change as compared to control ($P \geq 0{\cdot}05$).

2010) under similar media conditions when supplemented with 5 mmol l^{-1} tryptophan.

Siderophores are low molecular weight iron chelaters that are produced by many micro-organisms to scavenge ferric iron with high affinity and resulting ferric-siderophore complexes are shuttled back into cells via active transport mechanisms (Matzanke *et al.* 1989). *Streptomyces* species are known to produce hydroxymate type siderophores, which inhibit growth of phytopathogens by limiting iron in rhizosphere (Khamna *et al.* 2009). Microbial siderophores may also be utilized by plants as an iron source (Wang *et al.*, 1993). The highest siderophore production amongst the selected isolates was in *S. rochei* (*S. rochei* IDWR19) (hydroxymate type – 34·17 mg l^{-1}) (Table 1). The findings are similar to those reported for *Pseudonocardia halophobica* (hydorxymate type – 39·3 mg l^{-1}) (Nimnoi *et al.* 2010).

The test Actinomycetes were also screened for phosphate solubilization and a maximum of phosphate was solubilized by *S. thermolilacinus* (911·6 mg l^{-1}) on tris-(50 mmol l^{-1}) buffered tricalcium phosphate medium (Table 1). The main mechanism responsible for P-solubization is organic acid production by micro-organisms, which also promote the acidification of the microbial cell and its environment, consequently releasing Pi from mineral phosphate (Goldstein 1994). The organic acid secretion differs in various organisms. Actinomycetes are known to produce pyruvate, α-ketoglutarate, lactate, succinate, citrate, malate and oxalate in varying concentration (Rozycki and Strzelczyk 1986). However, Actinomycete phosphate solubilization via organic acid production is poorly reported because of insufficient concentration of these acids. It was found that *S. thermolilacinus* IDWR81 produced malic acid on TLC plates (data not shown), which lowered medium pH from 8 to 5·5 thus releasing free phosphates.

The rhizosphere competence of any micro-organism is dependent on its ability to utilize nutrients secreted by plant in the form of root exudates. The three isolates could utilize plant sugars under study signifying broader

carbon utilization ability. *Streptomyces rochei* IDWR19 had highest number of colonies on isolation agar, which also co-related with its higher growth on the tested plant sugars (Table 3).

Actinomycetes under study were further screened for production of soil enzymes under solid state fermentation. In SSF, the water is present in a complex form within the solid matrix or as a thin layer either absorbed to the surface of the particles or less tightly bound within the capillary region of the solid (Raimbault 1998) resembling closely to the soil conditions. The soil enzymes are essential to utilize the nutrients available in rhizosphere. These nutrients, majorly available from plants, are broadly termed as 'Rhizodeposition'. This includes wide processes by which C enters soil including lysis of root cells, flow of C through mycorrhizas, gaseous loses, root exudates, insoluble polymer secretion from living cells, etc (Jones *et al.* 2004). These factors contribute to construct a nutrient pool containing polymers, sugars, peptides and amino-acids, organic phosphates, etc in rhizosphere. The soil enzymes selected in the present study are the fertility markers (Cellulase, invertase: C-cycling; Protease, chitinase: N-cyling; Phytase: P-cycling). The Actinomycete isolates obtained in present study were capable of utilizing these nutrients by producing soil enzymes and in turn exhibit PGP activities that were extremely beneficial for plants. The data shows that soil extract and agrowaste in combination can be used to attain adequate enzyme production (Fig. 2). To the best of our knowledge, we are the first to report enzyme production by Actinomycetes under ssf using agrowaste as substrate matrix and soil extract as fermentation medium.

The most efficient PGP *Streptomyces* (isolate IDWR81 and IDWR19), when inoculated in pot experiment significantly improved plant growth and development in terms of roots and shoots length, number of branches and biomass as compared to uninoculated control plants under nonaxenic conditions (Table 4, Fig. 3). Our results are in line with the findings of Dobbelaere *et al.* (2002) who reported the inoculation of PGPR *Azospirillum brasilense* resulted in increased dry weight of the root and shoot parts. Similarly, promotion in plant height, plant dry weight and grain yields of various crop plants in response to inoculation with PGPR were reported by other workers (Chen *et al.* 1994; Khalid *et al.* 1997; Biswas *et al.* 2000; Hilali *et al.* 2001). The isolated strain *S. thermolilacinus* IDWR81 (Table 1), which was abundant in wheat rhizosphere, produced highest auxin and solubilized maximum phosphate also significantly promoted yield of trials. This may imply that the strain had competitive advantage and positively affected the growth of inoculated plants and can be tested as a potential biofertilizer in field trials.

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