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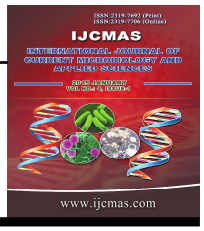

Original Research Article

Generic diversity and a comparative account on plant growth promoting characteristics of actinomycetes in roots and rhizosphere of *Saccharum officinarum*

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Keywords

Indole-3-acetic acid, Phosphate solubilization, Siderophores, Ammonia, Biocontrol, HCN, Actinomycetes, *S. officinarum*

A B S T R A C T

Actinomycetes are the important group of rhizosphere and endophytic microbial community. The objective of this study is to reveal diversity of rhizosphere and root actinomycetes associated with sugarcane and their screening and comparison for plant growth promoting traits production potential. Richness and diversity of actinomycetes in both environments were calculated and found higher in rhizosphere than on roots. All endophytes (22) were identified as *Streptomyces* whereas rhizospheric isolates (35) were from *Streptomyces*, *Streptosporangium*, *Micromonospora*, *Nocardiopsis actinomadura* and *Saccharopolyspora*. The 68% rhizospheric and 78% root actinomycetes produced indole-3-acetic acid ranging between 27 to 81µg ml^{-1} and 14 to 45 µg ml^{-1} respectively. Ammonia production was found in 19 rhizospheric and 5 root isolates whereas HCN production was detected only among rhizospheric actinomycetes (40%). The rhizospheric isolates released significantly higher amounts of soluble phosphate and had higher antimycotic efficacy than endophytes. Hydroxymate type siderophores were produced in the range of 6.09 to 43.21 µg ml^{-1} and 4.32 to 20.36 µg ml^{-1} by 19 rhizospheric and 9 root actinomycetes respectively. Catechol type siderophores were detected in 9 rhizospheric and only 5 endophytic isolates. The potential rhizospheric *Streptomyces*-SR 23 and endophytic *Streptomyces*-SE 22 were further identified at molecular level as *Streptomyces atrovirens* and *Streptomyces albus*. The study highlighted the significance of actinomycetes in plant growth promotion and indicated that adaptations made by rhizosphere and root actinomycetes in their natural environments results in their differing capacities to produce plant growth promoting substances.

Introduction

Rhizosphere microbial community represents a large spectrum of an important group of microorganisms that develops in response to plant metabolic products released in soil as root exudates. Endophytes represent a subgroup of the rhizobacterial communities which may have the ability to enter the roots of their host after the

rhizosphere is colonized (Rosenblueth and Martinez Romero 2006). The endophytic and rhizospheric environments are very different chemically, physically and biologically (Fageria and Stone 2006). Rhizosphere environments are nutritionally rich due to release of plant exudates containing amino acids, vitamins, sugars, plant hormones etc. This environment is influenced by external parameters including soil type, its moisture, pH, and nature of plants. In contrast endophytic environment is more stable and uniform and is less affected by environmental changes. The plant growth promotion made by endophytes or by rhizosphere organisms follows similar mechanisms. These include nitrogen fixation (Han *et al.*, 2005), solubilization of Mineral phosphates (Wakelin *et al.*, 2004), and production of growth hormones and enzymes involved in plant growth and biocontrol activity (Karthikeyan *et al.*, 2005).

A large population of plant growth promoting microbes is found both in rhizosphere and inside plants. Actinomycetes are the dominant group of rhizosphere microbial community and are also found within plants where they live as endophytes. These organisms are known for their prolific activity in nutrient cycling, nitrogen fixation, production of secondary metabolites and plant growth promotion (Lazarovitz and Nowak, 1997). Vast studies have been performed to prove applications of actinomycetes as biocontrol agents and some information is also available on the populations of actinomycetes in a range of soils and plant roots (Gorlach-Lira and Coutinho, 2007). However, the studies that have addressed the role of rhizosphere and endophytic actinomycetes related to sugarcane in plant growth promotion sugarcane are limited. *Frankia* was previously recognized as the only endophytic actinomycetes that resided in nodule of non legume plants and fixed nitrogen. Recently, research is more driven towards non-*Frankia* endophytic actinomycetes. They were isolated from crop plants such as wheat (Coombs and Franco, 2003) rice (Tian *et al.*, 2004) and from roots of *Acacia auriculiformis* (Bunyoo *et al.*, 2008) and *Aquilaria crassna* Pierre ex Lec (Nimnoi *et al.*, 2010). To harness the applications of these organisms in plant growth and development, it is necessary to find out the potential of actinomycetes to sustain in different environments and their ability to produce plant growth promoting substances.

Sugarcane (*Saccharum officinarum*) is the most important cash crop in several developing countries including India. The cultivation of sugarcane is mainly based on chemical fertilizers which not only makes its cultivation expensive but may also lead to the formation of toxic pollutants. In order to make its production cost effective and sustainable, there is need to understand the possible contributions of rhizosphere and endophytic microflora associated with sugarcane in improving its cultivation and yield.

This will be also helpful for better understanding of the adaptations made by these organisms that enable to colonize them multiple environments and their interactions with the surrounding environments. Keeping this aspect in view the present study was conducted to compare qualitatively and quantitatively the plant growth promoting activities of actinomycetes isolated from roots and rhizosphere of sugarcane. The ability of these organisms to produce indole-3-acetic acid (IAA), Hydrogen cyanide (HCN), ammonia, siderophores, to solubilize inorganic phosphate and potential to promote biocontrol of fungal phytopathogens were assessed.

Materials and Methods

Strain isolation and selection

Actinomycetes were isolated from rhizosphere and from within roots of sugarcane cultivated in agriculture fields of Nanded (M.S.), India (Latitude, 19^{o} 9'N and Longitude, 77^{o} 27'E) between Jan to April 2012. To obtain endophytic actinomycetes from roots, root samples were washed in tap water and surface sterilized with serial transfers in 70% to 90 % ethanol for 2 to 5 min. The root samples were macerated in 10 ml of sterile distilled water, 0.1 ml was taken and inoculated (triplicates) in starch casein broth.

The flasks were incubated at 30^{o}C on orbital shaking incubator (120 rev min^{-1}) for seven days. The flasks showing turbidity were used for isolating endophytic actinomycetes on starch casein agar (SCA, Mitra *et al.,* 2008) supplemented with 75µg ml^{-1} of each nystatin and cycloheximide, and 50 µg ml^{-1} nalidixic acid as antifungal and antibacterial agents respectively. The plates were incubated at 30^{o}C up to the development of colonies (three weeks approximately). The isolates showing distinct morphology were selected and subcultured individually. The surface sterilization procedure was verified by spreading 0.1ml aliquots of rinsed waster on SCA medium and then incubated at 30^{o}C for 5 days.

The isolation of rhizospheric actinomycetes was conducted by taking 1g roots with adhered soil into of 10 ml of sterile distilled water, through mixing by shaking at 150 rev min^{-1} for 30min. The 0.1ml of supernatant was plated on SCA medium and after incubation, morphologically different colonies were selected. The selected isolates were identified to genus level based on morphological characteristics including aerial mycelia, spore mass color, diffusible pigment production and spore chain morphology (Holt *et al.,* 1994) and analysis of cell wall compositions (Hasegawa *et al.,* 1983).

Screening of actinomycetes for plant growth promoting traits

The following methods used for assessing the characteristics reveled to plant growth promotion of the actinomycetes strains.

Culture media

IAA and HCN production was studied in SCA medium supplemented with 0.5 g l^{-1}of tryptophanand 4.4 g l^{-1} glycine respectively. Peptone water was used for ammonia determination and phosphate solubilization ability was detected using SCA medium containing 5% tricalcium phosphate. For antifungal assay Potato Dextrose Agar medium (PDA, HiMedia) was used.

IAA production

The selected isolate were grown individually in triplicate in modified SCA and after incubation at 30^{o}C for seven days, the cultures were centrifuged at 10,000 rev min^{-1} for 30 min and cell free supernatants (CFS) were used for IAA analysis. After addition of Salkowski's reagent development of pink color indicated positive results. The concentration of IAA produced was determined spectrophotometrically at 530 nm using standard IAA as reference (Gordon and Weber, 1951).

Hydrogen cyanide (HCN) production

On HCN screening medium, a Whatmann filter paper no. 1 soaked in 2% of sodium carbonate in 0.5% picric acid solution placed in the lid of the plate. Plates were

sealed with paraffin and incubated at 30°C for seven days development of orange red color indicated HCN production (Lorck, 1948)

Ammonia production

Actinomycetes isolates were grown in Peptone water and after incubation at 30°C for seven days, tested for ammonia production by using Nessler's reagent. Development of brown to yellow color was considered as positive test for ammonia production (Cappucino and Sherman, 1992). Ammonium chloride (NH_4Cl) in the range of 0–10 μg ml^{-1} was used to prepare a standard curve. The concentration of ammonia was determined by comparison with a standard curve.

Phosphate solubilization

The isolates were spot inoculated on tricalcium phosphate supplemented SCA plates and incubated at 30°C for seven days. Appearance of clear zones around the colony indicated phosphate solubilization. The isolates showing positive results were subjected for quantitative studies in liquid medium under similar incubation conditions. After incubation amount of phosphate solubilized in CFS was determined according to Jackson (1973).

Antimycotic potential

All isolates were screened for in vitro antagonism against *Alternaria solani* (NCIM 887), *Ustilago maydis* (NCIM 983), *Sclerotium rolfsii* (NCIM 1084), *Helminthosporium gramineum* (NCIM 1070) and *Rhizoctonia solani* (isolated from an infected potato tuber and identified at School of Life Sciences, Swami Ramanand Teerth Marathwada University, Nanded) on PDA plates using agar well diffusion assay (Cooper, 1955). The isolates were grown in soybean casein digest broth containing 1% dextrose at 30°C for seven days. The CFS obtained was tested for their antifungal efficacy.

Siderophore production

The siderophore production ability of rhizospheric and endophytic actinomycetes was detected by inoculating the isolates on Chrome Azurol S (CAS) agar (Schwyn and Neilands, 1987) and incubated at 30°C for seven days. The colonies showing orange zone production around their growth were selected as siderophore producing isolates and cultivated in starch casein broth for seven days at 30°C and with shaking at 150 rev min^{-1}. Arnow's (Arnow, 1937) and Csaky (Csaky, 1948) tests were used to detect and estimate catechol and hydroxymate type of siderophores respectively.

Identification of potential isolate

Based on the ability of isolates to produce plant growth promotion traits, the isolate Streptomyces SR-23 from rhizosphere and *Streptomyces* SE-22 from root were selected as promising strains and further identified at molecular level using 16S rRNA sequence analysis. The obtained sequences were aligned using BLASTN program to identify the isolate.

Statistical analysis

ANOVA is used to estimate the significance of statistical parameters and the richness (d) and diversity (H) indexes (Shannon- Weaver index) were calculated on the basis of total number of actinomycetes isolated from the rhizosphere and roots of sugarcane by using PAST statistical software.

Result and Discussion

Isolation and identification of root and rhizosphere actinomycetes

In the present study 207 rhizospheric and 49 endophytic actinomycetes strains associated with sugarcane were isolated. The effectiveness of surface sterilization procedure was confirmed as no microbial growth was observed on SCA medium after incubation at 30^{oC} for five days when inoculated with rinsed water sample. The diversity of representative actinomycetes isolates from sugarcane rhizosphere and root is shown by bubble plot in Fig.1. Richness and diversity of actinomycetes was higher in the rhizosphere (Shannon H index: 1.502, D index: 0.6918) than in the roots of sugarcane (Shannon H index: 0.69, D index: 0.5).

Total 57 morphological distinct isolates including 35 rhizospheric and 22 endophytic actinomycetes were selected for further study. Among the rhizospheric actinomycetes 14 isolates belong to *Streptomycete s*, 7 *Streptosporangium*, 4 *Micromonospora*, 3 *Nocardiopsis*, 3 *Actinomadura* and 4 isolates were *Saccharopolyspora*. However all selected endophytic isolates were from *Streptomycete s* group.

IAA production

The plant growth promoting activities of rhizosphere and root isolates are shown in Table 1, 2 and 3 respectively. IAA was produced by 17 endophytic and 22 rhizospheric strains representing approximately 78% and 68% of the strains from each group. The endophytic actinomycetes produced IAA between 14 to 45 μg ml^{-1}. The amounts produced by rhizospheric isolates were significantly greater ($p>0.05$) than those produced by endophytes and ranged between 27 to 81μg ml^{-1}.

The activity was found to be distributed among all identified genera isolated from rhizosphere; highest being in *Streptomyces* SR-11 (81 μg ml^{-1}) and lowest in *Saccharopolyspora* P2 (27 μg ml^{-1}). Among endophytes, *Streptomyces* SE-3 released highest amounts of IAA and least amounts were detected in *Streptomyces* SE-7.

Ammonia excretion and HCN production

There was significant difference ($p>0.05$) in the number of ammonia releasing actinomycetes among rhizosphere (55%) and endophytic environments (15%). The rhizospheric isolates released between 20 to 65 μg ml^{-1} ammonia where as endophytes produced between 12 and 27 μg ml^{-1}.

All four rhizospheric isolates belonging to *Micromonospora* were able to excrete ammonia whereas only one isolate each belonging to *Nocardiopsis* and *Actinomadura* respectively were able to show positive results. Detectable amounts were also observed in culture broths of 6 *Streptomyces* sp, 3 *Saccharopolyspora* sp and 4 isolates from *Streptosporangium*. 40% of rhizospheric isolates showed HCN production potential where as this activity was not observed among endophytes.

Phosphate solubilization

The considerable differences in the number of phosphate solubilizing isolates and in the amount of phosphate solubilized were observed among rhizospheric and endophytic actinomycetes ($p>0.01$). 23 rhizospheric (65.71%) and 7 (31.81%) of endophytic strains showed ability to solubilize phosphate. The amount of phosphate solubilized by rhizospheric

isolates was ranged between 30 to 157 µg ml^{-1} where as substantially lower concentrations of soluble phosphate (26 to 60 µg ml^{-1}) were observed among endophytes. The activity was 100% positive in *Micromonospora* and *Actinomadura* isolates from rhizosphere whereas its varied presence was observed among other genera. *Streptomyces* sp SE-22 isolated from roots showed higher efficiency to solubilize P (60 µg ml^{-1}) than other endophytic isolates.

Antimycotic potential

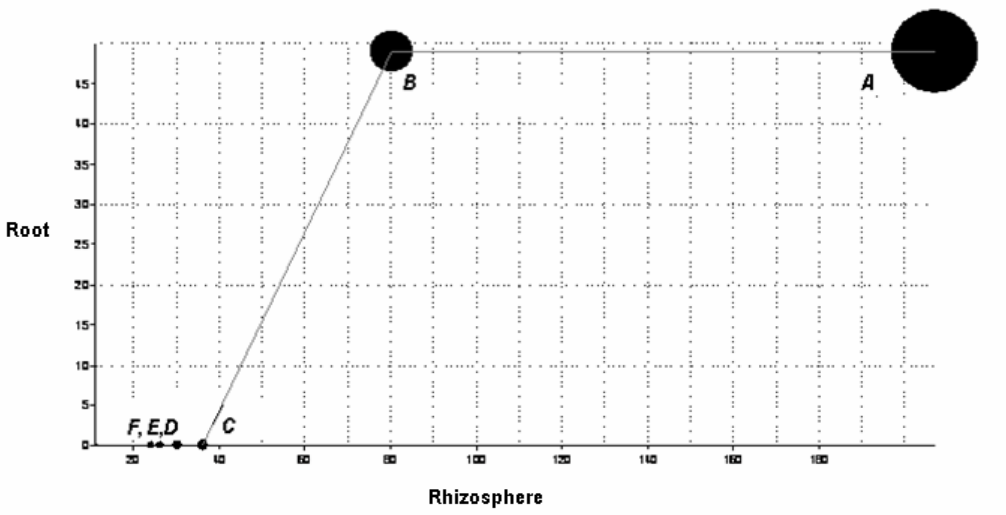
Antimycotic efficacy test conducted against *A. solani, U. maydis, S. rolfsii, H. gramineum* and *R. solani* indicated that the rhizospheric actinomycetes had greater inhibitory effect on fungi than endophytes (Table 4).

Siderophore production

Siderophore production was found in 19 (54.28%) and 9 (40.90) of the selected rhizospheric and endophytic actinomycetes isolates respectively. All 19 rhizospheric and 9 endophytic actinomycetes were able to produce hydroxymate type of siderophores whereas catechol type siderophores were detected only in 9 rhizospheric and 5 endophytic isolates.

The noticeable differences were observed in the amounts of siderophores produced among isolates of both environments ($p>0.05$). The rhizospheric actinomycetes including produced catechols in the range of 3.89 to 11.16 µg ml^{-1} and hydroxymates in the range of 6.09 to 43.21µg ml^{-1} (Table 5) whereas the respective amounts for endophytic isolates were 3.45 to 11.88 µg ml^{-1} and 4.32 to 20.36 µg ml^{-1} (Table 6).

Fig.1 Bubble plot indicating distribution of representative genera of actinomycetes from sugarcane rhizosphere and root



A: *Streptomyces sp*, B: *Streptosporangium sp*, C: *Saccharopolyspora sp*, D: *Micromonospora sp*, E: *Nocardiopsis sp*, F: *Actinomadura sp*. (Size of the bubble indicates the distribution of representative genus).

Table.1 Plant growth promoting activities of *Streptomyces* sp isolated from sugarcane rhizosphere

Isolate	Identified Genus	Plant Growth Promoting Trait			
		[a]IAA (µg ml^{-1})	[a]Ammonia (µg ml^{-1})	[a] Soluble P (µg ml^{-1})	HCN production
SR-1	*Streptomyces* -R_1	35±0.5	25±0.15	44±0.20	+
SR-2	*Streptomyces* -R_2	31±0.22	ND	ND	+
SR-4	*Streptomyces* -R_3	37±0.3	ND	ND	ND
SR-7	*Streptomyces* -R_4	55±0.5	42±0.22	ND	ND
SR-8	*Streptomyces* -R_5	ND	ND	70±0.5	+
SR-9	*Streptomyces* -R_6	ND	51±0.5	ND	ND
SR-14	*Streptomyces*-R_7	41±0.11	ND	34±0.35	ND
SR-16	*Streptomyces* -R_8	59±0.45	ND	84±0.65	ND
SR-19	*Streptomyces* -R_9	41±0.11	20±0.25	50±0.22	ND
SR-22	*Streptomyces* -R_{10}	40±0.10	ND	36±0.10	+
SR-23	*Streptomyces* -R_{11}	**81**±0.15	**65**±0.10	**157**±0.5	**+**
SR-28	*Streptomyces*-R_{12}	ND	ND	30±0.5	+
SR-29	*Streptomyces* -R_{13}	ND	41±0.15	72±0.33	+
SR-35	*Streptomyces* -R_{14}	ND	ND	ND	+

ND not detectable

a Average of three replicates ± standard deviation

Table.2 Plant growth promoting activities of non-*Streptomycete* actinomycetes isolated from sugarcane rhizosphere

Isolate	Identified Genus	Plant Growth Promoting Trait			
		[a]IAA (µg ml^{-1})	[a]Ammonia (µg ml^{-1})	[a] Soluble P (µg ml^{-1})	HCN production
SR-3	*Saccharopolyspora* -P_1	ND	35±0.5	40±0.6	+
SR-5	*Nocardiopsis*-S_1	ND	ND	50±0.5	ND
SR-6	*Streptosporangium*-N_1	30±0.15	ND	44±0.5	ND
SR-10	*Streptosporangium*-N_2	ND	ND	56±0.7	ND
SR-11	*Saccharopolyspora* -P_2	27±0.12	ND	60±0.5	ND
SR-12	*Streptosporangium* -N_3	31±0.23	27±0.35	ND	+
SR-13	*Micromonospora* -H_1	41±0.05	29±0.46	64±0.22	+
SR-15	*Nocardiopsis* -S_2	29±0.5	33±0.32	48±0.58	ND
SR-17	*Actinomadura* -M_1	55±0.22	ND	59±0.44	+
SR-18	*Nocardiopsis* -S_3	31±0.15	ND	ND	ND
SR-20	*Saccharopolyspora* -P_3	35±0.47	49±0.16	ND	ND
SR-21	*Streptosporangium*-N_4	53±0.26	37±0.79	ND	ND
SR-24	*Micromonospora*-H_2	31±0.11	29±0.44	146±0.5	ND
SR-25	*Saccharopolyspora* -P_4	29±0.31	32±0.22	80±0.5	ND
SR-26	*Streptosporangium* -N_5	32±0.05	ND	ND	+
SR-27	*Micromonospora* -H_3	ND	37±0.15	46±0.11	ND
SR-30	*Streptosporangium* -N_6	37±0.25	44±0.27	ND	ND
SR-31	*Actinomadura*-M_2	ND	ND	64±0.5	ND
SR-32	*Micromonospora* -H_4	ND	30±0.12	78±0.5	+
SR-33	*Streptosporangium* -N_7	ND	37±0.15	ND	ND
SR-34	*Actinomadura*-M_3	ND	40±0.23	66±0.5	ND

Table.3 Plant growth promoting activities of actinomycetes isolated from sugarcane root

Isolates	Identified Genus	Plant Growth Promoting Trait [a]		
		IAA ($\mu g\ ml^{-1}$)	Ammonia ($\mu g\ ml^{-1}$)	Soluble P ($\mu g\ ml^{-1}$)
SE-1	*Streptomyces*- E_1	27±0.11	12±0.2	26±0.44
SE-2	*Streptomyces* -E_2	33±0.25	18±0.5	ND
SE-3	*Streptomyces* -E_3	45±0.05	ND	28±0.35
SE-4	*Streptomyces* –E_4	20±0.05	ND	ND
SE-5	*Streptomyces* -E_5	22±0.5	ND	ND
SE-6	*Streptomyces* -E_6	ND	19±0.5	ND
SE-7	*Streptomyces* -E_7	14±0.12	ND	ND
SE-8	*Streptomyces* -E_8	ND	ND	ND
SE-9	*Streptomyces* –E_9	23±0.5	ND	ND
SE-10	*Streptomyces* -E_{10}	25±0.33	ND	ND
SE-11	*Streptomyces* -E_{11}	27±0.40	ND	ND
SE-12	*Streptomyces* -E_{12}	31±0.5	ND	ND
SE-13	*Streptomyces* -E_{13}	37±0.26	ND	36±0.21
SE-14	*Streptomyces* -E_{14}	24±0.47	17±0.35	ND
SE-15	*Streptomyces* -E_{15}	26±0.19	ND	28±0.5
SE-16	*Streptomyces* -E_{16}	ND	ND	ND
SE-17	*Streptomyces* -E_{17}	22±0.22	ND	ND
SE-18	*Streptomyces* -E_{18}	ND	ND	22±0.5
SE-19	*Streptomyces* -E_{19}	ND	ND	ND
SE-20	*Streptomyces* -E_{20}	24±0.49	ND	44±0.16
SE-21	*Streptomyces* -E_{21}	30±0.26	ND	ND
SE-22	*Streptomyces* -E_{22}	**20**±0.5	**27**±0.5	**60**±0.5

ND not detectable

[a] Average of three replicates ± standard deviation

Table.4 Antimycotic efficacy of rhizosphere and root actinomycetes

Isolate Environment	Test pathogen				
	A. Solani	*R. solani*	*S. rolfsii*	*U. maydis*	*H. gramineum*
Rhizosphere	13 (37.14)*	09 (25.71)	12 (34.28)	10 (28.57)	17 (48.57)
Root	05 (14.28)	02 (5.71)	00 (00)	00 (00)	03 8.57)

*Figures in the parenthesis indicate the percentage of isolates showing antimycotic activity.

Table.5 Siderophore production by rhizospheric actinomycetes

Isolates	Identified genus	Type of siderophore (µg ml^{-1})[a]	
		Catechol	Hydroxymate
SR-2	*Streptomyces*-R_2	ND	14.32± 0.5
SR-3	*Saccharopolyspora*-P_1	7.45±0.7	10.89±0.7
SR-7	*Streptomyces* -R_4	ND	6.34±0.6
SR-8	*Streptomyces* -R_5	ND	7.83±0.2
SR-9	*Streptomyces* -R_6	9.88±0.5	11.32±0.4
SR-12	*Streptosporangium* -N_3	6.04±0.5	17.20±0.5
SR-15	*Nocardiopsis* -S_2	ND	21.36±0.6
SR-19	*Streptomyces* -R_9	ND	12.78±0.6
SR-20	*Saccharopolyspora*-P_3	11.16±0.7	25.76±0.9
SR-21	*Streptosporangium*-N_4	ND	16.77±1.0
SR-23	*Streptomyces*-R_{11}	9.43±0.6	43.21±0.9
SR-24	*Micromonospora*-H_2	5.42±0.3	32.54±0.7
SR-25	*Saccharopolyspora*-P_4	ND	30.43±0.5
SR-26	*Streptosporangium*-N_5	ND	11.89±0.6
SR-29	*Streptomyces*-R_{13}	3.89±0.7	16.23±0.5
SR-30	*Streptosporangium*-N_6	4.54±0.6	26.45±0.9
SR-31	*Actinomadura*-M_2	ND	6.09±0.1
SR-32	*Micromonospora*-H_4	ND	10.35±0.7
SR-33	*Streptosporangium*-N_7	5.64±0.5	19.87±0.5

ND not detectable [a] Average± standard error obtained from triplicate results

Table.6 Siderophore production by root actinomycetes

Isolates	Identified genus	Type of siderophore (µg ml^{-1})[a]	
		Catechol	Hydroxymate
SE-3	*Streptomyces*-E_3	ND	4.32± 0.5
SE-4	*Streptomyces*-E_4	3.45±0.7	12.89±0.7
SE-7	*Streptomyces*-E_7	6.22±0.5	6.78±0.6
SE-9	*Streptomyces*-E_9	ND	11.83±0.2
SE-10	*Streptomyces*-E_{10}	11.88±0.5	10.32±0.4
SE-12	*Streptomyces*-E_{12}	7.04±0.5	18.20±0.5
SE-16	*Streptomyces*-E_{16}	ND	20.36±0.6
SE-19	*Streptomyces*-E_{19}	ND	9.78±0.6
SE-21	*Streptomyces*-E_{21}	6.54±0.7	15.76±0.9

ND not detectable; [a] Average± standard error obtained from triplicate results

Identification of potential strains

The rhizospheric isolate *Streptomyces*-R11 (SR 23) showed highest production of IAA and ammonia and phosphate solubilization activity. Promising antagonistic activity of SR 23 also revealed its significance in terms of HCN production and fungal inhibition (data not shown), hence it was selected as potential strain and identified as *Streptomyces atrovirens* based on its morphology, chemotaxonomy, biochemical characterization (Table 7) and 16s rRNA sequencing. The isolate showed 99 % similarity with *Streptomyces atrovirens* NRRL B-16357. Similarly, the root actinomycete isolate *Streptomyces* SE-22 was able to release IAA and ammonia, solubilized phosphate efficiently and showed inhibition of *U. maydis*, *H.*

gramineum and *R. solani* (data not shown). Hence, it was selected as potential root isolate and further identified at molecular level by 16s rRNA sequencing as *Streptomyces albus.* The characteristics of *S. albus* are shown in Table 7. The 16S rRNA sequences of the strain SR-23 as *Streptomyces atrovirens*-SR11 and SE-22 as *Streptomyces albus* SE-20 have been deposited in Gen Bank under the accession number KC710333 and KC710336 respectively.

The soil microbial communities play an integral and unique role in ecosystem functions and are among the most complex and diverse communities in the biosphere (Zhou *et al.*, 2003). The capacity to colonize host rhizosphere and internal tissues and contribute to plant growth promotion has made rhizosphere and endophytic microorganisms valuable for agriculture as a tool to improve crop performance. The beneficial plant microbe associations can be exploited to offer promising and ecofriendly strategies for conventional and organic agriculture worldwide. This is well studied in legumes (Alves *et al.*, 2003) as well as non-legumes such as sugarcane and rice (James, 2000).

Table.7 Morphological, chemotaxonomic and biochemical characteristics of *Streptomyces atrovirens* and *Streptomyces albus*

Test Character	*S. atrovirens* SR-11	*S. albus*SE-20
Spore chain morphology	Spiral	Spiral
Spore mass color	Grey	Yellow
Color of substrate mycelium	Light Brown	Light yellow
Diffusible pigment	-	-
Cell wall chemotype	I	I
Characteristic sugar in cell wall	No	No
Gelatin hydrolysis	+	+
Starch hydrolysis	+	+
Melanin production	+	-
Nitrate reduction	+	+
H_2S production	-	-
Tyrosinase	+	-
Lysozyme resistance	+	+
Utilization of carbon sources		
L-Arabinose	-	+
D-galactose	+	+
D -mannitol	-	+
D-Fructose	+	+
D-glucose	+	+
Meso-Inositol	-	-
Rhamnose	+	-
Sucrose	+	-
Salicin	-	+
L-Glutamine	+	-
L-Tryptophan	+	+
Tyrosine	+	-
Glycine	+	-
L-Asparagine	-	+
Cysteine	+	-
Phenyl alanine	+	+
Histidine	+	+
Hydroxyproline	+	-
L-Lysine	+	+

The present study demonstrated the plant growth promoting ability of both rhizospheric and endophytic actinomycetes associated with sugarcane. The release of substances such as IAA, ammonia, soluble phosphate and production of HCN, antifungal substances and siderophores by the isolates make them available to their environments which possibly contribute to growth promotion. However, the original location from which the actinomycetes were isolated, rhizosphere or internal tissues of plant resulted in varied numbers of isolates with the capacity to release IAA, ammonia, soluble phosphate, HCN, siderophores as well as to promote biocontrol. In our studies, a higher diversity of actinomycetes has been found in the rhizosphere than in the inner root tissues.

These results are in accordance with previous studies which also recognized more diverse microbial communities in rhizosphere than in endophytic environments of different crop plants (Rosenblueth and Martinez-Romero, 2004). However, Lopez-Fuentes *et al.* (2012) recognized higher bacterial density in roots than in rhizosphere of medicinal plant, *Hypericum silenoides.* The presence of different endophytic species in plants depends on plant genotype, plant age, tissue sampled and also on the season of isolation (Rosenblueth and Martinez-Romero, 2006). Both endophytic and rhizosphere populations are conditioned by biotic and abiotic factors (Seghers *et al.,* 2004) but endophytes might be better protected from biotic and abiotic stresses than rhizosphere organisms.

Of the 35 rhizosphere isolates, 14 were tentatively identified as *Streptomyces*, 7 as *Streptosporangium,* 4 as *Micromonospora*, 3 as *Nocardiopsis*, 3 as *Actinomadura* and 4 as *Saccharopolyspora* whereas all 22 endophytic actinomycetes belong to *Streptomyces* sp. This showed more diverse nature of actinomycetes in rhizosphere and dominance of *Streptomyces* genus in root environments of sugarcane. Appearance of *Streptomyces* in endophytic environments of different crops has been previously well documented from surface sterilized roots of 28 plant species in northwestern Italy (Sardi *et al.,* 1992), roots and leaves of maize in northeast Brazil (De Araujo *et al.,* 2000) and from roots of wheat (Coombs and Franco, 2003) and rice (Tian *et al.,* 2004). The overall dominance of *Streptomyces* sp associated with sugarcane is quite expected because this genus represents 70–90% of the actinomycetes found in natural environments (Kennedy, 1999).

Other actinomycetes were from rare group and could be isolated from selective environments and hence their prevalence was lower as compared with *Streptomyces* sp. The major key to succeed in isolating and studying endophytes is to ensure the sterility of the plant surface (Hallmann *et al.,* 1997). The surface sterilization protocol used in present study was effective in removing epiphytic microflora; however, considering the presence of slow growing organisms, we mentioned selected endophytes as putative endophytes.

The production of IAA by actinomycetes is a well reported phenomenon. Although considerable difference was observed in the amounts of IAA released by endophytic and rhizosphere actinomycetes, 78% of endophytic isolates showed this ability. In the rhizospheric environments, root exudates may provide

the tryptophan for microorganisms which may enhance the synthesis of indole acetic acid. It may be possible that the amount of tryptophan available in rhizosphere of sugarcane may be more than in roots which caused higher detection of IAA in rhizospheric isolates. However, detection of IAA in 78% of endophytic isolates indicated that this feature is not much affected by the location of the actinomycetes. The benefits of the application of endophytic and rhizosphere IAA producing strains to plant growth are documented in previous studies (Govindarajan *et al.,* 2008; Khan and Doty, 2009).

Availability of nitrogen is the critical factor in sugarcane development. The ammonia released by rhizobacterial strains plays a signaling role in interaction between PGPR and plants (Chitra *et al.,* 2002). In the present study, 55 % rhizosphere and 15% root actinomycetes were observed to produce ammonia and because ammonia is usually taken up by plants as a source of nitrogen for their growth, the promising isolates could have a beneficial role in plant development. The phosphate solubilization activity was observed in both endophytic and rhizosphere isolates.

Microbial solubilization of mineral phosphate might be either due to the acidification of external medium or the production of chelating substances that increase phosphate solubilization. Therefore phosphate solubilizing microbes play important role in agriculture via increasing efficiency of plants for phosphate utilization. Hamdali *et al.* (2008) reported high amount of Phosphate solubilization activity in *Streptomyces griseus, Streptomyces carocirensis* and *Micromonospora aurantica*.

HCN production ability was found only in rhizosphere isolates; none of the endophytic isolate was able to produce HCN. Some of the recent studies have indicated that the microbially produced metabolites such as HCN may enhance plant establishment.
The isolates from the rhizosphere soil of Chick pea were observed to exhibit HCN production potential which promoted plant growth directly or indirectly or synergistically (Joseph *et al.*, 2007).

The capacity to inhibit phytopathogenic fungi was observed among both endophytic and rhizosphere isolates. However, the activity was more remarkable in rhizosphere isolates. Actinomycetes- fungus antagonism has been demonstrated for wide variety of plant pathogens such *as Alternaria, Rhizoctonia, Verticillium, Fusarium, Phytopthora* and *Phytium* (Tian *et al.*, 2004; Fialho de Oliveira *et al.,* 2010). This important feature demonstrates the potential of studied isolates to indirectly promote plant growth.

The siderophore production potential was more distributed among rhizospheric isolates than in endophytic isolates. Generally, soil microorganisms produce siderophores to bind ferric ions from the environment, transport it back to the microbial cell and make it available for growth (Leong, 1996). Siderophore production may be important for the growth of rhizospheric soil actinomycetes than for endophytic actinomycetes because they have to face higher competition with other rhizosphere bacteria and fungi.

Probably, little competition is exerted in endophytic environments and hence the low amounts of siderophores are produced by endophytic actinomycetes. Further,

competition for iron may be a possible mechanism to control phytopathogens. Our results are in accordance with Khamna *et al.,* (2009) who also reported the siderophore production from several genera of rhizospheric actinomycetes, including *Streptomyces, Actinomadura, Microbispora* and *Nocardia.*

Conclusion of the present study is it demonstrated that the abilities of actinomycetes to produce plant growth promoting traits differ qualitatively and quantitatively according to their locations. Based on the available literature, we also conclude that this study possibly first time demonstrated the presence of *Streptomyces albus* in the roots of sugarcane plant. Further the strain of *Streptomyces atrovirens* reported here showed production of multiple plant growth promoting traits including IAA, ammonia, HCN and siderophore production along with capacity to solubilize inorganic phosphate and inhibit fungal phytopathogens. Although IAA and antifungal compound production by *Streptomyces atrovirens* was reported previously, the present study is specific in terms of its multiple plant growth promoting traits. The features such as IAA, ammonia and HCN production and release of soluble phosphate, siderophores and biocontrol potential may be adapted by actinomycetes to allow better interactions with their natural environments. At the same time these features of associated actinomycetes confer many advantages to the host plant and contribute directly or indirectly in plant growth promotion and development. However, the studies performed here are with pure cultures and under controlled conditions; hence further studies are needed in the direction to know whether these organisms are able to compete with other soil microorganisms and survive environmental conditions that might affect their efficacy as plant growth promoting agents.

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