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Endophytes from *Phyllanthus niruri*: Selection, Characterization and Metabolite Production

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ABSTRACT

Endophytes constitute an underexplored group of microorganisms with a rich potential for production of bioactive metabolites. *Phyllanthus niruri* is a plant with a rich ethnobotanical history and has not been comprehensively studied for its cultivable endophyte population. This study involved selectively isolating bacterial (inclusive of actinobacteria) and fungal endophytes from *P. niruri*, and investigating them for growth characteristics, antimicrobial activity, antioxidant capacity, production of siderophores and industrially important enzymes. A few actinobacterial endophytes were identified by 16S rDNA sequencing. Maximum diversity of isolates was obtained from fresh shoots. Some actinobacterial colonies exhibited diffusible pigment production, indicating possible bioactivity. The isolates were broadly able to tolerate a wide range of pH, temperature and salinity. Many isolates exhibited an alkaliphilic nature (growth at pH 11.0), and 83% isolates grew at 5% NaCl. All isolates were positive for siderophore production, indicating their role in plant growth promotion. Most of the isolates were active against Gram positive targets. All the isolates exhibited antioxidant production capacity, with IS-300 showing maximum antioxidant activity. A selection of isolates, identified by 16S rDNA sequencing, showed that they were potentially new taxa, related to *Streptomyces*. The diversity of endophytes obtained and their distinct antimicrobial and antioxidant activities, siderophore and enzyme production suggest that these can be potentially of use for scaled-up industrial applications.

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Introduction

Increasing multi-drug resistance amongst microorganisms, and lifestyle changes leading to metabolic disorders has led to renewed research focus on bioprospecting for novel natural products, with antimicrobial and antioxidant properties, considering that natural products form the backbone of major bioactive molecules and combinatorial chemistry approaches has resulted in only one new molecule [1]. Actinobacteria continue to be the major sources of antimicrobial and other bioactive compounds [2]. However, other bacteria and fungi are also known to produce such compounds, though with a much smaller repertoire. Current approaches propose to avoid re-isolation of known producer organisms and known compounds by bioprospecting from under-explored habitats such as extreme environments (desert, hot springs) and endophytes [3].

Plants and endophytes share a predominantly mutualistic relationship [4,5]. The plant growth promoting activity of endophytes and their biocontrol properties assist the host plant in its survival, protection and growth [6]. Endophytes, in return, are provided with a protected site for their establishment with direct accessibility to the nutrients [7]. Endophytes, being a relatively less studied domain, have a greater possibility of comprising new species of microorganisms and secreting potentially new bioactive compounds (production of growth promoting compounds, antagonistic compounds against competing or other organisms) such as Xylarphthalide A, an antibacterial compound from *Xylaria* sp. [8] and Phomopoxides, a group of cytotoxic and antifungal compounds from *Phomopsis* [9]. Production of secondary metabolites by endophytes is influenced by their niche, various stresses and their interaction with the host.

Ethnobotanical history of a plant can serve as a good indicator for isolation of potentially novel endophytes and thereby novel bioactive compounds. Going by this rationale, the present study was conceived with *Phyllanthus niruri*, which has a pronounced ethnobotanical history of usage in liver damage, malaria [10], with anti-oxidative, anti-bacterial, anti-inflammatory properties [11]. Endophytic fungi from *P. amarus* and their capability of producing phyllanthin and hypophyllanthin, which are hepatoprotectives have been documented [12]. Taware et al. (2014) [13] had purified Trichothecinol-A, an anticancerous and antifungal compound, from an endophytic fungus *Trichothecium* of *P. amarus*. Endophytic bacteria from *P. amarus* were shown to enhance growth promotion of host plants under salt stress [14].

However, there has been no comprehensive study on the total cultivable endophyte population, especially with focus on actinobacteria. Hence this study was undertaken with the following objectives: (a) To isolate endophytes (focus actinobacteria) from roots and shoots of *P. niruri* (b) To study the taxonomical features of the isolated endophytes and (c) To study the antimicrobial and plant growth promoting characteristics of the endophytes.

Experimental

Materials and Methods

Sample collection

The plant sample was collected from two locations in a community park in NOIDA (28°37'22.4"N 77°22'18.7"E), where it was found growing in competitive environment with weeds and other plants. The plant was authenticated

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based on taxonomic characters by Professor Arun K. Pandey, University of Delhi, India. A voucher specimen (DU14167) was deposited at the herbarium of Department of Botany, University of Delhi, India.

Isolation of endophytes

Ten plants (~200 g fresh weight) were excised into roots and shoots, washed thoroughly in running tap water and double distilled water. Some of the excised plant material was dried for 48 hours in shade. Then the fresh and dried roots and shoots were surface sterilized to eliminate possibility of isolation of epiphytic microorganisms [15]. Material was surface sterilized with 70% (v/v) ethanol for 1 min followed by 0.1% $HgCl_2$ (w/v) for 5 min, again washed with 70% (v/v) ethanol for 30 sec and then with sterile distilled water 5-6 times. Some of the surface sterilized plant material was placed intact on various media as control. 100 mg of shoot and 100 mg of root material was crushed in sterile quarter strength Ringer's solution using mortar and pestle and 500 µL spread plated onto actinobacteria-selective isolation media - Starch Casein Agar (SCA), Glucose Yeast Extract (GYE), Nutrient Agar (NA) for bacteria and Potato Dextrose Agar (PDA) for fungi [16]. All media were solidified with agar (15.0 g L^{-1}). The plates were then incubated at 30 °C for up to 2-3 weeks. The bacterial and fungal colonies were enumerated and pure cultures made of twenty four dereplicated colonies for further studies. Colonies showing actinobacteria-like morphology (filaments/fragmented filaments) were further grown on Bennett's media (in g L^{-1}: beef extract 1.0, yeast extract 1.0, glucose 10.0, peptone 2.0, casein 2.0, agar 15.0) while other bacteria and fungi were cultivated on NA and PDA respectively. Growth conditions were 30 ^oC for actinobacteria and 37 ^oC for others.

Characterization of endophytes

The endophytes were characterized for morphology (colony colour, pigment production, Gram reaction/lactophenol cotton blue staining), physiological/biochemical features (temperature, pH, salinity, carbon and nitrogen source utilization), and activity (antimicrobial, total antioxidant capacity, siderophore production, phosphate solubilization).

Isolates were tested for growth at different temperatures (4, 25, 30, and 40 ^oC), pH (3.0, 5.0, 7.0, 9.0, 11.0), salinity (0%, 0.1%, 0.5%, 1%, 2.5% and 5% NaCl), for carbon utilization (glucose, fructose, lactose, maltose, raffinose, sucrose) and nitrogen utilization (ammonium sulphate, potassium nitrate, ammonium chloride, alanine, proline, urea). Isolates were also tested for production of enzymes (protease, lipase, amylase, cellulase, gelatinase, urease) [16].

Antimicrobial activity was assessed against Gram positive (*Micrococcus luteus* (MTCC-106), *Brevibacterium linens* (MTCC-268), *Bacillus subtilis* (MTCC-121), *Staphylococcus epidermidis* (MTCC-435) and Gram negative *Escherichia coli* (MTCC-1679), *Pseudomonas fluorescens* (MTCC-2421) bacteria by seeding agar plug cultures of the endophytes in Mueller-Hinton agar and measuring the diameters of inhibition zones formed around test isolates [17]. *Streptomyces rimosus* (MTCC-7033) was used as positive control. Siderophore production was studied by the Chrome Azurol Sulfonate (CAS) assay and measuring the diameter of the orange halos surrounding the agar plugs of the endophytes [18], indicating production of siderophore. E. coli (MTCC-1679) was the positive control. Phosphate solubilization capability of the endophytes was studied by seeding the endophytes in wells on National Botanical Research Institute's Phosphate (NBRIP) growth medium (in g L^{-1}: Glucose 10, $Ca_3(PO4)_2$ 5.0, $MgCl_2.6H_2O$ 5.0, $MgSO_4.7H_2O$ 0.25, KCl 0.2, $(NH_4)_2SO_4$ 0.1, Agar 15.0), incubated at 30 °C for two weeks and clear zones around the colonies measured [19]. Pseudomonas putida (MTCC-2445) was positive control and E. coli (MTCC-1679) negative control. Total antioxidant capacity of the isolates was studied, using ascorbic acid as standard, and total antioxidant capacity was expressed as Ascorbic Acid Equivalents with equation (y = 0.062x - 0.059) derived from the calibration curve [20].

A selection of endophytes (IS-501, IS-516 and IS-520) was subjected to 16SrDNA identification [21] after genomic DNA isolation, amplification using universal primers 8F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R (5'-GGTTACCTTGTTACGACTTC-3'), quality analysis of sequenced fragments, and alignment of the sequences using the EzTaxon server (http://eztaxon-e.ezbiocloud.net) [22]. The phylogenetic tree was constructed using MEGA 6.0 software based on the neighbour joining tree algorithm [23]. The nearly complete 16S rDNA consensus sequences were deposited in the GenBank database.

Results and Discussion

Isolation of endophytes

Extracts of fresh and dried root and shoot material of *P. niruri* were plated on various media. Majority of colonies obtained were from those fresh root and shoot material (Table 1). Except for one colony obtained from dried root extract (on SCA), other dried material did not exhibit growth of microorganisms. The maximum number of colonies were obtained on nutrient agar from fresh root material (1.0 x 10^4), followed by that of shoot (1.44 x 10^3). Dereplication on the basis of colony characteristics and microscopic features resulted in thirty five distinct isolates, with most from fresh shoot extract on SCA. Further studies were carried out on twenty four isolates which could be maintained and sub-cultured.

Table 1: Endophyte isolates obtained from fresh and dried *P. niruri* plant material on various media

Media	Plant Material	Plant part	CFU (g FW^{-1}) or (g DW^{-1})	Dereplicated isolates
SCA	Dried	Root	40	1
		Shoot	0	0
	Fresh	Root	2.8 x 10^2	6
		Shoot	1.35 x 10^3	22
GYE	Dried	Root	0	0
		Shoot	0	0
	Fresh	Root	2.4 x 10^2	1
		Shoot	8	1
NA	Dried	Root	0	0
		Shoot	0	0
	Fresh	Root	1.0 x 10^4	2
		Shoot	1.44 x 10^3	1
PDA	Dried	Root	0	0
		Shoot	0	0
	Fresh	Root	0	0
		Shoot	40	1

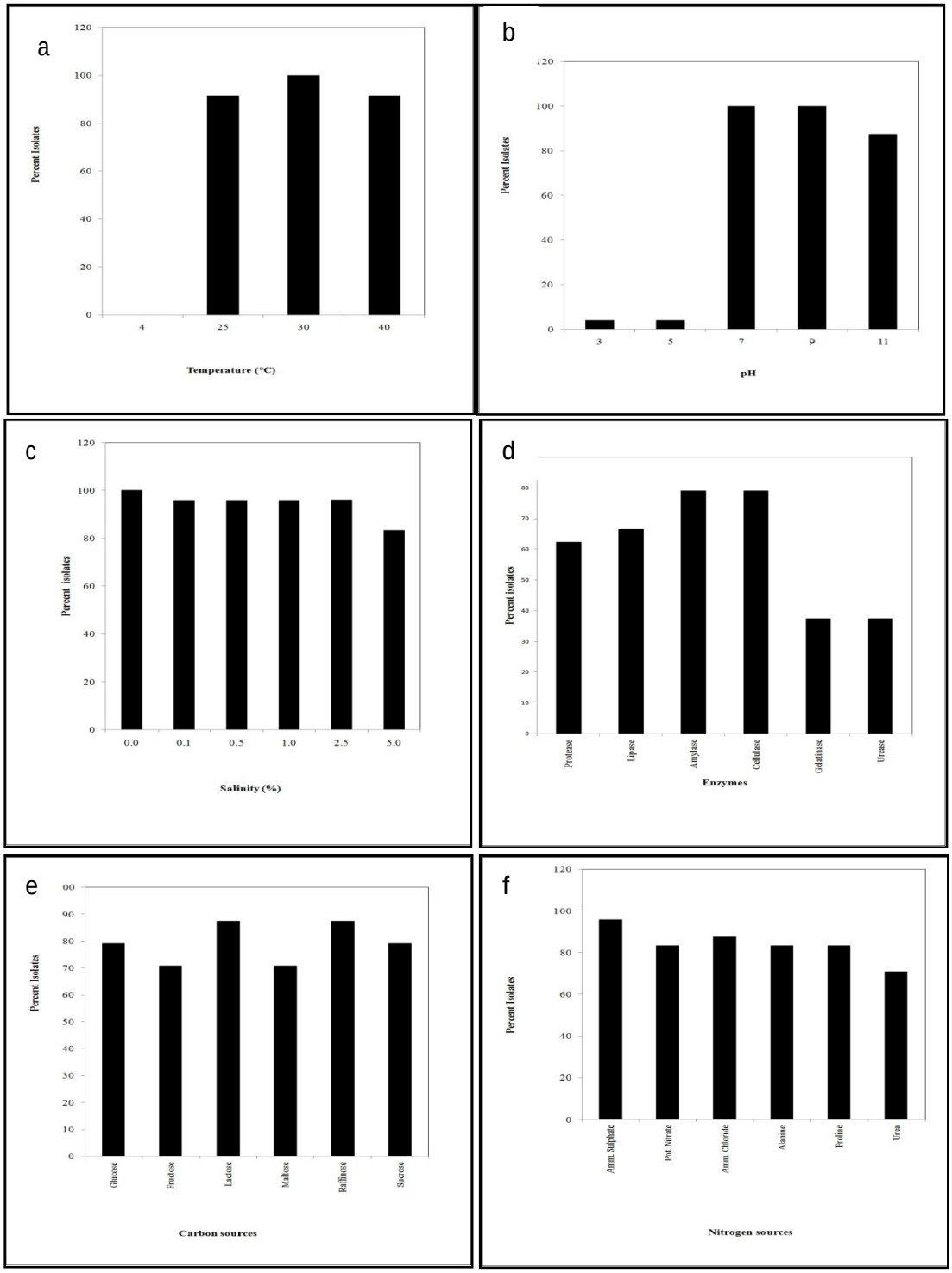

Figure 1: Percentage of endophytic isolates from *P. niruri* growing at various a. temperatures b. pH c. salinity d. enzyme production e. Carbon source utilization f. Nitrogen source utilization

Characterization of endophytes

Morphology

Amongst bacteria, all were Gram positive; 21% were cocci or with coccoid morphology, 12% each were bacilli or larger fragmented bacilli. Mycelial forms comprised 33% and 12% were of mixed cocci and bacilli which could not be purified further (Table 2). The morphology and colour of the mycelia-forming bacterial colonies ranged from white to grey to brown/pink and yellow; substrate mycelia also exhibited similar colour variations. The three fungal colonies exhibited distinct colony colour, yet sporangia/spore morphology was similar.

Biochemical, Physiological and Molecular Characteristics

The endophytes were characterized for growth at various temperatures, pH and salinity ranges and production of industrially important enzymes (Table 3). None of the isolates grew at 4°C, while all showed growth at 30°C and all isolates (except IS-501 and IS-513) could grow up to 40°C (Figure 1a). Except IS-800, none of the isolates

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Table 2: Characterization of endophytes obtained from fresh and dried *P. niruri* plant material on various media

Medium/ plant material	Isolate Number	Colony Morphology	Gram Reaction
SCA (Fresh root)	IS-001	Black circular	Fungi: Oval sporangia with septa
	IS-002	Green circular	Fungi: Oval sporangia with septa
	IS-003	Brown circular, small	Gram positive coccus
	IS-004	Yellow, Irregular	Gram positive coccus
	IS-005	Whitish yellow, Irregular	Gram positive coccus
	IS-006	White colored, Irregular	Gram positive short filaments and slightly bigger rods
SCA (Fresh shoot)	IS-501	Whitish pink aerial and substrate, circular	Gram positive mycelia
	IS-503	Light yellow, circular, slimy	Gram positive rods and coccus
	IS-504(1)	White aerial, cream substrate mycelia	Gram positive mycelia
	IS-504(2)	Greyish aerial, orange cream substrate	Gram positive mycelia
	IS-504(3)	Brownish aerial, substrate	Gram positive mycelia
	IS-505(1)	Light pink with dark pink center, small, circular	Gram positive mycelia
	IS-505(2)	Pinkish grey aerial, substrate	Gram positive mycelia
	IS-506	Peach, circular, shiny	Gram positive Fragmented filaments and rods
	IS-507	White, translucent, irregular	Gram positive coccus and elongated filaments
	IS-508	Dark yellow colony, granular	Gram positive thin filament and oval shaped cells
	IS-509	Yellow, circular	Gram positive rods
	IS-510	Yellow, circular	Gram positive curved rods
	IS-512	Dark yellow, irregular	Gram positive short rods
	IS-513	Orange, circular	Gram positive coccus
	IS-513(B)	Pink aerial, dark pink orange substrate, powdery	Gram positive coccus
	IS-514	Orange yellow, circular	Gram positive large rods, short filaments
	IS-515	Yellow, irregular	Gram positive oval shaped cells
	IS-516	Pink-greyish white aerial, cream substrate	Gram positive mycelia
	IS-517	Greyish pink aerial, pinkish cream substrate	Gram positive mycelia
	IS-518	Cream aerial and substrate	Gram positive mycelia
	IS-519	Pink aerial, pink orange substrate, leathery	Gram positive mycelia
	IS-520	Grey aerial, pink substrate	Gram positive mycelia
GYE (Fresh root)	IS-203	Cream, irregular	Gram-positive short filaments
NA (Fresh root)	IS-300	Cream, opaque, circular	Gram positive rods
	IS-301	Cream, translucent, circular	Gram positive rods and coccus
NA (Fresh shoot)	IS-700	White, translucent, irregular	Gram positive coccus
PDA (fresh shoot)	IS-800	Orange cream aerial and substrate	Fungi: Oval sporangia with septa

Table 3: Antimicrobial activity, siderophore production and phosphate solubilization by endophytes obtained from *P. niruri*

Plant part	Isolates	Antimicrobial activity Zone of Inhibition (mm)						Siderophore production (cm)	Phosphate solubilization
		B. linens	S. epidermidis	B. subtilis	M. luteus	P. fluorescens	E. coli		
Root	Control	21.6± 0.05	4.30±0.75	19.3±0.20	15.3±0.15	0	0	1.59 ± 0.09	
	IS-003	7.00±0.60	0	6.60±0.57	4.00±0.69	0	0	1.06 ± 0.05	-
	IS-004	13.6±0.11	0	13.3±0.11	0	7.00±0.60	0	1.16 ± 0.05	-
	IS-005	6.30±0.55	5.00±0.86	0	8.00±0.70	4.30±0.75	0	1.20 ± 0.00	-
	IS-203	8.30±0.73	0	0	20.3±0.25	7.60±0.66	0	2.13 ± 0.32	-
	IS-300	3.60±0.63	0	13.0±0.26	3.60±0.63	0	0	2.46 ± 0.30	-
Shoot	IS-501	10.6±0.05	0	3.60±0.63	11.3±0.05	14.0±0.43	0	1.53 ± 0.20	-
	IS-504(1)	10.3±0.89	12.0±0.10	7.00±0.60	11.0±0	11.0±0	0	1.36 ± 0.05	-
	IS-504(2)	3.30±0.57	3.30±0.57	15.0±0.20	0	3.60±0.63	0	1.40 ± 0.10	-
	IS-504(3)	15.0±0.55	0	8.00±0.70	0	3.30±0.57	0	1.33 ± 0.11	-
	IS-505(1)	5.00±0.86	0	8.00±0.69	0	5.30±0.92	0	1.33 ± 0.11	-
	IS-505(2)*	7.00±0.60	10.6±0.94	13.3±0.11	14.0±0.10	3.30±0.57	17.6±0.25	1.50 ± 0.20	+
	IS-509	7.30±0.64	8.30±0.72	0	14.3±0.20	4.00±0.69	0	1.20 ± 0.00	-
	IS-510	8.30±0.76	0	8.60±0.75	0	0	0	1.26 ± 0.15	-
	IS-512	15.6±0.32	4.30±0.75	17.6±0.20	0	0	0	1.06 ± 0.05	-
	IS-513	6.30±0.56	0	12.6±0.05	3.60±0.63	0	0	1.10 ± 0.17	-
	IS-513(B)*	14.3±0.11	12.3±1.06	15.0±0.17	14.6±0.15	17.3±0.15	17.6±0.11	1.13 ± 0.05	-
	IS-515	8.60±0.75	0	14.3±0.40	0	0	0	2.63 ± 0.32	+
	IS-516	8.60±0.77	0	19.0±0.17	0	25.0±0.70	0	1.23 ± 0.05	+
	IS-517*	10.6±0.11	4.30±0.75	12.0±0.17	22.0±0.17	12.0±0.10	15.3±0.15	1.76 ± 0.25	+
	IS-518*	10.3±0.05	12.6±1.10	13.6±0.37	17.3±0.32	7.60±0.66	17.6±0.15	1.50 ± 0.34	+
	IS-519*	10.0±0	17.0±0.17	11.0±0.20	13.6±0.15	10.3±0.05	20.3±0.20	1.93 ± 0.11	+
	IS-520*	17.6±0.05	6.00±1.03	19.0±0.17	11.6±1.02	20.3±0.05	19.0±0.17	1.26 ± 0.11	+
	IS-700	12.3±0.11	0	9.30±0.83	7.00±0.60	0	0	1.40 ± 0.17	-
	IS-800	0	0	0	0	0	0	2.46 ± 0.50	+

*Isolates exhibiting diffusible pigment production; + phosphate solubilization; - no phosphate solubilization

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tolerated acidic pH of 3.0 and 5.0. IS-800 tolerated a wide pH range from 3.0-11.0. All the isolates showed growth at pH 7.0 and 9.0, while 83% grew at pH 11.0 also, indicating their alkaliphilic nature (Figure 1b). With respect to salinity tolerance, IS-003 was most sensitive, showing inability to tolerate 0.1% NaCl. All other isolates could grow at up to 2.5% salinity, while 83% tolerated 5% (Figure 1c). Isolate IS-509 produced all six enzymes. 25% isolates produced five enzymes and 25% four enzymes. IS-515 did not secrete any of the extracellular enzymes. Amylase and cellulase were secreted by majority (79%) of the isolates (Figure 1d). Apart from this, 63% isolates were able to hydrolyse casein, 67% isolates hydrolysed tributyrin, and 38% hydrolysed gelatin and urea. Lactose and raffinose were the preferred carbon sources (Fig. 1e) utilized by 88% organism and ammonium sulphate the preferred nitrogen source (Fig. 1f) used by 96% isolates. Fourteen isolates used all carbon sources. Some of the actinobacterial isolates exhibited production of diffusible pigments (IS-505(2), IS-513(B), IS-517, IS-518, IS-519 and IS-520).

The endophytes were tested for antimicrobial activity, siderophore production and phosphate solubilization. As seen from Table 3, all isolates except IS-800 were able to show antimicrobial activity against at least two target organisms. IS-505(2), IS-513(B), IS-517, IS-518, IS-519, IS-520 showed antimicrobial activity against all the target organisms tested. IS-516 showed the maximum inhibition against *P. fluorescens* with an inhibition zone of 25.0±0.70 cm. Majority of the isolates were active against Gram positive targets, with 60 and 70% (from root and shoot respectively) active against both types of targets. All isolates were positive for siderophore production, with 25% isolates producing larger orange zones than control E. coli. IS-515 showed the maximum zone diameter (2.63±0.32 cm). Phosphate solubilization was exhibited by only 33% of the isolates. IS-300 showed highest antioxidant activity (280 AAE gm FW^{-1}), while other isolates such as IS-005, IS-509, IS-515 and IS-800 also exhibited comparable high antioxidant capacity ranging from 176-245 AAE gm FW^{-1} (Figure 2).

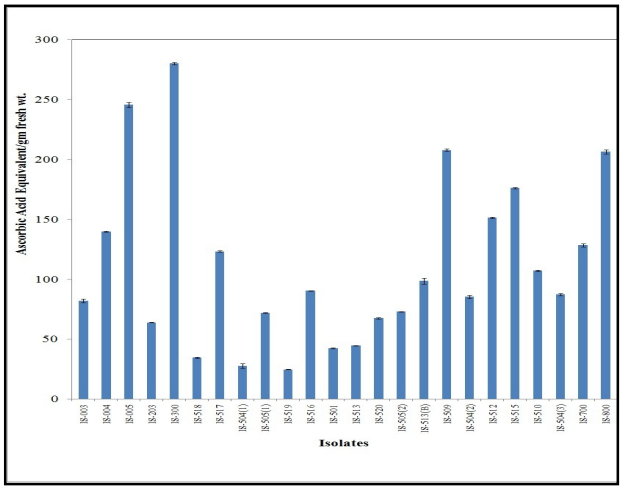

Figure 2: Total antioxidant capacity of endophytic isolates from *Phyllanthus niruri*

Isolates IS-501, IS-516 and IS-520 were identified by 16S rDNA sequencing (Figure 3), and after alignment with type sequences, and rooting with *Streptomyces griseus*, were found to be related to *S. violaceorectus*, with varying similarity percentages (96.81%, 99.71% and 96.93% respectively), but forming a distinct clade. There were nucleotide differences of 4/1364, 41/1285 and 39/1270 respectively. These sequences were deposited in GenBank with the accession numbers KT984382, KR606520 and KT988140 respectively.

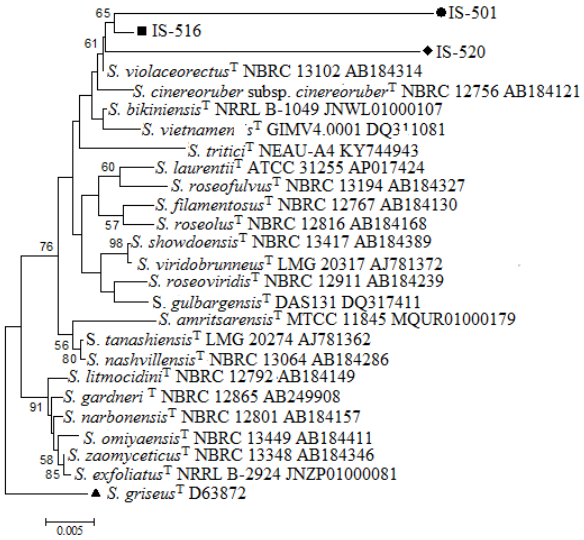

Figure 3: Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing relationships between isolates IS-501, IS-516 and IS-520 with the type strains of *Streptomyces*. Numbers at the nodes indicate levels of bootstrap support based on a neighbour-joining analysis of 1000 resampled datasets (values above 50% are shown). The scale indicates 0.005 substitutions per nucleotide site.

Discussion

The fresh plant extracts of *P. niruri* showed the presence of diverse and distinct endophytes. Only one type of colony was observed in case of dried plant material indicating that other endophytes were unable to survive in desiccated conditions. Out of the total endophytes obtained, 79% were isolated from root material of the plant and 21% were obtained from the shoot part suggesting that the plant harbours more of root associated endophytes. It has been suggested that endophytes typically colonize the root first, finding direct entry from the rhizosphere to the rhizoplane and subsequently enter into the host tissues [24,25]. Nevertheless, diversity of endophytes (maximum number of distinct isolates) was more from shoot extracts of *P. niruri*. It was also seen that most of the endophytes were bacteria and only three were fungi, in contrast to other studies which have reported more of endophytic fungi [12]. The endophytes exhibiting actinobacterial morphology showed wide-ranging bioactivities. Much of the antimicrobial activity exhibited was by the actinobacteria, tallying with other results which have shown them to be prolific producers of antimicrobial compounds [3,26]. This is not surprising considering that this group produces a wide number of diverse secondary metabolites, primarily due to the presence of the modular Non-Ribosomal Peptide Synthetase (NRPS) and Polyketide Synthase (PKS) biosynthetic pathways [27].

This actinobacterial group also showed maximum phosphate solubilisation activity, indicating their role in plant growth promotion activities. Jog et al. (2014) [28] had shown that endophytic and rhizospheric *Streptomyces*

isolated, from wheat fields exhibited such activity. Siderophore production is also well-documented amongst actinobacteria [29]. Siderophores are compounds with low molecular mass and act as iron chelator peptides. They form Fe^{3+}-siderophore complexes, which facilitate iron uptake from iron-deficient soils [30].

The biochemical and physiological profiles of the endophytes show that they are broadly able to tolerate a wide range of pH and temperatures, as also salinity (some up to 5%). In addition, these organisms also enlarge a number of enzymes, indicating that they provide support to their hosts in effecting breakdown of important carbohydrate, protein and lipid substrates [31]. They could be playing a bio-control role in assisting their hosts. These organisms also utilize a wide range of carbon and nitrogen substrates, exhibiting diversity in physiological profile. Production of diffusible pigments by IS-505(2), IS-513(B), IS-517, IS-518, IS-519 and IS-520 indicate potential production of bioactive compounds/contribution to antimicrobial activity [32].

Free oxygen radicals-induced oxidative stress is widely being assumed to be the causative factor for induction of many conditions such as cancer and ulcer. Many botanicals have been shown to have anti-oxidative properties [33]. Methanolic extracts of five *Phyllanthus* sp. have been earlier shown to possess antioxidant activities [34]. In this study, we show that the endophytes from *P. niruri* also exhibit antioxidant activities. It is quite probable that they contribute in a significant measure to their host capabilities.

While IS-516 showed relatively high similarity match percentage (99.71%) with *S. violaceorectus*, the correspondingly low similarity match percentages of IS-501 and IS-520 (96.81 and 96.93% respectively) with *S. violaceorectus* indicate that they need to be ascribed to new genera and species. Moreover, the colony characteristics of *S. violaceorectus* (grey to red aerial mycelium and greyish yellow to brown substrate mycelium) differ from those of the aerial and reverse substrate colour characteristics for IS-501 (white/pink), IS-516 (pink-grey/cream) and IS-520 (grey/pink) [35].

Considering the wide-ranging therapeutic properties associated with *P. niruri* in traditional medicine, it can be speculated that the activities are associated, at least to some extent, with their resident endophytes [36]. This study has clearly enunciated that considerable cultivable microorganisms exist as endophytes within *P. niruri*. Preliminary antimicrobial activity against clinical isolates suggests that they hold much promise. The identification of the exact compounds produced by these endophytes can comprehensively establish that the medicinal properties of the host plant are inherently due to their resident endophytes. This can further facilitate large-scale industrial production of the compounds, since microorganisms are more amenable to scale-up.

Conclusions

The diversity of endophytes obtained and their distinct properties (antimicrobial, siderophore, enzyme secretion) suggest that these can be of use for industrial applications. IS-501, IS-516 and IS-520 are potentially novel taxa (at genus or species levels), which further suggests novel chemodiversity.

Conflict of Interest

Authors declare no conflict of interest.

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