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Research Article

Investigation of Biochemical Properties and Biological Activities of Bacteria Endogenous to Carnivorous Plant *Drosera burmannii* Vahl.

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ABSTRACT:

The endosphere of the carnivorous plant *Drosera burmannii* Vahl., represents a novel niche for studying the microbial diversity. This study is aimed to investigate the diversity and biochemical potentials of bacteria endogenous to *D. burmannii* Vahl. tissues following a culture-dependent approach. A total of 12 phenotypically distinguishable bacteria endophytic to leaf, stem, root and flower were isolated and tentatively assigned to *Bacillus*, *Paenibacillus*, *Lactobacillus*, *Pseudomonas*, *Acetobacter*, *Alcaligenes* and *Kurthia* based on their morphological and physio-biochemical properties. While the colonization frequency and isolation rates were highest in the root, Shanon-Weaver diversity index was maximum in leaf segments. The biochemical potentials of these isolates along with their plant growth promoting properties, antimicrobial, and antioxidant activities as well as heavy metal tolerance have been evaluated. While isolates *Alcaligenes* DF 01 and *Kurthia* DL 08 produced significant amount of IAA, only *Acetobacter* DR 01 and *Pseudomonas* DS 01 could solubilise phosphate. Many of these isolates were able to produce iron-chelating siderophore and grow in nitrogen-free medium. The leaf isolate, *Pseudomonas* DL 06 showed a wide spectrum of antimicrobial activity but the highest DPPH scavenging activity (65%) was recorded in *Alcaligenes* DF 01. The metabolic potentials of these endophytes, therefore, deserve special attention in understanding their role in growth, development and environmental sustenance of *D. burmannii.*

Keywords: *Carnivorous plant, bacterial endophytes, Drosera burmannii, plant growth promotion, antimicrobial activity, antioxidants.*

[I] INTRODUCTION

Drosera burmannii Vahl., commonly known as 'tropical sundew' is a small, delicate herb, which inhabit infertile soil or marshy places and has spathulate leaves produced in a rosette. The ventral surface of the leaves contain characteristic tentacles, which secrete sticky fluid and help to capture, digest and absorb nutrients from insect prey. These animal-derived nutrients are believed to allow the carnivorous plants to grow successfully in the nutrient-deficient

environments [11]. Moreover, sustainable growth of *Drosera* spp. has also been demonstrated by root mineral nutrition stimulated by leaf nutrient absorption from insect prey [1].

Endophytes in general have been found to produce novel metabolites with diverse chemical nature [38] and exhibit antimicrobial, antioxidant, immunosuppressant, anticancer and plant growth promoting activities [6, 40]. Plant growth promoting traits of these microorganisms include production of indole acetic acid, solubilisation of insoluble phosphate [25] and production of iron-chelating compound, siderophore [23]. In addition, endophytes also promote endurance of host plant against pathogenic attack and tolerance to abiotic stress.

Though the carnivorous plants are generally regarded as non-mycorrhizal, there are several reports on the colonization of *Drosera* roots by mycorrhizal and dark-septate fungi [10, 13, 39]. In addition, fungal endophytes have been isolated from the roots of *D. spathulata* [9] and *D. rotundifolia* [31]. It is becoming apparent that fungal endophytes are ubiquitous and abundant [33] and may be responsible in conferring abiotic stress tolerance, facilitate nutrient acquisition and nutrient signaling [4, 30]. Contrary to the wide occurrence of fungal endophytes, only a few endophytic bacteria, including the diazotrophic species have been isolated from the surface-sterilized roots and leaves of *D. villosa* var. *villosa* [2] growing in oligotrophic habitats in Brazil. These nitrogen-fixing bacteria belonged to genera *Bacillus*, *Burkholderia*, *Paenibacillus*, *Pseudomonas* and *Sphingomonas*. Furthermore, it has been suggested that the diversity of the bacteria associated with *D. villosa* var. *villosa* may be related to some of the strategies that these plant uses to survive in locations of very poor nutrient availability. However, reports regarding the exploitation of endophytes of carnivorous plants for production of bioactive compounds are inadequate.

The present work was undertaken keeping in view the fact that the carnivorous plants represent a unique niche for endophytes. As such, we have attempted to isolate and characterize the culturable endophytic bacterial diversity of the carnivorous plant *Drosera burmannii* Vahl. and assess their plant growth promoting, antioxidant and antimicrobial properties.

[II] MATERIALS AND METHODS

2.1. Collection of plants

Drosera burmannii Vahl. (Droseraceae) was collected from the district of Midnapore, West Bengal, India during 2013-2014. Samples collected in zip-lock polythene bags were brought immediately to the laboratory, stored at 4°C until used for the isolation of bacterial endophytes.

2.2 Isolation and characterization of endophytic bacteria

Endophytic bacteria were isolated from leaf, stem, root, and flower of *D. burmannii* following surface sterilization as per the modified methods of Panchal and Ingle [26]. The collected plant parts were first washed thoroughly with tap water followed by surface sterilization in 0.05% sodium hypochlorite, washed thoroughly in sterile distilled water, aseptically cut into 2 mm segments and placed on Petri plates containing Nutrient agar, Tryptic Soy agar and Lindenbein Synthetic agar. Plates were incubated at 32°C for 2-4 days and observed for growth of bacterial colonies around the plant segments. Colonization frequency of the bacterial endophytes was calculated as the total number of plant segments yielding the bacteria divided by the total number of segments incubated. Isolation rate was determined as the number of bacterial isolates obtained from the plant samples divided by the total number of samples incubated. The Shanon-Weaver diversity index was calculated as $H= -\Sigma P_i \ln P_i$ where P_i is the species abundance. Pure cultures of bacterial endophytes were developed following dilution-streaking on the same agar media. The bacterial isolates were then

characterized following morphological and physio-biochemical analysis according to standard microbiological methods [14].

2.3 Evaluation of plant growth promoting traits

IAA production: The ability of the endophytes to produce indole-3-acetic acid (IAA) was assessed following Salkowski colorimetric assay [25]. Isolates were grown in tryptophan broth at 32º C for 5 days and the culture filtrate was separated by centrifugation at 10,000xg for 10 min. To 1 ml of the culture filtrate, 3 ml of Salkowski reagent and 2 ml of distilled water was added. After an incubation of 30 min in dark, the tubes were observed for the development of pink colour.

Phosphate solubilisation: The bacterial endophytes were screened for their ability to solubilise insoluble phosphate in Pikovskya's medium [28]. The plates were incubated at 32º C for 5-7 days and observed for development of clear zone surrounding the bacterial growth.

Growth in N_2-free medium: Cells from overnight grown bacterial endophytes were washed thoroughly in sterile water and inoculated in Norris nitrogen-free medium. The ability of the isolates to fix atmospheric nitrogen was indicated by their growth in N_2-free medium.

Siderophore production: Production of siderophore by the endophytic bacterial isolates was tested following the modified protocol of Schwyn and Neilands [34]. Isolates were grown in succinate agar medium at 32º C for 96 h. Fe-CAS indicator solution was then poured on the plates and observed for the change of colour from blue to orange around the bacterial growth.

2.4 Antimicrobial activity

Antimicrobial activity of the endophytic bacterial isolates was determined following the cross-streak method using *Bacillus subtilis*, *Staphylococcus aureus, Escherichia coli* and *Pseudomonas cepacia* as test bacterial strains, while the test fungi include *Penicillium citrinum*, *Aspergillus niger* and *Saccharomyces cerevisiae*. Tryptic soy agar plates were inoculated with bacterial endophytes as a single streak across the centre of the Petri plate and incubated at 32˚C for 72 h. Freshly prepared inoculum of test strains were streaked at right angles to the producer endophyte and incubated for 24-48 h at 32˚C. The length of inhibition zone of the test organisms was measured in nearest millimiter.

2.5 DPPH scavenging activity

DPPH scavenging activity of bacterial endophytes was determined following the modified method of Liu et al. [21]. Freshly prepared methanolic extract of bacterial biomass (0.5 ml) was mixed with 0.2 ml of 0.4 mM DPPH and 2.5 ml distilled water. The reaction mixture was incubated for 30 min and optical density was measured at 517 nm. The percentage of scavenging of free radical was calculated as:

% scavenging activity = $\{1- (A_1-A_2/A_0)\}$ X 100

Where, A_1 = O.D. of reaction mixture

A_2 = O.D. of reaction mixture without DPPH

A_0 = O.D. of reaction mixture with DPPH but without sample

2.6 Stress tolerance

Heavy metal tolerance: Heavy metal tolerance of bacterial endophytes was tested on nutrient agar plates supplemented with heavy metals nickel, cobalt, chromium, mercury and cadmium. While Ni, Co, Cd and Hg were used as chloride salts, Cr was used as $K_2Cr_2O_7$.

Antibiotic sensitivity: Antibiotic sensitivity of the isolates was determined following the Kirby Bauer disc-diffusion method [5] using antibiotic impregnated discs (HiMedia 6 mm dia.). Antibiotics used include penicillin G (10 units), methicillin (5 µg), bacitracin (10 units), vancomycin (30 µg), erythromycin (15 µg), kanamycin (30 µg), streptomycin (10 µg), gentamycin (10 µg), chloramphenicol (30 µg) and chlortetracycline (30 µg). Based on the diameter of the inhibition zone, the organisms were categorized as resistant, intermediate and sensitive following Himedia manual.

[III] RESULTS

3.1. Diversity of endophytic bacteria

Segments of surface sterilized leaf, stem, root and flower of *D. burmannii* Vahl. incubated on nutrient agar, tryptic soy agar and Lindenbein synthetic agar media showed growth of morphologically distinguishable bacterial colonies surrounding the plant segments after 48-96 h of incubation at 32°C. Based on the differences in colony morphology, a total of 12 bacterial endophytes were isolated in pure form from 84 segments. Colonization frequency was recorded to be low in stem (28.5%) and flower (12.5%) samples as compared to root (50%) and leaf (49.2%), while the isolation rates were 0.12, 0.29, 0.5 and 0.13 for leaf, stem, root and flower respectively. The Shanon-Weaver diversity index showed that leaves harbour most diverse types of endophytic bacteria compared to stem, root and flowers [Table-1].

Parameters	Plant tissue				Total
	Leaf	Stem	Root	Flower	
Number of samples used	65	7	4	8	84
Samples yielding endophytic isolates	32	2	2	1	37
Number of endophytic isolates	8	2	1	1	12
Colonizing frequency (%)	49.2	28.5	50.0	12.5	44
Isolation rate	0.12	0.29	0.5	0.13	0.14
Shannon Weaver diversity index	1.63	0.69	0	0	2.33

Table 1: Diversity of culturable endophytic bacteria in leaf, stem, root, and flower tissues of *D. burmannii* Vahl.

3.2. Characterization and identity of the isolates

The bacterial endophytes of *D. burmannii* were characterized based on morphological and physio-biochemical properties [Table-2]. Out of the 12 rod-shaped isolates, majority were motile, Gram-positive and only one (DS 01) produced light yellow pigment. Moreover, the isolates also showed a wide variation of tolerance to temperature, pH, and NaCl [Table-2]. Enzyme profile of the isolates indicated that all, but one produced catalase and was followed by predominance of proteolytic activities (83 and 75% of isolates showed production of caseinase and gelatinase respectively). Almost equal number of isolates (66.6%) produced amylase and pectinase, while production of lipase (33.3%), nitrate reductase (41.6%) and cellulase (25%) were not uncommon [Figure-1]. The isolates were also tested for their ability to utilize and ferment a number of sugars in phenol red agar medium. Glucose and fructose were best utilized by most of the isolates [Figure-2].

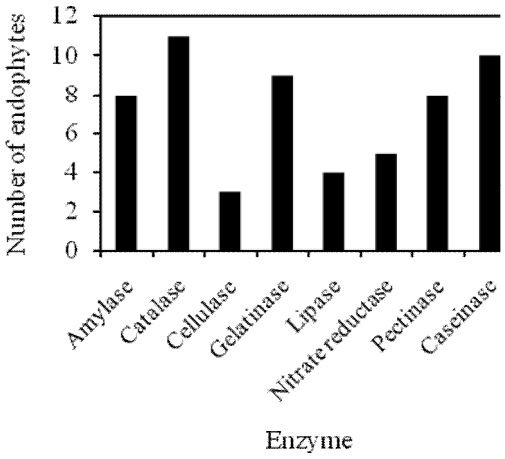

Fig: 1. Production of hydrolytic enzymes by bacterial endophytes isolated from *D. burmannii* Vahl.

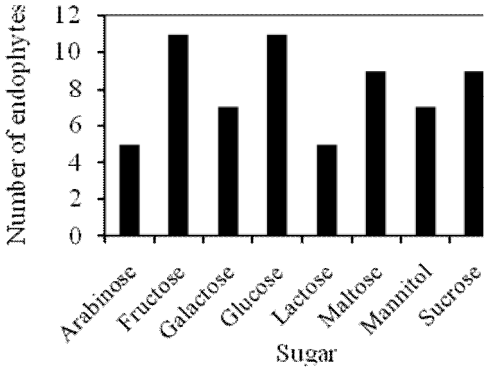

Fig: 2. Sugar fermentation capacity of bacterial endophytes isolated from *D. burmannii* Vahl.

Parameters	Endophytic bacterial isolate											
	DR 01	**DF 01**	**DL 01**	**DL 02**	**DL 03**	**DL 04**	**DL 05**	**DL 06**	**DL 07**	**DL 08**	**DS 01**	**DS 02**
Colony morphology	Small, white, mucoid	Small, spreading	White, smooth	Cream, smooth irregular	White, smooth	Smooth, raised, Light yellow	Light yellow smooth	Creamish, wrinkled, dry, raised	Creamish, smooth margin	Light yellow thick smooth	Wrinkled white, dry surface	Thick, yellowish, smooth
Shape and size (µm)	Rods, 1.2-1.6x0.6-0.8	Rods, 3.5-3.7x0.5-0.7	Rods, 1.8-2.0x0.5-1.0	Rods, 3.7-4x1.2-1.4	Rods, 2.5-3x1.6-1.8	Rods, 2.4-3x1.2-1.6	Rods, 2.5-3x1.2-1.6	Rods, 2.5-3x1.2-1.6	Rods in chain, 1-2x0.3-0.5	Rods, 3-4x2-3	Rods, 0.5-1x0.3-0.6	Rods, 2-3x1.2-1.7
Gram nature	-ve	-ve	+ve	+ve	+ve	+ve	+ve	-ve	+ve	+ve	-ve	+ve
Motility	-	+	+	+	+	+	+	+	-	+	+	+
Endospore	-	-	+	+	+	+	+	-	-	-	-	+
Diffusible pigment	-	-	-	-	-	-	-	-	-	-	light yellow	-
Temp. tolerance (ºC)	32-40	32-40	32-40	32-40	32-37	32-40	32-40	30-32	32-40	32-40	32-40	32-40
NaCl tolerance (%)	0-10	0-3	0-1	0-7	0-13	0-13	0-3	0-3	0-3	0-7	0-10	0-10
pH tolerance	5-10	7-8	5-10	5-8	5-7	6-8	6-9	6.5-7	6-9	5-10	6-10	6-10
Citrate utilisation	-	-	-	-	-	+	-	-	-	-	-	-
Growth in McConkey agar	±	-	-	-	-	±	-	-	+	-	+	-
Tentative generic identity	*Acetobacter*	*Alcaligenes*	*Bacillus*	*Paeni-bacillus*	*Bacillus*	*Paeni-bacillus*	*Bacillus*	*Pseudo--monas*	*Lacto--bacillus*	*Kurthia*	*Pseudo--monas*	*Bacillus*

+ = Positive response; - = Negative response; ± = Weakly positive response

Table 2: Morphological and physio-biochemical characteristics of the endophytic bacteria isolated from *D. burmannii* Vahl.

The morphological, physiological and biochemical characteristics [Table-2] of the endophytic isolates were compared with the standard descriptions in Bergey's Manual of Determinative Bacteriology [7] and the isolates were tentatively identified as members of the genera *Bacillus* (DL 01, DL 03, DL 05 and DS 02), *Paenibacillus* (DL 02 and DL 04), *Lactobacillus* (DL 07), *Pseudomonas* (DL 06 and DS 01), *Acetobacter* (DR 01), *Alcaligenes* (DF 01) and *Kurthia* (DL 08) [Table-2].

3.3 Evaluation of plant growth promoting traits

IAA production: When grown in tryptophan broth, the endophytic bacterial isolates showed the production of indole acetic acid (IAA) as revealed by the development of pink colour on treatment with Salkowski's reagent [Table-3]. Out of the 12 isolates, *Alcaligenes* DF 01 and *Kurthia* DL 08 were good IAA producers and were followed by isolates *Bacillus* DS 02 and DL 01 producing moderate amount of IAA. However, isolates DR 01, DL 06 and DS 01 have failed to produce IAA under the experimental conditions.

Phosphate solubilisation: The ability of the endophytic bacterial isolates to solubilise insoluble phosphate (as calcium phosphate), was considered as an important plant growth promoting trait. Phosphate solubilisation by the isolates was evident from the formation of clear zone surrounding the growth of the isolate on Pikovskya's medium [Table-3]. Only two isolates, *Acetobacter* DR 01 and *Pseudomonas* DS 01 could solubilise phosphate during the period of incubation.

Growth in nitrogen-free medium: Out of the 12 isolates inoculated in Norris nitrogen-free glucose medium, only four of them (*Acetobacter* DR 01, *Pseudomonas* DS 01, *Paenibacillus* DL 02, and *Bacillus* DL 03) could grow in absence of nitrogen in the medium [Table-3].

Siderophore production: Qualitative siderophore assay showed that the isolates *Acetobacter* DR 01, *Pseudomonas* DS 01 and DL 06, *Bacillus* DS 02, and *Lactobacillus* DL 07 could produce siderophore. This was evident because the blue colour of the Fe-CAS indicator surrounding the colonies turned orange due to production of

Bacterial isolate	IAA production	Phosphate solubilisation	Growth in N_2-free medium	Siderophore production
Acetobacter DR 01	-	+	+	++
Alcaligenes DF 01	+++	-	-	-
Bacillus DL 01	++	-	-	-
Paenibacillus DL 02	+	-	+	-
Bacillus DL 03	+	-	+	-
Paenibacillus DL 04	+	-	-	-
Bacillus DL 05	+	-	-	-
Pseudomonas DL 06	-	-	-	+
Lactobacillus DL 07	+	-	-	+
Kurthia DL 08	+++	-	-	-
Pseudomonas DS 01	-	+	+	+
Bacillus DS 02	++	-	-	+

+ = Positive response; - = Negative response

Table 3: Plant growth promoting traits of endophytic bacterial isolates of *D. burmanni* Vahl.

siderophore. However, more than 50% of the isolates appeared to be siderophore negative [Table-3] under the tested condition.

3.4 Evaluation of antimicrobial activity

Antimicrobial activity of the bacterial endophytes was assessed against the test organisms *B. subtilis, S. aureus, E. coli, P. cepacia, A. niger, P. citrinum* and *S. cerevisiae* following the cross-streak method [Table-4]. Out of the 12 endophytes screened, majority showed inhibitory activity towards *S. aureus* followed by *E. coli* and *B. subtilis*, while none of the endophytes were inhibitory to *P. cepacia* (data not shown). Isolates like *Bacillus* DS 02 and DL 01, *Paenibacillus* DL 04 and *Lactobacillus* DL 07, however, have failed to show any antibacterial activity. A few isolates (*Acetobacter* DR 01, *Pseudomonas* DL 06 and DS 01) were inhibitory only to *A. niger* and *S. cerevisiae*.

Bacterial isolate	Length of inhibition (mm)					
	Bacteria			Fungi		
	B. subtilis	*S. aureus*	*E. coli*	*P. citrinum*	*A. niger*	*S. cerevisiae*
Acetobacter DR 01	NI	16.5	6.5	NI	8.5	8.5
Alcaligenes DF 01	11.5	12.5	12.0	NI	NI	NI
Paenibacillus DL 02	10.0	12.0	10	NI	10	NI
Bacillus DL 03	12	13.5	12	NI	NI	NI
Pseudomonas DL 06	18	26	10	NI	18.5	12
Lactobacillus DL 07	NI	NI	NI	NI	15	NI
Kurthia DL 08	NI	5	NI	NI	NI	NI
Pseudomonas DS 01	NI	14	NI	7.0	7.5	7

NI= no inhibition

Table 4: Evaluation of antimicrobial activity of bacterial endophytes of *D. burmannii* Vahl.

3.5 DPPH scavenging activity

DPPH scavenging activity clearly indicates that the endophytic microorganisms have different degrees of (45-65%) scavenging activity. Among the tested isolates, *Alcaligenes* DF 01 showed the

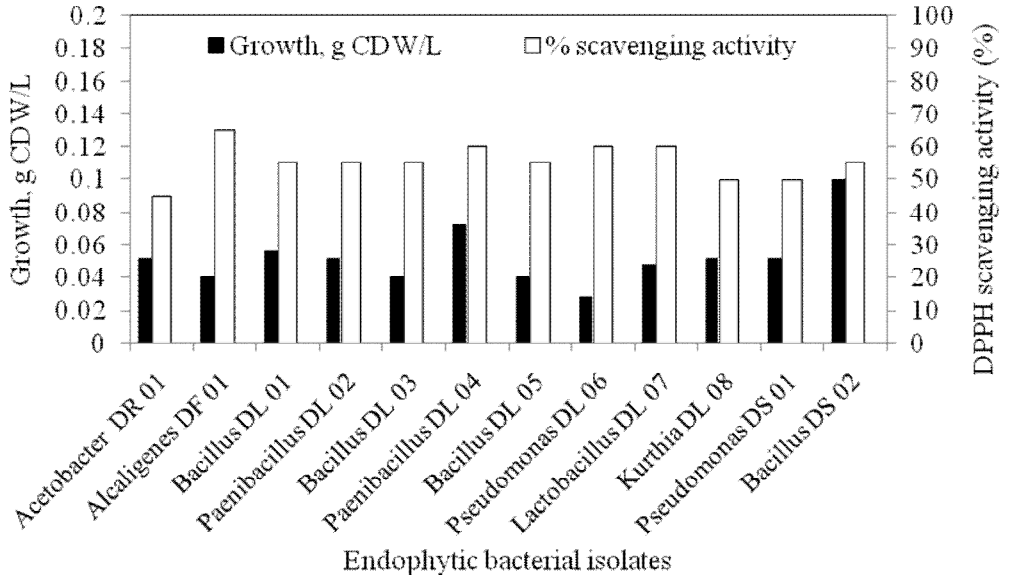

Fig: 3. Growth associated DPPH scavenging activity of the endophytic bacteria isolated from *D. burmannii* Vahl.

maximum DPPH scavenging activity (65%), while it was least (45%) in *Acetobacter* DR 01 [Figure-3].

3.6 Stress tolerance

Heavy metal tolerance: Minimum inhibitory concentration (MIC) of heavy metals like Ni, Co, Cr, Hg and Cd of the endophytic isolates as determined by solid plate assay [Table-5] have clearly indicated that the isolates were highly sensitive to Hg (0.001-0.01 mM) followed by Cd (0.02-0.1mM), and Co (0.5-2.7 mM), but were comparatively tolerant to Ni (0.1-2.8 mM) and Cr (0.1-4.0 mM).

Antibiotic sensitivity: Antibiotic susceptibility pattern of endophytic bacteria as tested against ten different antibiotics showed that the isolates were resistant to penicillin G, bacitracin and chloramphenicol, but were sensitive to streptomycin and gentamycin [Figure-4].

Isolate no.	Minimum inhibitory concentration (MIC), mM				
	Ni(II)	Co(II)	Cr(VI)	Hg(II)	Cd(II)
Acetobacter DR 01	1.0	1.0	1.0	0.005	0.05
Alcaligenes DF 01	1.0	0.5	1.2	0.005	0.1
Bacillus DL 01	2.0	1.0	2.6	0.002	0.05
Paenibacillus DL 02	2.5	1.0	2.8	0.005	0.05
Bacillus DL 03	2.8	1.0	3.4	0.002	0.06
Paenibacillus DL 04	2.0	1.0	4.0	0.01	0.04
Bacillus DL 05	2.2	1.0	2.6	0.001	0.05
Pseudomonas DL 06	1.0	2.3	0.1	0.002	0.02
Lactobacillus DL 07	2.5	1.0	2.0	0.001	1.0
Kurthia DL 08	2.5	2.7	2.0	0.005	0.05
Pseudomonas DS 01	2.6	2.6	1.0	0.005	0.07
Bacillus DS 02	2.5	2.6	3.0	0.002	0.06

Table 5: Heavy metal tolerance profile of endophytic bacteria isolated from *D. burmannii* Vahl.

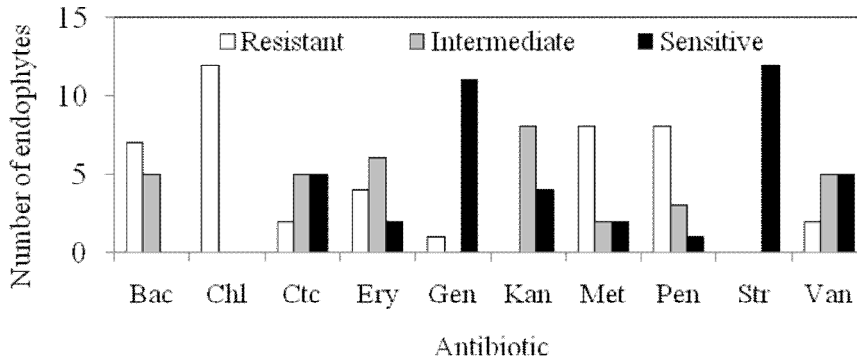

Fig: 4. Antibiotic sensitivity profile of bacterial endophytes isolated from *D. burmannii* Vahl. (Antibiotics: Bac = Bacitracin; Chl = Chloramphenicol; Ctc = Chlortetracycline; Ery = Erythromycin; Gen = Gentamycin; Kan = Kanamycin; Met = Methicillin; Pen = Penicillin G; Str = Streptomycin; Van = Vancomycin)

[IV] DISCUSSION

The diversity of endophytic fungi in a number of carnivorous plants [32, 15] including those of *Drosera* [31] have been investigated in the recent past. Similarly, the culturable diversity of bacteria associated with *Sarracenia* leaf [16, 27, 17] as well as pitcher fluid of *Nepenthes* spp. [35] and *Sarracenia minor* [36] have been explored.

The present culture-dependent study revealed that the internal tissues of *D. burmannii* Vahl. harbour a number of phenotypically distinguishable bacteria [Table-1]. Most of them were motile, rod-shaped, Gram-positive and tolerant to a temperature of 32º- 40ºC, pH 5-10 and 3-10 % NaCl in the growth medium [Table-2].

Though several reports have documented the production of hydrolytic enzymes by the endophytic fungi and bacteria [8, 24, 3], but production of such enzymes by endophytes of carnivorous plants are scanty. Bacterial endophytes of *D. burmannii*, however, have been found to produce hydrolytic enzymes such as protease, pectinase, lipase, and amylase [Figure-1]. The predominance of protease producers along with production of other hydrolytic enzymes by these endophytes might contribute to the digestion of insect prey by the host plant [15]. The isolates could also ferment various sugars, predominantly glucose and fructose [Figure-2]. The endophytic isolates were tentatively identified as members of *Acetobacter* (DR 01), *Alcaligenes* (DF 01), *Bacillus* (DL 01, DL 03, DL 05, DS 02), *Paenibacillus* (DL 02, DL 04), *Pseudomonas* (DL 06, DS 01), *Lactobacillus* (DL 07) and *Kurthia* (DL 08), based on their morphological and physio-biochemical characters, however an in depth studies are essentially needed to ascertain their species identity.

The endophytes are known to promote plant growth through production of IAA, iron-chelating siderophore, phosphate solubilisation and nitrogen fixation [40] and these traits are not uncommon to the endophytic bacteria of *D. burmannii* [Table-3]. As far as we are aware, these traits appear to be new records from endophytes of *D. burmannii*. The nitrogen fixing ability of the endophytic isolates *Acetobacter* DR 01, *Bacillus* DL 03, *Paenibacillus* DL 02 and *Pseudomonas* DS 01 as evident from the growth in Norris N_2-free medium [Table-3] are in conformity with the findings of the diversity of diazotrophic bacteria in *Drosera villosa* var *villosa* [2]. These observations are indicative of the fact that the plant growth promoting attributes of endophytic bacteria of *D. burmannii* could confer their host plant an ability to grow in nutrient-scarce environment.

In view of the ever increasing demand for novel antimicrobials, particularly against drug resistant pathogens, the endophytic bacteria could serve as sources of potent antimicrobials [19, 29].While the antimicrobial activity of *Drosera* spp. [12] along with in situ stimulation of antibacterial compound production [18] have been reported, the endophytic population of *Drosera* has not been explored for antimicrobial activity. Here in this study we report the broad-spectrum antimicrobial activity of endophytes from *D. burmannii* affecting both Gram-positive and Gram-negative bacteria as well as fungi [Table-4].

Several recent reports have established that the endophytic metabolites could be potent sources of novel natural antioxidants [22, 37]. The DPPH assay in the present study confirmed maximum free radical scavenging activity (65%) by the isolate *Alcaligenes* DF 01 along with few others [Figure-3]. These isolates, therefore, could be probable sources of natural antioxidants, which, however, need further investigation.

Endophytic bacteria have been shown to tolerate heavy metals [20], which might help them to survive and grow in the internal tissues of plants growing in soil contaminated with heavy metals. Most of the endophytic isolates of *D. burmannii* have acquired resistance to metals like Ni. Co, Cr, Cd [Table-5], but were sensitive to Hg. Moreover, the isolates were observed to be resistant towards antibiotics interfering bacterial cell wall and protein synthesis [Figure-4]. These findings might indicate not only the exposure of host plants to heavy metal contaminated soil, but also the contribution of the endophytes to host survivality in harsh conditions. However, antibiotic resistance development in endophytes

could possibly be explained by the inheritance of such traits by horizontal transfer of genes from the soil microbiota.

[V] CONCLUSION

The results are suggestive of the fact that the endophytic bacteria provide the plants competitive advantage in surviving under nutrient poor and abiotically stressful environments. Further, the predominance of production of proteolytic enzymes by the endophytes of *D. burmannii* indicates their possible involvement in the process of digestion of insect prey by the host plant.

FINANCIAL DISCLOSURE

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