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ENDOPHYTIC FUNGAL DIVERSITY FROM FOUR WOODY LIANAS PLANTS OF CHILKIGARH, WEST MEDINIPUR, W.B.

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Abstract: Four woody lianas- *Bauhinia vahlii*, *Celastruspaniculatus*, *Combretumroxburghii* and *Ventilagodenticulata* were studied for screening of endophytic fungal diversity of these plants. Aerial tissues (leaf, petiole and bark) were assessed for endophytic isolation. A total of 218 tissue segments out of 375 were infested with fungi and 245 endophytic fungi were isolated. Average colonization frequency (CF%) was 58.13% and petioles of the climbers were colonized by a great number of endophytic fungi. CF% is maximum in *Combretum* sp. (73.33%) and in *Bauhinia*sp, 67.2%. A total of 31 fungal genera with few unknown genera and few sterile fungi were isolated. Highest Shannon-Wiener index (2.494) was shown by *Bauhinia* sp. with highest Simpson's diversity (0,8926). It indicates great species specificity. *Celastrus* sp. And *Ventilago* sp. showed the highest similarity coefficient (34.95%). *Cylindrocladium* sp., *Pestalotiopsis* sp., *Aspergillus* sp., *Penicillium* sp., *Verticillium* sp., *Chaetomium* sp. are dominant endophytes.
Keywords- Endophytes, diversity, lianas, fungi, indices.

Introduction

The term endophytes include all the organisms that live symtomlessly within various plant tissues [1]. They are classified into two distinct groups- bacteria and fungi [2]. But the most frequently isolated endophytes are fungi [3]. Huge number of endophytes remain in association with shade and moisture-loving woody climbers in stand forest [4]. Carroll and Carroll (1988) reported that endophytes live asymptomatically and sometimes systematically within the plant tissues.

Endophytic fungi are very important in the biodiversity since they have an effect on structure and defence mechanism of plants and ultimately in the ecosystem [5]. Arnold et al (2000) isolated extremely abundant and very diverse group of endophytic fungi from plant tissues. Endophytic fungi are ubiquitous in distribution found within the tissues of plants, residing intercellular or intracellular, at least for a portion of their life cycle without causing apparent symptoms of diseases.

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Dreyfuss and Chapela (1994) estimated that there may be one million species of endophytic fungi in the world and only a few metabolites have been discovered from very few group of endophytes for pharmaceuticals.

Ganley et al (2004) reported that endophytes are the normal inhabitants of the plant tissues. They protect plants against pests [6] fungal pathogens [7] even enhance the defense mechanism of host plants against grazing animals [8] or increase the tolerance power of host against harsh environment [9].

Recent work has been started to explore and isolate the endophytic fungal symbionts and their diversity in four woody lianas- *Bauhinia vahlii* (Caesalpiniaceae), *Celastruspaniculatus* (Celastraceae), *Combretumroxburghii* (Combretaceae) and *Ventilagodenticulata* (Rhamnaceae). Fungal endophytes of woody lianas represent one class of microbial symbionts that have so far been neglected in diversity studies. These endophytes are estimated to occur in 25% to 60% of plant segments and can play important ecological roles in plant communities [10]. Endophytic fungi can also increase drought resistance [11] and enhance drought resistance [12]. Many symbiotic endophytic fungi have been isolated from three plants of Lamiaceae family [13].

Research Objectives

i) To isolate endophytic fungi from selected lianas plants of West Medinipur district.

ii) To identify the endophytic isolates.

iii) To study the variation of endophyticfungal infection and
colonization frequency in different genera of lianas plants.

iv) Determination of host and organ specificity in fungal endophytes.

Materials and Methods

(i) Study sites and collection of samples

The study was conducted in PaschimMedinipur district of West Bengal, India. (latitude 22°25′ to 22°57′North, longitude 87°11′East, altitude 2meters from the sea level). The climate is tropical, warm and humid with a mean temperature of 33°C and an average annual rainfall of 120cm. Four lianas plants (*Bauhinia* sp., *Celastrus* sp., *Combretum* sp., and *Ventilago* sp.) were selected from Chilkigarh for endophytic fungal screening. All sample types were collected from mature, healthy, disease free plants.

(ii) Sampling procedure

Plant samples (leaves, stems, petioles) were collected randomly from the location during winter. The samples immediately after collection were kept in zipper-lock plastic

bags, brought to the laboratory and stored at 4°C within 2-3 hours of collection until isolation procedure was accomplished.

(iii) Surface disinfection

Samples collected from different localities were thoroughly washed under running tap water before processing and following sequences were followed: leaf, petiole and stem samples were surface sterilized by sequentially dipping into 80% ethanol for 1 min, 1% sodium hypochlorite (NaOcl)(4% available chlorine) for 4 min, 90% ethanol for 20sec. Finally, samples were rinsed with sterile distilled water for 3 times, then allowed to surface dry under sterile condition.

(iv) Placing the samples in media

Sterile leaves were cut into pieces of about 1 square cm size by sterile scizor and placed in plate of water agar (WA), 5 samples in each, equidistant from each other. Similarly 5 sterile petioles of 0.5-1cm long were placed in another WA plate. Stem tissues were cut into short pieces of 4-5 cm long and after sequential sterilization, the outer layer was removed and inner tissues were peeled with sterile scalpel. Thin peels from various depth were placed on another WA plate. Thus, at least 5 replica plates for each sample from the plant were made.

(v) Isolation of endophytic fungi

Fungal growth was observed each and every day. Within 2-3 days fungal hyphae were in appearance. Some samples show more than one hyphal growth. From each sample fungal hypha was isolated and transferred to PDA media by cutting a square block of water agar. The plates were incubated in light chamber at 23°C. After 10-15 days huge mycelial and in some cases reproductive growth was observed. Culture slants were made and preserved for identification at 4°C and also for further work in future.

(vi) Identification of endophytes

The endophytic fungal organisms were studied under optical compound microscope. The fungal isolates were identified based on their morphological and reproductive characters using the standard identification manuals (Barnett and Hunter: Illustrated genera of Imperfect fungi, Gilman: A Manual of soil fungi).

(vii) Data analysis

The relative colonization frequency (CF%) was calculated as the number of sample segments colonized by at least a fungus divided by total number of segments plated x100 using the formula outlined by Hata and Futai: CF = (Ncol/Nt x100, where Ncol = number of segments colonized by at least a fungus, Nt = total number of segments plated. Dominant

endophytes were calculated as percentage of colony frequency divided by sum of percentage of colony frequency of all endophytes x100. Dominant endophyte percentage (D) = Ni/Ns x100, where Ni = percentage of colony frequency of individual endophytes, Ns = percentage of colony frequency of all endophytes. Using PAlaeontologicalSTatistics software package (PAST) [21], following diversity indices were calculated-

(a) Simpson's Diversity Index (1-Dominance) was calculated using the formula 1-D, where D = $\sum n(n-1)/N(N-1)$. Here, n = the total number of organisms of a particular species, N = the total number of organisms of all species.

(b) Shannon-Wiener diversity index was calculated using the following formula: Shannon-Wiener index (H′) = $-\sum s(Pi)(In\ Pi)$, where H′ = Symbol for the diversity in a sample of species or kinds, s = the number of species in the sample, Pi = relative abundance of ith species or kinds and measured by = n/N, N = total number of individuals of all kinds, ni = number of individuals of ith species, In = log to the base 2.

(c) Evenness was calculated using the following formula: Evenness (E) = H′/H′max, where H′max is the maximum value of diversity for the number of species.

Results and Discussion

All the four woody lianas were infested with huge number of endophytic fungi forming an symbiotic association. Altogether 245 fungal endophytes were isolated from 375 segments of leaf, petiole and stem from four lianas. The endophytes belong to 31 genera, few unknown and few sterile mycelia. Previous studies also showed that different number of endophytic fungi were isolated from woody lianas of different locations.

The highest number of fungal endophytes was isolated from *Combretum*sp. (CF=73.33%).*Bauhinia* sp. also nearer to it (CF=67.2%). Most of the endophytic fungi were colonized in petioles (CF=68.75%). In *Combretum* sp. Leaf shows maximum colonization frequency (88%) [1]. It is an evidence for the tissue specificity of endophytes. Previous researchers also observed tissue specificity of endophytes in their studies [13,23].

All the statistical analyses were made using the formulae stated by Raviraja (2005) [23] with the statistical software PAST. Species diversity of endophytes was determined using the Simpson's diversity index, Shannon-Wiener index, Fisher alfa index, Manhinif index etc. Bauhinia showed the highest Simpson's diversity (0.8926) with maximum Shannon-Weiner index (2.494) and highest Fisher alfa index (7.652). All these indices indicate great species specificity of endophytes.

Similarity coefficient was calculated to determine the colonization similarity of fungal endophytes in four different host plants. In all plants similarity coefficient ranges between 4.59% -34.95%. *Celastrus* sp. and *Ventilago* sp. showed the highest similarity coefficient (34.95%). In the present study *Cylindrocladium* sp., *Pestalotiopsis* sp., *Aspergillus* sp., *Penicillium* sp., *Verticillium* sp., *Chaetomium* sp. are the dominant endophytes in all four lianas plants.

Conclusions

There is a diverse groups of endophytes in lianas plants found from my study. Majority has been identified with some unknown genera and some mycelia sterilia. We may draw conclusion that there is a host specificity by endophytes and also they have organ and tissue specificity. The plant of *Bauhinia vahlii* shows maximum number of endophytes and *Ventilago denticulate* has minimum numbers. *Celastruspaniculatus* and *Ventilagodenticulata* show maximum similarity coefficient.

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Tables and Figures

Table 1. Colonization frequency of endophytes from different aerial segments of four woody lianas

Endophytic fungi	*Bauhinia* sp.			*Celastrus* sp.			*Combretum* sp.			*Ventilago* sp.		
	L	P	S	L	P	S	L	P	S	L	P	S
Acrocylindrium sp.	0	0	1	0	0	0	0	0	0	0	0	0
Apophysomyces sp.	0	0	0	0	0	1	0	0	0	0	0	0
Arthrinium sp.	0	0	0	2	2	0	0	0	0	0	0	0
Aspergillus sp.	0	0	4	2	2	1	3	1	3	0	0	0
Bispora sp.	0	1	0	0	0	0	0	0	0	0	0	0
Botryotrichum sp.	0	0	0	0	1	1	0	0	0	0	0	0
Chaetomium sp.	0	0	0	0	0	0	8	0	0	0	0	0
Chrysosporium sp.	0	0	0	0	1	2	0	0	0	0	0	0
Cladosporium sp.	0	0	1	0	0	0	0	0	0	0	0	0
Curvularia sp.	0	0	0	0	0	0	0	0	0	0	2	1
*Cylindrocladium*sp	7	4	0	0	0	0	0	0	0	0	0	0
Dicoccum sp.	0	0	1	0	0	0	0	0	0	0	0	0
Diplodia sp.	0	0	0	0	1	0	0	0	0	0	0	0
Fusarium sp.	0	0	0	0	0	1	0	0	0	4	7	21
Fusidium sp.	0	1	0	0	0	0	0	0	0	0	0	0
Geotrichum sp.	0	0	2	0	0	0	0	0	0	0	0	0

Humicola sp.	0	0	2	0	0	0	0	0	0	0	0	0
Hymenella sp.	1	0	0	0	0	0	0	0	0	0	0	0
Lasiodiplodia sp.	0	0	0	0	0	2	0	0	0	1	0	0
Mucor sp.	0	1	3	0	0	4	0	2	0	0	0	0
Murogenella sp.	0	0	0	0	0	0	0	0	0	0	0	1
Nigrospora sp.	0	0	0	0	3	1	0	0	0	0	0	0
Papulospora sp.	2	3	1	0	0	0	0	0	0	0	0	0
Penicillium sp.	0	0	10	1	0	0	0	1	0	0	0	0
Perisporium sp.	0	1	0	0	0	0	0	0	0	0	0	0
Pestalotiopsis sp.	2	2	10	0	0	0	0	0	0	0	0	0
Philophora sp.	0	0	1	0	0	0	0	0	0	0	0	0
Podospora sp.	0	0	0	0	0	0	4	0	0	0	0	0
Scopulariopsis sp.	0	0	0	0	0	0	0	2	0	0	0	0
Sterile mycelia	2	1	1	0	0	5	5	4	0	0	0	0
Torula sp.	1	1	2	0	0	0	0	0	0	0	0	1
Unidentified	4	6	5	5	2	7	5	14	1	0	0	0
Verticillium sp.	0	0	0	0	0	0	0	0	6	0	1	0

Table 2. Diversity indices, evenness and species richness of endophytic fungi isolated from the lianas

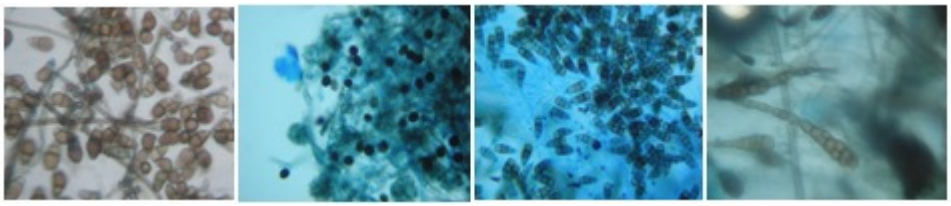
parameter	*Bauhinia* sp.	*Celastrus*sp.	*Combretum* sp.	*Ventilago* sp.
Taxa_S	19	13	9	6
Individuals	84	47	59	39
Dominance_D	0.1074	0.1426	0.1882	0.6818
Simpson_1-D	0.8926	0.8574	0.8118	0.3182
Shannon_H	2.494	2.239	1.891	0.7354
Evenness_e^H/S	0.6375	0.7215	0.7361	0.3477
Brillouin	2.202	1.901	1.68	0.597
Menhinick	2.073	1.896	1.172	0.9608
Margalef	4.062	3.117	1.962	1.365

Equitability_J	0.8471	0.8727	0.8606	0.4104
Fisher_alpha	7.652	5.945	2.959	1.98
Berger-Parker	0.1786	0.2979	0.339	0.8205
Chao-1	28.33	15	9	12

Table 3. Similarity coefficient of four woody lianas

	Bauhinia sp.	*Celastrus* sp.	*Combretum* sp.	*Ventilago* sp.
Bauhinia sp.	100%	23.14%	22.76%	4.59%
Celastrus sp.		100%	25.64%	34.95%
Combretum sp.			100%	9.21%
Ventilago sp.				100%

Figures: Few compound light microscopic pictures of endophytic fungal genera isolated by us.



i) *Curvularia* sp. ii) *Nigrospora* sp. iii) *Pestalotiopsissp*. iv) *Alternaria* sp.