Current Botany 2023, 14: 24-31
doi: 10.25081/cb.2023.v14.7210
https://updatepublishing.com/journal/index.php/cb

Research Article

ISSN: 2220-4822

Diversity of fungal endophytes at different maturity levels of *Cryptolepis buchanani* leaves

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ABSTRACT

A study on endophytic microflora diversity at each level and portion of the leaves of *Cryptolepis buchanani*, a medicinal plant used by tribals of Madhya Pradesh, India was carried out in the present investigation. As many as 383 isolates from 360 discs of leaves belonging to 17 fungal species and 9 isolates, which did not show any sporulation (sterile), were recovered. Among the 17 identified species, hyphomycetes is the dominant class and *Aspergillus, Colletotrichum and Khuskia* are the dominant genera. Colonization frequency (CF) was higher in mature leaves (78.3%) and comparatively lower in younger leaves (51.1%) and there was a marginal decrease in CF from the base of the leaf (66.7%) towards the leaf apex (62%). Statistical analysis revealed that level of the leaf had a significant effect on CF and diversity of fungal endophytes, while as leaf sub-parts had little influence. Biochemical characterization of the endophyte revealed the production of various enzymes viz. protease, amylase, lipase, cellulase, xylanase and pectinase. These fungal enzymes can be tapped for food, pharma, beverages, textiles, confectionaries, and leather industries. These bioactive natural products are easy to process as they are usually more stable than products obtained from other sources. The enzymatic activities also help to get a better insight into the host-endophyte relationship. However, the world of fungal endophytes needs to be researched extensively for production of plant based novel eco-friendly biomolecules in cost-effective manner.

Received: July 13, 2021
Revised: February 13, 2023
Accepted: February 15, 2023
Published: March 04, 2023

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KEYWORDS: Fungal endophytes, *Cryptolepis buchanani*, Medicinal plant, Leaf diversity

INTRODUCTION

Microorganisms are cosmopolitan and live in almost every perceptible niche on this planet. The microorganisms that inhabit plants are known as 'endophytes'. Endophytes are a fascinating group of microorganisms which are a subject of increasing interest to mycologists, plant pathologists, and ecologists (Schulz & Boyle, 2005). They usually live in the cells internally and it is interesting to note that the host plants remain symptomless (Bacon & White, 2000). The endophytes include an aggregation of microbes that perform different functions. After an endophytic phase of growth, these may grow as saprophytes on dead decaying plant matter, as latent pathogens and virulent pathogens. The host plant essentially gives sustenance and refuge to the endophytes and in turn gets increased hardiness from endophytes which produce unique utilitarian compounds (Tan & Zou, 2001).

Endophytes are hidden within the host plants, so they have been poorly investigated microorganisms. They are less explored as sources of natural products which can be exploited for medical and commercial use. The bacteria, fungi, and protists form a very mixed microbial body living as endophytes (Hardoim *et al.*, 2015). The endophytes present many intriguing possibilities, as they occupy distinctive biological spaces in plants growing in varied habitats. These endophytes produce bioactive metabolites with anti-tumor, antibiotic, antioxidant, anti-inflammatory activities (Owen & Hundley, 2004). The fungal endophytes residing in medicinal plants possess many unusual metabolites which may find their use in industry, pharmaceuticals, agriculture, and many more. The need of the hour is to focus on endophytic biodiversity, especially in medicinal plants. The interaction between the host and endophytes concerning the bioactivity of their metabolites and their interactions is one of the least studied biochemical systems. Endophytes present in medicinal plants have become relatively new hotbeds for the discovery of novel metabolites.

The fungal endophytes isolated from medicinal plants, which produce valuable bioactive phytochemicals, may produce exceptional and valuable compounds capable of modulating

metabolic processes akin to their host plants. Interestingly, it has been discovered that *Taxomyces andreanae*, an endophytic fungus present in *Taxus brevifolia* also produces taxol, a product that is normally obtained from the host plant. Taxol and its derivatives are a major group of drugs that are used in anticancer treatments can now be obtained from various fungal endophytes (Tan & Zou, 2001). Fungal endophytes show many symbiotic relationships with their hosts resulting in long-term relationships favorable for both partners (Card *et al*., 2016). The growth hormones which increase the growth of the host plants are found to be produced by a few endophytic partners (Waqas *et al*., 2012). The production of Huperzine, an important bioactive compound produced by *Huperzia serrata* is contemplated to be invigorated by an endophytic fungus, *Acremonium* sp. (Wang *et al*., 2011). The endophytes also play an effective role in protecting their host against abiotic stresses (Hardoim *et al*., 2015; Rho *et al*., 2018; Manasa *et al*., 2020; Sampangi-Ramaiah *et al*., 2020). It has been seen that ecological factors, such as the composition of the soil, climate, sunshine, and humidity etc., also modify secondary metabolites. For example, the endophyte population in the host plants with less duration and intensity of sunlight and high moisture level, resulted in the host medicinal plants producing more compounds that supplemented and supported fungal endophyte's growth (Wu *et al*., 2013). Biological control of diseases has been studied as an alternative to chemicals and endophytes could fill in this gap (Collinge *et al*., 2022). The endophytes have also been reported as immunomodulators, thereby increasing the resistance against various pathogens (Latz *et al*., 2020).

Understanding medicinal plant-endophyte relationships may provide help for isolating endophytic fungi producing various unique bioactive metabolites. This approach will help to save rare medicinal plants and reduce pressure on them for the extraction of useful secondary metabolites. Some endophytic fungi can promote the aggregation of many important metabolites of host plants, which in turn improve the nature of drugs (Chen *et al*., 2016). Endophytic fungi can produce phytohormones which improve crop growth and protect them against abiotic stresses (Khan *et al*., 2015). The microbial endophytes synthesize beneficial products that can be made available commercially at cost effective prices.

It is speculated that there may be many thousands of endophytes useful to mankind but they still remain to be explored. There is an urgent need to focus on the area of plant-endophyte relationship as biodiversity is being lost at an alarming rate and forests are shrinking. These endophytes need to be explored for their potential usefulness especially in health care, before they are wiped out from the face of the earth due to several reasons.

Cryptolepis buchanani also known as Indian sarsaparilla or Kala bel, is an important plant used in tribal medicine. It belongs to the family Asclepiadaceae (http://flora-peninsula-indica.ces.iisc.ac.in) and sub-family Periplocoideae (Paulo & Haughton, 2003). It is a rich resource of important phytocomponents like cryptosin (Venkateswara *et al*., 1989), sarverogenin, isosarverogenin glycosides (Purushothaman *et al*., 1988), new nicotinoyl glucoside (Sunil *et al*., 1980), cryptolepain (Pande *et al*., 2006), buchanin (Khare & Shah, 1983) and possess antioxidant, hepatoprotective (Padmalochana *et al*., 2013), analgesic, anti-inflammatory, chondroprotective (Hanprasertpong *et al*., 2014), immunomodulatory (Kaul *et al*., 2003), cardiotonic activities (Venkateshwer *et al*., 1989). *C. buchanani* has been used for long in Ayurveda in Madhya Pradesh, India as anti-bacterial, anti-ulcerative, anti-inflammatory agent, for purifying blood, treating cough, against diarrhea, and also for the cure of rickets in children (Kaul *et al*., 2003). An ethanolic extract of the stem is used as a paste for the treatment of arthritis and muscle pain (Panthong *et al*., 1986; Laupattarakasem *et al*., 2003).

MATERIALS AND METHODS

Collection of Leaves

Leaves were selected from three different levels (Lower part (L), Middle part (M), and Upper part (U) of *Cryptolepis buchananni* plant for isolation of endophytic fungi. Four leaves were collected from each level of the plant and each leaf was divided into 6 parts. The ventral side facing up the leaf was divided into the left and right sides of the midrib. The left and the right side was further subdivided into 3 sub-parts PLI (lower), PL II (Middle) and PL III (apical) and PRI, PRII and PR III respectively (Figure 1).

Removal of Surface Microbial Load from Leaf

For the removal of microbial load from the surface of *Cryptolepis* leaves cut discs of leaves (3mm/3mm) were washed in 70% ethanol, then 4% sodium hypochlorite, and rinsed three times in sterile distilled water. Duration of the wash was varied according to the Table 1. Then the leaf discs were placed over the surface of Potato Dextrose Agar medium (PDA) plates. From the last rinsed water, 100 µL was plated on a PDA medium to confirm the presence of epidermal microflora and a control was also maintained.

Isolation of Fungal Endophytes

PDA (39 gm/L of HiMedia Company) plates were used for the isolation of fungal endophytes. The media was amended by 0.15 gm/L of streptomycin sulphate to inhibit the growth of

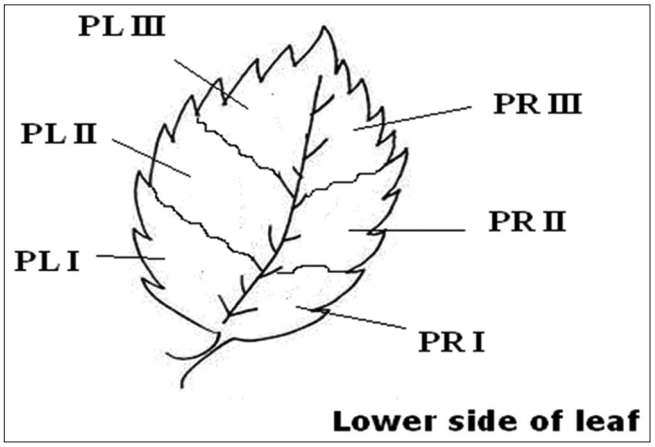

Figure 1: Leaf sub-parts for the isolation of endophytic fungi

Table 1: Effect of sterilant and duration on the removal of microbial load from leaf surface

Time (minutes)		100 μL of H_20 (last rinse) plated on PDA
70% ethanol	4% sodium hypo chloride	
1	1	Growth seen
1	1 ½	Growth seen
1	2	Growth seen
1	2 ½	Growth seen
2	2 ½	Growth seen
2	3	Growth seen
2	3 ½	Growth seen
2	3	Growth seen
3	2	Growth seen
3	2 ½	Growth seen
3	3	No growth
3	3 ½	No growth
3	4	No growth

bacteria. From each leaf subpart 5 discs of 3 mm/3 mm size were cut with a sharp sterilized cutter and sterilized leaves were plated on PDA plates and kept for incubation at 27° C till the endophytes appeared. At each level of the plant, 132 plates were kept for incubation and in all 396 plates were used for the isolation of fungi. The plates were observed for fungal growth at regular intervals. The tips of a hypha of actively growing fungi were then sub-cultured. The identification of the fungal endophytes was carried out by detailed microscopic study of their characters such as morphology of hypha, pigments developed, spore morphology and other unique structures etc. For the identification of the fungal endophytes, Standard Taxonomic Manuals were used. The slants with pure colonies of fungal endophytes were kept at 4°C for further use.

Diversity of Endophytes

Diversity and percent colonization frequency of endophytic fungi was determined by the following formulas:

Simpson's Index (λ) = $\sum pi^2$, where

$$P_i = ni/N$$

ni = number of individuals of i^{th} species
N = Total number of individuals of all species

Shannon's Index (H) = - $\sum pi^2$ In Pi, where

$$Pi = ni/N$$

ni = number of individuals of i^{th} species
N = Total number of individuals of all species

Evenness Index (E_1) = $H_{1/}I_n(S)$, where S is number of total species

Percent Colonization Frequency % CF = N_{col}/N_t X 100, where

N_{col} = Number of segments colonized by each fungi

N_t = Total number of segments
% CF of a given endophyte/Sum of %CF of all endophytesX100

Biochemical Characteristics of Endophytes

Protease activity

Skim Milk Agar Medium (4 gm Skim milk and 100 mL distilled water) (A). 1 gm Peptone Protease, 3 gm Agar and 100 mL distilled water (B) both the media (A) & (B) were separately autoclaved and mixed in sterile condition. The medium was poured into plates, fungal discs were placed at the center, and incubated at 27°C for 2 days. A clear zone appeared around the discs indicating positive protease activity.

Amylase activity

Starch agar medium (starch 1 gm, peptone 1 gm, yeast extract 1 gm, agar (2%), distilled water 1 L, pH 6.5) plates were prepared and discs of fungi were put in the center of the plate. After two days of incubation at 27°C, these incubated plates were inundated with iodine solution and rested for 15 minutes. Later plates were rinsed with distilled water. A clear zone indicates positive amylase activity.

Lipase activity

For the preparation of one litre medium, Mixed Peptone (10 gm), NaCl (5 gm), $CaCl_2$ $2H_2O$ (0.1 gm), Agar (2%) in 1L distilled water and the pH was adjusted at 6 (solution 1). Tween 20 (10 mL) is solution 2 . The solutions 1 and 2 were autoclaved separately. After autoclaving, peptone was added to solution 1. Both the solutions were then mixed in the laminar flow under sterile condition. The endophytes were plated and incubated for 2 days at 27° C. The appearance of white zones around the discs incubated showed presence of lipolytic activity.

Cellulase activity

Cellulose medium (for 300 mL –Na_2HPO_4-1.8 gm; KH_2PO_4-0.9 gm NaCl-0.15 gm, NH_4Cl-0.3 gm, $MgSO_4$-0.36 gm, $CaCl_2$-0.00042 gm, 300 mL distilled water, adjust pH-6.8, add 5% tryptone, 0.2% CMC and 1.5% agar) plates were prepared and endophytes discs were placed and incubated at 27°C for 2 days. These plates were flooded with Congo Red stain (0. 2%) for 30 minutes, drained and then 1 M NaCl was added and kept for 10-20 minutes. The development of yellow zones indicated positive cellulase activity.

Xylanase activity

Prepared M9 medium (300 mL) by mixing Na_2HPO_4 (3.6 gm), KH_2PO_4 (1.8 gm), NH_4Cl (0.6 gm), NaCl (3 gm), $MgSO_4$ (0.72 gm), $CaCl_2$ (0.0084 gm) in distilled water. The pH of the medium was adjusted at 6.8, to which agar (1.5%) and xylan (0.5%) was added. The endophyte discs were placed at the center of the plates and incubated for 2 days. Flooded the plates with Congo Red (0.1%) for 30 minutes and then drained. Finally, 1M NaCl treatment was given for 25 minutes. The

occurrence of clear zones around the discs indicated xylanase activity was present.

Pectinase activity

Prepared M9 medium - (300 mL) as in xylanase activity and added pectin (1.5 gm) instead of xylan. The endophyte discs were placed in the center of the plates and incubated for 2 days. The plates were flooded with CTAB (2%) and kept for 30 minutes. Development of clear zones around the endophyte discs indicated positive activity.

Siderophore activity

Chrome Azurol S (CAS) medium was used to study siderophore activity. The CAS medium (200 mL) involved preparation of different solutions. For the preparation of solution, A, 0.0303 gm CAS was dissolved in 25 mL of distilled water. Similarly, solution B was made by adding 0.5 µL of 1M $FeCl_3$, to 4.995 mL 0.01 M HCl. For solution C, 0.03645 gm of CTAB was dissolved in 20 mL in distilled water. Finally, solution A (25 mL) and solution B (5 mL) were mixed thoroughly and then solution C (20 mL) was added gently from the side. The final colour of the solution appeared greenish and the pH was adjusted at 7.0. In addition, 5.85 gm of Potato Dextrose Agar (HiMedia) was dissolved in 150 mL of distilled water.

These solutions were autoclaved separately, mixed gently in laminar flow and poured into petri plates. The endophyte discs were placed in the centre of the plates and kept at 27°c in the incubator for 3 days. A pink zone appeared around the discs which indicated positive lipolytic activity.

RESULTS AND DISCUSSION

For the present study on the endophytic fungi of *Cryptolepis buchanani*, the leaves (Figure 2) were used for isolation of endophytes. The removal of microbial load from the surface of *C. buchanani* leaves was achieved by cutting leaves into 3 mm discs, submerged in 70% ethanol for 3 minutes, immersed in 4% Sohypochloriteoride for 3 minutes and finally rinsed three times in sterile distilled water. The last rinsed water when plated on PDA medium showed no fungal growth (Table 1). A total of 360 segments from various regions of leaves were screened to check the growth of endophytic fungi.

As many as 383 fungal endophytes were isolated from different levels and different sub-parts of the leaves (Table 2). The distribution of endophytic fungi was observed at three levels and three sub-parts (Figure 2). The maximum numbers of fungal isolates were obtained from the lowermost leaves of the plant followed by the middle level leaves and the least number of fungi was obtained from the youngest leaves. Such results indicate that the age of the leaves plays an important role in the colonization by fungi. The possible reason for less distribution of fungal endophytes in upper young leaves may be due to due to more physiological activity in upper young leaves, and fungi may not be able to establish well there. The highest number of

Table 2: Frequency of various endophytes at different levels of maturity of leaves

Name of Endophyte	Total	L (Mature Leaves)	M (Middle aged leaves)	U (Young leaves)
Aspergillus flavus	47	10	27	10
A. niger	8	0	5	3
A. repens	1	0	1	0
A. sydowi	1	0	0	1
A. versicolor	1	0	0	1
Alternaria alternata	10	6	0	4
Cladosporium cladosporioides	20	2	10	8
C. herbarum	19	12	4	3
C. oxysporium	5	0	0	5
Chaetomium indicum	2	0	0	2
Colletotrichum gloeosporioides.	47	31	16	0
Corynospora cassicola	7	1	3	3
Fusarium micrococcus	20	11	9	0
Khuskia oryzae	43	17	19	7
Paecilomyces variotii	1	0	1	0
Periconia byssoides	5	2	0	3
Scytalidium lignicola	16	13	3	0
Mycelia sterilia 1	27	11	11	5
Mycelia sterilia 2	2	0	0	2
Mycelia sterilia 3	20	2	10	8
Mycelia sterilia 4	6	2	1	3
Mycelia sterilia 5	8	4	0	4
Mycelia sterilia 6	35	9	24	2
Mycelia sterilia 7	18	16	1	1
Mycelia sterilia 8	7	0	7	0
Mycelia sterilia 9	7	1	6	0
Total	383	150	158	75

Figure 2: *Cryptolepis buchanani* –a medicinal plant

mixed colonies of fungal endophytes was obtained in the middle level leaves compared to lower mature leaves and least in upper young leaves. Similar observations were reported by Rubina (2009). There was a marginal decrease in colonizing frequency from base of the leaf towards the leaf apex.

It was also observed that the CF of the left side of leaves was marginally higher than the right side of the leaves (Table 3). Colonization frequency of the endophytes increased with the age of leaves and reached the highest number when the leaves became senescent. Enzymes such as cellulases and

Table 3: Colonization frequency of different leaf sub-parts by fungal endophytes of *Cryptolepis buchanani*

Leaf Level →	L	L	L	L	M	M	M	M	U	U	U	U	CF	Total CF of sub parts
Leaf Level Nos.→ Sub level ↓	L1a	L1b	L1c	L1 d	L2a	L2b	L2c	L2d	L3a	L3b	L3c	L3d		
LI	5	4	3	4	5	3	3	3	5	1	2	2	66.7%	66.77%
RI	5	3	5	5	4	3	3	3	3	5	0	1	66.7%	
LII	5	4	4	4	5	4	1	4	5	1	3	0	66.7%	65.7%
RII	5	3	5	5	4	1	2	3	3	2	1	1	64.7%	
LIII	5	1	2	5	5	1	2	3	1	1	3	1	62.3%	62.0%
RIII	4	2	3	4	4	2	2	4	1	1	3	0	61.7%	
CF	96.6 %	56.7 %	73.3 %	86.6 %	86.6 %	46.6 %	43.3 %	66.6 %	60 %	36.6 %	40 %	16.6 %		

ligninases are produced by endophytic fungi responsible for the decomposition of leaves (Carroll & Carroll, 1978), phytohormones like gibberellins (Hamayun *et al*., 2009), many subsidiary metabolites (Strobel & Daisy, 2003) play an important role in plant physiology. It is seen that the number of endophytes that are present and isolated from the leaf tissue increases as the leaf matures and reaches senescence in several plant hosts (Stone, 1987; Okane *et al*., 1998; Taylor *et al*., 1999). Similarly, Arnold *et al.* (2003) explained that the presence of endophytes is lowest in young leaves and gradually increases with the development and maturity of leaves. This increase in the colonization of older leaves is because of the superinfection of older leaves over time by air-borne inoculums (Carroll *et al*., 1977; Rodrigues *et al*., 1993; Suryanarayanan & Vijaykrishna, 2001). Increased colonization of endophytes has also been attributed to the changes in structural and chemical properties of older leaves (Malinowski & Belesky, 2000; Arnold, 2005).

The isolates (383) obtained in the present study belonged to as many as 11 genera and 17 species. Among the isolated fungi the dominant class was Hyphomycetes. *Aspergillus flavus* and *Colletotrichum* glucosporioides were the dominant genera followed by *Khuskia oryzae* with a Colonization Frequency of 12.27%, 12.27% and 11.22% respectively.

Endophytic Diversity Analysis

For the measurement of diversity, species is taken as the fundamental unit and characterization of diversity within a given community is done customarily by using species richness i.e. the total number of species; species evenness i.e. the relative abundance of the species or indicators that combine these two dimensions (Lozupone & Knight, 2008).

In the present study, species richness and evenness was calculated for the endophyte diversity analyses. The diversity analyses covered all the samples (different leaf levels and their sub-parts) were done using Shannon and Simpson's indices. Diversity indices were calculated using software PAST 2.1 (PAleontological STatistics) as designed by Hammer *et al.* (2001).

A total of 383 endophytic isolates were collected from the leaves of *Crypolepis buchanani* belonging to 26 taxa (Table 2). It was found that the distribution of isolates among the 26 taxa (OUTs) approximated a log-normal pattern, with few common taxa (*A. flavus*, *Colletotrichum*, *Khuskia oryzae* and *Fusarium*) and few scarce taxa (*Paecilomyces*, *Chaetomium indicum*, *A. repens*, *A.sydowi*, *A. versicolor*, *C. oxysporium*, and *Periconia*) (Figure 3). *A. flavus* (12.27%), *Colletotrichum* (12.27%) and *Khuskia oryzae* (11.23%) dominated the fungal endophytic community in *C. buchanani*. Few isolates as *A.niger*, *Corynospora* sp., *C. oxysporium*, *Periconia*, *Chaetomium indicum*, *A. repens*, *A. sydowi*, *A. versicolor* and *Paecilomyces* were collected representing 2.09% to 0.26%.

The total species richness associated with *C. buchanani* was 26 and evenness of 0.64. Shanon and Simpson indices suggest a comparable fair diversity of the fungal endophytic community. The Shanon's (H) and Simpsons's (1-D) diversity indices were 2.82 and 0.92, respectively, indicating the fair diversity of fungal endophytic communities. Dominance (D) index has the value 0.07, indicating the dominance of few taxa.

Comparison between Leaf-level Diversity

The richness values at L, M, and U levels are 17, 18 and 19 respectively, suggesting relatively high diversity at the U level (young leaves). The evenness index at L, M, and U levels are 0.70, 0.66 and 0.82 respectively, suggesting the even distribution of taxa at the U level. Simpson and Shanon's indices suggested that the U level leaf harbor, greater endophytic diversity than L and M level leaves (Table 4).

The endophytic diversity was statistically significant at U level compared to L level and M level (Table 5). Table 6 presents the analyses of the effect of leaf level and sub-parts of leaves and their combined effect on endophytic diversity using Statistical Analysis of variance (Two-way ANOVA), Confidence level: 95%, Probability: 0.05. Factor 1: L (Level of Leaves) and Factor 2: P (Sub-part of Leaves). The result indicates that endophytic diversity is significant with reference to the level of leaves.

Enzymatic activity of endophytes was studied for six enzymes viz. protease, amylase, lipase, cellulase, xylanase, and pectinase.

Table 4: Diversity indices for endophytic fungi recovered from leaves of *C. buchananim*

Index	Whole Plant	Leaf Level		
	Frequency	L	M	U
No. of Taxa (S)	26	17	18	19
No. of Individuals	383	150	158	75
Dominance (D)*	0.07386	0.1028	0.09902	0.07449
Simpson (1-D)	0.9261	0.8972	0.901	0.9255
Shannon (H)	2.826	2.48	2.516	2.748
Evenness e^H/S	0.6488	0.7027	0.66879	0.8213

Table 5: Effect of leaf maturity level (L, M, U) on endophyte diversity

ANOVA: Single Factor						
SUMMARY						
Groups	Count	Sum	Average	Variance		
L	12	152	12.66667	18.06061		
M	12	158	13.16667	16.33333		
U	12	77	6.416667	16.08333		
ANOVA						
Source of Variation	SS	Df	MS	F	P-value	F crit
Between Groups	339.5	2	169.75	10.0887	0.000381**	3.284918
Within Groups	555.25	33	16.82576			
Total	894.75	35				

**Significant effect of leaf level on endophytes diversity at 95% confidence.

Table 6: Analysis of variance (Two way ANOVA) to show the effect of leaf level, sub-parts of leaves and their combined effect on endophytic diversity

	Analysis of Variance (Two Way ANOVA)					
	Source	Type III SS	df	Mean Squares	F-Ratio	p-Value
Factor: Level of Leaves	L	339.500	2	169.750	9.035	0.001**
Factor: Sub-parts of leaves	P	30.167	2	15.083	0.803	0.458
Combination	L*P	17.833	4	4.458	0.237	0.915
	Error	507.250	27	18.787		

**Significant at 95% level of confidence

Table 7 depicts the enzymatic activity of various endophytes studied. Among all taxa investigated, *Aspergillus repens showed positive enzymatic activity for all the enzymes tested except lipase. Similarly, Fusarium micrococcus* exhibited positive activity for all enzymes except cellulase. However, *Alternaria alternate* showed positive activity only for pectinase enzyme and negative for rest of enzymes studied. It was also observed that maximum isolates produced siderophore except for *Khuskia oryzae*, *Periconia*, and sterile isolates no. MS2, MS4, MS6 and MS7. The presence or absence of enzymatic activity data was converted to binary form to perform cluster analysis. Cluster analysis was performed using Jaccard's similarity coefficient on PAST Software. A cluster was separated according to the maximum similarity among the endophytic species (Figure 3). Endophytes like *Aspergillus*

Table 7: Enzymatic activity of endophytes based on plate assay

Name of Endophyte	Protease	Amylase	Lipase	Cellulase	Xylanase	Pectinase
Aspergillus flavus	+	+	-	-	+	+
A. niger	+	+	-	+	+	+
A. sydowi	+	+	-	-	+	+
A.repens	+	+	-	+	+	+
A. versicolor	+	+	-	-	+	+
Alternaria alterata	-	-	-	-	-	+
Cladosporium cladosporioides	+	+	-	+	+	+
C. herbarum	+	+	-	-	+	+
C. oxysporium	+	+	-	-	+	+
Chaetomium indicum	-	+	-	+	-	-
Colletotrichum glucosporiodes	+	+	-	+	+	+
Corynospora cassicola	+	+	+	-	-	+
Fusarium micrococcus	+	+	+	-	+	+
Khuskia oryzae	+	-	+	-	+	-
Paecilomyces variotii	-	+	-	-	-	-
Periconia byssoides	+	-	+	-	+	+
Scytalidium lignicola	+	+	-	-	-	+
Mycelia sterilia1	+	+	+	-	+	-
MS2	+	-	-	+	+	-
MS3	+	+	+	-	-	+
MS4	-	-	-	-	-	-
MS5	+	+	+	+	-	+
MS6	-	-	-	+	-	-
MS7	-	-	-	+	+	-
MS8	-	+	+	+	-	+
MS9	+	+	+	+	+	+

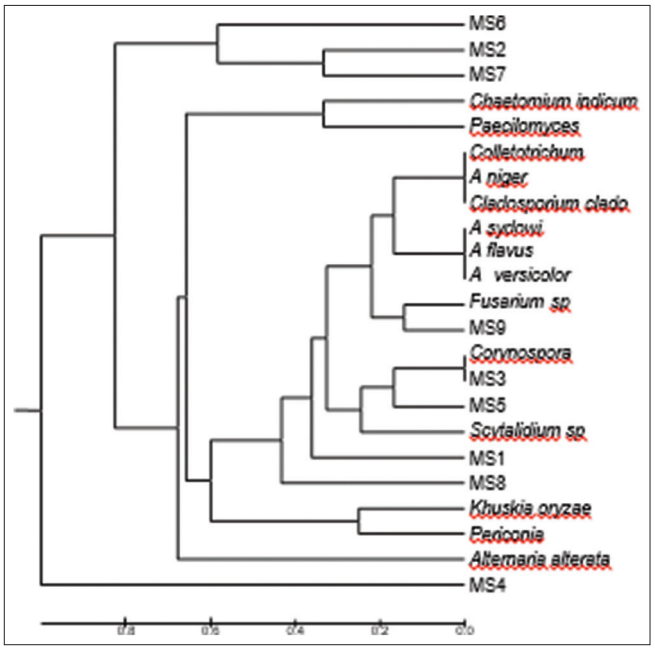

Figure 3: Cluster analysis of endophytic fungi based on UPGMA method, Model: Jaccard's Similarity coefficient, Binary data (Enzymatic activity)

species (sp), *Cladosporium* sp, *Colletotrichum* sp, *Scytalidium* sp, and a few sterile species were clustered together and showed

the maximum number of enzymatic activities which can be correlated to their dominant presence in symbiotic association.

CONCLUSION

Fungal endophytes are a diverse group of microorganisms that inhabit plants. They have a great impact on plants by increasing their fitness through various mechanisms. The present study reveals that the age of the leaf plays an important role in the colonization frequency and diversity of fungal endophytes in *Crypolepis buchanani*. The colonizing frequency and diversity was highest in lower mature leaves followed by the middle level leaves and least in upper young leaves. However, with reference to these parameters, leaf sub-parts showed only marginal difference.

The fungal isolates which were characterized showed positive enzymatic activities for most of the enzymes. Further investigations are required to test these taxa for more enzymatic activities. However, this untamed and unexplored domain of the world of endophytes needs to be researched and explored extensively for novel biomolecules for their potential use in various applications.

ACKNOWLEDGEMENT

The authors are grateful to Prof. C. Manoharachari and Prof. Indra Kunwar, Osmania University, Hyderabad, India for their help in the identification of fungal endophytes. Authors are thankful to the Principals of Bhaskaracharya College of Applied Sciences and Zakir Husain Delhi College, University of Delhi for their motivation and encouragement. The authors wish to thank Sh. Kishore Shinde for his help in statistical analysis.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest among them.

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