ISSN (Online) 2456 -1304

International Journal of Science, Engineering and Management (IJSEM)
Vol 5, Issue 7, July 2020

DNA Barcoding of Cynadon Dactylon and Molecular Characterization of Its Endophytic Fungi

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***Abstract*—Medicinal plant-like C*ynodon dactylon* has been used for millenary years in folk medicines and still have considerable value in the area of ethnomedicine. Prior to the study of the medicinal importance of the sample, identification of the plant sample as C*ynodon dactylon* was essential, which was done using the DNA barcoding technique, with the primer rbcl. DNA barcoding is the process of identification of species based on nucleotide diversity of short DNA segments. Investigation of this medicinal Durva plant shows that it harbors a wide variety of endophytes. Endophytes are widely distributed in nature and portray a balanced symbiotic continuum ranging from mutualism to parasitism in the course of a long period of co-evolution with contrasting kinds of ecological functions. Endophytic fungi from plants are exceptionally substantial due to the presence of certain novel bioactive compounds that bears promising application in the fields of agriculture, medicine and food industry. The identification of endophytic fungi present in the plant C*ynodon dactylon* was performed using the DNA barcoding technique. The paper also expounds on screening of the different kinds of extracellular enzymes secreted by the endophytic fungi isolated and identified.**

***Keywords*— C*ynodon dactylon*, endophytic fungi, metabolites, DNA barcoding**

I. INTRODUCTION

Durva or the *Cynodon dactylon* occupies a key position in the ethnomedicinal practice and traditional medical knowledge systems (Ayurveda, Unani, Nepalese, and Chinese) (Mishra, 2006). *Cynodon dactylon* (Linn.) Pers. Belonging to family Poaceae is an elegant, tenacious, perennial, creeping grass growing throughout the country have much medicinal value and may be applied both externally as well as internally (Animesh *et al*., 2012). The plant possesses antiviral and antimicrobial activity (Dhar *et al*., 1968). Decoctions of the root are used in the treatment of secondary syphilis and ease the irritation of urinary organs (Auddy *et al*., 2003). The plant is astringent, sweet, cooling, haemostatic, depurative, vulnerary, constipating, diuretic and tonic and is useful in impaired conditions of *pitta* and *kapha*, hyperdipsia, burning sensation, haemoptysis, haematuria, haemorrhages, wounds, leprosy, diarrhoea, dysentery, conjunctivitis, vomiting, etc. (Vijayalakshmi *et al*., 2011).

The novel technique of identifying biological specimens using short DNA sequences from either nuclear or organelle genomes is called DNA barcoding. The term 'DNA barcoding' though not a new concept, since it was first used in 1993, but is undoubtedly a technique in vogue for rapid and accurate characterization of species (Valentini *et al*., 2009). A DNA barcode, in its simplest definition, is one or shorter gene sequences taken from a standardized portion of the genome used to identify species. It is a short sequence of DNA, between 400 to 800 base pairs long, that can be easily isolated through various genetic techniques and are used to characterize all species of plants on the planet (Hebert *et al*., 2003). The significant aspects of a DNA barcoding technology is its universality, specificity on variation and simplicity in handling. This means that the gene segment to be used as a barcode should be befitting a wide range of taxa, bear high variation between species but conserved within the species, so that the intra-specific variation will be insignificant (Vijayan and Chih, 2010). By combining the strengths of molecular genetics, sequencing technologies and bioinformatics offer a quick and accurate means to recognize previously known, described and named species and to retrieve information about them. This tool also has the potential to speed the discovery of the thousands of plant species yet to be named, especially in tropical biomes (Cowan *et al*., 2006). DNA barcoding of plants is already being employed in a wide variety of applications.

Barcoding approaches have been used for the verification of plant products ranging from medicinal plants (Chen *et al*., 2011). For most of the land plants and fungi the standard region of COI is not suitable for use as a DNA barcode, due to the fact that the mitochondrial genes in these groups evolve too slowly to allow accurate discrimination between species. Top to that, in fungi, these genes are subject to duplications. Accordingly, other alternatives are sought. In case of fungi, the internal transcribed spacers (ITS) of nuclear ribosomal DNA have been selected as the best alternative DNA region, whereas in the case of land plants, two short coding regions of plastid DNA (matK and rbcL) are chosen. Genetic sequences obtained in the context of DNA barcoding have also been used to create phylogenetic

ISSN (Online) 2456 -1304

International Journal of Science, Engineering and Management (IJSEM)
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trees for use in phylogenetic community ecology (Kress *et al*., 2009).

On the other hand fungal taxonomy is traditionally based on comparative morphological features (Lodge *et al*., 1996; Zhang *et al*., 2009) but needs to take particular caution for closely related or morphologically similar species. Mycologists are facing a tough task to identify various endophytic fungi at genera or species level because they mainly depend only on morphological characteristics and are very time-consuming. Moreover, significant portions of endophytic isolates consist of sterile mycelia and cannot be identified based on traditional approaches. Modern molecular techniques exhibit high sensitivity and specificity for identifying and classifying microbial strains at diverse hierarchical taxonomic levels (Sette *et al*., 2006). Many phylogenetic studies involving fungi rely on the analysis of ribosomal DNA, in particular, the internal transcribed spacer (ITS) regions, to assist in separation at the genus and species levels (Beever and Weeds., 2004). Evidently, endophytic fungi possess the two types of extracellular enzymatic systems necessary to degrade the vegetal biomass: (1) the hydrolytic system responsible for polysaccharide degradation consisting mainly in xylanases and cellulases; and (2) the unique oxidative ligninolytic method, which degrades lignin and opens phenyl rings, comprises mainly laccases, ligninases, and peroxidases. (Corrêa *et al*., 2014). The enzymes function sin order to obtain nutrition from their host, hydrolyze food substances, and are involved in evoking immunity response against pathogens. There is an indispensable need to isolate and employ such diverse novel enzymes with high stability for industrial purposes (Uzma and Chowdappa, 2016).

Hence, this study focuses on the accomplishment of the novel technique of DNA barcoding in identifying the sample Durva grass as *Cynodon dactylon* and as well as the identification of the endophytes isolated as *Aspergillus aculeatus* and *Cladosporium oxysporum* respectively. The isolated endophytic fungi were later screened for the presence of few extracellular enzymes.

II. MATERIALS AND METHODS

Collection of Grass Sample:

Mature, healthy and asymptomatic grass leaves were collected from the campus premise of the University of Agricultural Sciences (GKVK, Bangalore). Samples were collected in sterile polythene bags and brought to the laboratory where they were sterilized and stored at 4˚C. Later, they have been used for plant genomic DNA extraction and to isolate endophytic fungi within 48 h of collection.

DNA extraction from grass sample:

The genomic DNA of grass was extracted following the CTAB method. Approximately 200mg of grass was ground with 1ml of preheated CTAB buffer using pestle and mortar. The homogenate was then subjected to incubation in a water bath at 60ºc for 30 mins. The content was allowed to cool down at room temperature and an equal volume of chloroform: isoamyl alcohol (24:1 ratio) was added and mixed gently. The mixture was centrifuged at 10,000 rpm for 10 min in a refrigerated centrifuge, the pellet was discarded and the supernatant was transferred to another fresh microtube to which equal volume of ice-cold isopropanol was added. This mixture was also centrifuged at 10,000 rpm for 10 min in a refrigerated centrifuge. Supernatant was discarded and 500µl of 70% ethanol was added to the pellet, followed by a quick spin at 10,000 rpm for 10 min in a refrigerated centrifuge. The supernatant was discarded and pellet was retained, which was allowed to dry for about 45 min, and then dissolved in 10µl TE buffer. This DNA was treated with RNAse and column purified to get rid of impurities.

Electrophoresis of the isolated DNA:

The isolated plant DNA was later subjected to electrophoresis for proper quantification. A 0.8% agarose solution in 1x TAE buffer was prepared for a volume of 100 ml, which was allowed to 40ºc, followed by addition of ethidium bromide solution (0.1g\ml). Once the gel is set 2µl of the extracted DNA solution was loaded along with 2µl of bromophenol dye and compared with standard1kb ladder. The Gel unit was run at 100 volts for 40 min.

PCR Amplification and Gel purification:

PCR amplification of the isolated plant DNA was done using primers:matK, petB, rpoB and rbcL. Amplification reaction was performed in 25µl of reaction mixtures, DNA sample 3µl and 5µl respectively. The reaction mixtures were prepared by adding 1µl of each primer and 25µl of the master mix that contains 1µl dNTPs, 0.5µl Taq DNA polymerases, 5µl 10x buffer and 14.5µl distilled water. DNA amplification was performed and DNA thermal cycler programmed for 35 cycles, the PCR amplification conditions consisted of an initial denaturation step and final denaturation step followed by annealing, extension or elongation, 1st cycle of 3.5 min at 94^{o}c, and 2nd cycle is 60^{o}c for 30 sec, and 3rd cycle for 72^{o}C for 1 min. The quality of PCR products was checked on 1 % agarose gel electrophoresis stained with ethidium bromide.

Sanger Sequencing:

Bidirectional sequencing of PCR products was carried in Sequencer- Applied Biosystems (Hitachi) 3130x1 Genetic Analyzer. The data from the sequencer was taken in storage drive and processed in software Finch TV. The electropherogram files which were in .AB1 format was converted to .pdf and fasta files using Sequence Scanner

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Software 2. The sequence data generated during this study were subjected of BLAST searches in the nucleotide database of GenBank (http://blast.ncbi.nlm.nih.gov/) to determine their most probably closely related taxa.

Construction of phylogenetic tree:

Usual methods of Phylogenetic inference involve computational approaches implementing the optimality criteria and methods of parsimony, and maximum likelihood (ML) which is based on Bayesian inference. All these depend upon an implicit or explicit model describing the evolution of characters observed. In the mid-20th century a technique called Phenetics was popular but now mostly obsolete, used distance matrix-based methods to construct trees based on overall similarity in morphology or other observable traits (i.e., in the phenotype, not the DNA). Hence this was often assumed to show approximate Phylogenetic relationships. Prior to 1990, Phylogenetic inferences were generally presented as narrative scenarios. Such methods are often ambiguous and lack explicit criteria for evaluating alternative hypotheses. Further, all the sequences obtained were aligned using the Clustal omega computer program to understand the relationship of unknown sequence with other related species. A phylogenetic tree was generated which informs us about evolutionary relationship and the phylogram to classify the organisms that are closely related to each other.

Isolation of endophytic fungi from *Cynadon dactylon*:

Leaf samples were washed thoroughly under running tap water for 10 min to remove the superficial debris adhered and finally washed with double distilled water to minimize the microbial load from the sample surface. The surface treatment was done and epiphytic mycelia were removed by immersing the tissues in 70% ethanol for 1–3 min and in an aqueous solution of sodium hypochlorite (4% available chlorine) for 2–5 min followed by washing with 70% ethanol for 5s. The tissues were then rinsed in sterile distilled water and allowed to surface dry in sterile conditions. The leaves are carefully dissected into small pieces (1.0 · 0.5 cm). The pieces were placed on petri dishes containing potato dextrose agar (PDA) medium and incubated for seven days at 25 ± 2˚C in the incubator. Tissues were observed for fungal growth at 2-day intervals for seven days. Actively growing fungal tips emmerging from plant tissues were sub-cultured on PDA Petri plates for identification.

DNA extraction, PCR amplification, and sequencing:

Genomic DNA was extracted from pure fungal cultures and was named as a black colony (Bc) and grey colony (Gc) by using the CTAB method followed by column purification. Polymerase chain reaction (PCR) was used to amplify partial gene regions of Internal Transcribed Spacers (ITS) universal fungal primer such as ITS1 and ITS4 were used. The total volume of PCR mixtures for amplification was 25 µL containing 16 µL nuclease-free water, 1 µL dNTPs, 3 µL of DNA template, 1 µl of each forward and reverse primers (10 pM), 2.5 µL of 5X buffer and 0.5 µL of Taq polymerase. PCR conditions were initial denaturation at 95˚C for 5 min, final denaturation at 95˚C for 30 s, annealing at 55˚C for 30 s, extension at 72˚C for 1 min and final extension at72˚C for 7 min. The quality of PCR products was checked on 1 % agarose gel electrophoresis stained with ethidium bromide. Purification and sequencing of PCR products were carried in Sequencer- Applied Biosystems (Hitachi)- 3130x1 Genetic Analyzer. The phylogenetic tree was constructed using a similar technique as explained above.

Screening of the endophytic fungal extracellular enzymes:

The screening was done by a qualitative method using agar plates. The functional role of extracellular enzymes by fungal endophytes was assessed by growing them on PDA for 6-7 days, incubated at 25˚C and placing 5 mm mycelial plugs on the solid media. After incubation, at room temperature, the zone of enzyme activity surrounding the fungal colony was measured. The qualitative estimation of Amylolytic, Proteolytic, Cellulolytic and Lipase activity was done.

Amylolytic Activity:

In the qualitative method, amylase activity is assessed by isolates that were inoculated in nutrient agar with 1% starch at pH 6.0. After incubation, the plates were treated with iodine. A clear zone around the active colonies is an indication of amylolytic activity.

Proteolytic Activity:

To determine protease activity qualitatively, the fungi were grown on Glucose Yeast extract Peptone Agar (GYP) medium (glucose-1g, yeast extract -0.1g, peptone-0.5g, agar -16g, distilled water-1L) containing 0.4% gelatin at pH-6. After 3-5 days of incubation for fungal colony growth, the plates were treated with saturated ammonium sulphate and the clear zone is an indication of proteolytic activity.

Cellulolytic Activity:

For qualitative analysis of cellulolytic activity, Yeast Extract Peptone Agar medium containing 0.5% Carboxy-methylcellulose(CMC) is used. After 3-5 days of incubation, the plates were flooded with 0.2% aqueous Congo red solution and destained with 1M NaCl for 15 minutes. Appearance of light pink areas around the fungal colony indicated cellulose activity.

Lipase Activity:

For lipase activity, the fungi were grown on Peptone Agar

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medium (peptone 10g, NaCl 5g, CaCl2. 2H2O 0.1g, agar-16g, distilled water-1L; pH 6.0) supplemented with separately sterilized Tween 20 and 1% added to the medium. After the incubation period, a clear zone formed around the active colony indicates lipase activity.

III. RESULTS AND DISCUSSION

DNA extraction and identification of grass sample:

DNA was isolated by CTAB method and the quality and quantity of the DNA was checked in spectrophotometer. The wavelength used was 260/280 nm and the concentration of DNA obtained was satisfactory to carry out other procedures as shown in **table 1**. After the end of electrophoresis, the gel was removed from the buffer and the DNA bands were observed under UV light, the concentration of which was estimated by comparing it with standard ladder. Using the genomic DNA PCR was performed using petB and rpoB primers, from which the rpoB primer produced proper band. The obtained PCR product was gel purified to get rid of salt contamination and other impurities. Similarly PCR was conducted with matK and rbcL primers and obtained products were gel purified to obtain pure template for sequencing as in **figure 1**. Sequencing files obtained were in .AB1 format which can be viewed by using software FinchTV as electropherogram peaks of individual nucloetide in its position. Quality of the obtained sequence can be observed through Electropherogram peaks as seen in **figure 2** and **figure 3**. FASTA sequences for further analysis were obtained by converting .AB1 file in Seq Scanner 2.0. Using BLAST server unknown sequences were identified based on query coverage, percentage identity and e-value. Further phylogenetic analysis was done to find out the evolutionary relationship of our query sequence, representative trees. The reverse primer of rbcL has given the sequence of length 445bp. When the FASTA file of this reverse rbcL was BLAST in NCBI blast, it gave the hits of *Cynodon dactylon* species with 99% similarity. Results from Finch TV .ab1 format were converted to .pdf and .fasta. These fasta sequences were used for constructing the phylogenetic tree using Clustal Omega as shown in **figure 4**.

Table 1: Showing concentration and purity of isolated DNA

Sample-ID	Scientific name	260/280	Conc(ng/µl)
CD1	*Zoysia pacifica*	1.79	71.8
CD2	*Cynodon dactylon*	1.72	93.4
CD3	*Cynodon nlemfuensis*	1.77	184.1

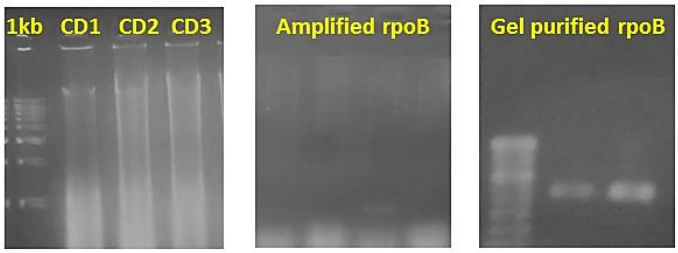

Figure 1: Showing genomic DNA bands on gel; amplified rpoB gene; and gel purified rpoB band.

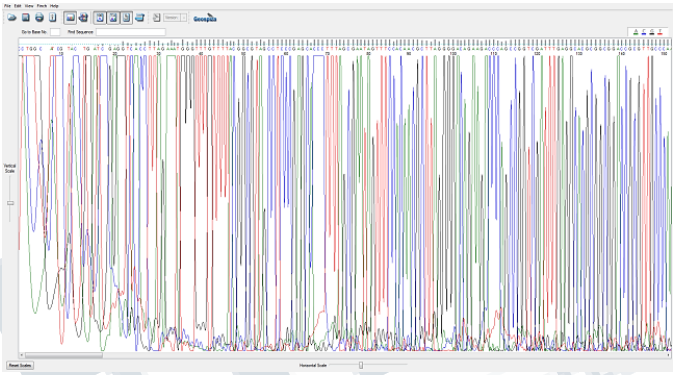

Figure 2: Representative Electropherogram of Rbcl sequence using FinchTV

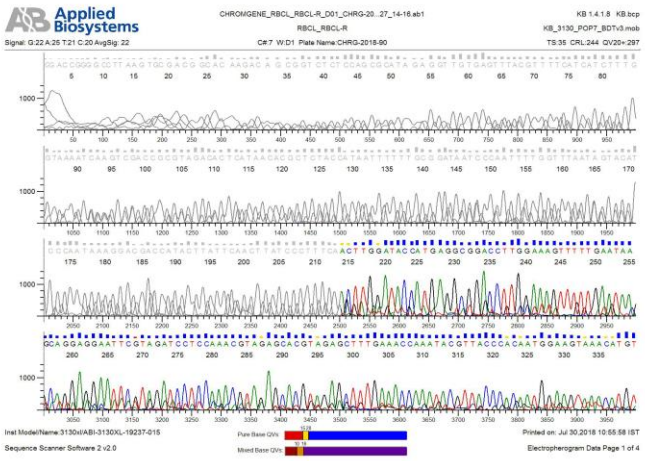

Figure 3: Sequence spread and length of Rbcl gene compacted in one file

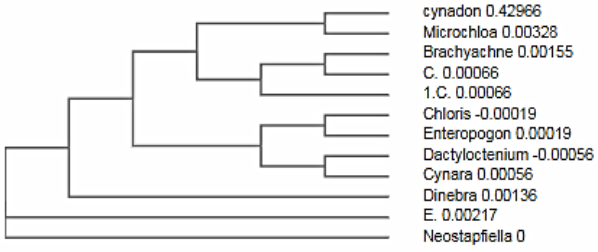

Figure 4: Phylogenetic tree showing *C.dactylon* has close relationship with Microchloa

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Isolation and characterization of endophytic fungi:

The fungal black colony and grey colony grew on PDA media were isolated and fungal DNA was extracted (**Figure 5**). The primer ITS1 and ITS4 were used in PCR to amplify the ITS region. Obtained PCR product of upto 700bp was gel purified to get pure template for Sanger sequencing (**Figure 6**). After sequencing the results procured from Finch TV .ab1 format were converted to .pdf and .fasta (**Figure 7**).

Figure 5: Endophytic fungi [black colony- Bc and grey colony- Gc]

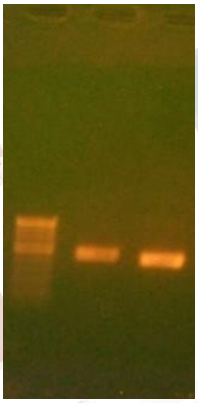
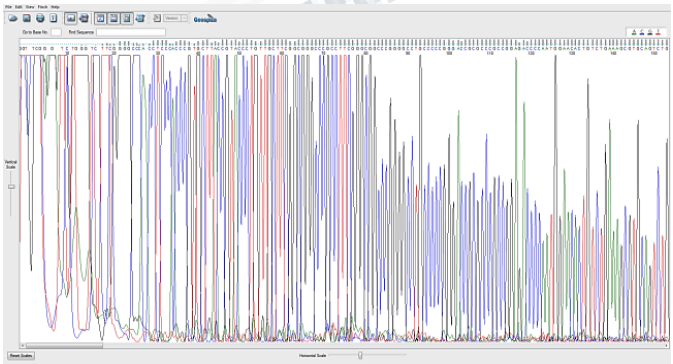


Figure 6: Gel purified samples of Bc and Gc PCR products.1st lane – Bc; 2nd lane – Gc



(a) (b)

Figure 7: Representative Electropherogram of ITS1 sequence of Black colony using FinchTV

Fasta sequences obtained for BLAST analysis are as below:

- **Black colony**

>BC_ITS_Query

GGTTCGGGTCTGGGTCTTCGGGGCCCACCTCCCACC
CGTGCTTACCGTACCCTGTTGCTTCGGCGGGCCCGC
CTTCGGGCGGCCCGGGGCCTGCCCCCGGGACCGCG
CCCGCCGGAGACCCCAATGGAACACTGTCTGAAAG
CGTGCAGTCTGAGTTGATTGATACCAATCAGTTAA
AACTTTCAACAATGGATCTCTTGGTTCCGGCATCGA
TGAAGAACGCAGCGAAATGCGATAACTAATGTGAA
TTGCAGAATTCAGTGAATCATCGAGTCTTTGAACG
CACATTGCGCCCCCTGGTATTCCGGGGGGCATGCC
TGTCCGAGCGTCATTTCTCCCCTCCAGCCCCGCTGG
TTGTTGGGCCGCGCCCCCCCGGGGGCGGGCCTCGA
GAGAAACGGCGGCACCGTCCGGTCCTCGAGCGTAT
GGGGCTCTGTCACCCGCTCTATGGGCCCGGCCGGG
GCTTGCCTCGACCCCCAATCTTCTCAGATTGACCTC
GGATCAGGTAGGGATACCCGCTGAACTTAAGCATA
TCAATAAACGGAGGAA

- **Grey colony**

>GC_ITS_Query

CCTGGCATCGTACTGATCGAGGTCACCTTAGAAAT
GGGTTTGTTTTACGGCGTAGCCTCCCGAGCACCCTT
TAGCGAATAGTTTCCACAACGCTTAGGGGACAGAA
GACCCAGCCGGTCGATTTGAGGCACGCGGCGGACC
GCGTTGCCCAATACCAAGCGAGGCTTGAGTGGTGA
AATGACGCTCGAACAGGCATGCCCCCCGGAATACC
AGGGGGCGCAATGTGCGTTCAAAGATTCGATGATT
CACTGAATTCTGCAATTCACATTACTTATCGCATTT
CGCTGCGTTCTTCATCGATGCCAGAACCAAGAGAT
CCGTTGTTAAAAGTTTTAATTTATTAATTAAGTTTA
CTCAGACTGCAAAGTTACGCAAGAGTTTGAAGTGT
CCACCCGGAGCCCCCGCCCGAAGGCAGGGTCGCCC
CGGAGGCAACAGAGTCAGACAACAAAGGGTTATG
AACATCCCGGTGGTTACACCGGGGTCACTTGTAGT
GATCCCTCCGCAGGTTCACCTACGAAAATCTTACA
AGTGACCCCGGTCTAACCACCGGGATGTTCATAAC
CCTTTGTTGTCGACTCTGTTGCCTCCGGGGCGACCC
TGCCTTCGGGCGGGGGGCTCGGGTGGACACTTCAA
ACTCTTGCGTAACTTTGCAGTCTGAGTAAACTTAAT
TAATAAATTAAACTTTTACAACGGATTCTTGGTTCT
GGCATCGATGAAAAACGCGCCAAATGCGATAAATA
ATGTGGAAAGTAGAAATACTGGGATCACCCATCTT
TTAACCCCTGTGCCCCCCGGTATTTCCGGGGGGAA
GCCGGTTTAACCTCTTTTTCCCTCCGCCCCCCCTGG
GTATTAGGGAAACGGTCGCCCCCCGGCTCAAAAAA
AACGCGGGGGGTCTGTCCTCTCCCAAAGTGTGTGT
AAACCCTAAAGAGGGCGGGGGGAGGCCCCACACA
AAACAACTCCCTATTTTTGTCTCTCCCCGTAGGGGG
GCGCCCTCCGCTATATATATATAATAGGAGAAGAA
AAGAAA

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Species were identified based on the highest similarity given by merging both forward [ITS1] and reverse [ITS4] primer sequences. The black colony was recognized as *Aspergillus aculeatus* with 100% similarity, and the grey colony was recognized as *Cladosporium oxysporum* with 99% similarity. The below **table 2** shows the sequenced lengths of ITS 1 and 4 primers used for fungal colonies.

Table 2: Showing Fungal colony identified and their length

COLONY	MICROORGANISM	LENGTH	
		ITS1	ITS4
Black colony	*Aspergillus aculeatus*	546bp	1012bp
Grey colony	*Cladosporium oxysporum*	526bp	1027bp

These fasta sequences were further used for constructing the phylogenetic tree to understand the evolutionary relationship. The cladogram obtained for Black colony showed that the query sequence has its similarity with *Aspergillus aculeatus* as shown in **figure 8**. Similarly the cladogram for Grey colony showed that the query sequence is closely related to *Cladosporium oxysporum* as shown in **figure 9.**

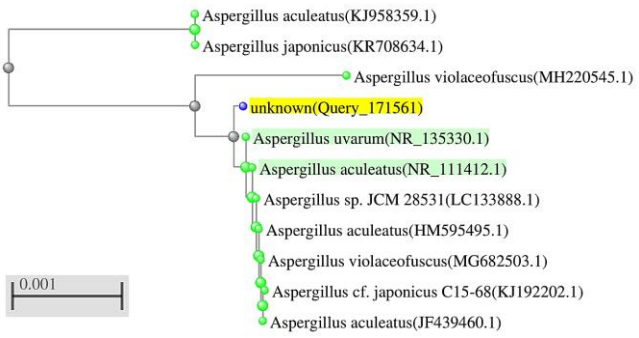

Figure 8: Distance tree results for Black colony fungi

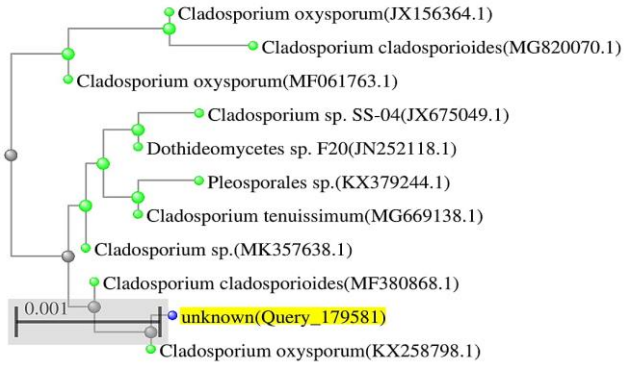

Figure 9: Distance tree results for Grey colony fungi

Screening of the endophytic fungal extracellular enzymes :

After incubation, the plates treated with iodine and saturated ammonium sulphate which gave clear zone around the active colonies, this indicates amylolytic activity and proteolytic activity of the endophytic fungi *Aspergillus aculeatus*, isolated from *Cynodon dactylon. Aspergillus aculeatus* showed positive extracellular proteolytic activity on Yeast extract Peptone Agar (GYP) medium containing 0.4% gelatin as shown in **figure 10**. To check the presence of Cellulolytic Activity, after fungal colony growth, the plates were flooded with 0.2% aqueous Congo red solution and destained with 1M NaCl for 15 minutes. A light pink area around the fungal colony was observed which indicated the cellulose activity of the endophytic fungi *Aspergillus aculeatus*, isolated from *Cynodon dactylon*. The results of which are shown in detail in **figure 11**. During Lipase activity there was no clear zone formed, which indicated the absence of lipase activity of the endophytic fungi, *Aspergillus aculeatus*, that was isolated from *Cynodon dactylon* leaves shown in **figure 12**.

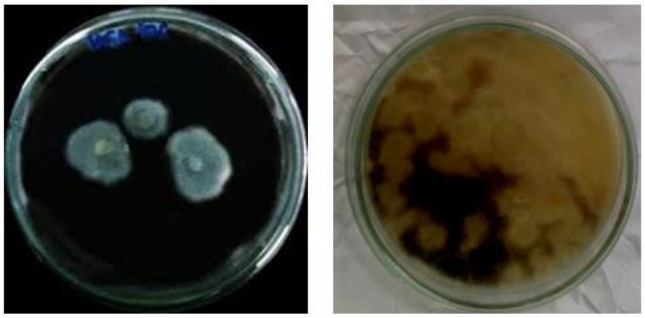

Figure 10: *Aspergillus aculeatus* showing Amylolytic Activity and Proteolytic Activity

Figure 11: *Aspergillus aculeatus* extracellular cellulolytic activity on Yeast Extract Peptone Agar medium containing 0.5% Carboxy-methylcellulose (CMC). (a) Before treating with congo red**, (b)** Plate flooded with aq congo red solution and **(c)**After 1M NaCl destaining

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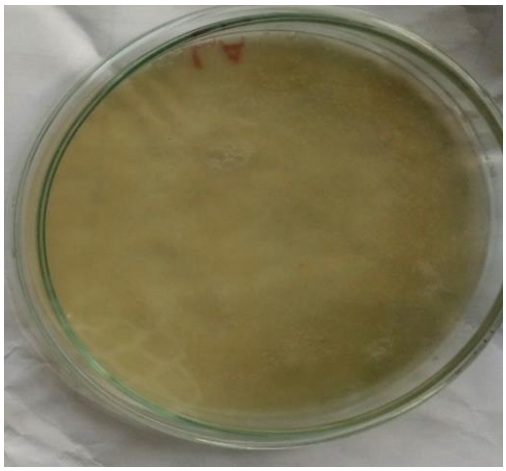

Figure 12: *Aspergillus aculeatus* extracellular lipase activity on Peptone Agar medium supplemented with separately sterilized 1% Tween 20

The endophytic fungal colonies obtained were subjected for analysis of secondary metabolites such as Amylolytic, Proteolytic, Celullolytic and lipolytic activity. The overall results are as tabulated in **table 3**.

Table 3: Positive test for qualitative analysis for Endophytic fungi isolated from the plant *Cynodon dactylon* leaves

Endophytic fungi	Activity	Amylolytic	Proteolytic	Celullolytic	lipolytic
Aspergillus aculeatus		+	+	+	-

DNA barcoding has been a standardized technique to identify and differentiate between species in recent years.. The usage of morphological traits for species identification is classic but has several shortcomings. For example, the misidentification of a taxon due to the phenotypic plasticity of the trait studied or the existence of cryptic taxa. Thus, an experienced proficiency is necessary to correctly identify species with precision is required in ecological studies. The DNA barcoding approach might currently represent the best solution. (Knowlton, 1993). In this study, first the plant sample was idenified as *Cynodon dactylon*(using rbcl) and the fungal endophytes isolated from its leaves were identified as *Aspergillus aculeatus* and *Cladosporium oxysporum*. The sequence analysis of ITS 1 and ITS 4 of both PCR products in BLAST NCBI showed significant similarity nearer to 100% of the species. The phylogenetic analysis highlighted the diversity between our sample and other known species. The data helped us to understand how close and similar our sample species are. Numerous studies have screened endophytes for the presensence of extracellular enzymes which are exploited for their versatile uses. The most common enzymes isolated are: Glucoamylase, cellulase, lipase, glucose oxidase, pectinase, laccase, catalase, phytase and proteases(Dicosimo *et al*., 2013). Owing to their special living environment the plant endophytic fungi may potentially produce many bioactive metabolites with diverse structural features. We have tried to assess few of these metabolites which hold pharmaceutical and ecological importance.

IV. CONCLUSION

The endophytic fungus *Aspergillus aculeatus* and *Cladosporium oxysporum* were isolated from the medicinal plant *Cynodon dactylon*. From the crude extract it has shown the presence of Amylolytic, Proteolytic, Celullolytic and lipolytic activity. Since last decades the endophytic fungi are representing new source of pharmacologically active secondary metabolites based on the underlying assumption that they live symbiotically within their plant host. In order to identify specific bioactive compounds, different methods should be developed in future to isolate secondary metabolites from plant endophytic fungi.

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