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Endophytic fungi from mangrove plant Avicennia marina and their enzyme cellulase activity

Kushwaha Vinodkumar, PG. Research Laboratory, Department of Botany, Smt. Chandibai Himatmal Mansukhani College. Ulhasnagar 421003, Thane Maharashtra, India
Sharma Monica, PG. Research Laboratory, Department of Botany, Smt. Chandibai Himatmal Mansukhani College. Ulhasnagar 421003, Thane Maharashtra, India
Kanade Pinky, PG. Research Laboratory, Department of Botany, Smt. Chandibai Himatmal Mansukhani College. Ulhasnagar 421003, Thane Maharashtra, India
Rana Tulsi, Chemical Laboratory, QC Department, Aquila Organic Pvt. Ltd. Thane 400604 Maharashtra, India.
Yadav Lal Sahab*, PG. Research Laboratory, Department of Botany, Smt.Chandibai Himatmal Mansukhani College. Ulhasnagar 421003, Thane Maharashtra, India
*Corresponding Author: lalsahablal@gmail.com

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Abstract: The most dominant Mangrove plant *Avicennia marina* has been studied for endophytic fungal biodiversity in various parts like pneumatophores, stem/twigs and leaves from Godrej mangrove forest, Mumbai- Maharashtra. The isolates were characterized for their enzyme cellulase production potential. A total of 25 different endophytic isolates were obtained during isolation from 12 different samples (three-time sampling) in three different seasons. The isolates were identified morpho-taxonomically. They were affiliated with 18 different genera. Genus *Chaetomium, Aspergillus, Penicillium, Fusarium,* and *Colletotrichum* were dominant in all parts. The maximum fungal emergence (colonization) was recorded from pneumatophore segments (85%) followed by leave (77.5%) and stem/twig 80%. In enzyme screening, 72% of isolates exhibited cellulase activity. The diversity of mangrove endophytic fungi has been studied by researchers. However, only a few mangrove plants are explored completely for their entophytic fungal association. This study provides a catalogue of endophytic fungi associated with *Avicennia marina* plant. The result of the enzyme study indicates that mangrove fungal endophytes are a very rich source of enzymes and they also may have some other active secondary metabolites for biotechnological applications.

***Keywords*:** Mangrove, Endophytic fungi, enzyme cellulose, secondary metabolites, etc.

Introduction

Mangrove areas are vital tropical habitats that offer a variety of ecosystem services and are important in mitigating the effects of climate change. These areas are also critical for sequestering carbon dioxide, with mangroves in the Asia-Pacific alone making up the majority of the planet's mangrove surface and being vital to carbon fluxes and stocks. Mangrove regions are vital areas for conservation efforts because they promote biodiversity and provide vital habitat for a variety of species, which highlights their significance even more. Additionally, millions of people directly depend on the products and services that these ecosystems supply, making mangroves of great biological, economic, and ecological significance on a worldwide scale. Mangrove areas are priceless ecosystems that offer a multitude of advantages to society, the economy, and the environment. Protecting and restoring these areas is essential for the preservation of biodiversity, reducing the effects of climate change, and ensuring the welfare of coastal populations.

Now, mangrove trees exert an important stabilizing influence, protecting the soil and land from waves and tides (Furkawa and Wolanski, 1996). They are unique for their well-known adaptation towards their extreme environmental conditions of high salinity, changes in sea level, high temperatures and anaerobic soils (Shearer et al., 2007). Mangrove plants include 9 orders, 20 families, 27 genera, and 70 species reported from 112 countries and dominate one-fourth of the world's coastline, a total covering an area of about 180,000 km2 (Li et al., 2016). Among them Genus *Avicennia* has a wide distribution, there are about 15 species of *Avicennia* found growing in the mangrove forests of intertidal zone throughout equatorial and moderate warm areas of the biosphere (Duke et al., 1998). *Avicennia marina* commonly known as grey mangrove or white mangrove is one of the dominant species of the Indian coastal region of mangrove forest which is characterized by thick, long, a bright leaf that are glossy green on the upper surface and silvery-white, or grey, with very small matted hairs on the lower surface. Different part of A. marina has been reported to display antimicrobial (S Thamizharasan & NA Saravanan, 2016), antioxidant (Ravindran et al., 2012) anti-cancer, antidiabetic, anti-inflammatory activities (Thatoi et al., 2016). Apart from focusing on the plant itself, several studies have been conducted on microorganisms associated with the genus *Avicennia*, including fungal endophytes.

Mangrove-associated fungi are increasingly recognized for their bioactive compound production due to extreme habitat. Mangrove endophytic fungi are considered a pool of active biochemical compounds such as phenols, alkaloids, steroids, isocoumarins, quinones, phenylpropanoids, lignans peptides, terpenoids, lactones, flavonoids and enzymes (Tan et al.,2001, Khalil et al. 2019) which are used for multiple applications such as antibiotics, agrochemicals, immunosuppressants, antiparasitic, antioxidants, antibacterial, antiviral, antifungal, anti-inflammatory and anticancer agents (Meena et al., 2017). Fungi are the main source of enzyme production at low cost and play an important role in biotechnological applications.(Ferreira Filho et al., 2012; Zhao et al., 2015 ; Regina et al.,2018). The most common enzymes produced by Mangrove endophytic fungi have been screened for enzyme are amylases, lipases, proteases, cellulases, and pectinase which are adaptive in nature (Lin et al., 2001). However, the report on enzymatic activities of various mangrove plants' associated endophytic fungi is still meagre. In this study, isolation of endophytic fungi from various parts of *A. marina* has been done and enzyme-cellulase profiling of endophytic fungi was screened for further characterization.

Materials and Methods

Study sites

The study was carried out at the Godrej Mangrove Forest, Vikhroli, India (21.62°N, 108.23°E). This site has a mean annual temperature is 27.2 °C and the average precipitation is 242.2 cm (95.35 inches) and soil salinity of 0.5–1.2%. In 2019-2020, we selected the dominant mangrove species *Avicennia marina* (Verbenaceae), from this site. A total of 4 mature and healthy individuals of *Avicennia marina* were randomly chosen, the selected plants were near about 50 m away from each other.

Sample collection

Twigs, leaves, and pneumatophores of *Avicennia marina* were sampled from selected healthy plants grown in the Godrej mangrove intertidal zone, Vikhroli, India. The samples were collected three times after four months of intervals. (May, 2018, August, 2018,

December, 2018 & March, 2019). The collected samples from selected plants were placed in plastic bags, labelled, and transported to the laboratory. Samples were stored at 4°C in the refrigerator and processed within 4 days. A total of 12 samples were processed for isolation of endophytic fungi.

Sample preparation

The collected plant parts from *Avicennia marina* were rinsed thoroughly in running water to remove the dust/mud and cut into small fragments before being surface sterilized. The fragments of samples were cut into 5mm long pieces and surface sterilized by immersing them sequentially in 70% ethanol for 3 min and 0.5% sodium hypochlorite for 1 min. (Guo et al., 2000) Thereafter, the pieces of samples were rinsed thoroughly with sterile distilled water and surface-dried. The sterilized samples were cut into many section segments before being placed onto culture media.

Isolation of fungi

Five sample segments were placed in each 90 mm Petri dish containing PDA, PCA, MEA, and CMA culture media. Streptomycin and chloramphenicol were added to suppress bacterial growth. Petri dishes were sealed and incubated for one months at room temperature and examined regularly for visible fungal growth from segments. When fungal hypha comes out from the segment, small portion of hyphae is transferred aseptically on fresh PDA plates. Central inoculation of pure isolates was done on PDA plates and growth pattern of isolate was recorded and photos of culture plates were taken.

Morphological characterization and Identification of isolates

Morphological characterization of isolates was performed on PDA media. All sporulated isolates were identified based on their morphological characteristics with the help of standard books and monographs Coelomycetes by Sutton (1980); Dematiaceous hyphomycetes. Ellis (1971, 1976), Ames, L.M. 1969; Seifert et al. (2011) and related references. The identified isolates were further sent to National Fungal Culture Collection of India, Pune (NFCCI) for confirmation and for Accession. Non-sporulated cultures were considered as Mycelia sterilia. The purified strains were transferred to slants for further study.

Data analysis

The colonization frequency (CF%) of endophytic fungi in various parts was calculated using the formula given by Deepthi et al. (2018).

Screening of isolates for their cellulolytic activity:

All isolates were under taken for screening of their enzyme cellulase producing ability. Isolates were grown on basal salt media supplemented with 5% carboxy methyl cellulose (Lingappa Y and Lockwood JL, 1962). The sterilized media was poured into Petri plates and allowed to solidify. Ten days old pure culture of isolates grown on PDA plate was used as inoculums. A disc of definite size cut from margins by cork borer and inoculated centrally onto to the plate contains CMC-Agar medium. The plates were incubated at room temperature (28 ± 2 °C) for seven days to allow fungal growth. After incubation plates were flooded with 1% Congo red dye for 15 minutes. After 15 min. Congo red solution discarded from the plates by decanting and 1N NaOH was added to the plates for 10 min. After 10

minutes NaOH decanted from the plates. The clear zone was observed around the colony and cellulolytic index was calculated. (Hankin L and Anagnostaksis L, 1975).

$$\text{Cellulolytic index} = \frac{\text{Clear Zone Diameter (mm) - Colony Diameter (mm)}}{\text{Colony Diameter (mm)}}$$

Production of Enzyme and Extraction

Submerged Fermentation (SmF): Submerged fermentation was carried out in 250 ml Erlenmeyer flasks containing 100 ml of fermentation medium. The composition of the fermented medium contained gram per litre of distilled water: Urea-0.3 g; (NH4)2SO4-1.4 g; KH2PO4, 2.0 g; CaCl2, 0.3 g; MgSO4, 0.3 g; Peptone, 1 g; FeSO4, 5.0 mg; MnSO4, 1.6 mg; ZnSO4, 1.4 mg; CoCl2, 2.0 mg and carboxymethyl cellulose (CMC) 10 g. pH of the medium were adjusted to 5. The medium was sterilized by autoclaving at 121ºC for 15 min. Each flask was inoculated with loop full of the above said inoculum. The cultures were incubated on a rotary shaker (120 rpm) at 30ºC for 7-8 days. (B. J. Akinyele, 2011).

Enzyme Extraction: The culture broth from the submerged fermentation was centrifuged at 6000 rpm for 15 minutes at the end of the fermentation and the supernatant was used as crude enzyme sample (P. Shobana, N. U. Maheswari,2013.)

Assay for Cellulase Activity

Endoglucanase activity- Carboxyl Methyl Cellulase (CMCase): The enzyme's cellulase activity was measured using 1% carboxy methyl cellulase as a substrate in sodium acetate buffer (0.2 M, pH 4.8). An aliquot of 0.5 ml of cell free supernatant was transferred to a clean test tube and 0.5 ml of CMC (1% w/v) in 0.05 M Sodium citrate buffer (pH 4.8) was added. The tubes were incubated in water bath at 55º C for 15 min. After incubation 3 ml of DNSA reagent was added to stop the reaction. The reactants in test tubes were incubated in boiling water bath for 10 min. After incubation liberated sugars were determined by measuring absorbance at 540 nm in colorimeter. Enzyme activity was determined in terms of International Unit (IU) which is defined as an amount of enzyme that produces 1µ mole of glucose per minute. (Sethi et al.2013).

Protein determination: The total protein content in cell free filtrate (extracted from flask) was determined by Lowry's methods of protein estimation in which crude enzyme extract was reacted with the Lowry's reagents and the absorbance obtained was compared with a standard graph plotted by reacting a standard protein bovine serum albumin (1mg/ml) with Lowry's reagents. Then a graph was plotted between concentration of standard protein (Bovine Serum Albumin) on X axis and absorbance at 660nm on Y axis. Enzyme activity is expressed as specific activity which is represented as UI/mg of protein. The experiments were carried out in triplicates and were statistically analysed (Lowry et al. 1951).

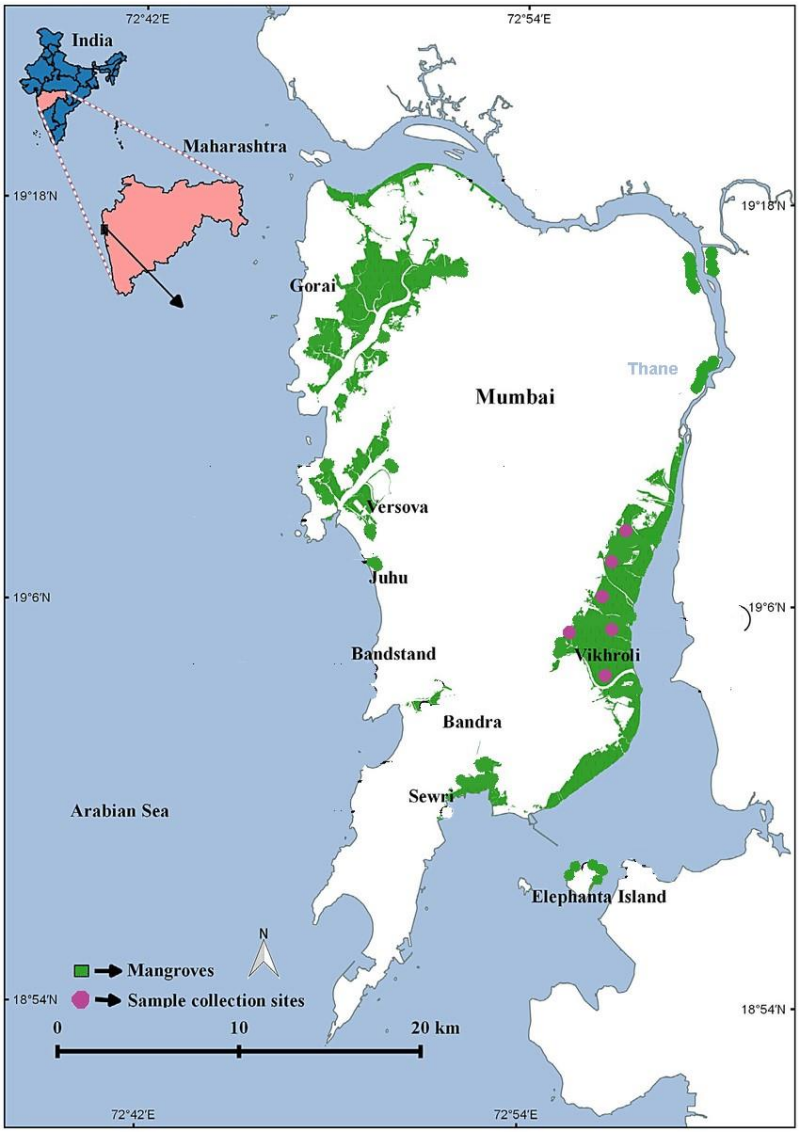
Results and Discussion

Isolation and Identification of Endophytic Fungi

A total of 240 sample segments were analyzed (80 segments from each Pneumatophore, stem/twig and leaves) out of 240 segments 194 segments showed fungal mycelial emergence. The overall emergence percentage was 80.8%. The percentage fungal colonization in four different A. marina plants was in range between 75% to 90% and the

number of isolates also varies in individual plant. This finding is well supported by Huang et al. (2008). He had reported similar trend of occurrence of endophytic fungi in medicinal mangrove plants. The differences in colonization percentage of endophytic fungi may be due to differences in age, phyto-chemical differences of plant and nutrient contents (Liu et al., 2019). The maximum fungal emergence recorded from pneumatophore segments (85%) followed by leave (77.5%) and stem/twig 80%. Soil is hub of microorganisms, microbial biodiversity in soil is always high, in this study maximum colonization was recorded in pneumatophore segments, it may be due to direct penetration of pneumatophore cells by soil fungi.

Fig.1 Map represent study area of Mangrove ecosystem at Mumbai: Sample collection sites in Godrej mangrove forest at Vikhroli.



A total of 25 endophytic fungal isolates were isolated from Pneumatophore, Stem/twig and leaves segments of *Avicennia marina.* During course of isolation of endophytic fungi, twelve different fungal species and one non- sporulating form were isolated from pneumatophore segments, nine different fungal species and three different non- sporulating forms isolated from twigs/stem, and 30 fungal species and one non-sporulating form isolated from leaves segments. Despite of many attempts using different

culture media, all six non-sporulating forms did not produce any spore and were considered as Mycelia sterilia, which were distinguished based on the color (white/ brown/black/orange/ yellow/ grey) and morphological aspect of the colony.

Fig.2 Plate-1: A- *Penicillium* sp. B-Cellulase activity on CMC plates, C-Conidiophore D-Conidia E- *Chaetomium* sp. 01 F- Cellulase activity on CMC plates, G-Perithecia H-Ascospores I- Asci J- *Chaetomium* sp. 02 K- Cellulase activity on CMC plates, L-Perithecia M-Ascospores N-Asci O-*Curvularia brachyspora* P- Cellulase activity on CMC plates, Q-Conidia & R- Conidiophore S-*Mycelium stericulia* F- Cellulase activity on CMC plates, U-Sterile mycelium.

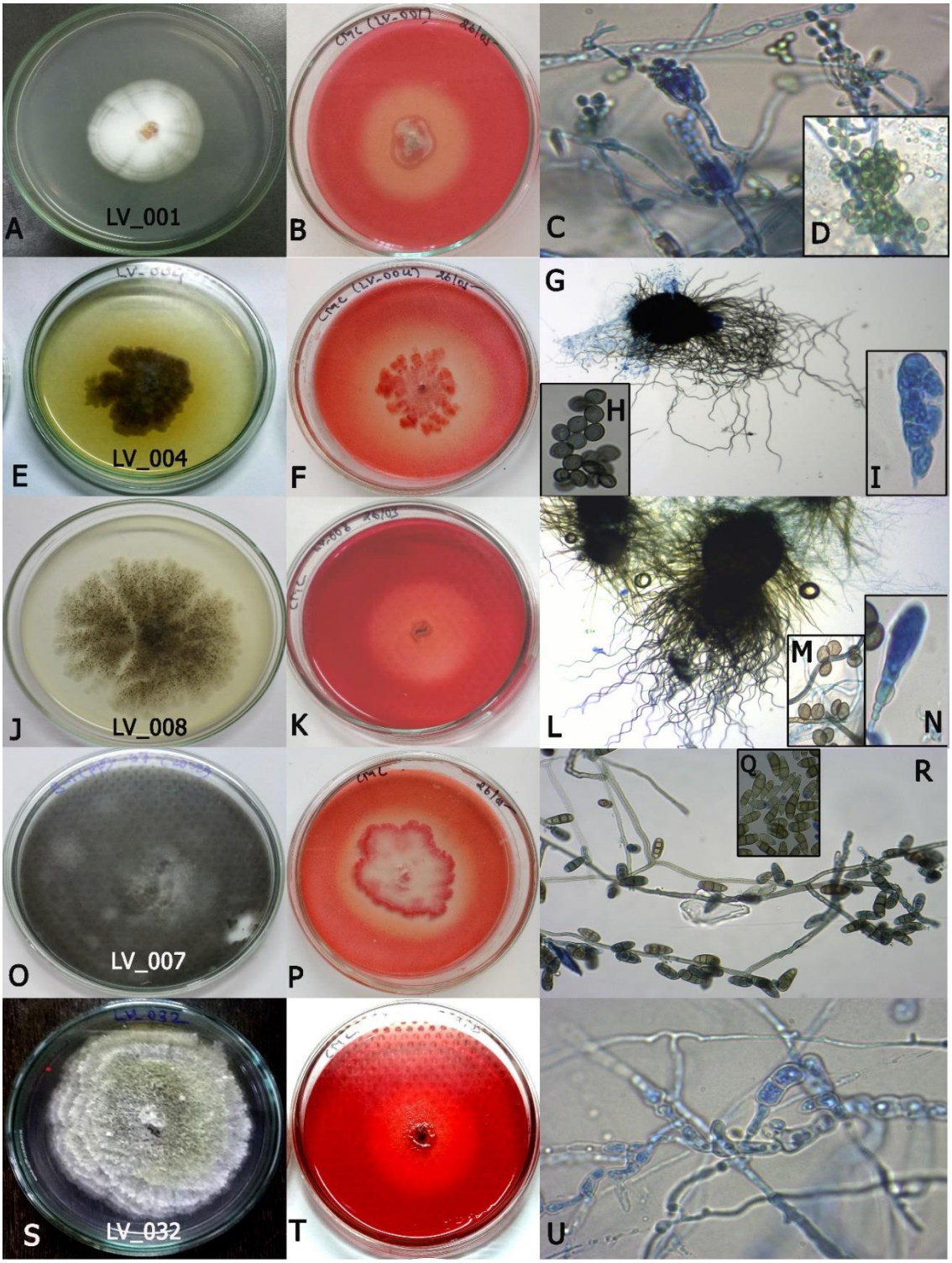
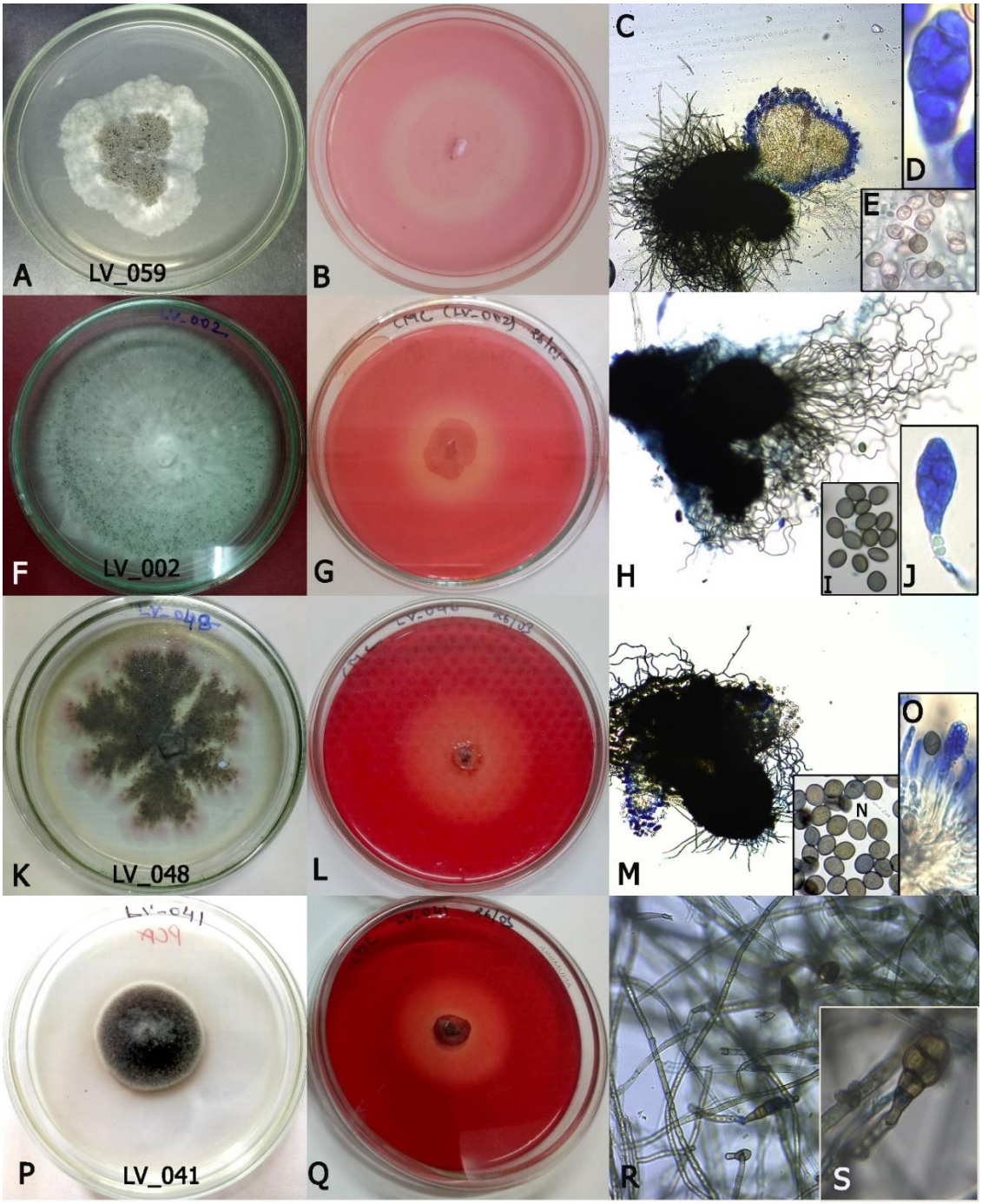


Fig.2 Plate-2: A- *Chaetomium* sp.03 B-Cellulase activity on CMC plates, C-Perithecia D-Asci & E-Ascospores F- *Chaetomium* sp.04 G- Cellulase activity on CMC plates, H-Perithecia I-Ascospores & J- Asci K- *Chaetomium* sp.05 L- Cellulase activity on CMC plates, M-Perithecia N-Ascospores O -Asci, P-*Alternaria* sp. Q- Cellulase activity on CMC plates, R-Conidiophore S-Conidia



Based on morpho-taxonomical identification approaches, morphological characters of fungi such as colony color texture, margins, reverse color, hyphae/mycelium, conidiophores/sporangiophores, conidia/spores, ascospores, ascoma morphology, conidial head, sporangia, color, and size and wall texture of all sporulating isolates were examined with the help of Olympus microscope CX21i-LED and identified using standard books/monographs. The isolates were belongs to 18 different genera and 25 species with a

majority belonging to the phylum Ascomycota (Table 1). He et al., 2012 and Koukol et al., reported that Ascomycetes are dominated group associated to mangrove plants. All pure and identified isolates were inoculated in the center of PDA plate and incubated at room temperature, photograph of fully grown culture was taken (Figure 2). The dominating genera were *Chaetomium* (3 species) *Aspergillus* (2 species) *Penicillium* (2species) *Fusarium* (2 species) and *Colletotrichum*(2 species), *Trichoderma* (2 species) whereas genera that were represented by single species were *Curvularia*, *Alternaria*, *Phoma*, *Nigrospora*, *Blastomyces*, *Pestalotiopsis*,, *Phomopsis Cephalosporium*, *Xylaria*, *Epicocum* and *Aureobasidum*. The relative frequency of *Chaetomium globosum, Aspergillus niger* and *Trichoderma harzianum* in pneumatophore were consistent in all four A. marina plant in all three attempts (Table 1). Different fungal genus and species isolated in this study showed different relative frequencies in different parts. Huang, 2008 reported similar finding from *Rhizophora mucronata* (red mangrove) and *Sonneratia alba* (mangrove apple). The endophytic fungal diversity in stem/twig and leaves was little different some foliar fungal pathogenic genera like *Colletotrichum,Pestalotiopsis, Phoma ,Fusarium* and *Alternaria* were encountered from all plants. In this study selected A.marina plants were found to harbor by some common endophytic fungi. Helen M. Kiti (2021) also reported that Endophytic fungi colonized the same mangrove species from different locations. Mangrove A. marina tolerates high salinity and extreme in intertidal region and also harbored by diverse group of fungi. This relationship may for their physiological adoption for such extreme condition.

Table 1. Endophytic fungal isolates from different parts of Avicenia marina Plant

Plant part	Sr. No.	Isolates name	AM-1	AM-2	AM-3	AM-4
Pneumatophores	1	*Chaetomium globosum* Kunze	✓**	✓***	✓**	✓*
	2	*Chaetomium humicola* Van Warmelo	-	✓**		
	3	*Chaetomium funicola* Cooke	✓*			✓*
	4	*Aspergillus nidulans*(Eidam)G.Winter	✓***	✓**	✓**	
	5	*Aspergillus niger* Gr.	✓**	✓**	✓*	✓**
	6	*Curvularia lunata* (Wakker) Boedijn	✓*		✓*	
	7	*Nigrospora* oryzae Berk. & Broome		✓***	✓**	✓**
	8	*Fusarium* sp.	✓**		✓**	✓*
	9	*Blastomyces* sp.		✓		
	10	*Penicillium chrysogenum* Thom	✓***		✓*	✓**
	11	*Penicillium verreculosum* Peyronel			✓*	
	12	*Trichoderma harzianum* Rifai	✓***	✓***	✓***	✓**
	13	Mycellia sterelia-1 (Black mycllium)	✓**	✓*	✓**	✓**
	14	Mycellia sterelia-2 (hailine mycelium)				
Stem/Twig	15	*Aspergillus nidulans*(Eidam)G.Winter	✓***	✓***	✓***	
	16	*Aspergillus niger* gr.	✓**	✓**	✓***	✓**
	17	*Penicillium chrysogenum* Thom.				✓**
	18	*Chaetomium globosum* Kunze	✓**	✓**		
	19	*Pestalotiopsis* sp	✓**		✓*	✓*
	20	*Phoma herbarum*. Westend.				✓**
	21	*Colletotrichum* Sp.			✓***	
	22	*Cephalosporium* sp.	✓***	✓*		
	23	*Fusarium verticillioides*.			✓**	✓**
	24	*Xylaria* sp.		✓*		
	25	Mycellia sterelia-3 (white mycelium)	✓*	✓*	✓*	✓*

	26	*Colletotrichum* sp.			✓**	
	27	*Phoma herbarum* Westend.	✓**			✓**
	28	*Phomopsis* sp.	✓*			
	29	*Pestalotia vaccinii*(Shear) Guba		✓*	✓*	
	30	*Chaetomium globosum* Kunze	✓**	✓**	✓**	
	31	*Penicillium chrysogenum* Thom				✓*
Leaf	32	*Epicoccum nigrum*	✓*			✓**
	33	*Alternaria alternata* (Fries) Keissler		✓**		
	34	*Cladosporium tenuissimum*.	✓**	✓**		✓*
	35	*Aureobasidum pullulans* (De Bary) G. A		✓**	✓*	
	36	Mycellia sterelia-4 (white mycelium)	✓**		✓*	✓**
	37	Mycellia sterelia-5 (Grey mycelium)	✓*	✓**		✓*

Cellulolytic activity of isolates.

All identified isolates were undertaken for screening of their cellulase enzyme production ability qualitatively using CMC agar plat. Out of 25 different species which were screened 18 species were showed positive result in primary screening on solid CMC Agar medium. The result of primary screening given in table-1, based on their cellulolytic activity marked as +, ++ & +++.

Table 2. Screening of cellulose activity of endophytic isolates

Isolates code	**Identified fungal sps.**	**Cellulase Enzymes activity**
LV_001	*Penicillium sp.*	++
LV_002	*Chaetomium sp. 04*	+++
LV_004	*Chaetomium sp. 01*	+
LV_005	*Aspergillus nidulans*(Eidam)G.Winter	+
LV_006	*Chaetomium sps.06*	+
LV_007	*Curvularia lunata*(Wakker)Boedijn	+
LV_008	*Chaetomium sps.02*	+
LV_009	*Collariella sp.*	+
LV_010	*Aspergillus flavus sp.*	-
LV_018	*Unidentified sp.*	-
LV_019	*Curvularia brachyspora* Boedijin	++
LV_020	*Trichoderma harzianum* Rifai	+
LV_022	*Alternaria tenuissiuma*(Kunze)Wiltshire	+
LV_028	*Phoma herbarum.*	-
LV_031	*Colletotrichum Sp.*	-
LV_032	*Mycelia stericulia*	+
LV_036	*Fusarium verticillioides.*	-
LV_040	*Xylaria sp.*	+
LV_041	*Alternaria sp.*	++
LV_043	*Phomopsis sp.*	-
LV_046	*Pestalotia vaccinii(Shear) Guba*	-
LV_048	*Chaetomium sp.05*	++
LV_053	*Curvularia lunata* (Wakker)Boedijn	-
LV_059	*Chaetomium sp.03*	+++
LV_076	*Aureobasidum pullulans (*De Bary) G. A	+

+, poor; ++ moderate**;** +++ high cellulose enzyme activity.

These studies have revealed that different endophytic fungi exhibit varying abilities in producing cellulases, highlighting the importance of evaluating their cellulolytic index

(Syamsia et al., 2019). One study conducted by Choi et al. 2005 investigated the cellulolytic index of endophytic fungi isolated from plant tissues. The study found that different endophytic fungi demonstrated varying abilities in producing cellulases. In this research Cellulolytic activity of fungal isolates was based on clear zone of degraded CMCase area around the colony. Cellulolytic activity test showed that isolate no. LV_002 and LV_59 has the largest cellulolytic index (1.76 and 2.80). followed by isolate no. LV_001, LV_041 and LV_048 has the medium cellulolytic index (0.60, 0.75 and 0.88) whereas LV_004, LV_007, LV_008, LV_018, LV_032 and LV_040 has smallest cellulolytic (0.48, 0.27, 0.40, 033,0.47,0.38 and 0.27) respectively. Based on cellulolytic index and growth isolate no. LV_002 and LV_059 were potential isolates.

Table 3: Cellulolytic index of isolated endophytic fungi

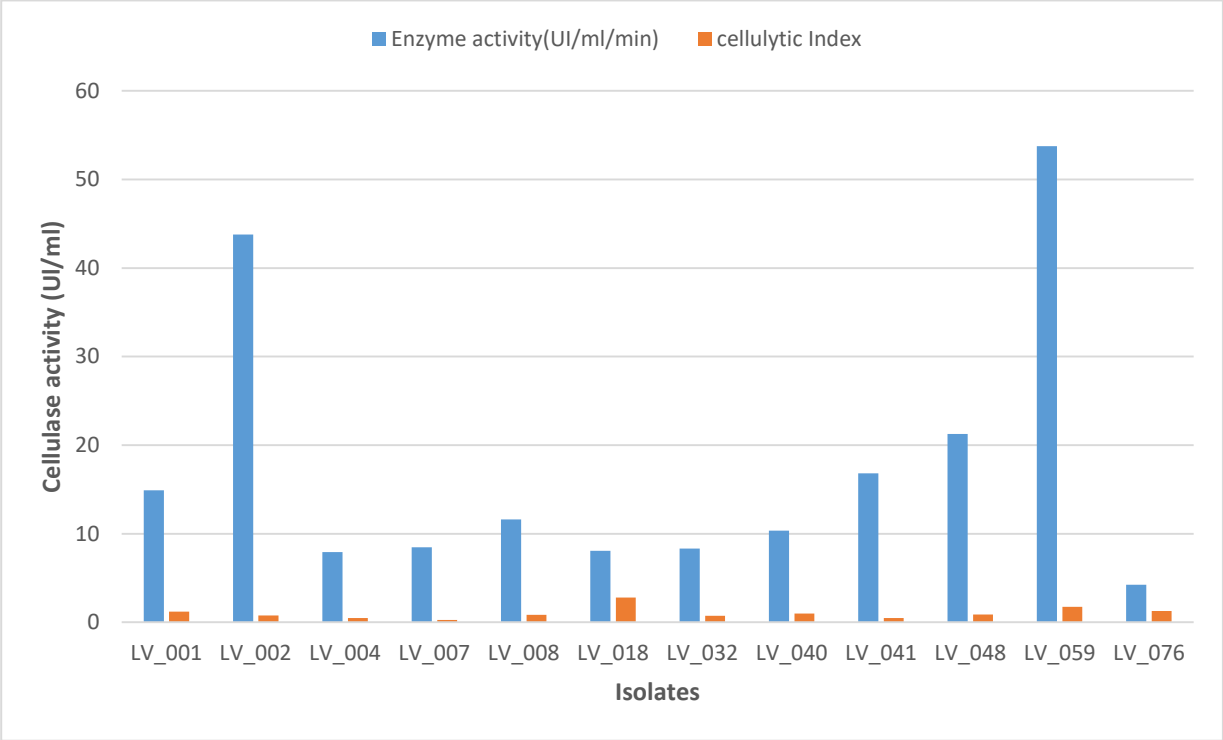
Isolates No.	Diameter of the Colony(mm)	Diameter of Cellulolytic Zone(mm)	Cellulolytic index
LV_001	30	48	0.60
LV_002	21	58	1.76
LV_004	35	52	0.48
LV_007	18	23	0.27
LV_008	25	35	0.40
LV_018	36	57	0.33
LV_032	38	56	0.47
LV_040	18	25	0.38
LV_041	32	56	0.75
LV_048	17	32	0.88
LV_059	15	57	2.80
LV_076	18	23	0.27

Enzyme Assay

All the pure isolates were primarily screened for cellulase production out of which 12 isolates shows positive detection (Table 2) for cellulase production during primary screening by plate assay (Fig.2) Further these isolates were identified (Table 1) and selected for secondary screening by submerged fermentation. For submerged fermentation under controlled conditions, the production media was prepared.

Total 12 endophytic fungal isolates showed cellulase production (Fig. 3). These fungal isolates were inoculated into production media with mycelium inoculate and incubated under controlled conditions for 7-8 days. On the 8th day, growth of fungal biomass was observed. The cell free filtrate was processed for determination of enzyme specific activity.

Fig.3: Cellulase activity



The production of cellulase by endophytic fungi has shown promising potential for various applications, including biodiesel production and the degradation of lignocellulosic residues (Lin et al., 2021). Cellulase is a group of enzymes that are responsible for breaking down cellulose, the main component of plant cell walls. Endophytic fungi have been found to have lignocellulolytic enzyme activity, making them ideal candidates for cellulase production (Syamsia et al., 2019). Choi et al. and Sunitha et al. reported that endophytic fungi have different abilities in producing enzymes, with the ability of the endophytic fungus *Acremonium* sp. to produce cellulase. So, in our research the specific activities (UI/ml) of cellulase for respective isolates were statistically calculated, are shown in the Figure 3. In the secondary screening, the specific activity of LV_002 and LV_059 showed highest concentration of an enzyme 8 days incubation periods 43.7899 UI/ml and 53.7675 UI/ml. Whereas other isolates LV_001, LV_041 and LV_048 showed concentration of an enzyme 8 days incubation 14.8946 UI/ml, 16.8103/ml and 21.2803UI/ml. Another isolates no. such as LV_004, LV_007, LV_008, LV_018, LV_032, LV_040 and LV_076 showed lowest concentration of enzyme at 8 days incubation 7.934 UI/ml, 8.477 UI/ml, 11.606 UI/ml, 8.0938 UI/ml, 8.3173 UI/ml, 10.3607 UI/ml and 4.2624 UI/ml. So, all the isolates need to be further investigated under optimum conditions for enhanced enzyme production as well as enzyme specific activity so that it can be used for scale up of cellulase production.

Conclusion

In this study it was investigated that the endophytic fungal community associated with leaves, twigs, and Pneumatophore of *Avicennia marina* mangrove species from Godrej Mangrove Forest. The fungal endophytes of mangrove Avicenna marina were diverse in forms. It also showed depicting host recurrence and spatial heterogeneity. Overall, the colonization rate of endophytic fungi was higher in Pneumatophore than twigs and leaves. Some endophytic fungi showed certain host and tissue preferences. The most dominant and widely distributed endophytic fungal genus were *Chaetomium*, *Aspergillus Penicillium*,

Fusarium and *Colletotrichum*. Most of the fungal genera were found in all parts of A. marina and, they may be host specific despite differences in location they exist in all selected plant and hence exhibiting host-specificity. Cellulases are hydrolytic enzymes which cleave cellulose into sugar subunits like glucose. There is a great demand of cellulases in food, beverages, and textile, laundry, paper and pulp industries. The high colonization of mangrove A. marina by diverse fungal endophytes coupled with the highly cellulolytic isolates indicates that the mangrove associated fungi are having biotechnological potentials, these endophytes can further be explored as sources of novel bioactive compounds.

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