BIOSCIENCES BIOTECHNOLOGY RESEARCH ASIA, December 2022. Vol. **19**(4), p. 1113-1122

Diversity and Extracellular Enzyme Production of Fungal Endophytes from the Genus *Ocimum* L.

M. Karthika and A.R. Rasmi*

Department of Botany, Government Victoria College (Affiliated to University of Calicut), Palakkad, Kerala – 678001, India.

http://dx.doi.org/10.13005/bbra/3060

(Received: 25 May 2022; accepted: 19 November 2022)

***Ocimum tenuiflorum*, *O. gratissimum,* and *O. basilicum* are medicinal plants extensively used in the traditional medicine of Kerala. The study is aimed at investigating the endophytic mycoflora associated with these *Ocimum* species and their ability to produce enzymes *in vitro*. A total of 149 fungal endophytes were isolated from roots, stems, and leaf segments from July to November 2021. They were grouped into 27 morphotypes, including five non-sporulating taxa. The highest number of isolates were obtained from the plant *O. basilicum*. An equally lower number of isolates were obtained from *O. gratissimum* and *O. tenuiflorum*. A greater number of fungal endophytes were obtained from the leaf segments of *O.basilicum* and least number of isolates obtained from the leaf segments of O.gratissimum. Isolates of *Aspergillus niger* complex, *Diaporthe* sp., and Daldinia *eschscholtzii* showed the highest colonizing frequency. *In vitro* analysis for enzyme production by all morphotypes was done and, except for laccase, all tested enzymes showed positive results.**

Keywords: Diversity; Enzyme Activity; *Ocimum* plants.

Fungal endophytes are a heterogeneous group of organisms, mainly belonging to Ascomycotina and Deuteromycotina. Petrini [1] defined endophytes as organisms which colonize symptomless in the internal tissues of host plant at some stages of their life cycle. The interaction between endophyte and host depends on the mode of infection and the defensive reaction of the host plant. The colonization could be local or systemic, inter or intracellular [2]. In endophytic association, the interaction between the host and fungal partner depends on three factors: the aggressiveness of fungi, the developmental stage of both host and fungi, and the host plant's susceptibility to pathogenicity. So that the symbiotic nature of endophytic relations is in a continual flux. They may enter into mutualistic symbiosis or pathogenicity [3] .

Fungal endophytes that were associated with their host plants influenced them. They help to increase fitness by conferring abiotic and biotic stress tolerance, reducing water consumption, increasing overall biomass, etc.[4] .The ability of endophytes to produce secondary metabolites and enzymes is directly correlated with their ecological importance. Endophytes produce xylan, pectin, peroxidases, laccases, cellulase, hemicellulose, and other enzymes necessary for effective colonization[5]. The structure and functional properties of bioactive secondary metabolites produced by fungal

*Corresponding author E-mail: rasmibotany@gmail.com

This is an Open Access article licensed under a Creative Commons license: Attribution 4.0 International (CC-BY).
Published by Oriental Scientific Publishing Company © 2022

endophytes were comparable to those of secondary metabolites produced by plants. As a result, endophyte-derived metabolites are widely studied[6]. Important compounds obtained from fungal endophytes include vincristin, podophyllotoxin, camptothecin, naphthodianthrones, and palcitaxol etc.[7,8,9,10,11].

The genus *Ocimum,* which has high therapeutic potential, belongs to Lamiaceae family. They are commonly used in traditional Ayurvedic and Unani medicine [12]. The high medicinal effect of *Ocimum* species is due to their richness in secondary metabolites, the majority of which are essential oils. *Ocimum* plants have been studied pharmacologically and have been found to have antiviral, larvicidal, antinociceptive, anti-inflammatory, antipyretic, antibacterial and antifungal activities. They are frequently employed in the treatment of blood related illnesses, respiratory conditions, feverish illness, nausea, migraine, abdominal cramps, gonorrhoea, dysentery, headache, dizziness, piles, cough, paralysis, nervous temperament, muscle cramps, insect bites and diabetes [13,14].

The variety of fungi that live inside the *Ocimum* species and their capacity for bio-prospecting have been the subject of extensive research in recent years. Numerous endophytic fungi, such as *Colletotrichum* sp., *Fusarium* sp., *Alternaria* sp., *Penicillium* sp., *Nigrospora* sp., etc., are frequently found in association with *Ocimum* plants [15,16,17]. Rajagopal et al. [17] reported a high number of fungal isolates from *O. basilicum* and *O. tenuiflorum*, representing greater diversity of their endophytes compared to other plants (*Coleus aromaticus* and *Tridax procumbens*), thus lending credence to our premise that these *Ocimum* species are rich in fungal endophytes and that it is important to investigate their biological potential.

In Kerala, *Ocimum tenuiflorum, Ocimum gratissimum*, and *Ocimum basilicum* are quite common and extensively dispersed. Although the diversity of endophytic fungi has been explored, there are no investigations on their capacity to produce extracellular enzymes. Endophytic fungus produces a variety of enzymes that have a significant impact on the food, pharmaceutical, and other industries. These enzymes are utilized in many different industries, including as pulp and paper production, textile production, brewing, laundry, and food processing. This study aims to describe the diversity of fungal endophytes present in the roots, stems, and leaf segments of *O. tenuiflorum, O. basilicum*, and *O. gratissimum.* Additionally, it evaluates their ability to produce extracellular enzymes including tyrosinase, amylase, cellulase, and asparaginase as well as other enzymes like protease and laccase.

MATERIAL AND METHODS

Sample collection

Roots, stem, and leaves of *O. tenuiflorum, O. basilicum,* and *O. gratissimum* plants were sampled for the isolation and investigation of endophytic fungal communities. The study was conducted during the period July to November 2021. Three randomly selected locations in the Palakkad district of Kerala, India (10°472 102 2 N, 76°392 62 2 E; 11°02 262 2 N, 76°232 572 2 E; and 10°472 352 2 N, 76°212 522 2 E) were sampled. From each site, a total of nine plants were collected (three plants of each species), and from each plant, three segments of root, stem, and leaves were used for isolation. All collected samples were kept in sterilized Ziplock plastic bags and placed into Potato Dextrose Agar (PDA) medium within six hours of being collected.

Surface sterilization and isolation

Before isolation, the samples were subjected to surface sterilization. The plant material was rinsed in running tap water to remove dust and debris for 2 to 3 minutes. After clear washing, the samples were cut into small pieces and further processing was done under aseptic conditions. surface sterilization is performed by the immersion of plant segments in sequence of ethanol, diluted sodium hypochlorite, and ethanol (each set of plant material was treated with 70% ethanol for 1 minute followed by immersion in 4% sodium hypochlorite for 3 minutes and again in 70% ethanol for 30 seconds). Lastly, the segments were rinsed three times with sterile (autoclaved) distilled water [5]. All surface-sterilized segments were dried on sterile blotting paper. After proper drying, each plant segment was placed onto sterilized PDA media supplemented with 50 mg/L ampicillin to prevent bacterial contamination. Petri plates were incubated for 4 to 7 days at 25±1°C. As fungal hyphae emerged, they were sub cultured and maintained

on freshly prepared PDA slants. Colonization frequency and isolation rate were also recorded using the following formula [18].

$$CF\% = [\text{ Number of segments colonized by the fungi/ Total number of segments observed }] \times 100$$

$$IR\% = [\text{ Number of isolates obtained from tissue segment / Total number of segments}] \times 100$$

Identification of fungal endophytes

All endophytic isolates obtained were characterized morphologically by analyzing the characteristics of the fungal colony and spores and the identification confirmed by available mycological literature [19,20,21].

Molecular analysis was performed to confirm identification of isolates showing the highest activity in each type of enzyme, we adapted the procedure of White et al.[22] with minor modification. The DNA was isolated by using the NucleoSpin® Plant II Kit (Macherey-Nagel). The sequencing reaction was done in a PCR thermal cycler (GeneAmp PCR System 9700, Applied Biosystems) using the Big Dye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, USA) following the manufacturer's protocol. The primers used were forward ITS1(F)-5'-TCCGTAGGTGAACCTGCGG-3' and reverse ITS-4(R)3'-TCCTCCGCTTATTGATATGC-5'. The Sequence Scanner Software V1 (Applied Biosystems) was used to check the sequence quality. Sequence alignment and required editing of the obtained sequences were carried out using Bioedit. Online BLAST searches were conducted on the GenBank sequence database (http://www.ncbi.nlm.nih.gov/BLAST/). The megablast ITS rDNA queries identified GenBank accessions with E-values of zero.

Enzyme Activity

All fungal endophytic morphotypes were inoculated with appropriate broth at 25±1 °C to analyse extracellular enzyme production. In this study, we considered only amylase, cellulase, protease, asparaginase, tyrosinase, and laccase for evaluating the enzyme production by fungal endophytes. The diameter of the hydrolysis and the fungal colony were measured after 5 to 7 days of incubation at 25±1 °C, and the enzyme index was calculated [23]. All experiments were done in triplicates.

$$\text{Enzyme index} = \text{Diameter of hydrolysis / Diameter of fungal colony.}$$

Asparaginase

The cultures were inoculated into a modified Czapex Dox agar medium containing 2.0 g/L glucose, 1.52 g/L KCl, 0.52 g/L $MgSO_4.7H_2O$, 0.001 g/L $ZnSO_4.7H_2O$, 0.001 g/L $FeSO_4.7H_2O$, and 16 g/L Agar with 10.0 g/L L-asparagine. Phenol red solution (3ml) were added to the media before sterilization. Asparaginase activity was indicated by the formation of a pink colour in colourless media [24].

Amylase

The cultures were inoculated on to autoclaved Glucose Yeast Extract Peptone (GYP) agar (1 g/L glucose, 0.1 g/L yeast extract, 0.5 g/L peptone, 16 g/L agar, pH 6) supplemented with 2% soluble starch to detect amylase activity. Following incubation, the plates were flooded with a freshly prepared iodine solution. A white or clear halo indicates amylase activity [25].

Cellulase

Cellulase activity was detected by inoculating each culture onto the GYP agar with 0.5% sodium carboxymethyl cellulose (CMC). After the incubation period, the cultures were flooded with 0.2% Congo red, washed with 1 M sodium chloride (NaCl), and allowed to stand for 15 min. The appearance of a yellow halo around the colony signifies that cellulase has been produced [25].

Laccase

Cultures were inoculated on to GYP agar medium with 0.005% of 1-naphthol. By laccase enzyme oxidation of 1-naphthol, colourless media will be changed to blue [26] .

Tyrosinase

Cultures were incubated with GYP medium to test for tyrosinase activity. The colony was overlaid with 0.11% p-cresol and 0.05% glycine and left for 24 hours. Positive tyrosinase activity can be detected by a reddish-brown zone surrounding the colony[25] .

Protease

The proteolytic activity of the isolates was determined by growing them on GYP medium

supplied with 0.4% gelatin. Separately, 8 g of gelatin in 100 mL of distilled water was added to the sterilized media [26]. Following incubation, the culture was flooded with saturated aqueous ammonium sulphate. Protease positive activity was observed around the colony in the form of a clear zone.

RESULTS AND DISCUSSION

A total of 149 endophytes were isolated from 243 segments of three *Ocimum* species studied. Among this isolates, 39 cultures were obtained from *O.tenuiflorum*, 71 fungal isolates from *O.basilicum* and 39 fungi were obtained from *O. gratissimum* (Table 1). The highest numbers of isolates were obtained from the leaves of *O. basilicum*, stems of *O. tenuiflorum* and roots of *O. gratissimum*. This suggests that the isolation rate is not purely dependent on the tissues but may be the function of fungal taxa involved. In contrast to this result, Banerjee et al.[27] showed that a high number of fungal endophytes were obtained from O. *tenuiflorum* as compared to *O. basilicum.*

Based on morphological features such as colony characters and conidial and spore characters, all 149 fungal isolates were separated and grouped into 27 morphotypes, with 22 sporulating and 5 non-sporulating cultures. Among them, five morphotypes belonged to Xylariales and Hypocreales each, three morphotypes belonged to Eurotiales, Diaporthales, and Botryospheriales each, two morphotypes come under the order Pleosporales and Glomerellales with one morphotype. The highest number of non-sporulating isolates was obtained from the stem segments of *O. tenuiflorum*. From *O. basilicum*, only a non-sporulating isolates was obtained, which was isolated from leaf segments, and from *O. gratissimum*, also only one isolate was obtained and it was recovered from both root and stem segments.

The highest colonization frequency was obtained from *Aspergillus niger* complex with 44.4%, which was isolated from all host plant and root and stem segments. *Diaporthe* sp. (culture code: MBLC2), showed 25.9% of colonization frequency which also had occurrence in all host plants, but they were obtained only from leaf segments. The third highest colonization frequency (18.5%) was reported with *Daldinia eschscholtzii* obtained from the stem segments of *O. gratissimum* (Table. 2). Earlier studies reported that, species of *Aspergillus* and *Diaporthe* were the most dominant endophytes, with a high rate of colonization [16,28,29] .Present study also reflect the same, along with *Daldinia eschscholtzii.* Isolates of *Aspergillus niger* complex showed more dominance over the others and they isolated from stem and root segments of all host plants under study. *Aspergillu*s sp. are well known for their pathogenicity [30], but previous studies revealed that, *Aspergillus* sp. can occur as endophytes with high bioprospecting ability [31,32,33].

Prior studies on endophytic fungi have revealed their affinity for particular tissues, demonstrating how well-adapted they are to tissues [16,34]. Tissue base specificity was also observed in root stem and leaf segments of *O. tenuiflorum, O. basilicum,* and *O. gratissimum*. *Neopestalotiopsis, D.liquidambaris, Ectophoma multirostrata, Trichoderma amazonicum complex,* and *F.solani complex* and *A.flavus complex* were isolated only from root segments. *Diaporthe sp., Simplicillium obclavatum,* and *Daldinia eschscholtzii* and three non - sporulating morphotypes were purely colonized on stem segments, and *Nigrospora oryzae, Pestalotiopsis microspora ,Nodulisporium gregarium, Lasiodoplodia citricola* and a non -sporulating morphotype were obtained from leaf segments only. Along with the tissue specificity, endophytes showed host preference. *F. incarnatum complex* and a *Fusarium* sp. were isolated from stem segments and *M. phaseolina* was isolated

Table 1. Total number of fungal isolates obtained from *Ocimum tenuiflorum, O,gratissimum* and *O.basilicum*

Host plant	Root segment	Stem segment	Leaf segment
O.tenuiflorum	12	14	13
O. gratissimum	18	15	6
O.basilicum	18	22	31

Table 2. Details of morphotypes obtained from *Ocimum* species

Order	Name code		Colonization frequency (CF) (%)									
			O.tenuiflorum		*O.basilicum*			*O.gratissimum*			Total	
			Root	Stem	Leaves	Root	Stem	Leaves	Root	Stem	CF	
Xylariales	PSRC2	*Neopestalotiopsis sp.*	14.8	-	-	-	-	-	-	-	-	14.8
	PSLC1	*Nigrospora oryzae* (Berk. & Broome) Petch	-	-	11.1	-	-	-	-	-	-	11.1
	PSLC2	*Nodulisporium gregarium* (Berk. & M.A. Curtis) J.A. Mey.	-	-	11.1	-	-	-	-	-	-	11.1
	PGSC1	*Daldinia eschscholtzii* (Ehrenb.) Rehm	-	-	-	-	-	-	-	18.5		18.5
	PBLC4	*Pestalotiopsis microspora* (Speg.) G.C. Zhao & N. Li	-	-	-	-		3.7	-	-	-	3.7
Hypocreales	PBSC3	*Fusarium incarnatum complex*	-	7.4	-	-	7.4	-	-	-	-	7.4
	PSSC5	*Fusarium sp.*	-	3.7	-	-	3.7	-	-	-	-	7.4
	PGRC2	*Fusarium solani complex*	-	-	-	-	-	-	7.4	-	-	7.4
	MBSC2	*Simplicillium obclavatum* (W. Gams) W. Gams	-	-	-	-	11.1	-	-	-	-	11.1
	MBRC1	*Trichoderma amazonicum complex*	-	-	-	14.8	-	-	-	-	-	14.8
Eurotiales	PBRC2	*Aspergillus niger complex*	-	18.5	-	18.5	-	-	7.4	-	-	44.4
	MGSC1	*Penicillium cataractarum complex*	-	-	-	-	-	-	3.7	3.7	3.7	11.1
	PBRC1	*Aspergillus flavus complex*	-	-	-	3.7	-	-	-	-	-	3.7
Diaporthales	MBLC2	*Diaporthe sp.*	-	-	11.1	-	-	11.1	-	-	3.7	25.9
	PSSC3	*Diaporthe liquidambaris* (C.Q. Chang, Z.D. Jiang & P.K. Chi) Udayanga & Castl.	3.7	-	-	-	-	-	-	-	-	3.7
	PSSC7	*Diaporthe sp.*		3.7	-	-	-	-	-	-	-	3.7
Botryosphaeriales	MGRC2	*Macrophomina phaseolina* (Tassi) Goid.	7.4	-	-	3.7		-	-	-	-	11.1
	MGLC1	*Lasiodiplodia citricola* Abdollahz., Javadi & A.J.L. Phillips		-	-			-	-	-	11.1	11.1
	LT	*Lasiodiplodia theobromae* (Pat.) Griffon & Maubl.	3.7	3.7	3.7	-	-	-	-	-	-	11.1
Pleosporales	PSRC3	*Ectophoma multirostrata* (P.N. Mathur, S.K. Menon & Thirum.) Valenz.-Lopez, J.F. Cano, Crous, Guarro & Stchigel	3.7	-	-	-	-	-	-	-	-	3.7
	PSRC4	*Paraphoma radicina* (McAlpine) Morgan-Jones & J.F. White	7.4	7.4	-	-	-	-	-	-	-	11.1
Glomerellales	MBLC1	*Colletotrichum orbiculare complex*	-	-	-	3.7	3.7	3.7	-	-	-	11.1
	PBLC1	*Non sporulating*	-	-	-	-	-	11.1	-	-	-	11.1
	PGRC4	*Non sporulating*	-	-	-	-	-		11.1	3.7	-	14.8
	PSSC6	*Non sporulating*		3.7	-	-	-	-	-	-	-	3.7
	PSSC2	*Non sporulating*	-	3.7	-	-	-	-	-	-	-	3.7
	PSSC1	*Non sporulating*	-	14.8	-	-	-	-	-	-	-	14.8

from root segments of *O. tenuiflorum* and *O. basilicum*. *Penicillium cataractarum* complex is obtained only from *O.gratissimum*, but *L. theobromae* were isolated from all segments of *O. tenuiflorum* and *C. orbicularae* complex from *O. basilicum*. root and stem segments of *O. tenuiflorum* yielded *P.radicina* while *O. gratissimum* root and stem segments yielded a non-sporulating isolate. Fungi, however, including *A. niger* complex and *Diaporthe* sp. (MBLC2), showed occurrence in all host plants. Among the total isolates, *S. obclavatum*, *N. gregarium*, *P. radicina*, *D. eschscholtzii, Pestalotiopsis microspora*, *E. multirostrata*, and *Trichoderma amazonicum* complex were reported new to *Ocimum* sp., whereas species of *Colletotrichum*, *Penicillium*, *Aspergillus*, *Nigrospora*, *Fusarium*, *Macrophomina*, *Diaporthe*, and *Lasiodiplodia* were already reported species from *Ocimum* plants [35,36,37,16].

Enzyme activity

The extracellular production of amylase, cellulase, laccase, tyrosinase, protease, and asparaginase was screened for all isolated endophytic fungi. Among the 27 isolates, 17 cultures were positive for any of the tested

Table 3. Enzyme activity of endophytic fungi from *Ocimum* species

Name	Enzyme index					
	Amylase	Cellulase	Protease	Tyrosinase	Asparaginase*	Laccase
Pestalotiopsis microspora	1.12	-	-	-	-	-
Aspergillus flavus complex	1.06	-	-	-	-	-
Non sporulating (PSSC1)	1.09	-	1.06	-	-	-
Diaporthe sp. (MBLC2)	1.07	-	-	-	-	-
Penicillium cataractarum complex	1.09	-	1.13	-	-	-
Diaporthe sps. (PSSC7)	-	1.1	-	1.11	-	-
Aspergillus niger complex	-	1.1	-	-	-	-
Simplicillium obclavatum	-	1.16	-	-	-	-
Colletotrichum orbiculare complex	-	1.1	-	-	-	-
Macrophomina phaseolina	-	1.06	-	-	-	-
Diaporthe liquidambaris	-	1.1	-	-	-	-
Paraphoma radicina	-	1.06	-	-	-	-
Fusarium sp. (PSSC5)	-	1.1	-	-	-	-
Non sporulating (PSSC6)	-	1.05	-	-	-	-
Fusarium solani complex	-	-	-	1.16	-	-
Lasiodiplodia citricola	-	-	-	-	+++	-
Lasiodiplodia theobromae	-	-	-	-	++	-

* - for asparaginase production, the colour of the media changes from yellow to pink, +++ indicates the pinkish colour intensity which means they are potentail producer of asparagianse

Table 4. Molecularly identified morphotypes with accession number

Culture Number	Identified Name	Accession Number	Reference accession number	Percentage of Similarity	Reference
MBSC2	*Simplicillium obclavatum* (W. Gams) W. Gams	ON344838.1	MT487854.1	100 %	Unpublished
MGLC1	*Lasiodiplodia citricola* Abdollahz., Javadi & A.J.L. Phillips	ON319006.1	MT587428.1	100 %	Zhang et al. [50]
MGSC1	*Penicillium cataractarum complex*	ON319003.1	MK534497.1	99.77 %	Unpublished
PBLC4	*Pestalotiopsis microspora* (Speg.) G.C. Zhao & N. Li	ON342908.1	ON796998.1	100 %	Unpublished
PGRC2	*Fusarium solani complex*	ON319007.1	MT293620.1	100 %	Unpublished

enzymes. Amylase activity was found in 18.5%of the tested isolates. A significant level of amylase activity was found in the isolate *Pestalotiopsis microspora* from the leaf of *O. basilicum* and in *A. flavus complex* and *Penicillium cataractarum complex.* Previous studies have reported *in vitro* extracellular amylase activity for *Aspergillus* spp*., Diaporthe spp., Penicillium* spp., and *Pestalotiopsis microspora* [38,39,40]. Nine of the tested cultures produced cellulase enzyme. The overall percentage of cellulase activity found was 33.3%. The highest activity was obtained from the fungus *S. obclavatum*, isolated from the stem segments of *O. basilicum.* Of all the isolates studied, 7.4% were found to be protease producers. The highest activity was obtained from *Penicillium cataractarum complex*, which was recovered from the stem segment of *O. gratissimum*. Studies by Bezerra et al.[41] also reported the protease production ability of *Penicillium* species as an endophyte. Two isolates were positive for tyrosinase. The percentage of positivity was 7.4%. The highest activity was shown by *F. solani complex* which was isolated from the root segment of *O. gratissimum*. In the case of asparaginase activity, the colour of the inoculated medium turned pinkish only in 7.4% of the isolates. Asparaginase was positive only for *Lasiodiplodia* sp. *L. citricola* showed higher activity than *L. theobromae* (Table. 3). Due to the fact that fungal asparaginase is produced extracellularly and is a very easy enzyme to purify, it has acquired considerable importance. According to other studies, *Alternaria* spp., *Aspergillus* spp*., Cylindrocarpon* spp*., Mucor* spp*., Fusarium* spp*., Penicillium* spp., and *Cladosporium* spp. are the most common asparaginase-producing fungi [42,43,44].

No enzyme activity was detected at *Neopestalotiopsis* sp*., T. amazonicum complex, D. eschscholtzii, N. oryzae, N. gregarium, F. incarnatum complex, E. multirostrata, A. niger complex* and non-sporulating isolates PBLC1, PSSC2 and PGRC4. Laccase enzyme activity was not reported. Previous studies reported the laccase producing ability of the taxon *Trichoderma* [45, 46,47]. It has been reported that *Trichoderma harzianum* can produce laccase enzyme by using wheat bran under solid state fermentation and by response surface approach[48,49]. In contrast to previous results, *Trichoderma* associated with *Ocimum* spp. does not show any laccase activity.

Based on enzyme activity, five cultures namely *S. obclavatum, L. citricola, Penicillium cataractarum complex, Pestalotiopsis microspora* and *F. solani complex* were showed higher activity for cellulase, asparaginase, amylase, protease and tyrosinase respectively were selected and they were characterized molecularly (Table.4)

CONCLUSION

In this study, 27 endophytic fungi were identified from *Ocimum tenuiflorum*, *O. gratissimum*, and *O. basilicum*, including five non-sporulating. These fungi were able to produce extracellular enzymes such as amylase, cellulase, tyrosinase, protease, and asparaginase. The study emphasizes the importance of fungal endophytes of *Ocimum* plants and their role in extracellular enzyme production. However, our study is preliminary and by intensive analyzing with different growth media and sterilization protocol, more diversity of endophytic mycobiota of the genus *Ocimum* may be detected. Advanced studies on enzyme production and quantification are also important to assess the potential significance of these endophytes.

ACKNOWLEDGMENT

The authors are grateful to the University Grand Commission for Junior Research Fellowship for financial support and the Post Graduate & Research Dept. of Botany, Government Victoria College, Rajiv Gandhi Centre for Biotechnology (RGCB), Thiruvananthapuram-Kerala, Omics Gen Life Science Pvt. Ltd., and National Fungal Culture Collection of India (NFCCI)- Agharkar Research Institute (ARI) Pune for the lab facility.

Conflict of Interest

There is no conflict of interest.

Funding Sources

There is no finding sources.

REFERENCES

1. Petrini O, Fungal endophytes of tree leaves, In Microbial ecology of leaves Springer (1991); pp. 179–197.
2. Boyle, C., Gotz, M., Dammann-Tugend, U., Schulz, B, Endophyte-host interactions III. Local vs. Systemic colonization, *Symbiosis* (2001);

31(4):259-281.

3. Schulz, B., Guske, S., Dammann, U, Endophyte-host interactions. II. Defining symbiosis of the endophyte-host interaction, Symbiosis (1998);25:213-227.
4. Rodriguez, R. J., White Jr, J. F., Arnold, A. E., Redman, A. R. A, Fungal endophytes: diversity and functional roles, New phytologist (2009); 182(2): 314-330.
5. Petrini, O., Sieber, T. N., Toti, L., Viret, O, Ecology, metabolite production, and substrate utilization in endophytic fungi, Natural Toxins (1993); 1(3): 185–196.
6. Aly, A. H., Debbab, A., Kjer J., Proksch P. Fungal endophytes from higher plants: A prolific source of phytochemicals and other bioactive natural products, Fungal Diversity (2010); 41(1): 1–16.
7. Amna, T., Puri, S. C., Verma, V., Sharma, J. P., Khajuria, R. K., Musarrat, J., Spiteller, M., Qazi, G. N. Bioreactor studies on the endophytic fungus *Entrophospora infrequens* for the production of an anticancer alkaloid camptothecin, Canadian Journal of Microiology (2006); *52*(3) :189–196.
8. Lingqi, Z., Bo, G., Haiyan, L., Songrong, Z., Hua, S., Su, G., Rongcheng, W, Preliminary study on the isolation of endophytic fungus of *Catharanthus roseus* and its fermentation to produce products of therapeutic value Zhong Cao Yao, Chinese Traditional and Herbal Drugs (2000); 31(11): 805–807.
9. Puri, S. C., Nazir, A., Chawla, R., Arora, R., Riyaz-Ul-Hasan, S., Amna, T., Ahmed, B., Verma, V., Singh, S., Sagar, R, The endophytic fungus *Trametes hirsuta* as a novel alternative source of podophyllotoxin and related aryl tetralin lignans, Journal of Biotechnology (2006); 122(4): 494–510.
10. Strobel, G. A., Torczynski, R., Bollon, A, *Acremonium* sp.—A leucinostatin A producing endophyte of European yew (*Taxus baccata*), Plant Science (1997); 128(1): 97–108. https://doi.org/10.1016/S0168-9452(97)00131
11. Strobel, G., Yang, X., Sears, J., Kramer, R., Sidhu, R. S., Hess, W. M, Taxol from Pestalotiopsis microspora, an endophytic fungus of *Taxus wallachiana*, Microbiology (1996); 142(2): 435–44
12. Prakash, P.& Gupta, N, Therapeutic uses of *Ocimum sanctum* Linn (Tulsi) with a note on eugenol and its pharmacological actions: A short review, Indian Journal of Physiology and Pharmacology (2005); 49(2): 125.
13. Ahmed, D., Aujla, M. I., *Ocimum basilicum*: A review on phytochemical and pharmacological studies, Pakistan Journal of Chemistry (2012); 2: 78–85.
14. Bunrathep, S., Palanuvej, C., Ruangrungsi, N, Chemical compositions and antioxidative activities of essential oils from four *Ocimum* species endemic to Thailand, Journal of Health Research (2007); 21(3): 201–206.
15. Atiphasaworn, P., Monggoot, S., Gentekaki, E., Brooks, S., Pripdeevech, P. Antibacterial and antioxidant constituents of extracts of endophytic fungi isolated from *Ocimum basilicum var. Thyrsiflora* leaves, Current Microbiology (2017); 74(10): 1185–1193.
16. Chowdhary K.& Kaushik N, Fungal endophyte diversity and bioactivity in the Indian medicinal plant *Ocimum sanctum* Linn, Public Library of Science ONE (2015); 10 (11). e0141444. https://doi.org/10.1371/journal.pone.0141444
17. Rajagopal, K., Kalavathy, S., Kokila, S., Karthikeyan, S., Kathiravan, G., Prasad, R., Balasubraminan, P, Diversity of fungal endophytes in few medicinal herbs of South India, Asian Journal of Experimental sciences (2010); 1(2): 415–418.
18. Petrini, O., Fisher, P. J, Fungal endophytes in *Salicornia perennis*, Transactions of the British Mycological Society (1986); 87(4): 647–651.
19. Barnet, H. L.& Hunter B. B, Illustrated genera of imperfect fungi 4, The American Phyto pathological Society Press. St. Paul, Minnesota (1998); 218.
20. Subramanian, C. V, Hyphomycetes. Taxonomy and Biology, Hyphomycetes. Taxonomy and Biology (1983);502.
21. Sutton, B. C, The Coelomycetes. Fungi imperfecti with pycnidia, acervuli and stromata., Commonwealth Mycological Institute (1980); 696
22. White, T. J., Bruns, T., Lee, S. J. W. T., Taylor, J, Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. PCR protocols: a guide to methods and applications, Academic Press, San Diego (1990);18 (1): 315-322.
23. Florencio, C., Couri, S., Farinas, C. S, Correlation between agar plate screening and solid-state fermentation for the prediction of cellulase production by Trichoderma strains, Enzyme Research (2012): 1-7.
24. Theantana, T., Hyde, K. D., Lumyong, S, Asparaginase production by endophytic fungi isolated from some Thai medicinal plants, Current Applied Science and Technology (2007); 7(1–1): 13–18.
25. Maria, G. L., Sridhar, K. R., Raviraja, N. S, Antimicrobial and enzyme activity of mangrove endophytic fungi of southwest coast of India, Journal of Agricultural Technology (2005); 1

(1): 67-80.

26. Sunitha, V. H., Devi, D. N., Srinivas, C, Extracellular enzymatic activity of endophytic fungal strains isolated from medicinal plants, World Journal of Agricultural Sciences (2013); 9 (1): 01-09.
27. Banerjee, D., Manna, S., Mahapatra, S., Pati B, Fungal endophytes in three medicinal plants of Lamiaceae, Acta microbiologica et immunologica Hungarica (2009); 56 (3) 243-250.
28. Rashmi, M., Kushveer, J. S., Sarma, V. V, A worldwide list of endophytic fungi with notes on ecology and diversity, Mycosphere (2019); 10 (1): 798-1079.
29. Ilyas, M., Kanti, A., Jamal, Y., Hertina, H., Agusta, A, Biodiversity of endophytic fungi associated with *Uncaria gambier* Roxb. (Rubiaceae) from West Sumatra, Biodiversitas Journal of Biological Diversity (2009); 23-28: 10(1).
30. Hocking, A. D, *Aspergillus* and related teleomorphs, Food spoilage microorganisms (2006); 451-487.
31. Maamoun, H. S., Rabie, G. H., Shaker, I., Alaidaroo, B. A., El-Sayed, A. S, Biochemical properties of tyrosinase from *Aspergillus terreus* and *Penicillium copticola;* undecanoic acid from *Aspergillus flavus*, an endophyte of *Moringa oleifera*, is a novel potent tyrosinase inhibitor, Molecules (2021); 26 (5).
32. Toghueo, R. M. K, Endophytes from *Gingko biloba*: The current status, Phytochemistry Reviews (2020); 19(4): 743–759.
33. Waqas, M., Khan, A. L., Kamran, M., Hamayun, M., Kang, S. M., Kim,Y.H., Lee, I. J, Endophytic fungi produce gibberellins and indoleacetic acid and promotes host-plant growth during stress, Molecules (2012); 17(9): 10754–10773.
34. Huang, W.Y., Cai, Y. Z., Hyde, K., Corke, H., Sun, M, Biodiversity of endophytic fungi associated with 29 traditional Chinese medicinal plants, Fungal Diversit*y* (2008); *33*: 75.
35. Shoeb, M., Hoque, M. E., Thoo-Lin, P. K., Nahar, N, Anti-pancreatic cancer potential of secalonic acid derivatives from endophytic fungi isolated from *Ocimum basilicum*, Dhaka University Journal of Pharmaceutical Sciences (2013); 12 (2): 91–95.
36. Shukla, S. T., Kulkarni, V. H., Habbu, P. V., Jagadeesh, K. S., Patil, B. S., Smita, D. M, Hepatoprotective and antioxidant activities of crude fractions of endophytic fungi of *Ocimum sanctum* Linn. in rats, Oriental Pharmacy and Experimental Medicine (2012); 12 (2): 81-91.
37. Bodhankar, M. G, Antimicrobial activity of endophytic fungi isolated from *Ocimum sanctum* Linn, American International Journal of Contemporary Scientific Research (2014); 1 (3): 17–22.
38. Toghueo, R. M. K., Zabalgogeazcoa, I., De Aldana, B. V., Boyom, F. F, Enzymatic activity of endophytic fungi from the medicinal plants *Terminalia catappa*, *Terminalia mantaly* and *Cananga odorata*, South African Journal of Botany (2017); 109: 146-153.
39. Yasser, M. M., Mousa, A. S. M., Marzouk, M. A., Tagyan, A. I, Molecular identification, extracellular enzyme production and antimicrobial activity of endophytic fungi isolated from *Solanum tuberosum* L. Egypt, Biosciences Biotechnology Research Asia; Bhopal (2019);16 (2): 135-14.
40. Joel, E. L.& Bhimba, B. V, Production of alpha amylase by mangrove associated fungi *Pestalotiopsis microspora* strain VB5 and *Aspergillus oryzae* strain VB6, Indian Journal of Geo -marine Science (2012); 41 (3): 279-283.
41. Bezerra, V. H. S., Cardoso, S. L., Fonseca-Bazzo, Y., Silveira, D., Magalhães, P. O., Souza, P. M, Protease produced by endophytic fungi: A systematic review, Molecules (2021); 26 (22): 7062.
42. Krishnapura, P. R., Belur, P. D., Subramanya, S, A critical review on properties and applications of microbial L-asparaginases, Critical Reviews in Microbiology (2016); 42(5): 720-737.
43. Moubasher, H. A., Balbool, B. A., Helmy, Y. A., Alsuhaibani, A. M., Atta, A. A., Sheir, D. H., Abdel-Azeem, A. M, Insights into Asparaginase from Endophytic Fungus *Lasiodiplodia theobromae*: Purification, Characterization and Antileukemic Activity, International Journal of Environmental Research and Public Health (2022);19 (2): 3-14.
44. Lee, J. M., Tan, W. S., Ting, A. S, Revealing the antimicrobial and enzymatic potentials of culturable fungal endophytes from tropical pitcher plants (*Nepenthes* spp.), Mycosphere (2014); 5 (2): 364-377.
45. Chakroun, H., Mechichi, T., Martinez, M. J., Dhouib, A., Sayadi, S, Purification and characterization of a novel laccase from the ascomycete *Trichoderma atroviride*: Application on bioremediation of phenolic compounds, Process Biochemistry (2010); 45(4): 507–513.
46. Gochev, V. K.& Krastanov, A. I, Isolation of laccase producing *Trichoderma* spp,Bulgarian Journal of Agricultural Science (2007);13(2): 171.
47. Savoie, J.M., Mata, G., Billette, C, Extracellular laccase production during hyphal interactions

between *Trichoderma* sp. And Shiitake, Lentinula edodes, Applied Microbiology and Biotechnology (1998); 49(5): 589–593.

48. Gao, H., Chu, X., Wang, Y., Zhou, F., Zhao, K., Mu, Z., Liu Q, Media optimization for laccase production by *Trichoderma harzianum* ZF-2 using response surface methodology, Journal of Microbiology and Biotechnology (2013); 23(12): 1757–1764.
49. Bagewadi, Z. K., Mulla, S. I., Ninnekar, H. Z. Purification and immobilization of laccase from *Trichoderma harzianum* strain HZN10 and its application in dye decolorization, Journal of Genetic Engineering and Biotechnology (2017); 15(1): 139–150.
50. Zhang, W., Groenewald, J. Z., Lombard, L., Schumacher, R. K., Phillips, A. J. L., Crous, P. W, Evaluating species in Botryosphaeriales, Persoonia-Molecular Phylogeny and Evolution of Fungi (2021); 46(1): 63-115.