Repository of fungal endophytes at VINSTROM, Chennai: waiting to be harnessed

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Our thirty years of research at Vivekananda Institute of Tropical Mycology (VINSTROM) on fungal endophytes of terrestrial and aquatic plants have yielded many interesting insights into this ecological group of fungi, a constituent of the plant microbiome. Having pioneered the study of fungal endophytes in India, we have established a culture collection of these fungi in VINSTROM. It now has about 1700 fungal endophyte isolates obtained from plants of different ecosystems in different parts of India. Endophytes produce an extraordinary array of bioactive compounds, novel industrial enzymes and enhance the fitness of the plants they colonize by elevating their tolerance to abiotic and biotic stressors. Considering such desirable traits of fungal endophytes, VINSTROM's culture collection is a unique repository that needs to be explored for the technological use of endophytes.

Keywords: Bioprospecting, bioactive compounds, culture collection, industrial enzymes.

ENDOPHYTES are bacteria and fungi which reside inside plant tissues[1]. They include mutualists, commensals, temporary residents, latent pathogens and saprotrophs[2]. Of the fungal endophytes (FE) that are horizontally transmitted, those belonging to the phylum Ascomycota predominate and are associated with terrestrial and aquatic plants (freshwater and marine) as well as with lichens. Although all the fungi that grow out from surface-sterilized plant tissues qualify to be termed endophytes, this definition has to be broadened when the existence of non-cultivable species is considered[3]. FE, whose association with plants dates back to 400 million years[4], are a vital part of a plant microbiome. FE have gained attention in recent years due to their ability to enhance the resistance of their host plants to biotic[5,6] and abiotic stressors[7], and to produce novel bioactive compounds[8] and industrial enzymes[9].

Fungal endophytes as a research focus

Although known for more than a hundred years, FE drew the attention of mycologists only in the last four decades mainly owing to the expectation that as an ecological guild, they may be harbouring many hitherto unknown species. Three decades ago, the existing fungal species was predicted to be 1.5 million even though only 74,000 had been identified[10]. FE of tropical plants were believed to be hyperdiverse and thus a reliable surrogate to reflect global fungal diversity[11–15]. This motivated us to survey endophytes residing in the leaves of mangroves[16] and trees of different forest types of the Western Ghats[17]. However, after studying more than 200 plant species, it turned out that although no plant could be free of FE, the species diversity of these fungi is limited due to the wide plant host range of a few fungal genera[18,19]. During these studies, we also showed that phytophagous insects disperse endophyte inoculum[20] and resident endophytes in a plant tissue deter infection by alien endophytes due to their higher competitive ability[21].

Table 1. Endophytic fungal isolates in VINSTROM's culture collection

Genus	No. of isolates in culture
Alternaria	36
Aspergillus	57
Aureobasidium	18
Chaetomium	63
Cladosporium	39
Colletotrichum	169
Corynespora	18
Curvularia	40
Drechslera	14
Fusarium	111
Glomerella	21
Humicola	14
Lasiodiplodia	51
Nigrospora	33
Nodulisporium	40
Penicillium	38
Periconia	17
Pestalotiopsis	57
Phomopsis	172
Phyllosticta	79
Sordaria	17
Sporormiella	14
Trichoderma	54
Xylaria	165
Sterile forms	95
Genera whose isolates are < 10 in number	300
Total	1732

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Table 2. VINSTROM's endophytic fungi and gene sequences deposited in national culture collection facilities and GenBank

Fungus	Deposition number	GenBank accession number	Trait(s)
Acremonium sp.	NFCCI 2443		Acetylcholinesterase inhibitor[44]; anti-algal[44]; anti-bacterial[44]; anti-fungal[44]; anti-oxidant[44]; insecticidal[44]
Alternaria sp.		KJ398150	Furaldehyde utilization[45]
Aspergillus amstelodami	NFCCI 2439		Anti-bacterial[44]; anti-oxidant[44]
Aspergillus terreus	MTCC 10346		Anti-algal[24]; anti-bacterial[24]; anti-cancer[46]; anti-fungal[24]; anti-malarial[47]; cellulase[#]; insecticidal[24]
Aspergillus terreus	NFCCI 2444		Acetylcholinesterase inhibitor[44]; anti-algal[44]; anti-bacterial[44]; anti-fungal[44]; anti-oxidant[44]; insecticidal[44]
Aspergillus terreus		KP122964	β-glucosidase[#]; cellulase[#]; xylanase[48]; xylosidase[48]
Bartalinia sp.	NFCCI 2307	HQ909075	Thermotolerant spore[49]
Botrytis cinerea	MTCC 8659		
Chaetomella raphigera	NFCCI 2308	HQ909076	Thermotolerant spore[49]
Chaetomium globosum		KF135624	Anti-algal[24]; anti-malarial[47]; insecticidal[24]
Chaetomium sp.	MTCC 8385		Anti-cancer[33]
Chaetomium sp.	NFCCI 2437		
Chaetomium sp.	MTCC 8658		Anti-bacterial[#]
Chaetomium spirochaete		KF135618	Anti-malarial[47]
Colletotrichum gliosporiodes	MTCC 8460		
Colletotrichum sp.	MTCC 10339		Anti-malarial[47]
Colletotrichum sp.	MTCC 8480		
Corynespora sp.	MTCC 8466		
Corynespora sp.	MTCC 8467		
Curvularia lunata	MTCC 10334		Anti-malarial[47]
Curvularia sp.		KF135619	Anti-malarial[47]; proteases[50]
Curvularia sp.	MTCC 10337		Anti-bacterial[24]; anti-malarial[47]
Curvularia sp.	NFCCI 2311	HQ909079	Thermotolerant spore[49]
Curvularia tuberculata	MTCC 10340		Anti-cancer[46]; anti-malarial[47]; cellulase[#]
Curvularia tuberculata	MTCC 9394		
Curvularia tuberculata	NFCCI 2434	KP122965	Anti-algal[#]; anti-bacterial[51]; anti-oxidant[51]; β-glucosidase[#]; cellulase[#]; insecticidal[#]; novel curvularin[#]; xylanase[48]; xylosidase[48]
Diaporothe pseudomangiferae	NFCCI 4403	MH371242	
Diaporothe pseudomangiferae	NFCCI 4404	MH371241	
Diaporothe pseudomangiferae	NFCCI 4405	MH371244	
Diaporthe discoidispora	NFCCI 4408	MH371247	
Diaporthe discoidispora	NFCCI 4410	MH371249	
Diaporthe discoidispora	NFCCI 4414	MH371253	
Diaporthe eucalyptorum	NFCCI 4411	MH371250	
Diaporthe hongkongensis	NFCCI 4412	MH371251	
Diaporthe hongkongensis	NFCCI 4413	MH371252	
Diaporthe kyushuensis	NFCCI 4406	MH371245	
Diaporthe liquidambaris	NFCCI 4415	MH371255	
Diaporthe longicolla	NFCCI 4402	MH371243	
Diaporthe perseae	NFCCI 4416	MH371254	
Diaporthe pseudomangiferae	NFCCI 4409	MH371248	
Diaporthe tectonae	NFCCI 4417	MH371256	
Drechslera papendorphii	MTCC 10338		Anti-malarial[47]; cellulase[#]
Drechslera sp.	NFCCI 2442		Anti-algal[44]; anti-bacterial[44]; anti-fungal[44]; insecticidal[44]
Exserohilum rostratum	NFCCI 2312	HQ909080	Thermotolerant spore[49]
Fusarium mangiferae	NFCCI 3032		Mycotoxin[#]
Fusarium oxysporum		KF135622	Anti-algal[24]; anti-bacterial[24]; anti-malarial[47]; xylanase[48]
Fusarium pallidoroseum	NFCCI 3031		Mycotoxin[#]
Fusarium sp.	MTCC 8461		
Fusarium sp.	MTCC 8666		
Fusarium sp.	NFCCI 2436		Anti-algal[44]; anti-bacterial[44]; anti-fungal[44]
Hypoxylon sp.		HQ435664	Anti-algal[52]; anti-bacterial[52]; anti-fungal[52]; enzymes[52]

(*Contd*)

Table 2. (*Contd*)

Fungus	Deposition number	GenBank accession number	Trait(s)
Lasiodiplodia theobromae	MTCC 10347		Chitin modifying enzymes[53]; weedicidal[19]
Lasiodiplodia theobromae	NFCCI 2435		Anti-bacterial[44]
Leptosphaerulina crassiasca	MTCC 6977		
Leptosphaerulina sp.		HQ909074	Thermotolerant spore[49]
Neopestalotiopsis australis		KT589411	
Neopestalotiopsis cubana		KT589402	
Neopestalotiopsis cubana		KT589406	
Neopestalotiopsis cubana		KT589419	
Neopestalotiopsis ellipsospora		KT589414	
Neopestalotiopsis mesopotamica		KT589400	
Neopestalotiopsis mesopotamica		KT589407	
Neopestalotiopsis mesopotamica		KT589412	
Neopestalotiopsis piceana		KT589404	
Neopestalotiopsis saprophytica		KT589408	
Nigrospora oryzae	MTCC 10348		Anti-cancer[33]
Nigrospora oryzae	MTCC 8465		
Nigrospora oryzae	MTCC 8660	KF135620	Anti-malarial[47]
Nodulisporium sp.	MTCC 8665		
Penicillium sp.	MTCC 8664		
Penicillium sp.	NFCCI 2445		Anti-bacterial[44]; anti-oxidant[44]; insecticidal[44]
Periconia sp.	MTCC 8663		
Pestalotiopsis menezesiana		KT589405	
Pestalotiopsis microspora	NFCCI 2309	HQ909077	Thermotolerant spore[49]; furaldehyde utilization[45]
Pestalotiopsis microspora	NFCCI 2313	HQ909082	β-glucosidase[#]; cello biohydrolase[#]; thermotolerant spore[49]; weedicidal[#]
Pestalotiopsis microspora		KT589401	
Pestalotiopsis microspora		KT589403	
Pestalotiopsis microspora		KT589409	
Pestalotiopsis microspora		KT589410	
Pestalotiopsis microspora		KT589415	
Pestalotiopsis microspora		KT589416	
Pestalotiopsis microspora		KT589417	
Pestalotiopsis microspora		KT589418	
Pestalotiopsis microspora		KT589420	
Pestalotiopsis parva		KT589397	
Pestalotiopsis sp.	MTCC 10336	KY024221	β-glucosidase[#]; chitin deacetylase[#]; thermotolerant spore[#]; weedicidal[19]
Pestalotiopsis theae		KT589413	
Pestalotiopsis vismiae		KT589393	
Pestalotiopsis vismiae		KT589394	
Pestalotiopsis vismiae		KT589395	
Pestalotiopsis vismiae		KT589396	
Pestalotiopsis vismiae		KT589398	
Pestalotiopsis vismiae		KT589399	
Phoma sp.	NFCCI 2310	HQ909078	Thermotolerant spore[49]
Phomopsis heveicola	NFCCI 4407	MH371246	
Phomopsis sp.	MTCC 10345	KP204449	Anti-bacterial[24]; anti-malarial[47]; β-glucosidase[#]; xylanase[48]
Phomopsis sp.	MTCC 8464		
Phomopsis sp.		DQ 235667	
Phomopsis sp.		DQ 235668	
Phomopsis sp.		DQ 235669	
Phomopsis sp.		DQ 235670	
Phomopsis sp.		DQ 235671	
Phomopsis sp.		DQ 235672	
Phomopsis sp.		DQ 235673	
Phomopsis sp.		DQ 235674	
Phomopsis sp.		DQ 235675	
Phomopsis sp.		DQ 235676	
Phomopsis sp.		DQ 235677	
Phomopsis sp.		KJ398147	β-glucosidase[#]; furaldehyde utilization[45]

(*Contd*)

Table 2. (*Contd*)

Fungus	Deposition number	GenBank accession number	Trait(s)
Phomopsis sp.		KJ398149	Furaldehyde utilization[45]
Phyllosticta capitalensis	MTCC 10343		Chitosanase[53]
Phyllosticta capitalensis	MTCC 4651	AF532314	
Phyllosticta capitalensis	MTCC 4652		
Phyllosticta capitalensis	MTCC 4653		
Phyllosticta capitalensis	MTCC 4654		
Phyllosticta capitalensis	MTCC 4870		
Phyllosticta capitalensis	MTCC 4871		Melanin[54]
Phyllosticta capitalensis	MTCC 8468		
Pithomyces chartarum	MTCC 8657	KJ398148	Anti-malarial[47]; furaldehyde utilization[45]
Pithomyces sp.	MTCC 9398	HQ909081	Thermotolerant spore[49]
Sordaria sp.	MTCC 10342		Chitin modifying enzymes[53]
Spegazzinia sundara	MTCC 9597		Furaldehyde utilization[45]
Talaromyces stipitatus	NFCCI 4222	MG996147	Acetylcholinesterase inhibitor[#]; anti-algal[#]; anti-fungal[#]; anti-oxidant[#]; enzymes[#]; proteases[50]; salt tolerant chitinase[36]
Thielaviopsis sp.	NFCCI 2438		
Trichoderma harzianaum	MTCC 10344	KF135622	Anti-algal[24]; anti-bacterial[24]; anti-fungal[24]; anti-malarial[47]; Cellulase[#]; insecticidal[19]; ionic-liquid tolerant xylosidase[37]; salt-tolerant xylanase[48] and xylosidase[48]; thermotolerant spore[#]; weedicidal[19]
Trichoderma sp.	MTCC 8662		
Trichoderma sp.	NFCCI 2440		Anti-algal[44]; anti-bacterial[44]
Tritirachium	NFCCI 2441		Anti-bacterial[44]; insecticidal[44]
Xylaria psidii	MTCC 10840	HQ435673	Anti-algal[52]; anti-bacterial[52]; anti-fungal[52]; enzymes[52]
Xylaria sp.	MTCC 10736	HQ435661	Anti-algal[52]; anti-bacterial[52]; anti-fungal[52]; enzymes[52]
Xylaria feejeensis	MTCC10735	HQ435672	Anti-algal[52]; anti-bacterial[52]; anti-fungal[52]; enzymes[52]
Xylaria laevis		HQ435669	Anti-algal[52]; anti-bacterial[52]; anti-fungal[52]; enzymes[52]
Xylaria psidii	MTCC 10737	HQ435662	Anti-algal[52]; anti-bacterial[52]; anti-fungal[52]; anti-malarial[47]; enzymes[52]
Xylaria sp.	MTCC 10734	HQ435666	Anti-bacterial[52]; enzymes[52]
Xylaria sp.	MTCC 10838	HQ901081	Anti-algal[52]; anti-bacterial[52]; anti-fungal[52]; enzymes[52]
Xylaria sp.	MTCC 10839	HQ435659	Anti-algal[52]; anti-bacterial[52]; anti-fungal[52]; enzymes[52]
Xylaria sp.		HQ435660	Anti-algal[52]; anti-bacterial[52]; anti-fungal[52]; enzymes[52]
Xylaria sp.		HQ435663	Anti-algal[52]; anti-bacterial[52]; anti-fungal[52]; enzymes[52]
Xylaria sp.		HQ435670	Anti-algal[52]; anti-bacterial[52]; anti-fungal[52]; enzymes[52]
Xylaria venosula	MTCC 11040	HQ435671	Anti-algal[52]; anti-bacterial[52]; anti-fungal[52]; enzymes[52]

NFCCI, National Fungal Culture Collection of India, Pune; MTCC, Microbial Type Culture Collection and Gene Bank, Chandigarh; [#]unpublished.

FE from marine and non-angiosperm source

VINSTROM then proceeded to study FE assemblages of seagrasses including *Halophila ovalis*[22], *Cymodocea* spp., *Halodule* spp., *Syringodium* sp., *Enhalus acoroides* and *Thalassia* spp.[23], and various seaweeds such as green, brown and red algae[24,25]. We also surveyed lichens for their endophyte (endolichenic fungi) assemblages[26,27].

FE as a source of novel bioactive metabolites

Many studies attest to the extraordinary ability of FE to elaborate an impressive range of secondary metabolites possessing interesting bioactivities[28,29]. The synthetic ability of FE is more when compared to their conspecifics isolated from other ecological niches[30] and some of them even produce their plant host's secondary metabolites[31,32].

The bioactivities exhibited by the secondary metabolites of various FE include anti-bacterial/-fungal/-cancer agents[33], anti-plant pest metabolites and weedicides[19].

FE and industrial enzymes

FE also produce diverse plant biomass degrading enzymes aiding them to survive as pioneer plant litter degraders as they switch over to a saprotrophic mode of life in abscised plant parts[34]. They have been shown to produce amylase, cellulase, chitinase, laccase, lipase, protease and tyrosinase[35]. FE isolated from marine plants produce salt tolerant[36] and ionic liquid-tolerant enzymes[37]. Some FE also produce therapeutic enzymes possessing desirable qualities such as l-asparaginase enzyme devoid of glutaminase action for possible use in curing acute lymphoblastic leukaemia in children[38]. Despite their extraordinary species diversity, only five species of fungi such as *Aspergillus*, *Humicola*, *Penicillium*, *Rhizopus* and *Trichoderma* are being used as enzyme source in industries[39]. This underscores the need to explore FE for novel enzymes for industrial use.

FE culture collection

The need to explore and conserve fungi for technological applications, especially in India, cannot be overstressed[14,15]. VINSTROM now has about 1700 FE cultures including those isolated from the mangroves and marine algae of the Andaman Islands and the Tamil Nadu coast, trees of the dry deciduous, dry thorn, semi-evergreen and montane forests of the Western Ghats, orchids and bamboos of Arunachal Pradesh, and lichens of Uttarakhand and the Western Ghats. This FE culture collection is perhaps one of the largest collections in Asia (Table 1) and a few of these have been screened for different technological traits (Table 2). The strenuous task of maintaining a culture collection compounded by the requirement of space and labour was overcome by following the method of storing the cultures under water[40] or in Eppendorf tubes[41]. Apart from being the least expensive culture preservation method, preserving FE in water reduced the period required for sub-culturing and thus preserving their desirable traits and minimizing storage space.

FE are very attractive as candidates for technological exploitation due to their extraordinary ability to: (i) synthesize novel bioactive metabolites and enzymes, and (ii) increase the ecological fitness of their host plants[42]. Exploring them to better understand their interactions with their plant hosts/co-occurring microbes and with the environment[43] and mining them for exploitable traits has far-reaching economic potentials and would strengthen the bio-economy of the country. In this context, VINSTROM would be ready to share its FE cultures to institutions addressing such activities.

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ACKNOWLEDGEMENTS. I thank Dr V. Muruganandam, Dr J. P. Ravishankar, RKM Vidyapith, Chennai and all research collaborators for their unstinted support. I appreciate the efforts of my students which helped immensely in creating this genetic resource. I also thank the DBT, DST and MoEF&CC for funding various research projects without which this work would not have progressed.

Received 1 July 2019; accepted 24 July 2019

doi: 10.18520/cs/v117/i9/1469-1474