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Extracellular Enzymatic Activity of Endophytic Fungal Strains Isolated from Medicinal Plants

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Abstract: Endophytic fungi exhibit a complex web of interactions with host plants and have been extensively studied over the last several years as prolific sources of new bioactive natural products. Fungal enzymes are one of them which are used in food, beverages, confectionaries, textiles and leather industries to simplify the processing of raw materials. They are often more stable than enzymes derived from other sources. Enzymes of the endophytes are degraders of the polysaccharides available in the host plants. The use of simpler solid media permits the rapid screening of large populations of fungi for the presence or absence of specific enzymes. Fifty fungal strains, isolated from medicinal plants (*Alpinia calcarata*, *Bixa orellana, Calophyllum inophyllum* and *Catharanthus roseus*) were screened for extracellular enzymes such as amylase, cellulase, laccase, lipase, pectinase and protease on solid media. Sixty four percent of fungi screened for enzymes showed positive for lipase, 62% for amylase and pectinase, 50% showed for lipase, 32% showed for cellulase, 30% for laccase and only 28% showed positive for protease. The array of enzymes produced differs between fungi and often depends on the host and their ecological factors.

Key words: Endophytic Fungi · Extracellular Enzymes · Medicinal Plants

INTRODUCTION

Fungi have proven themselves as invaluable sources of natural products for industrial as well as biomedical development for decades [1]. Endophytic fungi live inside plant tissues for at least part of their life cycle without causing any disease symptoms in their host. Within hosts, fungi inhabit all available tissue including leaves, petioles, stems, twigs, bark, root, fruit, flower and seeds. A variety of relationships exist between fungal endophytes and their host plants, ranging from symbiotic to antagonistic or opportunistic pathogenic [2, 3]. They improve the resistance of host plants to adversity by secretion of bioactive metabolites. These metabolites are of unique structure, including alkaloids, benzopyranones, chinones, flavonoids, phenolic acids, quinones, steroids, terpenoids, tetralones and xanthones [4]. They find wide-range of application in agrochemicals, industries, antibiotics, immunosuppressants, antiparasitics, antioxidants and anticancer agents [5]. Like other organisms invading plant tissues, endophytic fungi produce extracellular hydrolases as a resistance mechanism against pathogenic invasion and to obtain nutrition from host. Such enzymes include pectinases, cellulases [6], lipases [7], laccase from the endophytic fungus *Monotospora* sp. [8], xylanase [9], α-1, 4- glucan lyase [10], phosphotases [11] and proteinase [12, 13]. The failure of exploiting the endophytic fungi depends on our current poor understanding of the evolutionary significance of these organisms and their dynamic interaction with their respective hosts [14]. Caldwell *et al*. [6] reported the ability of dark septate root endophytic fungi, *Philaophora finlandia* and *P. fortinii* isolated from alpine plant communities were able to breakdown the major polymeric forms of carbon, nitrogen and phosphorus found in plants. Maria *et al.* [15] studied the enzyme activity of endophytic fungi from mangrove angiosperm *Acanthus ilicifolius* L. and mangrove fern, *Acrostichum aureum* L. of southwest coast of India. Choi *et al.* [16] screened the endophytic fungi for their ability to produce lignocellulases, amylase, cellulase, ligninase, pectinase and xylanase. Although the

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mechanism is unclear, endophytic fungi actually played an important role in local ecology [17, 18]. These qualitative assays help us in understanding whether fungi can change their mode of life from an endophyte, to a saprobe or pathogen.

The production of extracellular enzymes for penetration and limited colonization of selected plant cell is a common trait of endophytic fungi. In literature, the main studies on endophytic fungi include screening for secondary metabolites, with antimicrobial and antioxidant activity. Not many have explored the possibility of endophytic fungi as biotechnological sources of industrially relevant enzymes. Hence they occupy a relatively unexplored site and can represent a new source in obtaining different enzymes with potentialities. The present study was carried out to find new sources of valuable extracellular enzymes from endophytic fungi and to understand their functional role in the host.

MATERIALS AND METHODS

Sources of Endophytes: Endophytic fungi were isolated from fresh material of healthy wild medicinal plants-*Alpinia calcarata* Roscoe (RRCBI, Mus/No. 09), *Calophyllum inophyllum* L. (FRLHT, Coll. No. 74058), *Bixa orellana* L.(FRLHT, Coll. No. 74059) and *Catharanthus roseus* (L) G. Don (FRLHT, Coll. No. 74061) collected from the Charaka Vana, situated in Jnana Bharathi Campus. An authenticated voucher specimen of the plant herbarium is deposited in National Ayurveda Dietetics Research Institute and Institute of Ayurveda and Integrative Medicine (IAIM), Bangalore.

Isolation and Culture of the Endophytic Fungi: Different plant parts from the medicinal plants such as leaves, midrib, petiole and stem were cut with knife disinfected with 70% ethanol, brought to the lab and surface sterilized according to Hegde *et al.* [19]. The effectiveness of the sterilization procedure was confirmed by the vitality test [20]. Fifteen leaf segments from each individual part were placed in a Petri dish (9cm) containing Potato dextrose agar (PDA) and incubated in a light chamber at 25°C. Regular observations were done from the second day onwards for a period of 3-4 weeks for fungal growth [21]. The fungi growing from internal tissues were checked for purity, transferred to fresh PDA slants and stored at 4°C. Identification was based on the cultural characteristics and direct microscopic observations of the fruiting bodies and spores using standard manuals [22-24]. Non sporulating strains were induced for sporulation by culturing them on different media such as Potato Sucrose Agar (PSA), Potato Carrot Agar (PCA) and Water Agar (WA). Those cultures which failed to sporulate were grouped under mycelia sterilia. This is the common problem concerning with the identification of endophytes [25, 26].

Detection of Extracellular Enzyme Production: A survey for extracellular enzyme by endophytic fungi using the qualitative techniques helps us to screen a large number of fungi in a relatively short time. Twenty two isolates of *Alpinia calcarata,* twenty isolates from *Calophyllum inophyllum,* four isolates from *Bixa orellana* and *Catharanthus roseus* respectively were screened for extracellular enzymes. Screening of fungal extracellular enzymes typically involved growth on specific indicative media as mentioned by Hankin and Ananostakis [27]. The functional role of extracellular enzymes by fungal endophytes was assessed by growing them on PDA for 6-7days and placing 5mm mycelial plugs on the solid media with dissolved substrates. After incubation for 3-7days at room temperature, the zone of enzyme activity surrounding the fungal colony was measured.

Amylolytic Activity: Amylase activity was assessed by growing the fungi on Glucose Yeast Extract Peptone Agar (GYP) medium (glucose-1g, yeast extract -0.1g, peptone-0.5g, agar -16g, distilled water-1L) with 0.2% soluble starch pH 6.0. After incubation, the plates were flooded with 1% iodine in 2% potassium iodide (Fig. 1).

Lipolytic Activity: For lipase activity, the fungi were grown on Peptone Agar medium (peptone 10g, NaCl 5g, $CaCl_2$ $2H_2O$-0.1g, agar- 16g, distilled water-1L; pH 6.0) supplemented with Tween 20 separately sterilized and added 1% to the medium. At the end of the incubation period, a visible precipitate around the colony due to the formation of calcium salts of the lauric acid liberated by the enzyme indicated positive lipase activity (Fig. 2).

Pectinolytic Activity: Pectinolytic activity was determined by growing the fungi in Pectin Agar medium (Pectin -5g, yeast extract-1g, agar- 15g pH 5.0 in 1L distilled water). After the incubation period, the plates were flooded with 1% aqueous solution of hexadecyl trimethylammonoium bromide. A clear zone formed around the fungal colony indicated pectinolytic activity (Fig. 3).

Fig. 1: Amyloytic activity on Starch medium

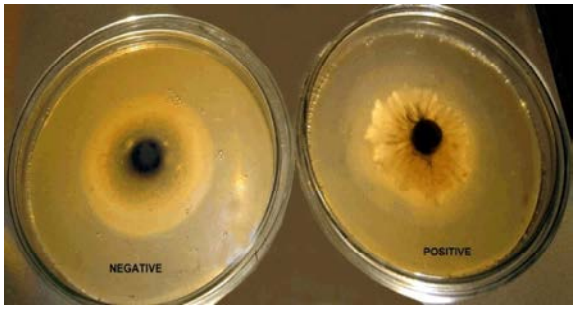

Fig. 2: Lipolytic activity on Peptone medium

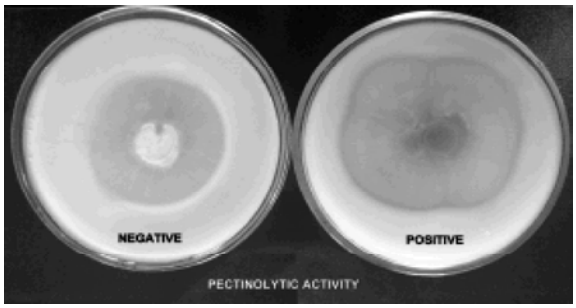

Fig. 3: Pectinolytic activity on Pectin Agar medium

Fig. 4: Cellulolytic activigty on GYP medium

Fig. 5: Proteolytic activity on Gelatin Agar medium

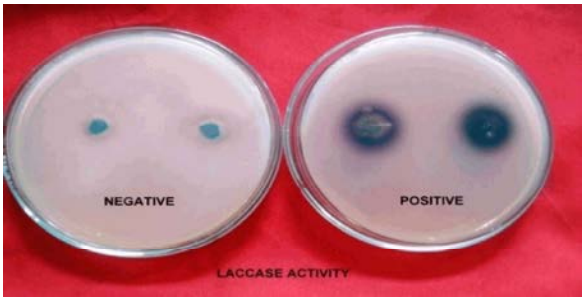

Fig. 6: Laccase activity

Cellulase Activity: Glucose Yeast Extract Peptone Agar medium containing 0.5% Carboxy-methylcellulose was used. After 3-5days of fungal colony growth, the plates were flooded with 0.2% aqueous Congo red solution and destained with 1M NaCl for 15minutes. Appearance of yellow areas around the fungal colony in an otherwise red medium indicated cellulase activity (Fig. 4).

Proteolytic Activity: Glucose Yeast Extract Peptone Agar medium with 0.4% gelatin (pH 6.0) was used. 8% of gelatin solution in water was sterilized separately and added to GYP medium at the rate of 5mL per 100mL of medium. After incubation degradation of the gelatin was seen as clear zone around the colonies. The plate was then flooded with saturated aqueous ammonium sulphate, which resulted in formation of a precipitate. This made the agar opaque and enhanced the clear zone around the fungal colony (Fig. 5).

Laccase Activity: Glucose Yeast Extract Peptone Agar medium with 0.05g 1-napthol L^{-1}, pH 6.0 was used. As the fungus grows the colourless medium turns blue due to oxidation of 1-napthol by laccase (Fig. 6).

Statistical Analysis: All the experiments were performed in triplicates and the means were analyzed statistically with the SPSS program version 20. The analyses of variance were carried out according to the rules of the ANOVA. The significant differences between the means were determined through Duncan's Multiple range Test (DMRT) [28].

RESULTS AND DISCUSSION

The fifty strains of endophytic fungi tested were able to produce one or the other extracellular enzymes (Table 1). In the present study, none of the strain was able to produce all six enzymes tested. The production of

Table 1: List of endophytic fungi screened for enzymes on solid media

Sl No.	Code No.	Endophytic Fungi	Amylase	Cellulase	Pectinase	Proteinase	Lipase	Laccase
1	Ac 3	*Fusaruim* sp.	1^{lm}	0^{i}	0^{m}	0^{h}	2^{j}	0^{i}
2	Ac 4	*Chaetomium* sp.	0^{m}	0^{i}	0^{m}	0^{h}	7.33^{fg}	4.33^{c}
3	Ac 5	*Colletotrichum* sp.	8.67^{e}	4^{fg}	0^{m}	0^{h}	8.67^{f}	2^{g}
4	Ac 6	*Aspegillus flavus*	0^{m}	0^{i}	2^{kl}	0^{h}	0^{k}	0^{i}
5	Ac 7	*Cylindrocephalum* sp.	13^{cd}	0^{i}	14^{b}	0^{h}	3.33^{ghi}	0^{i}
6	Ac 10	*Coniothyrium* sp.	8.67^{e}	2^{h}	4.67^{ghi}	0^{h}	0^{k}	0^{i}
7	Ac 11	*Phoma* sp.	0^{m}	0^{i}	10^{de}	0^{h}	12.67^{de}	0^{i}
8	Ac 12	*Aspegillus niger*	0^{m}	0^{i}	0^{m}	0^{h}	11.3^{e}	1.67^{g}
9	Ac 14	*Colletotrichum* sp.	1.33^{klm}	0^{i}	0^{m}	0^{h}	2^{j}	0^{i}
10	Ac 16	*Mycelia sterilia* sp.	8^{ef}	0^{i}	0^{m}	0^{h}	0^{k}	0^{i}
11	Ac 18	*Aspergillus fumigatus*	5^{ghi}	9.33^{d}	10^{de}	0^{h}	0^{k}	0^{i}
12	Ac 19	*Alternaria* sp.	2^{jklm}	17^{b}	0^{m}	18^{d}	17^{b}	0^{i}
13	Ac 20	*Colletotrichum gleosporoides*.	1^{lm}	0^{i}	8.67^{ef}	0^{h}	2^{j}	0^{i}
14	Ac 21	*Colletotrichum* sp.	4^{ghijk}	0^{i}	8.67^{ef}	18^{d}	0^{k}	0^{i}
15	Ac 22	*Myrothecium* sp.	0^{m}	3^{gh}	14^{b}	0^{h}	0^{k}	0^{i}
16	Ac 23	*Fusaruim chlamydosporum.*	3.33^{ghijkl}	0^{i}	6^{g}	0^{h}	0^{k}	2^{g}
17	Ac 25	*Xylaria* sp.	3^{hijkl}	4^{fg}	3.33^{ijk}	19.33^{bcd}	0^{k}	0^{i}
18	Ac 26	*Fusicoccum* sp.	4^{ghijk}	4.67^{fg}	15.33^{b}	11.33^{f}	0^{k}	7.3^{b}
19	Ac 31	*Mycelia sterilia* sp.	6^{fg}	0^{i}	0^{m}	20.67^{ab}	0^{k}	0^{i}
20	Ac 32	*Aspergillus* sp.	0^{m}	0^{i}	10.67^{cd}	20^{ab}	0^{k}	0^{i}
21	Ac 34	*Pestalotiopsis* sp.	0^{m}	0^{i}	$9.33d^{ef}$	0^{h}	0^{k}	0^{i}
22	Ac 36	*Colletotrichum* sp.	0^{m}	0^{i}	2.67^{jkl}	18.67^{cd}	0^{k}	0^{i}
23	Ci 1	*Talaromyces emersonii*	15^{c}	22.67^{a}	17^{a}	0^{h}	0^{k}	0^{i}
24	Ci 3	*Pyllosticta* sp.	4.33^{ghij}	0^{i}	4^{hij}	0^{h}	0^{k}	0^{i}
25	Ci 4	*Pestalotiopsis* sp.	4.33^{ghij}	0^{i}	0^{m}	0^{h}	4^{gh}	4^{cd}
26	Ci 5	*Discosia* sp.	23.67^{a}	16.67^{b}	0^{m}	0^{h}	0^{k}	9.33^{b}
27	Ci 10	*Aspergillus* sp.	13.33^{cd}	0^{i}	8.67^{ef}	21^{a}	0^{k}	0^{i}
28	Ci 11	*Mycelia streilia* sp.	0^{m}	6^{e}	0^{m}	0^{h}	4.33^{gh}	2^{g}
29	Ci 12	*Isaria* sp.	17.33^{b}	6.33^{e}	0^{m}	20^{ab}	20.67^{a}	4^{d}
30	Ci 13	*Xylaria* sp.	0^{m}	0^{i}	4.33^{ghij}	0^{h}	12^{de}	3.67^{e}
31	Ci 14	*Phoma* sp.	0^{m}	0^{i}	4.33^{ghij}	0^{h}	4.33^{gh}	0^{i}
32	Ci 15	*Pestalotiopsis disseminata*	13.67^{cd}	0^{i}	3.67^{ijk}	1.67^{h}	0^{k}	1.67^{g}
33	Ci 16	*Fusarium oxysporum*	0^{m}	4.33^{fg}	12^{c}	0^{h}	0^{k}	0^{i}
34	Ci 17	*Paecilomyces variotii*	0^{m}	0^{i}	2^{jkl}	0^{h}	8.67^{f}	0^{i}
35	Ci 19	*Fusarium chlamydosporum*	5.33^{ghi}	0^{i}	10.67^{cd}	0^{h}	15^{c}	0^{i}
36	Ci 20	*Acremonium implicatum*	6^{fg}	0^{i}	10.67^{cd}	0^{h}	2.67^{hi}	0^{i}
37	Ci23	*Nigrospora sphaerica*	0^{m}	0^{i}	10.67^{cd}	0^{h}	0^{k}	0^{i}
38	Ci 24	*Fusarium solani*	4.67^{ghi}	3.67^{fg}	0^{m}	15.67^{e}	0^{k}	0^{i}
39	Ci26	*Penicillium* sp.	2.67^{ijklm}	0^{i}	0^{m}	0^{h}	2.67^{hi}	2^{g}
40	Ci29	*Mycelia sterilia* sp.	2.67^{ijklm}	0^{i}	0^{m}	0^{h}	0^{k}	0^{i}
41	Ci30	*Phoma* sp.	0^{m}	0^{i}	7.67^{f}	0^{h}	0^{k}	3^{f}
42	Ci31	*Basidiomycetes* sp.	11.33^{d}	0^{i}	42^{hij}	0^{h}	6.67^{fg}	0^{i}
43	Bo 4	*Colletotrichum falcatum*	4^{ghijk}	12^{c}	0^{m}	14.33^{e}	12.33^{de}	0^{i}
44	Bo 13	*Phomopsis longicolla*	0^{m}	0^{i}	2^{kl}	0^{h}	3.67^{ghi}	10^{a}
45	Bo 21	*Fusarium oxysporum*	0^{m}	0^{i}	3.67^{ijk}	0^{h}	4^{gh}	0^{i}
46	Bo 26	*Colletotrichum gleosporoides*	0^{m}	0^{i}	13.6^{b}	0^{h}	5^{g}	0^{i}
47	Cr1	*Colletotrichum truncatum.*	4.33^{ghij}	0^{i}	0^{m}	0^{h}	12.33^{de}	0.833^{h}
48	Cr2	*Drechsclera* sp.	5.67^{fgh}	12.33^{c}	8^{f}	0^{h}	13.67^{cd}	0^{i}
49	Cr3	*Cladosporium* sp.	0^{m}	0^{i}	0^{m}	20^{ab}	0^{k}	0^{i}
50	Cr10	*Myrothecium* sp.	5^{ghi}	4^{fg}	0^{m}	8.67^{g}	0^{k}	0^{i}

Ac-*Alpinia calcarata*, Ci-*Calophyllum inophyllum*, Bo-*Bixa orellana* and Cr-*Catharanthus roseus*

Values followed by the same lower case alphabets in the same column are statistically equivalent ($P<0.05$) according to the Duncan multiple range test.

these enzymes also varied from 3-8 days of incubation. Only 62% of the endophytes were able to produce amylase unlike the previous reports of Choi *et al*. [16] where all strains were able to degrade starch. The maximum production of amylase was from the isolates of *Calophyllum inophyllum*. *Discosia* sp. (Ci5), followed by *Isaria* sp. (Ci12) and *Talaromyces emersonii* (Ci1) exhibited significant extracellular amylase activity. *Cylindrocephalum* sp. (Ac7) from *Alpinia calcarata* exhibited maximum amylolytic activity, however the isolates of *Bixa orellana* and *Catharanthus roseus* were weak producers of the enzyme. Amirita *et al*. [29] also reported 72% of positive amyloytic isolates from the medicinal plants such as *Adhatoda vasica, Costus igneus, Coleus aromaticus* and *Lawsonia inerims.* Maximum amylolytic activity were observed by Venkatesagowda *et al*. [30] in *Rhizopus stolonifer* and *Lasiodiplodia theobromae*, followed by *Aspergillus niger* and *Penicillium variotii,* while other isolates produced moderate- to-low amylolytic activitiy except *Phyllosticta occulta*, tested negative. The amylolytic potential of these endophytes may help them to degrade starch which is available when the plant senesces.

Cellulolytic activity was prominent in *Talaromyces emersonii* (Ci1), followed by *Discosia* sp. (Ci 5), of *Calophyllum inophyllum* and *Drechsclera* sp. (Cr2) from *Catharanthus roseus*. The production of cellulase was not significant from isolates of other two plants. Only 32% of the endophytes tested in our study were able to produce cellulase. Similar result was reported by Maria *et al*. [15] from mangrove angiosperm isolates. However, 100% cellulolytic activity was reported by Gessner [31], in leaf inhabiting salt marsh fungi and 66% by Choi *et al.* [16] from isolates of *Brucea javanica*. Bezerra *et al.* [32] reported 53.84% cellulolytic activity of endophytes from *Opuntia ficus-indica* Mill., *Cladosporium cladosporioides* with maximum activity. In our study *Cladosporium* sp. (Cr3) isolated from *C. roseus* was negative for cellulase. Venkatesagowda *et al*. [30] reported high cellulolytic activity from *Colletotrichum gloeosporioides*, *Aspergillus versicolor* and *Cladosporium cladosporioides* in endophytic fungi inhabiting seven oil-seeds.

Maximum pectinase activity was observed in *Taloromyces emersonii* (Ci1), followed by *Fusarium oxysporum* (Ci16) of *Calophyllum inophyllum*. Among *Alpinia calcarata* isolates, *Fusicoccum* sp. (Ac26), *Myrothecium* sp. Ac22) and *Cylindrocephalum* sp. (Ac7) were the significant producers of pectinase activity. *Colletotrichum gleosporoides* (Bo4) of *Bixa orellana* also exhibited moderate pectinase activity. Pectic enzymes are induced in the presence of pectic substances by both pathogenic and endophytic fungi. Microbial pectinases are important in the phytopathologic process, plant-microbe symbiosis and in the decomposition of dead plant material [33]. Degradation of host tissue by phytopathogens generally begins with the production of pectinolytic enzymes, which are the major enzymes involved in plant attack [34, 35]. If an endophyte can degrade pectic substances, this implies that the fungus is likely to be a latent pathogen. [16]. Schulz *et al*. [2] hypothesize that the fungal endophyte-plant host interaction is characterized by equilibrium between fungal virulence and plant defense, If this balance is disturbed by either a decrease in plant defense or an increase in fungal virulence, disease develops.

Among the tested organisms, maximum extracellular protease activity was observed in *Aspergillus* sp. (Ci10), *Isaria* sp. (Ci12) followed by *Fusarium solani* (Ci24) of *Calophyllum inophyllum*. *Aspergillus* sp. (Ac32) and *Mycelia sterilia* sp. (Ac31) from isolates of *Alpinia calcarata*, *Cladosporium* sp. (Cr 3) from *Catharanthus roseus* also produced significant amount of protease. *Xylaria* sp. (Ac25), *Colletotrichum* sp. (Ac36) and *Alternaria* sp. (Ac19) of *Alpinia calcarata* followed by *C. falcatum* (Bo4) from *Bixia orellana* exhibited moderate protease activity. Amirita *et al.* [29] reported protease activity by *Colletotrichum carssipes*, *C. falcatum, C. gleosporioides, Curvularia vermiformis* and *Drechslera hawaiiensis* and Xylariales from 4 medicinal plants such as *Adhatoda vasica, Costus igneus, Coleus aromaticus* and *Awsonia inerims.* Bezerra *et al.* [32] reported 88% of positive pectinolytic isolates from *Opuntia ficus-indica* Mill. Venkatesagowda *et al.* [30] reported high proteolytic activity of endophytic *C. gloeosporioides* followed by *Aspergillus niger, Penicillium citrinum* and *Pestalotiopsis palmarum.* Jalgaonwala and Mahajan [36] reported *Mycelia sterilia* from roots of *Catharanthus roseus* had greater proteolytic activity than other tested fungal isolates. Protease activity was also seen in some of the endophytes by Maria *et al.* [15] and Reddy *et al.* [12]. Ng'ang'a *et al*. [37] also reported positive protease activity from sixteen strains of *F. oxysporum* isolated from banana, where as in our studies *F. oxysporum* and *F.chlamydosporum* showed negative proteolytic activity, *F.solani* exhibited a moderate proteolytic activity.

The isolate, *Isaria* sp. (Ci12) of *C. inophyllum* was the maximum producer of lipase activity followed by the *Alternaria* sp. (Ac19) of *Alipnia calcarata, Colletotrichum truncatum* (Cr1) from *Catharanthus. roseus* and *C. falcatum* (Bo4) from *B. orellana* also

produced significant lipolytic activity. Amirita *et al*. [29] reported lipolytic activity of *Curvularia brachyspora, C. vermiformis, Drechslera hawaiiensis, Colletotrichum. falcatum* and *Phyllosticta* sp. isolated from medicinal plants. In our studies *Dreschslera* sp. also showed moderate lipolytic activity. Venkatesagowda *et al*. [30] found 40 isolates with strong lipolytic activity, among the 1,279 endophytic fungi isolated from the seven different oil-bearing seeds. Colen *et al.* [38] found *C. gloeosporioides* as the best of producer of alkaline lipase and also was able to hydrolase wide range of oils. Lipases were also produced by mangrove endophytic fungi of southwest coast of India such as *Acremonium* sp., *Alternaria chlamydosporus*, *Alternaria* sp., *Aspergillus* sp., *Fusarium* sp. and *Pestalotiopsis* sp. The high lipase activity suggests their ability to use fats as energy source [15]. Panuthai *et al.* [39] found 100% lipase production among 65 endophytic fungi isolated from healthy leaf of *Croton oblongifolius* Roxb. (Plao yai) in Thailand among them *F.oxysporum* was potential isolate, whereas in our studies *F.oxysporum* showed negative for lipolytic activity.

Phomopsis longicolla (Bo13) of *Bixa orellana,* was significantly highest producer of laccase enzyme followed by *Discosia* sp. (Ci5) from *Calophyllum inophyllum* and *Fusicoccum* sp. (Ac26) followed by *Chaetomium* sp. (Ac 4) from *Alpinia calcarata*. Very few endophytic strains in the present study were able to produce laccase, similar to the results on marine fungi. [40-42]. However Maria *et al.* [15] reported none of the isolated endophytic fungi were able to produce laccase. The endophytic nature of these fungi might be the reason for the lack of laccase activity, since an active enzyme might damage the host plant.

Fusicoccum sp. (Ac26) and *Isaria* sp. (Ci12) were able to produce four of the extracellular enzymes including amylase, cellulase, proteinase and laccase. Apart from these enzymes, *Fusicoccum* sp. (Ac26) produced pectinase and *Isaria* sp. (Ci12) exhibited lipolytic activity. *Discosia* sp. (Ci5) and *T. emersonni* (Ci1) from *C. inophyllum* produced both amylase and cellulase along with laccase and pectinase respectively. *Aspergillus* sp. (Ci10) and *Pestalotiopsis* sp. (Ci 4) produced extracellular amylase, pectinase and proteinase. *Pestalotiopsis disseminata* (Ci 15) also exhibited laccase activity. *C. falcatum* (Bo4), *Drechslera* sp. (Cr2), *F. solani* (Ci24), *F. chlamydosporum* (Ci19) and *F. oxysporum* (Ci16) showed amylolytic, pectinolytic, cellulolytic activity which is the major enzymes for plant polysaccharide degradation along with proteolytic activity. The production of extracellular cellulases together with that of pectinases in endophytes could imply that the fungus is well equipped for both penetration of living cells and decomposition of dead tissues [43]. The ability of endophytic fungi to produce these extracellular enzymes may provide a resistance mechanism to the host against pathogenic invasion, to get nutrition from the host or to be a latent pathogen [16, 44, 45].

In order to establish the functional role of endophytes it would be useful to establish their patterns of substrate utilization and which enzymes they produce [46]. If they are weak parasites or latent pathogens they may produce proteinase and pectinase [47, 12], while if they are mutualistic, eventually being saprobes they are likely to produce cellulase, mannanase and xylanase [48]. The role of endophytic fungi in plant decomposition has been demonstrated over several years, since these fungi are present in the senescent tissue these are the first ones to initiate plant decomposition than the ubiquitous saprobic fungi [49]. Promputtha *et al.* [50] have provided phylogenetic evidence indicating that the endophytes were found to produce the same degrading enzymes as their saprobic counterparts. The capability of endophytes to produce different enzymes should have an important role as saprobes, since they do not decompose the host living tissue. These degrading enzymes are important factors affecting the lifestyle of endophytes in becoming saprobes or pathogens. Sun *et al.* [51] indicated that endophytes generally decompose dead host leaves, not only as single species, but also as communities. This is prominent from their studies of saprobes on fallen leaves, where invariably several species can be found on a single decaying leaf.

Priest [52] showed that there are several possible regulatory mechanisms in the enzymes production including induction. The action of such enzymes gives rise to the possibility that the “genetic recombination” of the endophyte with the host which occur in evolutionary time. This may be the reason why some endophytes can produce some phyto-chemicals originally characteristic of the host. Maccheroni *et al.* [53] reported endophytic and pathogenic isolates of *Colletotrichum* sp. from Banana, Sweet orange and Key lime revealed distinct patterns of pH-regulated enzyme secretion. Amylase activity was not observed at alkaline pH, but starch had been degraded at neutral and acid pH. Lipase was not produced at acidic pH and secreted at neutral and alkaline pH, whereas cellulase was secreted preferentially at neutral and alkaline pH. Tran *et al*. [54] also observed that *Aureobasidium pullulans* isolated from *Acacia baileyana* (S6E3) and *A. floribunda* (S8E6) exhibited amylase activity, however they were not genetically identical.

In addition, the studies of endophytic fungi and their relationships with the host plants will shed light on the ecology and evolution of endophytes and their hosts; the evolution of endophyte - plant symbiosis; the ecological factors that influence the strength of endophyte-host plant interaction [44, 45].

CONCLUSION

The most significant inference from the current study is the period and degree of variability of enzyme production by the endophytic fungi isolated from medicinal plants. This indicates that the enzyme production differs between fungi and often corresponds to the requirements of its habitat. This may be due to the many factors changing in the host as related to age, environmental factors such as, climatic condition and geographical location may influence the biology of the fungi. However, knowledge of the types, amounts and characteristics of enzymes produced by endophytic fungi cited above would be useful for selecting organisms best suited for industrial requirements. The potential endophytic fungi are being investigated quantitatively for extracellular enzyme production in liquid media.

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