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# FATTY ACID PROFILING AND CLASSICAL TAXONOMY FOR CHARACTERIZATION AND IDENTIFICATION OF ENDOPHYTIC COELOMYCETES

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## ABSTRACT

This study aimed to analyze the endophytic coelomycetes under direct and indirect methods from stems of *Mangifera indica*, *Rhododendron arboreum*, *Taxodium distichum* and *T. mucronatum*. Both direct and indirect methods were effective for isolation, and identifying endophytic coelomycetous fungi such as *Botryodiplodia* sp., *Colletotrichum* sp., *Pestalotiopsis* spp., *Bartalinia* sp., *Monochaetia* spp., *Seimatosporium* sp. and *Truncatella* sp. All the fungi are reported from this study has been identified up to species level based on classical taxonomy using microscopic techniques and chemotaxonomy. This study demonstrates first time that fatty acid profile can be used for chemotaxonomical identification of coelomycetous fungi, *Pestalotiopsis* spp. and other genera. In addition, the fatty acid profile has not only facilitated to determine chemotaxonomy distribution, also provided an evidence for further research in fungal secondary metabolites and mycodiesel technology.

**Keywords:** Endophytic fungi, Coelomycetes, *Pestalotiopsis* spp., Ascomycotina, Deuteromycotina.

## INTRODUCTION

The term endophyte (Gr. Endon - within; phyton - plant) was first introduced by De Bary (1866) who defined it as 'organisms that colonize internal plant tissue'. The biological association between the fungus and its host is considered to be pathogenic (Sieber, 2002), mutualistic (Redman *et al*., 2002), commensalistic (Deckert *et al*., 2001) and latent pathogenic (Schulz *et al*., 1998). It was estimated that there are 1.5 million species of fungi on Earth; of which, only 75,000 species have been described so far (Hawksworth, 2001). Nevertheless, several novel or new fungal genera and species are hidden; it is possible to locate them by identifying their habitats (Hyde, 2001). The internal tissues of plants harbouring endophytes may well account for a substantial number of such new reports. Approximately 300,000 plant species growing in unexplored area on the Earth are host for number of endophytes (Strobel and Daisy, 2003). Earlier reports indicated that endophytic coelomycetous fungi were studied for over three decades (Webber and Gibbs, 1984; Stone *et al*., 1996; Suriyanarayanan and Vijaykrishna, 2001). The term coelomycete was mainly used to indicate that conidia are formed within a cavity lined by fungus or fungal host tissue. Hence, it may be considered as coelo-holo, mycetes-fungi. The growth of coelomycetous fungi mainly depends upon the host specificity and it may emerge as saprophytic or endophytic organisms for ecological commensalisms (Kirk *et al*., 2001). The diversity of endophytic coelomycetes from various tree species was subjected to biotechnological approach because of the colony frequencies accumulated over the time; the age of leaves and needles are strongly correlated with the fungal colonization (Helander *et al*., 1993; Carroll, 1995; Magan *et al*., 1996). Considering that, only few studies were carried out on endophytic coelomycetes (Lehtijarvi and Barklund, 2000; Poteri *et al*., 2001; Arnold and Herre, 2003). A recent report has motivated to evaluate and elucidate the potentials of these microorganisms applied on biotechnological processes focusing on the production of bioactive compounds (Pimentel *et al*., 2011). The production of bioactive substances by endophytic fungi is directly related to the independent evolution of these microorganisms, which may have incorporated genetic information from higher plants, allowing them for better adaptation to plant host and carry out some functions such as protection from pathogens, insects, and grazing animals (Strobel *et al*., 1996a). Endophytes are chemical synthesizer inside plants in other words; they play a role as a selection system for microbes to produce bioactive substances with low toxicity towards higher organisms (Strobel *et al*., 1996b). However, attempt has not been made so far to find out the

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endophytic coelomyceteous fungi from Chennai and Ootcamund in the Indian state of Tamil Nadu by direct method. Therefore, the present investigation has attempted on isolation of endophytic coelomyceteous fungi from inner stems of *Mangifera indica*, *Rhododendron arboreum*, *Taxodium distichum* and *T. mucronatum* using direct method and indirect method as well. In addition, this investigation reporting first time fatty acid profile can be used in chemotaxonomical identification of the endophytic fungi.

## MATERIALS AND METHODS

### Plants collection and sampling methods

The plant samples were collected from forest at Tamil Nadu Botanical Garden (2623 m above sea level) in Ootcamund, Nilgiri district is located between 10-38 and 11-49 North Latitude and between 76-0 and 77-15 East Longitude in South India.

Stems from the healthy plants, *Mangifera indica*, *Rhododendron arboreum*, *Taxodium distichum* and *T. mucronatum* were collected during four different season such as South West monsoon (July-September), North East monsoon (October-Navember), monsoon season (December-February) and summer season (April-June) between 2008 and 2009. In addition, leaflets and petioles were collected and brought to laboratory in separate plastic bags. All the samples were processed within 24 h. The bark and stem samples were subjected for surface sterilization as described by Kamalraj *et al*. (2008). One hundred and fifty small pieces of stems are approximately 0.5 cm diameter were cut from the upper, middle and lower portions of stems with the aid of a flame-sterilized scalpel blade.

The leaf were surface sterilized by immersion in 70% ethanol for 5 sec followed by 4% sodium hypochlorite for 90 sec and then rinsed in sterile distilled water for 10 sec. After surface sterilization, the tissues were dried on sterilize tissue paper.

### Examination of endophytic fungi in stem by light microscopy using direct method

The stem samples fixed using 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.2) at 4°C for overnight. The fixed specimens were rinsed with the 0.1 M phosphate buffer for 3 times and then dehydrated by ethanol dilution series about 30%, 70%, 83%, 95% and 100% for 2 sec (Gao *et al*., 2005). The thin sections of healthy stems about 5-10 µm were taken manually by using sterile razor blade and placed in the sterile water. Then, the stem sections were taken with the help of a single eye lash brush and placed on a glass slide with a drop of sterile distilled water. The slides were then placed on the slide warmer at 50°C for 1 min for adhesion of the sample on to the slide. The sections were further stained with 2, 3, 5-triphenyltetrazolium chloride and 1% aniline blue and mounted using DPX mount. The sections were photomicrographed using phase contrast microscope (Carl Zeiss Axiostar plus-photomicroscope) with camera by using Konica films and Nikon HFX Labophot bright field microscope.

### Isolation and identification of endophytic fungi from leaves by indirect method

The surface sterilized leaf segments were evenly placed in Petri dishes containing potato dextrose agar (PDA) media amended with chloramphenicol 150 mg $l^{-1}$ and incubated at 26 ± 1°C in a light chamber (Bills and Polishook, 1992). The growth of endophytic fungal colonies from the leaf segments were monitored every day. The hyphal tips, which grew out from the leaves segments were isolated and subsequent sub-culture was made to bring as a pure culture. The purified endophytic fungi were identified to species level by using standard keys by microscopy characteristic features (Guba, 1961; Sutton, 1980; Nag Raj, 1993). All the purified fungal cultures are preserved using 15% glycerol and stored at -80 °C.

### Illustration and photomicrographs of endophytic fungi using light microscope

Photomicrographs of conidiomata, conidiogenous cells and conidia were taken using phase contrast microscope with camera by using Konica films and Nikon HFX Labophot light microscope. The vertical sections of the conidiomata were taken and the conidia were observed under oil immersion microscope (100X, 320X and 700X) and photographed.

### Identification of endophytic fungi

The microscopic characteristics of the isolated endophytic fungi were observed by microslide preparation of the fruiting bodies and conidia. The endophytic fungi were identified on the basis of the structure of fruiting body (conidiomata), conidiogenous cells and conidia (Sutton, 1980). As, the presence of appendage on the conidia is now being recognized as an important taxonomic criterion (Guba, 1961; Sutton, 1973a; 1973b), the present study is also critically examined the appendage on the conidia of the isolated endophytic fungi.

Identification of coelomycetes up to different morphospecies levels were carried out by using standard mycology manuals (Guba, 1961; Sutton, 1980; Nag Raj, 1993). Descriptions of the coelomycetous fungi are included in the present study along with the photomicrographs.

### Scanning electron microscopy studies on endophytic fungi

The surface morphology of the fungal spore samples has been analyzed by using Scanning Electron Microscope (SEM) (Hitachi S-3400N, USA) employing

cathedoluminescence detector (CLD) to distinguish conidia, spores, septa and appendages were measured for the selective fungal samples (*Bartlinia robillardoides, Seimatosporium mariae, Monochaetia circularis, M. kansensis, Truncatella angustata* and *Pestalotiopsis mangiferae*). The sample preparation of endophytic fungal spores for SEM studies has followed described by Abdel-Kareem and Szostak-Kotowa (1999).

**Fatty acids isolation from endophytic fungi**

All the fungal pure cultures were grown in 250 ml Erlenmeyer flask containing 50 ml of potato dextrose broth (PDB) at 27 ± 2°C for 15 days under static condition. After incubation, the mycelial mat of each culture was filtered using filter paper and washed with sterile distilled water. The excess water was drained out by pressing the mycelial mat using blotting paper. The mycelial mat of each fungus was lyophilized (FlexiDry™) for desiccation.

**Chemotaxonomy of *Pestalotiopsis* spp. and other genera**

Two grams of each fungal mycelial mat were ground separately with sterile mortar and pestle by adding ether (100 ml). The mixture was shaken on a flatbed orbital shaker for 12-16 h, which was allowed to settle out until clear. Then, an aliquot of 10 ml was removed and diluted with 10 ml of 5% saline solution and extracted with hexane. The hexane fraction was allowed to dry under vacuum at 40°C. The each sample was added to a glass column (10×250 mm) packed with 1 g of dry silica gel (60-120 A°) and then it was eluted with a mixture of 100 ml chloroform and methanol (2:1). The collected sample was allowed to dry in rotary evaporator under vacuum at 45°C. The dried sample was re-dissolved in hexane and then it was subjected to GC-MS analysis.

## RESULTS AND DISCUSSION

The endophytic flora differs on the type and number in their host depends upon the host geographical position (Arnold and Herre, 2003; Gange *et al*., 2007). In the present investigation, a total of 74 different morphospecies of coelomycetes have been isolated and identified from 14,400 segments of healthy tissues collected from four plants such as *M. indica, R. arboreum, T. distichum* and *T. mucronatum*. During the isolation of endophytes, some hyphomycetous fungal colonies were also obtained. The phylloplane fungi such as *Alternaria* sp., *Aureobasidium* sp. and *Cladosporium* sp. were routinely isolated as endophytes from the wide range of plant species (Bills, 1996). Moreover, the phylloplane fungi are capable of penetrating the superficial layers of the leaf or may be localized in the substomatal chambers (Cabral *et al*., 1993). Earlier reports on leaf and stem endophytes were largely based on cytological and visual examination (Stone, 1985), however in the present study, spores of coelomyctes were observed using light microscopy; *Botryodiplodia* sp., and *Colletotrichum* sp., mycelia were observed below epidermis and cortex regions in the young stems (Fig. 1). Very few mycelia with spores were also observed inside the xylem parenchymatous ray cells in *T. mucronatum* by direct section method. The mycelium infects single cells and establishes small intracellular thalli (Baayen *et al*., 2002). However, many fungi reported as endophytes on a broad range of hosts, but only few are achieved sporulation (Baayen *et al*., 2002). Among the host plants, the *T. mucronatum* exhibited three interesting appendage bearing coelomycetous fungi *P. microspora, M. circularis* and *T. angustata* followed by *T. distichum* (*M. karstenii* and *S. mariae*), *M. indica* (*P. microspora*) and *R. arboretum* (*B. robillardoides*). The results are clearly revealed that *T. mucronatum* bearing rich diversity of endophytic coelomycetous fungi (Kamalraj and Muthumary, 2013).

Fungal systematic is an essential part of biological research especially in the context of the ecological, economical and biotechnological approaches. Such important fungi is studied in this investigation such as *Pestalotiopsis* sp. MUBL1002, *Bartalinia* sp. MUBL1096, *Monochaetia* sp. MUBL 1100, *Seimatosporium* sp. MUBL1107 and *Truncatella* sp. MUBL1109 for species level identification and their antimicrobial properties. *Monochaetia* sp. MUBL 1101 and *Pestalotiopsis* sp. MUBL1013 are producing anticancer drug Taxol (Unpublished data). The *Pestalotiopsis* spp. were noted in the wanted list of fungal culture in American Mycological Society since 2006 (http://MSAfungi.org). *Pestalotiopsis* spp. is heterogeneous group of coelomycetous fungi and their inter-specific delineation of the genus has been based on the conidial morphology (Guba, 1961; Nag Raj, 1993), conidiogenesis (Sutton, 1980) and the telomorph associations (Zhu *et al*., 1991; Metz *et al*., 2000). There has been considerable ambiguity and confusions in inter-generic classification, which has been dealt in different aspects by various researchers (Guba, 1961; Sutton, 1980; Nag Raj, 1993; Jeewon *et al*., 2002). In addition, taxonomy of the coelomycetous fungi, *Pestalotiopsis* spp. and other closely related genera based on the morphological characters has also been equivocal. Therefore analysis of molecular data combined with morphological data resolves many disputes in the identification of coelomycetous fungi (Jeewon *et al*., 2002). However, this study used both classical- and chemo- taxonomy for the identification of *Pestalotiopsis* spp. and other allied genera. Similarly, Jeewon *et al*. (2002) and Li *et al*. (2013) applied chemotaxonomy along with classical taxonomy for identification of *Pestalotiopsis* and allied genera. In the classical taxonomy of this study, the size of conidia, length of appendages, pigmentation of the septa and cells, and appendages

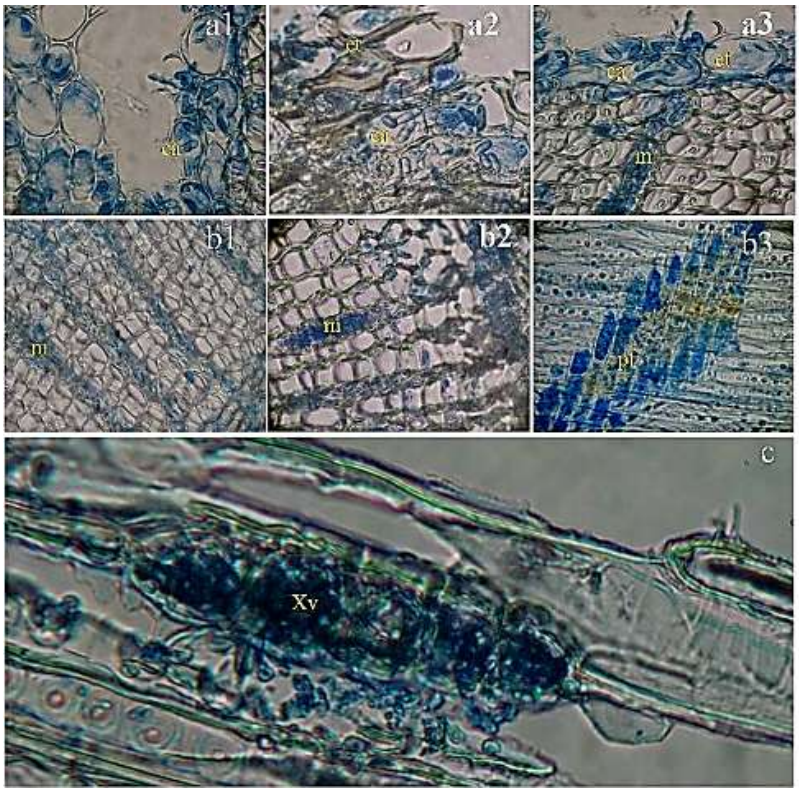


Fig. 1. Microscopic examination of fungal spores and mycelia from the plant samples.
a1-a3: Cross section of bark; b1-b3: Cross section of stem; c: Xylem vessels; ca: Conidia; et: Epidermis tissue; m: Mycelium; pt: Phloem tissue; Xv; Xylem vessels.

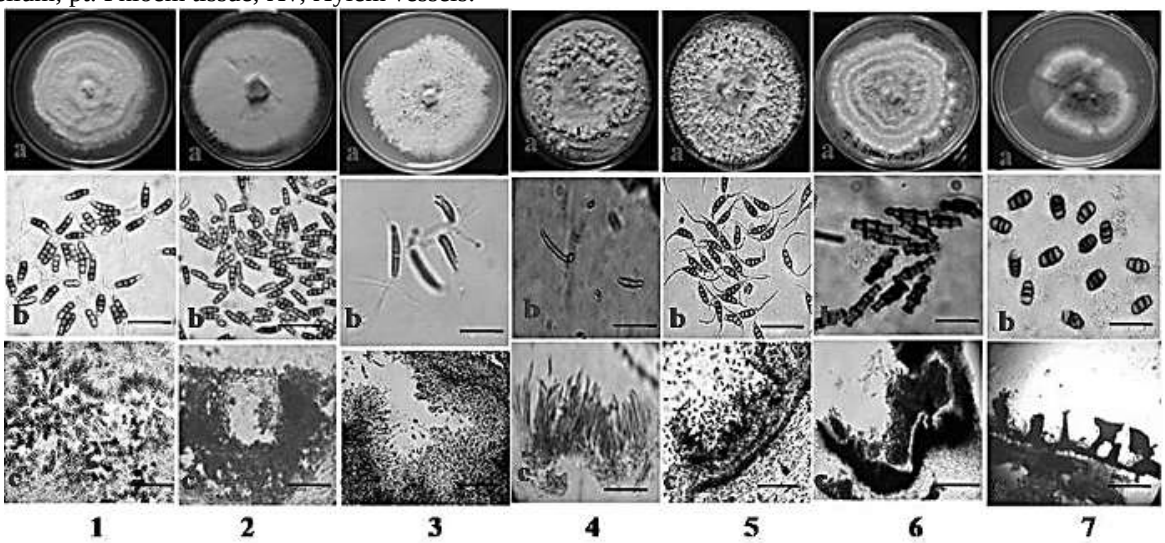


Fig. 2. Culture plate, size of conidia, length of appendages, pigmentation of the septa and cells, and appendages origination of *Pestalotiopsis* spp. and allied genera.
a. culture, b. conidia, c, conidiomata
1: *P. mangifera* ; 2: *P. microspora*; 3: *B. robillardoides*; 4: *M. circularis*; 5: *M. karstenii*; 6: *S. mariae*; 7: *T. angustata*

origination with mycelial morphology (Fig. 2) were analyzed for the identification. The characteristics such as number of median cells, which may or may not be pigmented, presence of apical and basal appendages and hyaline apical or basal cells have carefully been observed in the present study for coelomycetous fungi identification of *Pestalotiopsis* spp. and other genera as described by Jeewon *et al*. (2002). The characteristic feature of each fungus is given below:

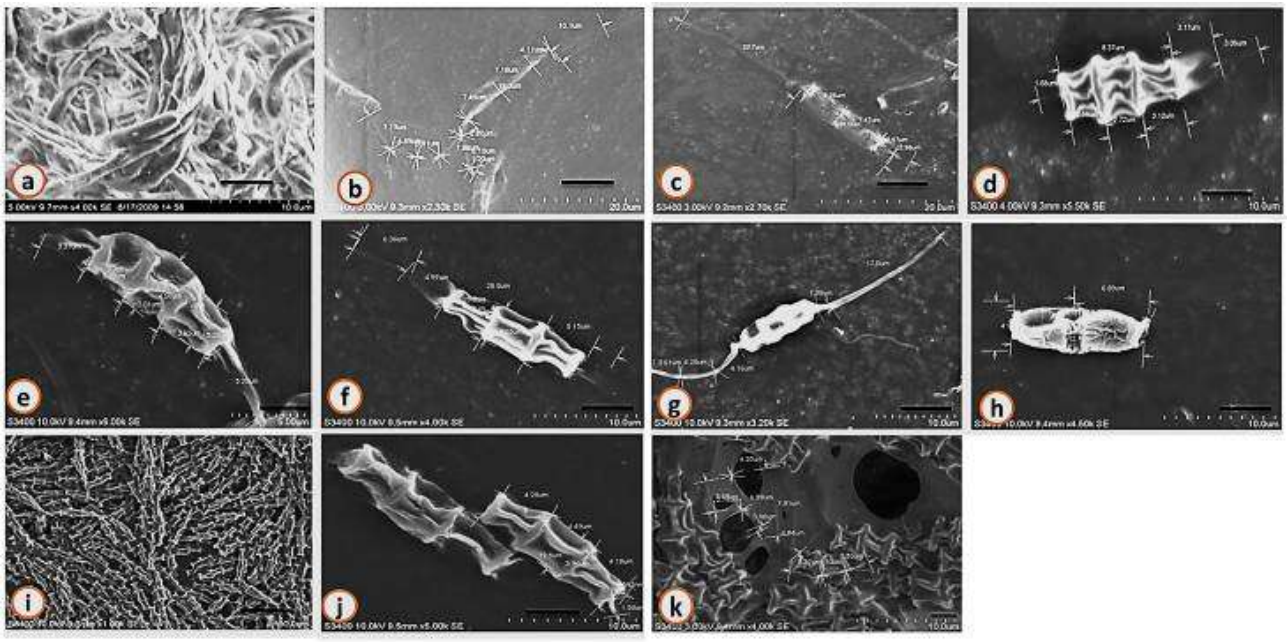

Fig. 3. Scanning electron microscopy of conidial surface morphology of *Pestalotiopsis* spp. and allied genera.
a-k: Conidial surface morphology of *Pestalotiopsis* spp. and allied genera; a-c: *B. robillardoides*; d: *S. mariae*; e-f: *M. circularis*; g: *M. karstenii*; h: *T. angustata*; i-k: *P. mangiferae*

*Pestalotiopsis* sp. MUBL1002/ *P. mangiferae* (Henn.): Conidia 5-celled, oblong-clavate 22-26 × 6-8 µm; colored cells 15-18 µm long, upper two of them amber to fuliginous or slightly darker than the lowest olivaceous colored cell, subglobose, the septa and walls sometimes black, indistinct; appendages 3, widely divergent, 19-26 µm long; pedicels short 6-7 µm long. Based on the microscopic results *Pestalotiopsis* MUBL1002 may be *Pestalotiopsis mangiferae* (Ell and Ev.) Steyaert (Fig. 3). *Isolated as endophyte from:* Leaves, stems and barks of *M. indica* from Botany Field Lab, University of Madras, Maduravoyal, Chennai, Tamil Nadu, India.

*Pestalotiopsis* sp. MUBL1013/ *P. microspora* (Speg.): Conidia clavate-fusoid, broad, tapering towards the base, 5-celled, straight, 15.69- 29 × 6.73-9.5 µm; intermediate colored cells guttulate, amber or olivaceous, equally colored, lowest colored cell sometimes slightly paler, 15-20 µm long, slightly constricted at septa. Apical appendages 1-2; 5-6 µm, and basal appendage 1, 2.92-4.5 µm long (Fig. 3). *Isolated as endophyte from*: Leaves, stems and barks of *T. mucronatum* from Government Botanical Garden, Ootacamund, Tamil Nadu, India.

*Bartalinia* sp. MUBL1096/ *B. robillardoides* (Tassi.): Conidia clavate to cylindrical, 4-septate, wall smooth, slightly constricted at septa, 15-20 × 2.5-3.5 µm with appendages, apical cell conic, colourless, 2.0-3.0 µm long, three apical appendages unbranched, attenuated towards the tip, flexuous, divergent, 8-18 µm long; median cells 3, cylindrical or subcylindrical, hyaline to pale brown, wall thicker than the apical and basal cells, together 11-15×2.5 – 30 µm; lower median cells longer than the upper cell, basal cell obconic with truncate base, hyaline, 2.5-3.0 µm long, with an excentric, filiform, unbranched, flexuous appendage, 3-5 µm long (Fig. 3). *Isolated as endophyte from*: Leaves, stem and bark of *R. arboreum* from Bryant Park, Kodaikanal, Tamil Nadu, India.

*Monochaetia* sp. MUBL1100/ *M. circularis*: Conidiomata acervular, irregular to subglobose, 250-300 µm diameter, wall composed of brown, pseudoparenchymatous cells. Conidiogenous cells lining the conidiomatal cavity, subcylindrical to irregular, hyaline, smooth. Conidia fusiform, 4-septate, 20-25×5-6 µm with three median cells, an apical and basal cell, apical and basal appendage, 10-15 µm long (Fig. 3). *Isolated as endophyte from*: Leaves, stem and bark of *T. mucronatum* from Government Botanical Garden, Ootacamund, Tamil Nadu, India.

*Monochaetia* sp. MUBL1101/ *M. karstenii* (Sacc. and P. Syd.): Conidia 4-celled, occasionally 2-celled, due to degeneration of end cells, slightly constricted at the median septum, 14 (12-16) × 6.5 (5.5-7) µm, two middle cells with thick walls, brown, (9-12) µm long, apical cell thin-walled, hyaline, with a short apical appendage, basal cell thin-walled, hyaline, sometimes with a minute central appendage, both hyaline cells sometimes collapsing (Fig. 3). *Isolated as endophyte from*: Leaves, stem and bark of *T. distichum* from Government Botanical Garden, Ootacamund, Tamil Nadu, India.

*Seimatosporium* sp. MUBL1107/ *S. mariae* (Clinton): Conidia are variable and cylindrical fusiform. 2-5 septate, 4 celled, lacking of appendage (Fig. 3). *Isolated as endophyte from*: Leaves, stem and bark of *T. distichum* from Government Botanical Garden, Ootacamund, Tamil Nadu, India.

*Truncatella* sp. MUBL1109/ *T. angustata* (Persoon: Fries): Conidia ellipsoid to fusiform, 4-celled, 18-25 × 5.5-7.5 µm, two median cells brown, 8-20 µm long, apical and basal cells hyaline, hemispherical, smaller than the median coloured cells, apical cell with 1-4 simple or 2-4 irregularly or dichotomously branched appendages, 10-30 µm long, basal cell with a simple, unbranched appendage, 2-3 µm long, which disappears at maturity of conidium (Fig. 3). *Isolated as endophyte from*: Leaves, stem and bark of *T. mucronatum* from Government Botanical Garden, Ootacamund, Tamil Nadu, India.

In taxonomy, the validity and delimitation of these genera are problematic (Steyeart, 1949). Concurrently, Fuckel's Fungi imperfecti concept and Saccardoan system of classification (Deuteromycetes) based on conidial and conidiophores morphology resulted in a comprehensive yet practical solution for classifying "asexual" fungi. The system was designed for convenience rather than for phylogenetic inference (Shenoy *et al*., 2007). In the present study, *Pestalotiopsis* spp. and other genera were identified up to species level. The conidia with non-pigmented median cells, axial single basal appendages arising from the particular locus and branches tubular outgrowth of the wall were found in *B. robillardoides* and *M. karstenii* (Muthumary, 1986). The *Bartalinia* spp. was characterized by spores having almost hyaline median cells with apical appendages arising from a particular locus and not separated by a septum; *Truncatella* spp. has two pigmented median cells and *Seimatosporium* spp. has two or three pigmented median cells with a single apical appendage and basal appendages that are excentric. *Monochaetia karstenii* was characterized by simple or complex eustromatic conidiomata and holoblastic conidia of variable morphology, mostly 3-euseptate, 2 unequal median cells olivaceous or very pale brown, the end cells hyaline, the apical one extended into a cellular appendage, sometimes the basal one with a central endogenously formed appendage (Muthumary, 1986). *Monochaetia circularis* differs slightly in morphology from *Pestalotiopsis* spp., in which, the spores possess median cells that have slightly thickened walls and are doliform shape, mostly verrucose in ornamentation and guttulate. They also have a slower growth rate on synthetic media compared to *P. mangiferae* and *P. microspora*. Most probably, the median cells have thicker walls and somehow distoseptate results in perplex during identification. The obtained result coincides with previous morphological hypothesis (Sutton, 1980; Nag Raj, 1993), in which, the median cells of *Pestalotiopsis* species such as *P. mangiferae* and *P. microspora* are not distoseptate as in *M. circularis*.

In contrast, Guba (1961) adopted a wide generic concept of the genus Pestalotia synonymizing *Pestalotiopsis*, Truncatella synonymized with Pestalotia and *Seiridium* synonymized with *Monochaetia*. Von Arx (1981) treated the genus, *Bartalinia* as a synonym of *Seimatosporium*, but, based on the morphology, however, Nag Raj (1993) disagreed as these two anamorphic genera are quite distinct whether the proposed synonym of von Arx (1981) was highly controversial. However, the findings are partially similar with Steyeart's view, which is an agreement with the commonly accepted taxonomic classification (Sutton, 1980; Nag Raj, 1993). The core of this study was to test the morphology-based hypothesis of Steyeart (1949) and Guba (1961) using molecular data. The present study opposes the concept proposed by Guba (1961) on wide generic treatment of *Bartalinia* and *Truncatella* as synonyms of *Seimatosporium* (1981). Traditionally, *Seimatosporium* has been regarded as having 3-5 euseptate conidia (the median cells of which are pigmented), usually a single apical cellular appendage and a single basal cellular appendage that is formed just above the truncate, unthickened basal scar.

According to Steyeart's concept, it is necessary to re-assign all four-septate conidia (three median cells) to *Pestalotiopsis*, a view supported by Sutton (1980; 1969) but Nag Raj (1993) preferred to adopt a wider generic concept of *Pestalotiopsis* by including three-septate (two median cells) form was originally assigned to *Truncatella* (Sutton, 1969). The classical taxonomy hypothesis of conidia with non-pigmented median cells and axial single basal appendages arising from the particular locus and branches tubular outgrowth from the wall was found in *B. robillardoides* and *M. karstenii* reported by Muthumary *et al*. (1986).

Chemotaxonomy is traditionally restricted to comprise fatty acids, proteins, carbohydrates and secondary metabolites. The researchers have suggested that volatile fungal metabolites, detected by chemical methods, might be used in the classification of fungi (Larsen and Frisvad, 1995a,b). Further, chemotaxonomic investigations were performed to confirm morphology-based identification (Fisher *et al*., 1996). The present study also applied chemotaxonomy for identification of *Pestalotiopsis* spp. and other genera. Intracellular fatty acids were analyzed and interpreted with the chemical library construction by using GC-MS analysis. Interestingly, total number of fatty acid peaks in *P. mangiferae, P. microspora, M. circularis, B. robillardoides, M. karstenii, S. mariae* and *T. angustata* were found to be 22, 21, 09, 07, 21, 13 and 27, respectively (Table 1). Each peak was represented to be a particular type of fatty acid derivatives in all the respective isolates. However, the fatty acid 2, 4-Di-

Table 1. Fatty acid profile of *Pestalotiopsis* spp. and other genera using GC-MS analysis.

| S. No. | Mycelial fatty acid | Strain (Absorption area %) | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | *Pm* | *Pmi* | *Mc* | *Br* | *Mk* | *Ta* | *Sm* |
| 1 | n-[2-(3-Bromo-phenyl)-1-(morpholine-4-carbonyl)-vinyl]-4-methyl-benzamide | - | - | - | 9.94 | - | - | - |
| 2 | Purin-2,6-dione, 1,3-dimethyl-8-[2-[3,4-dimethoxyphenyl]ethenyl]- | - | - | - | 16.84 | - | - | - |
| 3 | 1-Methylbutyl docosanoate | - | - | - | 8.64 | - | - | - |
| 4 | 2,6-Dimethylheptan-4-one | - | - | - | 11.22 | - | - | - |
| 5 | 2,4-Ditert-butylphenol | - | - | - | 36.06 | - | - | - |
| 6 | 4-Ethoxy-N-(1-methylacetonyl)amphetamine | - | - | - | 17.31 | - | - | - |
| 7 | Hexanoic acid | 4.99 | 2.12 | - | - | - | 2.53 | 1.36 |
| 8 | Nonanal | 1.53 | 0.64 | - | - | 0.21 | 0.58 | 0.26 |
| 9 | Octanoic Acid | 3.19 | 1.85 | 0.29 | - | 0.96 | 0.83 | 1.30 |
| 10 | Dodecane | 1.62 | 0.06 | 0.21 | - | 0.13 | 0.07 | - |
| 11 | Decanal | 0.58 | 0.20 | - | - | - | 0.07 | - |
| 12 | 1,3-Di-tert-butylbenzene | - | - | - | - | 0.18 | - | - |
| 13 | (2e)-2-Decenal | - | - | - | - | - | - | 2.29 |
| 14 | 4-Hydroxy-3-nonenoic acid lactone | 0.18 | - | - | - | - | - | - |
| 15 | Nonanoic acid | 7.13 | 2.87 | - | - | 1.35 | 1.28 | 1.28 |
| 16 | 8-Methyl-1-undecene | 0.86 | 0.55 | - | - | - | - | 0.33 |
| 17 | 2-Undecen-1-al | - | 0.05 | - | - | - | - | - |
| 18 | Tetradecane | 0.46 | 0.26 | 0.30 | - | 2.37 | 0.26 | 0.34 |
| 19 | Eicosane | 0.30 | - | - | - | 0.07 | - | - |
| 20 | 2,4-Di-tert-butylphenol | 0.72 | 0.27 | 0.46 | 0.26 | 0.41 | 0.48 | 1.30 |
| 21 | Heptadecane | 0.36 | - | - | - | 0.23 | - | - |
| 22 | 8-Pentadecanone | 0.24 | - | - | - | - | - | - |
| 23 | 2-Pentadecanone | 0.67 | - | - | - | 0.14 | 0.19 | - |
| 24 | Tetradecanoic acid | 0.14 | 0.21 | 1.38 | - | 2.38 | 1.10 | 1.10 |
| 25 | Fumaric acid, di(trans-4-tert-butylcyclohexyl) ester | 0.28 | - | - | - | - | - | - |
| 26 | Methyl tridecyl ketone | 0.36 | 0.21 | - | - | - | - | - |
| 27 | Hexadecanoic acid, methyl ester | 1.77 | 0.54 | - | - | - | 0.38 | 0.52 |
| 28 | 1,2-Benzenedicarboxylic acid, bis(2-methoxyethyl) ester | 1.77 | - | - | - | - | - | - |
| 29 | n-Hexadecanoic acid | 51.03 | 78.74 | 96.75 | - | 91.14 | 87.18 | 71.94 |
| 30 | Octanal | - | 1.20 | - | - | - | - | - |
| 31 | 7-Bromobicyclo[4.2.0]octa-1,3,5-triene | - | 1.61 | - | - | - | - | - |
| 32 | (2e)-2-Decinal | - | 0.49 | - | - | - | - | - |
| 33 | 1,13-Tetradecadien-3-one | - | 0.20 | - | - | - | - | - |
| 34 | (E)-2-Dodecen-1-al | - | - | - | - | - | 0.20 | - |
| 35 | 3-Nonen-2-one | - | - | - | - | - | 0.27 | - |
| 36 | (2z)-3-methyl-2-undecene | - | - | - | - | - | 0.19 | - |
| 37 | 9-Oxononanoic acid | - | - | - | - | - | 0.20 | - |
| 38 | 2-Butyloctan-1-ol | - | - | - | - | - | 0.81 | - |
| 39 | n,n-Bis(2-hydroxyethyl)dodecanamide | - | - | - | - | - | 0.11 | - |
| 40 | 3-Methylpentadecane | - | - | - | - | - | 0.12 | - |
| 41 | (1-Ethyloctadecyl)benzene | - | - | - | - | - | 0.43 | - |
| 42 | 1,5-Dodecadiene | - | - | - | - | - | 0.13 | - |
| 43 | Octadecanal | - | - | - | - | 0.14 | - | - |

*Continued..*

Table 1 continue…

| S. No. | Mycelial fatty acid | Strain (Absorption area %) | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | *Pm* | *Pmi* | *Mc* | *Br* | *Mk* | *Ta* | *Sm* |
| 44 | Trans-2-Decenal | - | - | - | - | 0.19 | - | - |
| 45 | 2,6,11-Trimethyldodecane | - | - | - | - | 0.04 | - | - |
| 46 | Decanoyl chloride | - | - | - | - | 0.27 | - | - |
| 47 | (2e)-3,7-Dimethyl-2,6-octadien-1-ol, [13c] isotopic labeled | - | - | - | - | 0.12 | - | - |
| 48 | 2,6,11,15-Tetramethylhexadecane | - | - | - | - | 0.34 | - | - |
| 49 | Octadec-9-enoic acid | - | - | - | - | 0.65 | - | - |
| 50 | 5-Ethyl-2-methyloctane | - | - | 0.05 | - | - | - | - |
| 51 | Docosane | - | - | 0.05 | - | - | - | - |
| 52 | Hexadecane | - | - | 0.24 | - | - | - | - |
| 53 | Cyclohexylmethylamine | - | - | - | - | - | - | 1.08 |
| 54 | (2z)-2-Decenal | 0.23 | - | - | - | - | - | - |
| 55 | 2- Undecenal | - | - | - | - | - | 0.35 | - |
| 56 | n- Decanoic acid | - | - | - | - | 0.35 | 0.21 | - |
| 57 | Octaiucanoic acid | - | 0.37 | - | - | 0.95 | 0.96 | 1.30 |
| 58 | 1,2-Bezenedicarboxylic acid, bis(2-methoxyethyl) ester | 0.84 | 0.41 | - | - | - | 0.48 | - |
| 59 | Pentadecacoic acid | - | 0.84 | - | - | - | 0.91 | - |
| 60 | 2-Bromo dodecane | - | - | - | - | - | 0.28 | - |

Pm: *P. mangiferae* ; Pmi: *P. microspora*; Mc: *M. circularis*; Br: *B. robillardoides*; Mk: *M. karstenii*; Ta: *T. angustata*; Sm: *S. mariae*

tertbutylphenol was observed in all the isolates. The tetradecanoic acid, n-hexadecanoic acid and octanoic acid were observed in *T. angustata, P. mangiferae, P. microspora, M. karstenii, S. mariae* and *M. circularis* except in *B. robillardoides*. The nonanal and nonanoic fatty acids were found in *T. angustata, P. mangiferae, P. microspora, M. karstenii* and *S. mariae* excluding in *M. circularis* and *B. robillardoides* (Table 1). The absorption of area percentage was calculated in GC-MS analysis. Significantly, 2, 4-Ditert-butylphenol was observed at 36.06 area percentage in *B. robillardoides* (Table 1). Interestingly n-hexadecanoic acid was exhibited in all strains except *B. robillardoides* and the absorption area percentage was calculated as 51.03, 78.74, 96.75, 91.14, 71.94 and 87.18 respectively, in *P. mangiferae, P. microspora, M. circularis, M. karstenii, S. mariae* and *T. angustata* (Table 1). Based on literature survey, the cell wall fatty acid analysis in *Pestalotiopsis* spp. and other genera are not reported so far. However the present study applies fatty acid profile for the first time in identification of coelomycetous fungi, *Pestalotiopsis* spp. and other genera. Nevertheless, the secondary metabolites were already reported in *Pestalotiopsis* spp. and *Bartalinia* spp. (Gangadevi and Muthumary, 2009a,b), *Monochaetia* spp., *Seiridium* spp. and *Truncatella* spp. (Lee *et al*., 1996).

## CONCLUSION

The present study reveals that both direct and indirect methods were efficient for isolating the endophytic coelomycetous fungi. Both classical- and chemo-taxonomy are used in this study for the species-level identification of *Pestalotiopsis* spp. and other allied genera. Further the fatty acid profiling used first time by this study for identification of coelomycetous fungi. Our findings indicates that the obtained fatty acids in the present study are the evidence of chemotaxonomy, which is strongly indicate that the distinguished relationship between *Pestalotiopsis* and other genera. Isolation of potential secondary metabolites and lipids from the isolated fungi obtained from this study would be taken for further research.

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