Morphotaxonomy of endophytic fungi on Cissus quadrangularis from Amravati (MS) India

Abstract

Endophytes play significant role to establish fungal diversity. The research interest has been increasing in ecology, biology and applications of endophytic fungi. It is believed that endophytic fungi are diverse in those areas where diversity of plants are diverse and certainly Melghat Forest is one among these areas which has huge plant diversity but studies on endophytic fungal diversity from this region extremely inadequate. In the present study widely used medicinal plant Cissus quadrangularis was investigated to isolate the endophytic fungi. Total eight endophytes were observed from different parts (stem, leaf and petiole) of the host by using standard methods. For the specific identification of species; morphological characters and dimensions of various fruiting bodies were studied microscopically.

Keywords: taxonomy, fungal endophytes

Introduction

The word ‘endophyte’ means “inside the plant” (endon Gr.=within, phyton=plant) and term was coined by De Bary in 1866 to define all microbes (including fungi, bacteria, cyanobacteria and actinomycetes) that reside within plant tissue.

The association between fungal endophytes and host plants may be symbiotic or antagonistic or slightly pathogenic in nature.1 Major impact of endophytes is observed on ecology, distribution and physiology along with immunity of plants. Almost all the plant species (~400,000) harbour one or more endophytic organisms.2 To date, only a few plants have been extensively investigated for their endophytic biodiversity and their potential to produce bioactive secondary metabolites. It is, therefore, important to determine endophytic biodiversity of medicinal plants. Identification, taxonomic position and mapping of fungi are challenging tasks. Out of many fungi about 70,000 fungal species had been isolated, identified and characterized.1,3 Manoharachary et al.,4 are of the opinion that about 27,000 fungal species have been described in India. Scientific community is continuously striving to search into the diversity of fungi and their natural potentials. The multiplicity of endophytic fungi and their role in various biochemical processes occupy most important place in the biological world and India has been the cradle for such fungi.

During mycological investigation of Melghat Forest of Amravati District several author collected and reported many rare and interesting fungal forms they are new to Maharashtra.5-12 The traditional taxonomy of fungi is based on morphological features like shape, size and colour of various fruiting bodies.13 In the present study isolated fungal species were also identified morphotaxonomically by studying their macroscopic and microscopic characteristics.

Materials and methods

Collection of plant samples

The plant samples were collected from Melghat forest of Amravati district. The samples were collected in sterilized polythene bags. The collected samples were brought to the laboratory and processed within 24hrs of collection.

Isolation of the endophytic fungi

Surface sterilization were done according to the method described by Suryanarayanan14 (Table 1) to remove the epiphytes. The surface sterilized explants then inoculated at 26±2°C into the Petri dishes containing potato dextrose agar (PDA). The plates were periodically observed for fungal growth.

Table I Surface sterilization of explants

| Chemicals | Concentration | Time |
|-----------|---------------|------|
| Ethanol   | 70%           | 1min |
| SDW       | -             | 3min×4times |
| NaOCl     | 4%            | 30sec |
| SDW       | -             | 3min×4times |
| Ethanol   | 70%           | 30sec |
| SDW       | -             | 3min×4times |

SDW, sterile distilled water; NaOCl, sodium hypochlorite

Microscopic observation

Permanent slides were prepared from pure colonies of isolated endophytic fungi. Morphological characters such as pycnidia, conidia and conidiogenous cells (Coelomycetes); conidia and conidiophores (Hyphomycetes) were studied under Carl Zeiss, Trinocular Research Microscope (Axioscope-A-1) with magnification of 5x, 10x, 40x and 100x.

Mountants and stain

In the present study microscopic observation of isolated endophytic fungi was initially done in water mountant. However, various fruiting structures were observed by mounting in lactophenol-cotton blue. This stain-cum-mounting medium has been used for different taxonomic groups of fungi.14,16
Identification of endophytic fungi

All the endophytic isolates were identified morphologically and placed in appropriate genera and species of fungi using standard taxonomic keys and monographs\(^{17-21}\) were referred for identification of endophytes.

Observations and results

**Arthrinium hydei** Crous and Groenewald (Plate I, Figure 1)

Mycelium smooth, hyaline to pale brown, branched, septate, 2-3μm diameter. Conidiophores pale brown smooth, cylindrical, septate, branched, 22-34×3-5μm. Conidiogenous cell aggregated in clusters on hyphae, smooth, hyaline, doliiform. Conidia unicelled, brown, globose to lenticular with pale equatorial slit 10-22μm diameter in side view.

Remark: The species under study matched with *A. hydei*, and new to this region. *A. hydei* was isolated and cultured on PDA. Colonies developed were olive white with patches of grey to black.

**Arthrinium phaeospermum** (Corda) Ellis (Plate I, Figure 2)

Colonies dark brown to greenish, round, oval or irregular in shape. Mycelium hyaline to pale brown, smooth hyphae, 3-4μm in diameter. Conidiophores are cylindrical, narrow, erect or flexuous, straight, simple, smooth, hyaline 5-12×3-5μm thick, dark brown with transverse septa 48-120μm long,2-4.5μm in diameter between septa, basal cell somewhat flattened and round or irregular in shape. Conidia sessile or sometimes borne on short hyaline pegs along the sides of the conidiophores, which are somewhat flattened, lemon shape in surface view, triangular in side view but outer edge is curve and the corners round, brown pale at tips, smooth 10-16μm long,4-7μm wide in surface view.

Remark: Isolated specie cultured on PDA. The colonies were dark brown to greenish in color, round, oval and irregular in shape (Table 2).

| Species                        | Colony character(color)         | Conidiophores | Conidia         |
|--------------------------------|---------------------------------|---------------|-----------------|
| *A. caricola* Kunze ex Fries   | Black, pulvinate                | 150μm×4μm     | 36-54μm×9-12μm  |
| *A. hydei* Crous                | Olive white with patches of grey to black | 21.65-33.22μm×3.2-4.8μm | 10-22μm |
| *A. marii Larrondo and Calvo*   | Whitish- black to olivaceous grey | 6-10×2.5×4μm  | 7-10μm         |
| *A. phaeospermum* (Corda) Ellis | Dark brown to greenish in color  | 4.7-11.6μm×2.8-5.1μm | 10-16μm×3.9-7.2μm |

Dense mycelial growth on PDA, colonies with uniform edges, color ranges from dark purple red to green brown in PDA. Sporodochia develop after approximately after 20days on PDA. Sporodochia powdery, brownish to grey black in color. Mycelium hyaline, smooth, septate, brown on maturation. Short conidiophores originated on hyphae in clusters. These conidiophores branched repeatedly and are visible as dense masses. Conidiophore hyaline, claviform, 1-3 septate, smooth up to 9-11μm, producing a single dark gangliospore terminally. Young conidia rounded, non septate and pale in color. Mature gangliospore golden brown or brown or olivaceous or black, 40 globose or pyriform or sometimes of irregular angular shape, septate, muriform, verrucose, 15-54μm in diameter. Mature conidia contain multiple transverse and vertical septa.

Remark: The characters of the specimen understudy were allied with *Epicoccum nigrum*, hence assigned to the said species (Table 3).

**Epicoccum andropogonis** (Ces) Schol- Schwarz

Greyish black sometime radish grey 9×12μm 22-28μm

**Epicoccum nigrum** Ehrenb.Ex.Schlecht.

Dark purple red to green brown 8-11.5μm 14.5-53.9μm

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Nigrospora oryzae (Berk & Br.) Petch. (Plate I, Figure 4)

Mycelium septate, branched, brown in colour. Conidiophore short, ampiliform, somewhat brown in colour, bearing single conidium at the tip. Conidia borne singly at the tip of the 113 conidiophores, globose or somewhat flattened, absolutely opaque black, with hyaline membrane on the upper side 24-28×21-24μm.

Figure 3 Mycelium with conidia of E. nigrum.

Table 4 Comparison between species of Nigrospora

| Species                                  | Colony character (color) | Conidiophores          | Conidia            |
|------------------------------------------|--------------------------|------------------------|--------------------|
| Nigrospora oryzae (Berk & Br) Petch.     | Brown                    | Short, ampiliform      | 24-28μm×21-24μm    |
| Nigrospora panici Zimm.                  | Yellowish                | Short, slightly inflated| 25-30μm×22-25μm    |
| Nigrospora padwickii Prasad, Agnihotri and Agarwal | Pale brown               | Short, swollen below apex | 33.5-41.8μm×31.8-40.2μm |

Table 5 Comparison between species of Pestalotiopsis

| Species                                  | Colony character(color) | Apical appendages | Conidia            |
|------------------------------------------|-------------------------|-------------------|--------------------|
| Pestalotiopsis funerea Stey.             | Dark brown              | 2-Jan             | 15.5-28.5×6.6-9.2μm |
| Pestalotiopsis guepinii (Desm) Stey.     | Olivaceous brown        | 3-Jan             | 12-15μm×5.5-6.6μm  |
| Pestalotiopsis maculans (Corda) Nag Raj  | Pale brown to moderate brown | 3-Feb            | 10-15.5μm          |

Pestalotiopsis funerea Stey (Plate I, Figure 5).

Pithomyces chartarum (Berk and Curtis) Ellis (Plate I, Figure 6)

Colonies effused faint yellow, olive-green, shiny. Mycelium composed of a network of brown, smooth or rough walled hyphae. Conidiophores short peg like, 2-4μm wide, arising laterally on hyphae, subhyaline. Conidia produced singly as blunt out ends at the apex of conidiophores, oval, elliptical, obtuse, pale brown at young stage, dark brown at mature stage and often one or more oblique or longitudinal septa, 21-32μmlong, 12-35μm wide, each conidium carrying away a part of conidiophores.

Remark: On comparison with known species, the present species proved to be P. funerea (Table 5).

Table 6 Comparison between species of Pithomyces

| Species                                  | Colony character(color) | Apical appendages | Conidia            |
|------------------------------------------|-------------------------|-------------------|--------------------|
| Pithomyces chartarum (Berk and Curtis) Ellis | Dark brown              | 2-Jan             | 15.5-28.5×6.6-9.2μm |
| Pithomyces guepinii (Desm) Stey.         | Olivaceous brown        | 3-Jan             | 12-15μm×5.5-6.6μm  |
| Pithomyces maculans (Corda) Nag Raj      | Pale brown to moderate brown | 3-Feb            | 10-15.5μm          |

Pithomyces chartarum (Berk and Curtis) Ellis (Plate I, Figure 6).
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**Table 6** Comparison between species of *Pithomyces*

| Species                       | Colony character(color) | Conidiophores       | Conidia                                      |
|-------------------------------|-------------------------|---------------------|----------------------------------------------|
| *Pithomyces chartarum* (Berk and Curtis) Ellis | Olive-green             | 1.8-3.9µm           | 20.6-31.8µm×11.7-34.9µm                     |
| *Pithomyces atro-olivaceous* (Cooke & Harkn) Ellis | Dark olivaceous brown   | 1-5µm×1-3µm         | 15-35µm×7-10µm                              |
| *Pithomyces flavus* Berk.& Br. | At first yellow to olive green later dark olivaceous | 2-5µm×1.5-2µm       | 28-45µm×15-26µm                             |

*Pithomyces chartarum* (Berk and Curtis) Ellis (Plate I, Figure 6)

**Stachybotrys nilgirica** Subram. (Plate I, Figure 7)

Mycelium composed of creeping hyaline or pale colored, branched hyphae. Conidiophores (phialophores) scattered on substratum, erect, straight, hyaline, slightly narrow above, 2-3 septate (septa 21.28-39.9µm apart), swollen at base, 68.7-93.1µm long smooth, terminating in a cluster of about 6-7 phialides, apical cell of philophore 13.3-17µm long and 2-6µm wide and subhyaline. When young pale olive green, at maturity conidia borne singly acrogenously at the tip of phialides, 1-celled, globose, tuberculate, dark greenish black 15.3-27.8µm in diameter.

**Table 7** Comparison between species of *Stachybotrys*

| Species                      | Colony characters                               | Conidiophore | Phialides | Conidia                                      |
|------------------------------|------------------------------------------------|--------------|-----------|----------------------------------------------|
| *Stachybotrys chartarum* (Ehrenb.) Hughes | Colorless or whitish then becoming black       | 64-109µm×5µm | 11-19µm×4.5-6.5µm | 15-16.5µm×3.2-7.3µm                           |
| *S. chlorohalonata* Andersen and Thrane | Colonies thick dark black coloured             | 73-91µm×13-16µm | 11-16µm×4-6µm | 11-16µm×4-6µm                                 |
| *S. nilgirica* Subram.       | Black                                           | 68.7µm-93.1µm | 13.3-17µm×2-6µm | 15.3µm-27.9µm                                 |

**Trimmatostroma hughesii** Rao and Subhedar (Plate I, Figure 8)

Colony olivaceous, brown, conidiophores macronematous, septate, sporogenous cell bears on top of conidiophores, 40-156µm long and 4-7µm wide. Conidiophores simple, sptate, hyaline. Conidia small, simple, catenulate, only one longitudinal septa over single transverse septa, 6-11µm×4-9µm.

Remark: Characters of the present specimen match with *Trimmatostroma hughesii*, hence assigned to the same (Table 8).

**Table 8** Comparison between species of *Trimmatostroma*

| Species                          | Colony character(color) | Conidiophores | Conidia |
|----------------------------------|-------------------------|---------------|---------|
| *Trimmatostroma eriodictyonis* (Dearn. and Barthol) Ellis | Dark brown             | 45-68µm       | 18-57µm×5-26µm |
| *Trimmatostroma hughesii* Rao and Subhedar | Olivaceous, brown       | 39.6-155.7µm×3.8-7.2µm | 6.2-11.4µm×3.9-8.8µm |
| *Trimmatostroma scutellare* (Berk and Br.) Ellis | Pale brown to moderate brown | 30-50µm long, 2-4µm | 13.5-32.3µm×29-39.5 |

*Trimmatostroma hughesii* Rao and Subhedar (Plate I, Figure 8)

**Table 9** Comparison between species of *Trimmatostroma*

| Species                          | Colony character(color) | Conidiophores | Conidia |
|----------------------------------|-------------------------|---------------|---------|
| *Trimmatostroma eriodictyonis* (Dearn. and Barthol) Ellis | Dark brown             | 45-68µm       | 18-57µm×5-26µm |
| *Trimmatostroma hughesii* Rao and Subhedar | Olivaceous, brown       | 39.6-155.7µm×3.8-7.2µm | 6.2-11.4µm×3.9-8.8µm |
| *Trimmatostroma scutellare* (Berk and Br.) Ellis | Pale brown to moderate brown | 30-50µm long, 2-4µm | 13.5-32.3µm×29-39.5 |

**Table 10** Comparison between species of *Trimmatostroma*

| Species                          | Colony character(color) | Conidiophores | Conidia |
|----------------------------------|-------------------------|---------------|---------|
| *Trimmatostroma eriodictyonis* (Dearn. and Barthol) Ellis | Dark brown             | 45-68µm       | 18-57µm×5-26µm |
| *Trimmatostroma hughesii* Rao and Subhedar | Olivaceous, brown       | 39.6-155.7µm×3.8-7.2µm | 6.2-11.4µm×3.9-8.8µm |
| *Trimmatostroma scutellare* (Berk and Br.) Ellis | Pale brown to moderate brown | 30-50µm long, 2-4µm | 13.5-32.3µm×29-39.5 |

**Table 11** Comparison between species of *Trimmatostroma*

| Species                          | Colony character(color) | Conidiophores | Conidia |
|----------------------------------|-------------------------|---------------|---------|
| *Trimmatostroma eriodictyonis* (Dearn. and Barthol) Ellis | Dark brown             | 45-68µm       | 18-57µm×5-26µm |
| *Trimmatostroma hughesii* Rao and Subhedar | Olivaceous, brown       | 39.6-155.7µm×3.8-7.2µm | 6.2-11.4µm×3.9-8.8µm |
| *Trimmatostroma scutellare* (Berk and Br.) Ellis | Pale brown to moderate brown | 30-50µm long, 2-4µm | 13.5-32.3µm×29-39.5 |
Discussion

Medicinal plants are reported as great reservoir of endophytes. One to several fungi could be isolated from single host. In the present study total eight endophytic fungi were isolated from single host. Previous study proved that out of the total groups of fungi, anamorphic fungi are prevalent as endophytes in the plants screened throughout the world. The fungi found in the present investigation also showed dominance of anamorphic fungi.

Taxonomy is the discipline of classifications i.e. the assemblage of organisms into definite category (taxa). Morphological characters are very much useful in the field of taxonomy to give the special identity to the organism. In the fungal taxonomy morphological as well as microscopical characters play an important role to identify them. Colony morphology, type of hypha, spore and reproduction are characteristics which can used to identify the fungi.

Arthrinium genus observing similarities in all the two species studied and some specific differences in structure and colour etc of conidia, the investigator through same may assist in finding morphological differences. Conidiogenesis is particularly interesting. Conidigenous cells tend to be dolliform to subcylindrical, pale brown with clear periclinal thickening. On further development these cells become ampulliform, with a prominent elongated neck. The neck can terminate in conidia either sympodially or in some species percurrently while in others with annelation etc. One must note that variation in conidiogenesis makes it difficult to compare these characters among taxa, as conidiophores can either be hyphae with lateral loci or be reduced to dolliform conidigenous cells. Conidial ontogeny is holoblastic. The apical holoblastic conidium, initially spherical, changes to lenticular. When conidium matures the neck of the conidiophores becomes narrower and a circular breaking can be seen on the outer wall conidiophores (this is initial of basauxic growth). This holoblastic nature and sizes of conidia vary as per species. The conidia of Arthrinium can develop a spontaneous break of the wall, thus, releasing protoplastic contents.

Epicoccum nigrum is an anamorphic ascomycete distributed globally which colonizes on different types of soil and different host plants. Morpho-cultural characters make two groups of E. nigrum. The first group is showing yellow to orange mycelium while second group shows grey, pink, red or brown mycelium. The fungi found in the present investigation aims to study basic taxonomic study of endophytic fungi from Cissus quadrangularis from Melghat Forest of Amravati district of Maharashtra. Study concluded that several endophytes can associated with single host. The present findings reported first time from this region.

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Conflict of interest

The author declares no conflict of interest.

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