Fungal Endophytes of Some Green Leafy Vegetables

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Abstract: Twenty one species of endophytes were recorded from three green leafy vegetables viz., Amaranthus dubius, Hibiscus sabdariffa and Moringa oleifera. Aureobasidium sp. 1, Phyllosticta sp. and Nigrospora sp. were dominant forms. Seven sterile forms were recorded as endophytes. The similarity coefficient of endophyte assemblages between any two hosts was low (maximum being 0.16). Endophytes from green leafy vegetables inhibited the growth of some of the plant pathogens in vitro. The endophytes were isolated from apparently healthy leaves that we normally consume. Therefore, this study paves way for further investigation to understand the role of this group of organisms in enhancing the nutrient content of vegetable that we consume.

Key words: Fungal endophytes • Amaranthus • Hibiscus • Moringa • Phyllosticta

INTRODUCTION

Endophytes are organisms that inhabit plant organs that at some time in their life can colonize internal plant tissues without causing apparent harm to their host [1]. Fungal endophytes mainly belong to Ascomycetes or Mitosporic fungi. Very few Basidiomycetes occur as endophytes [2]. Coelomycetes such as Pestalotiopsis, Phomopsis and Phyllosticta are frequently isolated as endophytes and are called as “almost exclusive” endophytes [3]. Ascomycetes such as Chaetomium, Sordaria, Sporormiella etc. have been reported as endophytes from several hosts although species belonging to these genera are known to be coprophilous [4].

Fungal endophytes are ubiquitous and have been isolated from algae, pteridophytes, gymnosperms and angiosperm members. Endophytic fungal diversity is more in tropical forests where woody angiosperm diversity is higher [5]. Investigations on endophyte assemblages have shown that a large number of fungal taxa can be isolated from a single host species however, only one or a few fungal species dominate in a host plant. Some endophytes appear to be specific to their host. Fisher and Petrini [6] reported that the genus Camarosporium was the dominant endophyte in tissues of Suaeda fruticosa growing in Chesil beach in Dorset. The same genus was found to be dominant in Suaeda maritima growing in India [7].

Endophytes are considered plant mutualists as they receive nutrition and protection from the host plants while the host plant may benefit from enhanced competitive ability. Evidence suggests that plants infected with endophytic fungi have distinguishable advantage against stress (biotic and abiotic) over non-endophytic counterparts [8, 9]. Endophytic fungi are increasingly recognized as a group of organisms that are likely to be sources of new metabolites useful to mankind [10]. Selim et al. [11] showed that fifty-five of 99 tested endophytes of medicinal plants showed a broad spectrum of inhibitory activity against different pathogenic bacteria and yeasts. Novel antibiotics, antymycotics, immunosuppressants and anticancer compounds are only a few examples of what has been found after the isolation and culturing of individual endophytes followed by purification and characterization of some of their natural products [12].

The association of endophytes from various groups of plants including medicinal plants, mangroves, tropical forest trees and grasses have been done but there are no studies on the endophytes of green leafy vegetables, especially, those sold in the market. Therefore, we studied the endophyte associations of these plants that we consume.
MATERIALS AND METHODS

Location: The plants for studying fungal endophytes were obtained from Puducherry (situated between 11° 46'-12° 43' N and 79° 36'-79° 52' E; 15 m, MSL) that lies in the east-coast of India 140 km south of Chennai.

Collection of Samples: Three green leafy vegetable species viz., Amaranthus dubius Mart., Hibiscus sabdariffa L. and Moringa oleifera Lam. were studied for their endophyte fungal presence or association. Five plants of each host were chosen and were transported in separate, closed, sterile polythene bags and processed within 24 hours of collection. In the case of slow growing endophytes, a mycelia plug of the endophyte was placed on the medium and incubated for 3-4 days before placing the mycelia plug of a pathogen. The interaction between an endophyte and a pathogen was classified following the method of Schoeman et al. [16].

RESULTS

A total of 21 species of endophytes were recorded from the three host plants studied (Table 1). A total of 37 isolates belonging to 11 species were isolated as endophytes Amaranthus dubius. Hyphomycetes dominated the endophyte assemblage of this plant and Aureobasidium sp. 1 was dominant endophyte species. A total of 14 isolates belonging to 5 species were isolated as endophytes from Hibiscus sabdariffa. Coelomycetes dominated the endophyte assemblage of this plant and Phyllosticta sp. was the dominant endophytic species. A total of 23 isolates belonging to 12 species were isolated as endophytes from Moringa oleifera. Hyphomycetes dominated the endophyte assemblage of this plant and Nigrospora sp. was dominant endophyte species (Table 1).

<table>
<thead>
<tr>
<th>Endophyte</th>
<th>A. dubius</th>
<th>H. sabdariffa</th>
<th>M. oleifera</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspergillus fumigatus</td>
<td></td>
<td></td>
<td>2</td>
</tr>
<tr>
<td>A. glaucus</td>
<td>1</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>A. niger</td>
<td>-</td>
<td>-</td>
<td>3</td>
</tr>
<tr>
<td>Aspergillus sp.</td>
<td></td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>Aureobasidium sp.</td>
<td>24</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Aureobasidium sp. 2</td>
<td>2</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Colletotrichium sp.</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Curvularia lunata</td>
<td>-</td>
<td>-</td>
<td>3</td>
</tr>
<tr>
<td>Fusarium sp.</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Nigrospora sp.</td>
<td></td>
<td>-</td>
<td>5</td>
</tr>
<tr>
<td>Penicillium sp.</td>
<td>3</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>Phialophora sp.</td>
<td>1</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Phyllosticta sp.</td>
<td>1</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td>Sporormiella sp.</td>
<td>-</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Sterile forms</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>PYE1</td>
<td>1</td>
<td>-</td>
<td></td>
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<tr>
<td>PYE2</td>
<td>1</td>
<td>-</td>
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<td>PYE3</td>
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<td>-</td>
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<tr>
<td>PYE4</td>
<td></td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>PYE5</td>
<td></td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>PYE6</td>
<td></td>
<td>-</td>
<td>2</td>
</tr>
<tr>
<td>PYE7</td>
<td></td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>Total number of isolates</td>
<td>37</td>
<td>14</td>
<td>23</td>
</tr>
<tr>
<td>Number of species</td>
<td>11</td>
<td>5</td>
<td>23</td>
</tr>
</tbody>
</table>
Table 2: Similarity coefficients of endophyte assemblages for different host plants

<table>
<thead>
<tr>
<th></th>
<th>A. dubius</th>
<th>H. sabdariffa</th>
<th>M. oleifera</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. dubius</td>
<td>1.00</td>
<td>0.16</td>
<td>0.10</td>
</tr>
<tr>
<td>H. sabdariffa</td>
<td>1.00</td>
<td></td>
<td>0.16</td>
</tr>
<tr>
<td>M. oleifera</td>
<td></td>
<td>1.00</td>
<td></td>
</tr>
</tbody>
</table>

An attempt was made to find out the degree of specificity of the endophytes to different host plants. The similarity coefficient for the endophyte assemblages were calculated as mentioned under materials and methods. Maximum coefficient of similarity between any two hosts was 0.16 (Table 2).

Endophytes are known to inhibit phytopathogenic fungi. Therefore, interactions between endophytes from green leafy vegetables and phytopathogenic fungi, *Alternaria alternata* and *Rhizoctonia solani* were carried out. The method adopted is given under materials and methods. The mycelial interactions between the fungi were observed after 5 days and were categorized according to Schoeman *et al.* [16] (Table 3 and Fig. 1).

**DISCUSSION**

Mitosporic fungi (Hyphomycetes and Coelomycetes), Ascomycetes and sterile forms were present as endophytes in the green leafy vegetables.

![Fig. 1: Interaction of endophyte with plant pathogen. i) Nigrospora sp. (A) inhibiting *Alternaria alternata* (B, plant pathogen), ii). Phyllosticta sp. (C) inhibiting *A. alternata*](image)
Basidiomycetes and Oomycetes were not encountered as these fungi rarely occur as endophytes [2]. The contribution by the different groups of fungi to the endophyte assemblages varied with the host plant. Hyphomycetes dominated the endophyte assemblages of *Amaranthus dubius*, *Moringa oleifera* and Coelomycetes dominated the assemblage in *Hibiscus sabdariffa*.

The presence of sterile forms as endophytes continues to frustrate mycologists because of their uncertain taxonomy [17]. However, it is possible to distinguish them from each other by taking into account their culture characteristics [18]. In the present study also we obtained sterile forms as endophytes that were distinguished based and the culture characteristics and given code numbers. Sterile forms have been recorded from many other angiosperm hosts also [19, 20]. In some cases molecular sequence data from the nuclear ribosomal internal transcribed spacer region have been used to identify sterile cultures [21].

Some of the sporulating genera such as *Aspergillus*, *Colletotrichum*, *Penicillium*, *Phyllosticta* and *Sporormiella* occurred in more than one host of the three host species studied (Table 1). These are among the genera of endophytes isolated frequently from tropical hosts [22]. *Phyllosticta* features among the endophyte flora of almost all the host plants investigated [14, 23] and more specifically *P. capitalensis* is known to occur in different host species [24, 25]. The host range of this endophyte genus is now widened as it occurred in green leafy vegetables too. The fact that these fungi occur in many tropical plant species shows that these are well adapted to an endophytic mode of life. Bills and Polishook [3] consider these genera as ‘almost exclusive’ endophytes; *Phyllosticta* sp. showed the maximum density of colonization in *H. sabdariffa* (Table 1).

Some hyphomycete genera such as *Curvularia* is common phylloplane fungi, but, are known to occur as endophytes (even in the present study, this fungus occurred as endophyte). However, they are capable of penetrating the superficial layers of the leaf, when they do so, they survive the rigorous surface sterilization procedures used for isolating endophytes and grow out as colonies in plates [26, 27]. Several workers have reported the occurrence of phylloplane fungi as endophytes from diverse groups of plants [28, 29]. O’Donnell and Dickinson [30] suggest that phylloplane fungi might resort to an endophytic mode of life to overcome adverse environmental conditions such as desiccation.

Intriguingly, some coprophilous genera such as *Podospora*, *Sordaria* and *Sporormiella* are frequently isolated as endophytes [2, 4, 28, 31, 32]. In the present study, *Sporormiella* sp., a coprophilous form, occurred as endophyte in all the three hosts.

The age of the leaf tissue is known to influence the endophyte assemblage [33]. The number of endophytes that can be recovered increases with the age of the leaf tissue in *Trachycarpus fortunei* [34] and *Azadirachta indica* [35]. We found that the density of colonization and species composition of the endophytes was less in the case of present study. This could be due to fact the plants or their twigs obtained from the market were brought within a month of their growth, thus reducing the chance of repeated re-infection of the leaf over time from air borne inoculum.

Investigations carried out by Espinosa-Garcia and Langenheim [36] showed that within a given site, individuals of host species show some qualitative difference in their endophyte assemblages. In the present study, we observed that the endophyte assemblages of the three host species showed very less similarity (Table 2) suggesting some host preference.

Apart from trying to understand the biology of endophytes, another motivation for mycologists to study these fungi is their ability to produce novel bioactive compounds [37] such as antibiotics [38], anti-insect chemicals [39] and anticancer compounds [40]. Our study showed that some of the endophytes obtained in the present study could inhibit the growth of plant pathogenic fungus (Table 3; Fig. 1). Hence, it is likely that these endophytes also produce some bioactive compounds.

This study adds on to our existing knowledge of fungal endophytes with regard to their occurrence in green leafy vegetables. The endophytes were isolated from apparently healthy leaves that we normally consume, therefore, it paves way for further studies to understand the role of this group of organisms in enhancing the nutrient content of vegetable that we consume.

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REFERENCES


