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# Endophytic Fungi of Bitter Melon *(Momordica Charantia)* in Guangdong Province, China

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# Endophytic Fungi of Bitter Melon (*Momordica charantia*) in Guangdong Province, China

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**Abstract**

Endophytic fungi can mutualistically interact with their host plants by deterring herbivores. Overall 1172 endophytic fungal isolates were recovered from roots, stems, leaves, flowers and fruits of bitter melon, *Momordica charantia,* at five sites in Guangdong Province. These isolates were identified to 25 genera using morphological and molecular characteristics. The endophyte communities at the five sites were similar. *Alternaria alternata*, *Aspergillus* spp., *Cladosporium* spp., *Colletotrichum* spp., *Nigrospora* spp., *Penicillium* spp., *Arthrinium* spp., *Chaetimium* spp., *Curvularia* spp., *Fusarium* spp., *Phoma* spp., and *Phomopsis* spp. were isolated from at least three of the five sites. The coefficient of similarity for endophytes ranged from 60.6% to 83.3% between any two sites. There were significant differences in the species composition of endophytes recovered from different tissues of bitter melon. *Fusarium* spp. was the most frequent in root and stem samples, *Colletotrichum* spp. in leaf samples, *A. alternata* in flower samples, and *Cladosporium* spp. in fruit samples. The coefficients of similarity for endophytes were between 42.9% and 80.0% from any two tissues. We found that the composition of endophytes of bitter melon was relatively stable across sites, but differed greatly among tissues. We also found that there were fewer insects such as aphids (Homoptera: Aphididae), leafminers (Lepidoptera, Gracillariidae), and cotton leafworms *Spodoptera litura (*Fabricius) (Lepidoptera: Noctuidae) collected from the leaves of bitter melon at the Huadu site compared to those collected at the Yunfu site. Whether this is related to the endophyte communities isolated from different sites requires further research.

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Endophytic fungi are thought to interact mutualistically with their host plants mainly by deterring herbivores (Faeth and Hammon 1996, Li et al. 2004, Rodriguez et al. 2009). Most notably, endophytes produce toxic alkaloids that can deter insect herbivores or reduce insect herbivore performance (Clay and Schardl 2002, Kerri et al. 2010). Endophytic fungi can grow within their host plants without causing any noticeable symptoms of disease (Arnold et al. 2000, Suryanarayanan et al. 2002, Promputtha et al. 2007, Rosa et al. 2009). They are of interest because of their rich diversity and because they provide an excellent potential source of novel biologically-active compounds (Hyde 2001, Photita et al. 2001). There have been numerous studies on endophytic communities in various plants, including vegetables (D'Amico et al. 2008), cacti (Suryanarayanan et al. 2005), palms (Fröhlich et al. 2000), wheat (Larran et al. 2007), lichens (Li et al. 2007), meadow ryegrass (Lehtonen et al. 2006) and banana (Photita et al. 2001). However, there has not been a detailed study on endophytes of a medicinal food plant such as bitter melon (*Momordica charantia*), which is known to contain

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charantin (a steroidal glycoside), vicine (a glycoalkaloid) and polypeptide 'p' (a 166 residue insulinomimetic peptide) (Fernandes et al. 2007).

Bitter melon is widely cultivated in tropical climates for use as a vegetable and in medicine (Chang et al. 1996). Extracts of bitter melon have been found to possess medicinal properties such as antitumor, antioxidant, antivirus and antidiabetes (Limtrakul et al. 2004). Endophytic fungi in *M. charantia* may take part in the synthesis or transformation of such medicinal materials. The basic aim of the present study was to investigate the diversity and distribution of endophytic fungi isolated from *M. charantia*. A secondary aim was to collect cultures of endophytic fungi for further screening of new bioactive compounds that may provide protection mediated by fungal alkaloids to the host against insects and pathogens.

## Materials and Methods

**Sample collection.** Asymptomatic bitter melon plants were sampled from five sites in Guangdong Province in southern China: 1) the Huadu Station of the Guangzhou Vegetable Science Institute; 2) the Forecast Station of Crop Diseases and Pests in Zhaoqing city; 3) one site in Shitang town, Renhua county, Shaoguan city; 4) one site in Xiangqiao district, Chaozhou city; and 5) one site in Ducheng town, Yunan county, Yunfu city. Each site had fields of bitter melon at least 100 $m^2$ in size. Five randomly selected plants were collected from each site. These were brought back to the laboratory in sealed polythene bags and processed within 24 h of collection.

**Isolation and culture of endophytic fungi.** Samples were first washed in running water. Leaf discs (5 × 5 mm), segments of stem, root, flower, and fruit (all 5 × 5 mm) were then cut from the washed plant tissues in the laboratory. A total of 30 fragments of each tissue type were processed per site. All fragments were then surface sterilized using 75% ethanol for 1 min, autoclaved water for 30 s, 3% NaClO for 1 min, autoclaved water for 30 s, and then dried on autoclaved paper.

Five surface-sterilized leaf discs or other organ segments were evenly spaced on 2% potato dextrose agar (PDA) medium amended with streptomycin sulfate (50 mg/l) in Petri dishes, isolates were held in a growth incubator, incubated for 2 months at 25°C, and examined periodically. Surface-sterilized tissue segments were pressed onto the surface of fresh antibiotic-amended PDA to check the efficacy of the surface sterilization procedure. The absence of microbial growth on the medium from the tissue surfaces confirmed that the surface sterilization procedure was effective against surface fungi (Suryanarayanan et al. 2005).

When colonies grew out from plant tissues on amended media, subcultures were transferred onto new malt extract agar (MEA, 2%) plates. Subcultures were then incubated on different media which included PDA, corn meal agar (CMA, 2%), and tap water agar (TWA, 0.8%). Isolates were incubated at 25°C under cool white fluorescent lights with 12 h light / 12 h dark to induce sporulation in culture. Isolates from this study have been deposited in the Institute of Plant Pathology, Zhongkai University of Agriculture and Engineering.

**Endophyte identification.** Sporulating isolates were identified to genus and to species when possible using traditional morphological techniques. Sequencing of the ribosomal DNA internal transcribed spacer (ITS) region was used to confirm the identity of most endophytic fungi. DNA was extracted from fresh mycelium according to procedures of Lee and Taylor (1990). DNA samples were then checked for purity and integrity by electrophoresis in 1% (w/v) agarose with ethidium bromide (10 mg/mL) in 1 × TAE buffer before storing at 4°C. The reaction mix for polymerase chain reaction (PCR) amplification of the rDNA consisted of: 0.2 mM each of the primer pair, 0.2 mM dNTP, 10-50 ng DNA, 0.04 U of Taq polymerase (Promega, WI, USA), and 1 × PCR buffer mix in a 25 µl

volume. The primers ITS4 and ITS5 (White et al. 1990) were used to amplify the ITS region. The thermal cycling program was as follows: 3 min preheating at 94°C followed by 35 cycles of 94°C denaturing for 30 sec, annealing at 55°C for 30 sec, extension at 72°C for 1 min with a final 7 min extension at 72°C.

**Insect Collection.** Sampled plants at each site were examined for the presence of insects. All insects were identified to family and tallied.

**Statistical Analyses.** Isolation rate was calculated as follows: (Total No. of isolates yielded by a given sample) / (Total No. of segments in that sample). Isolation rates were used as a measure of fungal richness at a given site / tissue (Fröhlich et al. 2000). Species abundance was expressed as relative frequency of isolation, which was calculated by using the number of discs colonized by a given fungus divided by the total number of discs infected, expressed as percentage (Photita et al. 2001).

To make comparisons among the fungi recovered from different sites or tissues, the coefficient of similarity of the number of genera of endophytic fungi from different sites or tissues was calculated for all the possible pairs of sites or tissues according to the formula: 2j / (A+B), where A is the total number of fungal species isolated from one site or tissue, B from another site or tissue, and j is the number of fungal species found in common (Pielou 1975). The results were expressed as percentages.

## Results

**Composition of endophytic fungi.** A total of 1172 endophytic fungal isolates were separated from bitter melon. According to morphological and molecular features, they were finally classified to 25 genera (Table 1). The ITS1-5.8S-ITS2 partial sequences of 21 representative isolates were submitted to the GeneBank to obtain their access numbers, and the closest related species were got by BLAST analysis (Table 2).

**Impact of location on the number of isolates and generic diversity of endophytic fungi in *M. charantia*.** Various numbers of endophytic fungi were obtained from the five sites (Table 1). Of these 18 genera were from Huadu, 15 from Zhaoqing, 16 from Chaozhou, 13 from Shaoguan, and 11 from Yunfu (Table 3). *A. alternata*, *Aspergillus* spp., *Cladosporium* spp., *Colletotrichum* spp., *Nigrospora* spp., and *Penicillium* spp. occurred in all the five sites examined. *Arthrinium* spp., *Chaetimium* spp., *Curvularia* spp., *Fusarium* spp., and *Phoma* spp. were isolated from four sites examined. *Phomopsis* spp. was separated from three sites. The relative frequency of *Penicillium* spp. (19.3%) was the highest among the isolates separated from Zhaoqing, and *Cladosporium* spp. (20.1%) was the highest from Shaoguan. The relative frequency of *Colletotrichum* spp. were 21.4%, 18.2%, and 18.9%, which were the highest from Chaozhou, Huadu, and Yunfu, respectively. *Annulophloxylon* spp., *Aureobasidium* spp., *Botryosphaeria* spp., *Rhizoctonia* sp., and *Stemphylium solani* were only isolated from Zhaoqing. *Ceratobasidium* spp., *Exserohilum rostratum*, *Rhizopycnis* spp., and *Stagonospora* spp. were separated from Huadu only. *Paecilomyces* spp. and *Trichoderma* spp. were specific species from Chaozhou, and *Ascochyta* spp. from Shaoguan (Table 1). The isolation rate of endophytic fungi of Shaoguan (0.98) was the highest among the five sites and in the other sites the isolation rates of endophytic fungi from high to low were Chaozhou (0.93) > Yunfu (0.80) > Huadu (0.47) > Zhaoqing (0.44) (Table 3).

The coefficient of similarity for endophytes ranged from 60.6% to 83.3% between any two sites (Table 4). The highest similarity (83.3%) of endophytic communities was between Shaoguan and Yunfu, and the lowest similarity (60.6%) between Huadu and Zhaoqing. Similarities (>80%) of endophytic communities were high between Huadu and Chaozhou, Chaozhou and Yunfu, Chaozhou and Shaoguan, and Shaoguan and Yunfu.

**Table 1.** Relative frequency (%) of endophytic fungi in bitter melon from different sites.

| Taxa | Huadu % | Zhaoqing % | Chaozhou % | Shaoguan % | Yunfu % |
|---|---|---|---|---|---|
| *Alternaria alternata* | 11 | 2.1 | 9.6 | 8.6 | 3.1 |
| *Annulophloxylon* spp. | | 1.4 | | | |
| *Arthrinium* spp. | 1.3 | | 1.8 | 6.0 | 9.4 |
| *Ascochyta* spp. | | | | 0.9 | |
| *Aspergillus* spp. | 0.7 | 6.2 | 4.2 | 2.6 | 3.1 |
| *Aureobasidium* spp. | | 0.7 | | | |
| *Botryosphaeria* spp. | | 1.4 | | | |
| *Ceratobasidium* spp. | 0.7 | | | | |
| *Chaetomium* spp. | 7.1 | | 4.2 | 5.6 | 4.2 |
| *Cladosporium* spp. | 14.3 | 16.6 | 9.2 | 20.1 | 9.4 |
| *Colletotrichum* spp. | 18.2 | 7.0 | 21.4 | 6.4 | 18.9 |
| *Curvularia* spp. | 3.6 | 2.1 | 1.3 | 0.9 | |
| *Didymella* spp. | 7.8 | | 1.6 | | |
| *Exserohilum rostratum* | 2.6 | | | | |
| *Fusarium* spp. | 13.0 | | 14.3 | 14.5 | 2.6 |
| *Nigrospora* spp. | 3.3 | 1.4 | 4.0 | 0.9 | 2.6 |
| *Paecilomyces* spp. | | | 0.7 | | |
| *Penicillium* spp. | 0.7 | 19.3 | 7.8 | 6.4 | 4.2 |
| *Phoma* spp. | 2.6 | 0.7 | 1.8 | 1.3 | |
| *Phomopsis* spp. | 1.3 | 2.1 | 1.1 | | |
| *Rhizoctonia* sp. | | 2.1 | | | |
| *Rhizopycnis* spp. | 0.7 | | | | |
| *Stagonospora* spp. | 5.8 | | | | |
| *Stemphylium solani* | | 0.7 | | | |
| *Trichoderma* spp. | | | 0.2 | | 1.1 |
| Unidentified | 5.8 | 36.6 | 16.7 | 26.1 | 41.4 |

**Effect of tissue on the number of isolates and genera diversity of endophytic fungi in *M. charantia*.** The number of endophytic fungal isolates varied among different tissues. Fifteen genera were from roots, 13 from stems, 13 from leaves, 8 from flowers, and 17 from fruits (Table 5). *Cladosporium* spp. and *Penicillium* spp. occurred in all the five tissues. *Aspergillus* spp., *Colletotrichum* spp., *Nigrospora* spp., and *Fusarium* spp. were isolated from four tissues examined. *Alternaria alternata*, *Arthrinium* spp., *Chaetomium* spp., *Didymella* spp., *Phoma* spp., and *Phomopsis* spp. were separated from three tissues examined. The relative frequency of *Fusarium* spp. were 26.0% and 30.0%, which were the highest among the isolates separated from roots and stems, respectively. *Colletotrichum* spp. (41.1%) was the highest from leaves, *A. alternata* (36.7%) from flowers, and *Cladosporium* spp. (28.5%) from fruits. *Ceratobasidium* spp., *Paecilomyces* spp., *Rhizoctonia* spp., and *Rhizopynis* spp., were only isolated from roots, *Annulophloxylon* spp., *Ascochyta* spp., and *Stemphylium solani* were separated from leaves only, *Aureobasidium* spp. and *Stagonospora* spp. were specific species from fruits (Table 6). The isolation rate of endophytic fungi from stems (0.90) was the highest among the five tissues followed by flowers (0.82), then leaves (0.71), then fruits (0.65), and finally roots (0.58) (Table 5).

The coefficients of similarity for endophytes ranged from 42.9% to 80.0% between any two tissues. The highest similarity (80.0%) of endophytic communities was between stems and fruits, and the lowest similarity (42.9%) between roots and leaves. Lower similarities (<43.5%) of endophytic communities were found between roots and leaves, and between roots and flowers (Table 7).

**Table 2.** Closest relatives of the fungal endophyte isolates based on BLAST analysis.

| Fungal isolate | GeneBank accession number | Closest related species | Similarity (%) |
|---|---|---|---|
| 138 | AF455539.1 | *Alternaria alternata* | 99 |
| 236 | EU272517.1 | *Annulophloxylon stygium* | 99 |
| 225 | AM176711.1 | *Arthrinium* sp. | 99 |
| 122 | EU833207.1 | *Aspergillus japonicus* | 99 |
| 240 | EF197817.1 | *Aureobasidium pullulans* | 99 |
| 140 | EF423547.1 | *Botryosphaeria rhodina* | 99 |
| 141 | DQ102433.1 | *Ceratobasidium* sp. | 99 |
| 163 | AJ279468.1 | *Chaetomium* sp. | 93 |
| 151 | EF405864.1 | *Cladosporium cladosporioides* | 99 |
| 216 | AJ301979.1 | *Colletotrichum gloeosporioides* | 98 |
| 186 | AF212308.1 | *Curvularia brachyspora* | 97 |
| 200 | AB266850.1 | *Didymella bryoniae* | 100 |
| 156 | AJ853741.1 | *Exserohilum rostratum* | 100 |
| 8 | EF423517.1 | *Fusarium* sp. | 100 |
| 153 | DQ219433.1 | *Nigrospora oryzae* | 96 |
| 143 | AB353909.1 | *Penicillium marneffei* | 98 |
| 172 | EF423518.1 | *Phoma* sp. | 100 |
| 136 | EF488377.1 | *Phomopsis* sp. | 99 |
| 169 | DQ682600.1 | *Rhizopycnis* sp. | 99 |
| 206 | AM262366.1 | *Stagonospora* sp. | 99 |
| 152 | AF203451.1 | *Stemphyllium solani* | 100 |

**Table 3.** Isolation rates and generic diversity for endophytic fungi in *M. charantia* from different sites.

| Sites | No. of segments or discs | No. of isolates | Isolation rate | No. of genera |
|---|---|---|---|---|
| Huadu | 330 | 154 | 0.47 | 18 |
| Zhaoqing | 330 | 145 | 0.44 | 15 |
| Chaozhou | 480 | 448 | 0.93 | 16 |
| Shaoguan | 240 | 234 | 0.98 | 13 |
| Yunfu | 240 | 191 | 0.80 | 11 |

**Table 4.** The similarity coefficients for generic diversity of endophytic fungi of bitter melon among the different sites.

| Site | Zhaoqing | Chaozhou | Shaoguan | Yunfu |
|---|---|---|---|---|
| Huadu | 60.6 | 82.4 | 77.4 | 69.0 |
| Zhaoqing | | 64.5 | 64.3 | 61.5 |
| Chaozhou | | | 82.8 | 81.5 |
| Shaoguan | | | | 83.3 |

**Table 5.** The isolation rates and genera diversity of endophyte fungi in different tissues in bitter melon.

| Tissue | No. of segments or discs | No. of isolates | Isolation rate | No. of genera |
|---|---|---|---|---|
| Root | 180 | 104 | 0.58 | 15 |
| Stem | 300 | 271 | 0.90 | 13 |
| Leaf | 440 | 314 | 0.71 | 13 |
| Flower | 170 | 139 | 0.82 | 8 |
| Fruit | 530 | 344 | 0.65 | 17 |

**Table 6.** Relative frequency (%) of endophytic fungi in different tissues of bitter melon.

| Taxa | Root | Stem | Leaf | Flower | Fruit |
|---|---|---|---|---|---|
| *Alternaria alternata* | | | 4.8 | 36.7 | 6.7 |
| *Annulophloxylon* spp. | | | 0.6 | | |
| *Arthrinium* spp. | | 2.6 | 4.8 | | 5.8 |
| *Ascochyta* spp. | | | 0.6 | | |
| *Aspergillus* spp. | 1.9 | 6.6 | | 11.5 | 1.5 |
| *Aureobasidium* spp. | | | | | 0.3 |
| *Botryosphaeria* spp. | | 0.4 | | | 0.3 |
| *Ceratobasidium* spp. | 1.0 | | | | |
| *Chaetomium* spp. | 15.4 | | 3.2 | | 7.3 |
| *Cladosporium* spp. | 5.8 | 4.4 | 6.7 | 10.8 | 28.5 |
| *Colletotrichum* spp. | 3.9 | 12.9 | 41.1 | | 4.9 |
| *Curvularia* spp. | 1.9 | | | 7.9 | |
| *Didymella* spp. | | 0.4 | | 10.1 | 1.2 |
| *Exserohilum rostratum* | 1.0 | 1.1 | | | |
| *Fusarium* spp. | 26.0 | 30.0 | 2.2 | | 3.2 |
| *Nigrospora* spp. | | 1.1 | 3.5 | 7.9 | 2.0 |
| *Paecilomyces* spp. | 2.9 | | | | |
| *Penicillium* spp. | 6.7 | 14.4 | 3.2 | 2.9 | 7.9 |
| *Phoma* spp. | 3.8 | 0.7 | | | 2.9 |
| *Phomopsis* spp. | | 0.7 | 1.9 | | 0.6 |
| *Rhizoctonia* spp. | 2.9 | | | | |
| *Rhizopynis* spp. | 1.0 | | | | |
| *Stagonospora* spp. | | | | | 2.6 |
| *Stemphylium solani* | | | 0.3 | | |
| *Trichoderma* spp. | 1.0 | | | | 0.6 |
| Unidentified | 25.0 | 24.7 | 27.1 | 12.2 | 23.8 |

**Table 7.** Similarity coefficients of generic diversity for endophytic fungi in different tissues of bitter melon.

| Tissues | Stem | Leaf | Flower | Fruit |
|---|---|---|---|---|
| Root | 57.1 | 42.9 | 43.5 | 56.3 |
| Stem | | 61.5 | 57.1 | 80.0 |
| Leaf | | | 47.6 | 66.7 |
| Flower | | | | 56.0 |

## Discussion

In the present study, 25 genera of endophytic fungi were isolated from bitter melon. Similar results were obtained in previous studies for other plants. For instance, Suryanarayanan et al. (2005) identified 900 endophyte isolates belonging to 22 fungal species from cacti in Arizona. Paul et al. (2007) reported that 24 fungal genera were isolated from roots of *Aralia elata* and *A. continentalis*. Lin et al. (2007) investigated the endophytic fungi in *Camptotheca acuminata* and obtained 18 taxa. Sun et al. (2008) used morphological characters to identify 973 endophyte isolates of 21 taxa from 6 medicinal plant species.

The endophyte communities isolated from the five sites in this study were similar, despite the fact that varieties of bitter melon were quite different. *A. alternata*, *Aspergillus* spp., *Cladosporium* spp., *Colletotrichum* spp., *Nigrospora* spp., *Penicillium* spp., *Arthrinium* spp., *Chaetimium* spp., *Curvularia* spp., *Fusarium* spp., *Phoma* spp., and *Phomopsis* spp. were separated from at least three sites. Similar results were obtained in previous studies. Taylor et al. (1999) reported several taxa such as *Glomerella cingulata*, *Phomopsis* spp. and *Guignardia cocogena* dominated the assemblages of endophytic fungi at each site associated with the temperate palm, *Trachycarpus fortunei*, and they pointed out that the diversity at each site was similar in number, but the abundance of isolates varied. Swart et al. (2000) isolated endophytic fungi from *Protea cynaroides*, *Leucospermum cordifolium* and *Leucadendron salignum* × *laureolum* in three locations in the Western Cape province of South Africa, and they found the endophytic fungi at three locations were remarkably similar.

The results of this study revealed differences in the species composition of endophtes recovered from different tissues of bitter melon. *Fusarium* spp. was the most frequent in root and stem samples, *Colletotrichum* spp. in leaf samples, *A. alternata* in flower samples, and *Cladosporium* spp. in fruit samples. Petrini et al. (1992) concluded that different plant tissues and organs may resemble distinct microhabitats. Santos et al. (2003) investigated the endophytic fungi in *Melia azedarach* and obtained similar results with regard to tissue specificity of different fungal taxa. Gond et al. (2007) reported that the bark, leaf and root samples of *Aegle marmelos* differed in their endophytic fungal colonization. Different tissues can provide different substrates to support the survival of endophyte assemblages. Tissue specificity among the endophytes of bitter melon might be a reflection of tissue preferences of individual dominant taxa.

Endophytes belonging to potentially pathogenic species were also isolated, such as species of *Colletotrichum*, *Fusarium* and *Rhizoctonia*. Their presence as endophytes in bitter melon was interesting since these might be latent infections. Similar results have been obtained in previous studies. Some of the endophytes isolated from asymptomatic tissue of banana were known as pathogens of banana, such as *Deightoniella torulosa*, *Cordana musae*, *Colletotrichum musae*, *Guignardia musae* and *Pyriculariopsis parasitica* (Photita et al. 2001). Species of *Fusarium* and *Trichoderma* were obtained as endophytic fungi of *Melia azedarach* (Santos et al. 2003). Fisher and Petrini (1992) reported that *Fusarium equiseti*, *F. oxysporum* and *Phoma sorghina* were isolated from asymptomatic rice plants. Shamoun and Sieber (2000) pointed out that scientists should concentrate on finding the mechanisms which are responsible for causing a fungus to switch from asymptomatic endophytic to symptomatic pathogenic mode of colonization.

*Alternaria alternata* was one of the most frequently isolated species in bitter melon. Sun et al. (2008) pointed out that *A. alternata* was one of the common species among six plants studied. *A. alternata* was dominant in *Pinus tabulaeformis* (Guo et al. 2004), in *Camptotheca acuminata* (Lin et al. 2007), in a mangrove community (Kumaresan and Suryanarayanan 2001) and in wheat (*Triticum aestivum*) (Larran et al. 2007). The above results indicated that *A. alternata* is not host specific and can be isolated from tissues of different host plants.

Interestingly, we found that there were fewer insects such as aphids (Homoptera: Aphididae), leafminers (Lepidoptera, Gracillariidae), and cotton leafworms *Spodoptera litura (*Fabricius) (Lepidoptera: Noctuidae) collected from the leaves of bitter melon of Huadu than those of Yunfu. Kerri et al. (2010) reported that insect herbivores showed a significant preference for endophyte-free plant material for the majority of native grasses, with up to three times lower insect herbivores for endophyte-symbiotic plants, both in the field and in experimental trials. Tintjer and Rudgers (2006) found that deterrence of insect herbivory depends on the fungal strain and growth stage of the plant. Whether this is caused by the endophyte communities isolated from different sites deserves further research.

This study was the first to examine endophytes of bitter melon, a medicinal food plant with very bitter charantin, vicine and polypeptide 'p'. It revealed that both isolates and generic diversity of endophytes of bitter melon varied by site and tissue. There is growing interest in endophytic fungi as potential producers of novel, biologically-active products (Sun et al. 2008; Petrini et al. 1992; Monaghan et al. 1995). Investigations on the endophytic fungi of bitter melon will be of great value to our understanding of ecology and pharmacology. Further research is needed to screen endophytic fungi for antitumor, anti-insect and antifungal activity, and to investigate the roles of strong bioactive isolates including charantin, vicine and polypeptide 'p'.

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