Int. J. Environ. Sci. Technol. (2015) 12:3781–3790
DOI 10.1007/s13762-015-0802-5

ORIGINAL PAPER

Fungi colonizing wood sticks of Chinese fir incubated in subtropical urban soil growing with *Ficus microcarpa* trees

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Received: 1 October 2013 / Revised: 3 January 2014 / Accepted: 15 March 2015 / Published online: 9 April 2015

Abstract Fungi colonized on wood sticks of the Chinese fir *Cunninghamia lanceolata* after incubation in soil around the *Ficus microcarpa* trees were isolated. In total, 1189 wood sticks from 28 *F. microcarpa* trees were sampled and analyzed in this study. A combined approach of culture morphological observation and PCR amplification of ITS of purified fungal isolates in revealing restriction fragment length polymorphism patterns was applied to group the purified fungal isolates for further species identification. Fungal species were identified based on phylogenetic analysis of ITS and β-tubulin gene-amplified sequences. Overall, 25 species belonging to 17 genera from four classes were identified, and all of them were Ascomycota. Among them, *Penicillium* spp., *Fusarium* spp., and *Phoma insulana* were the most frequently isolated. In this study, *Dichotomonyces cejpii*, *Fusicolla acetilerea*, and *Purpureocillium lilacinum* are recorded in Hong Kong for the first time. Some species of the fungal cultures obtained in this study may have potential application as biocontrol agents to control tree disease.

S. Ding and H. Hu contributed eqaually to this work and therefore they should be treated as co-first authors.

Electronic supplementary material The online version of this article (doi:10.1007/s13762-015-0802-5) contains supplementary material, which is available to authorized users.

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Keywords ITS · RFLP · Soil fungi · Taxonomy · Urban environment · Incubation

Introduction

Fungi play an important role in maintaining the carbon and nutrient cycling of the urban ecosystem, and they are also crucial in either maintaining the health or causing diseases of the trees (Burke et al. 2011). The natural community of fungi can form a barrier to resist the invasion of exotic fungi, which may threaten local fungal diversity and also the health of local trees (Berendsen et al. 2012). The balance of different fungal species in the form of higher diversity may prevent some opportunistic fungal species turning into pathogens. However, when the trees are senescent and/or weak, opportunistic pathogenic fungi may invade and cause disease and damages to the trees (Gazis and Chaverri 2010).

In urban environment, biodiversity of fungal community in trees grown soils is relatively low, which may be caused by the intense human impact, poor growing condition, environmental pollution, transplantation of seedlings, which may carry pathogens to trees, and low biodiversity of roadside trees (Newbound et al. 2010). The low diversity of the fungal community may result in a lack of antagonistic fungal species, therefore, allowing pathogens including opportunistic pathogens to cause infection of the trees and further damage of the physical property of the trees. Under such circumstance, the existence of antagonistic fungi is extremely important to the maintenance of tree health. Therefore, it is important to know the fungal communities in the urban soil and the fungal species that can cause significant damage of the trees.

The conventional culture-dependent methods usually end up with a large number of isolates. Identification by checking the morphology of each culture strain can be tedious. An alternative can be molecular approaches, such as restriction fragment length polymorphism (RFLP), denaturing gradient gel electrophoresis (DGGE), and temperature gradient gel electrophoresis (TGGE), which are usually applied in ecological and biodiversity studies of fungi by culture-independent method (Viaud et al. 2000). These methods could be used to group fungal isolates according to DNA band patterns of electrophoresis. Species identification within each group of identical electrophoresis position can be achieved selectively with phylogenetic analysis of the obtained DNA sequences through comparison with relevant sequences from GenBank, and morphological characteristics could also be applied as confirmation of the individual species. Such combined approach of methods provides a faster and more accurate means to identify fungal species, despite the subtle culture morphological similarities of different species or significant culture morphological differences of the same species or the failure of sporulation in the culturing process on agar plates.

In this study, both culture-dependent and molecular methods were combined to investigate the composition of fungal community on wood sticks incubated in soils along the Nathan Road, Kowloon, Hong Kong; to interpret their possible function in the urban ecosystem; and to explore and test the application of molecular approaches in study of fungal community in urban environment. This study was conducted in urbanized Kowloon of Hong Kong in February 2011.

Materials and methods

Sampling site information

Nathan Road is a main road at Tsim Sha Tsui in Kowloon of Hong Kong, which connects several different commercial centers. It is one of the busiest roads with high volume flow of both vehicles and visitors. Both sides of the road are mostly planted with the Chinese banyan trees *Ficus microcarpa*. These trees have been grown there for several decades and are delightful signs of the cosmopolitan against the background skyscrapers and the crowd of locals and visitors (Supplement Fig. S1). However, some of the *F. microcarpa* are showing decline of health not only because of senescence, but also due to the restricted living environments and poor condition for growth. Walls made of bricks/stone and cement were built around the base of the trees with soil filling into the area. Various underground utilities also create barrier to the growth of the roots for extension. These conditions restrict the spreading and growth of the banyan roots for access of water and nutrients. In addition, growing urban pollution, improper trimming, and the invasion of plant pathogens also contribute to the health declination of the trees. In order to better understand the microflora that trees are exposed to and take more effective proactive mitigation plans to protect the trees and improve their health conditions, it is crucial to gather information on the fungal community in the soils grown with *F. microcarpa*.

Study sites and sample collection

The study was carried out on Nathan Road in Kowloon of Hong Kong in February, 2011. Wood sticks of the Chinese fir *Cunninghamia lanceolata* were used as baits for the soil fungi to colonize. The wood sticks (1 × 1 × 70 cm) were inserted into the soil about 60–65 cm in depth surrounding a *F. microcarpa* tree in 16 directions and four at each direction with 1 m of interval away from the tree according to the space availability (Fig. 1). Theoretically, there were 64 wood sticks for each tree, and the actual number was less than this due to the availability of space in all four directions for any of the 28 trees. They were incubated in the soil for at least 20 days, then retrieved, sealed in individual plastic bags, and brought back to the laboratory for further examination and isolation of fungi.

Isolation and purification of fungal isolates

Isolation of fungi was carried out in the sterile laminar-flow hood. Four segments (2 × 2 mm) were cut from each of

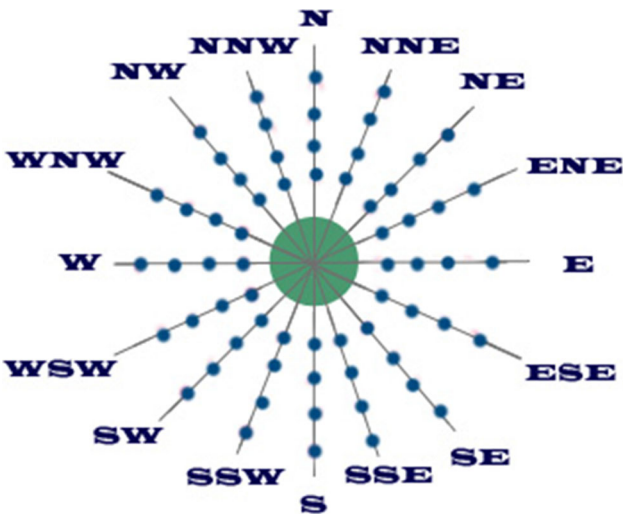

Fig. 1 Schematic diagram of maximum wood sticks deployments surrounding each *F. microcarpa* tree. Due to space availability, the actual number varied

the wood sticks, soaked in 75 % ethanol for 1.5 min, and then rinsed three times in sterile water before placed on malt extract agar (MEA) (BD Difco™) plate, which consists of 20 g/l malt extract, 20 g/l agar, 10 mg/l benomyl, 10 mg/l dicloran, 100 mg/l ampicillin, and 500 mg/l gallic acid (Chang 1995). Then the plates were incubated at room temperature (25 °C) for 4 weeks. Colonies developed on the plates were further transferred to potato dextrose agar (PDA) (BD Difco™) plates, containing 0.05 % streptomycin, and incubated at room temperature for purification. Subculture on PDA plates was repeated until the structure and color of the culture on agar plate were identical to those on the initial plates.

PCR-RFLP

Total genomic DNA of each purified isolates was extracted directly by grinding approximately 0.5 g mycelia (wet wt) in a mortar with liquid nitrogen and then transferring to a sterile 1.5-ml Eppendorf tube. CTAB method was used for DNA extraction (Lacap et al. 2003). The specific steps were as the followings. First, 600 µl of 2× CTAB was added into the tube and then incubated at 65 °C for 1 h, during which vortex was made every 10 min. Then 600 µl of phenol/chloroform (1:1, v/v) was added, mixed, and then centrifuged at 14,000 rpm for 25 min, and the upper aqueous layer was transferred into a new Eppendorf tube. This step was repeated twice. Then 900 µl of absolute ethanol was added, and the tube was kept at −20 °C over night and centrifuged at 11,000 rpm at 4 °C for 45 min. The supernatant was discarded, and the DNA pellet was washed with 70 % ethanol twice. After drying up in the sterile hood, the DNA pellet was dissolved in 100 µl of 10× TE without RNase and stored at 4 °C for further experiments.

PCR was carried out with universal primers ITS1F (5′-CTT GGT CAT TTA GAG GAA GTA A-3′) (Gardes and Bruns 1993) and ITS4 (5′-TCC TCC GCT TAT TGA TAT GC-3′) (White et al. 1990). Maxime PreMix (Intron Biotechnology) was used during the PCR amplification with 1 µl of ITS1F (20 µM), 1 µl of ITS4 (20 µM), 2 µl of template DNA (approximately 10 ng), and 16 µl of sterilized water (total volume of 20 µl). The protocol of thermal cycling parameters (Cai et al. 2005, 2006) with modification included an initial denaturation at 95 °C for 3 min, followed by 30 cycles consisting of denaturation at 95 °C for 1 min, annealing at 52 °C for 1 min, and extension at 72 °C for 1 min. A final extension at 72 °C for 10 min was added at the end of the thermal cycling. PCR products were visualized on 1 % agarose electrophoresis gels stained with GelRed™ nucleic acid gel stain (Biotium) to check for product purity and size. PCR products were purified by using GFX™ PCR RNA and Gel Band Purification Kit (Amersham Biosciences) under the guide of the manufacturer's protocol.

The purified PCR products of ITS rDNA were treated in two ways. On the one hand, some of the purified PCR products were digested with two restriction enzymes *Hae*III and *Hinf*I (Takara) following the manufacturer's instruction. Each reaction contained 5 µl of purified PCR product (about 5–10 ng), 1 µl of buffer, 0.5 µl of restriction enzyme (50 µM), and 3.5 µl of sterile water (total volume of 10 µl). Then the mixture was incubated at 37 °C for 2 h. The RFLP results were checked on 2 % agarose electrophoresis gels stained with GelRed. The pattern of the RFLP results was recorded and grouped. On the other hand, in each identified different group, one or several purified PCR products were sequenced at Genome Research Centre, The University of Hong Kong).

For certain groups of fungi which could not be identified to species level or did not achieve high branch support with ITS rDNA, β-tubulin gene was amplified with the primer pair T1 (5′-AAC ATG CGT GAG ATT GTA AGT-3′) and T2 (5′-TAG TGA CCC TTG GCC CAG TTG-3′) (O'Donnell and Cigelnik 1997). The PCR amplification using Maxime PreMix in a total volume of 20 µl was carried out with 1 µl of T1 (20 µM), 1 µl of T2 (20 µM), 2 µl of template DNA (approximately 10–15 ng), and 16 µl of sterilized water. The protocol of thermal cycling parameters following that for ITS rDNA with modification included an initial denaturation at 95 °C for 3 min, followed by 30 cycles consisting of denaturation at 95 °C for 1 min, annealing at 54 °C for 1 min, and extension at 72 °C for 1 min. A final extension at 72 °C for 10 min was added at the end of the thermal cycling. The subsequent steps were the same as that for ITS rDNA.

Phylogenetic analysis

The obtained sequences were checked with BioEdit (Hall 1999), mainly for the sequence accuracy, deleting varied characters at the beginning and the end and modifying the varied characters in the sequences. Each sequence was BLASTed in NCBI (http://www.ncbi.nlm.nih.gov/) to confirm the sequence validity. Together with other closely related sequences (Supplement Table S1 and S2), the sequences were aligned using CLUSTALX (1.83) (Thompson et al. 1997). Manual adjustments were made in BioEdit by inserting gaps to improve the alignments. Then the alignments were converted to NEXUS files using CLUSTALX for phylogenetic analysis in PAUP (Phylogenetic Analysis Using Parsimony) v.4.0b10 (Swofford 2002). Any ambiguously aligned regions were excluded from phylogenetic analyses.

Data sets were initially analyzed using maximum parsimony (MP). The heuristic search option was used,

ignoring constant and uninformative characters. Random addition of sequences for 5000 replicates with tree bisection–reconnection (TBR) branch swapping was performed. MulTrees option was in effect, and zero-length branches were collapsed. Gaps were treated as missing data. Tree scores, including tree length (TL), consistency index (CI), retention index (RI), rescaled consistency index (RC), and homoplasy index (HI), were also calculated for all the trees generated under different parameters. Branch support and stability for all MP analyses were assessed by bootstrap analysis (Felsenstein 1985). Bootstrap analysis was carried out based on 1,000 resampled data sets analyzed with random addition of taxa.

MrModeltest 2.2 (Nylander 2004) was used to get the best-fit model of substitution for maximum likelihood (ML) analyses and MrBayes analyses. ML analyses were carried out using heuristic search, with addition sequence set to "asis" and TBR branch swapping algorithm. Bayesian posterior probability was calculated by using MrBayes v3.0 (Huelsenbeck and Ronquist 2001). The implemented model was the same as that for ML analysis. One million generations were run for six chains and sampled every 100th generations resulting in 10,000 trees. The first 2,000 trees were discarded because they represented the burn-in phase of the analyses, and the remaining 8,000 trees were used to calculate the posterior probabilities in a consensus tree. Trees were viewed in Treeview (Page 1996).

The PCR-amplified sequences of ITS and β-tubulin genes of this study are assigned with accession numbers KF596337-KF596438 and KF596439-KF596512.

Results and discussion

Fungal colonization on wood sticks

In total, 1189 wood sticks were deployed and incubated around 28 *F. microcarpa* trees along the Nathan Road. All the 1189 of them were retrieved successfully after at least 20 days of incubation, but 166 of them were analyzed in this study due to the low diversity of fungal isolates obtained through increasing the number of samples analyzed. Because of this, no further examination of the remaining samples was carried out. These wood sticks were all visually intact without indication of apparent decomposition. However, all the sticks showed discoloration to some extent, either in forms of spots or patches (Supplement Fig. S2). Staining colors ranged from gray-blue to black. Wood sticks retrieved from the tree OVT35 appeared to be more intense coloration than the others.

Conventional isolation and culturing technique were used to isolate and culture the fungi from the wood sticks. After isolation and purification, 324 fungal isolates were obtained from 166 wood sticks, about 14 % of all the wood sticks incubated initially. Of the 166 wood sticks, approximately two isolates were obtained from each stick on average. Wood sticks retrieved from trees OVT35 and OVT36 showed significantly more isolates, about 3.45 and 4.25 isolates per stick on average, respectively. This uneven distribution of fungal colonization may be related to the urban environment (Newbound et al. 2010). Though all of the trees are grown along one road in a city center, the fungal communities obtained from the soil of each tree were not uniform (Fig. 2). Such unevenness is largely caused by the highly variable underground conditions due to soil type and the construction of utilities and telecommunication. Wood sticks retrieved from OVT35, the tree with pipes constructed underneath, were heavily colonized, and as a result, more fungal isolates with higher species richness were obtained. Similar results were also evident from OVT34 and OVT36, which were on each side of OVT35, forming a straight line with only a few meters apart. Other factors, such as the health condition of the individual tree, are the most important one contributing to the different fungal community around each tree. Several trees such as OVT1, 2, 7, 8, 11, and 12 are seriously

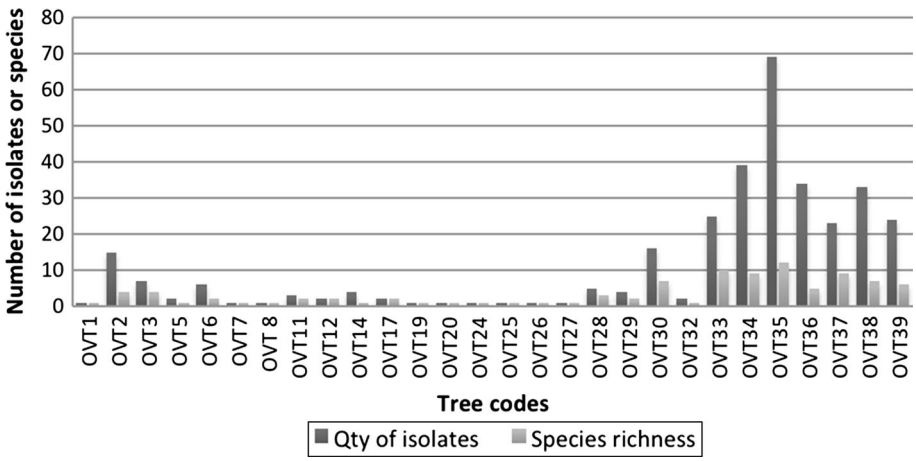

Fig. 2 Number of fungal isolates and species richness obtained from each tree

Table 1 A list of identified fungal species and their abundances on the wood sticks in this study

Species	Isolates number	Percentage of each species (%)	Occurrence (number of trees)	Occurrence rate (%)
Amorphotheca resinae	15	4.63	3	13.04
Aspergillus niger	1	0.31	1	4.35
Bionectria ochroleuca	27	8.33	12	52.17
Candida parapsilosis	2	0.62	1	4.35
Cochliobolus geniculatus	1	0.31	1	4.35
Cylindrocladiella lageniformis	3	0.93	3	13.04
Dichotomomyces ceijpii	6	1.85	5	21.74
Fusicolla acetilerea	1	0.31	1	4.35
Fusarium oxysporum	32	9.88	9	39.13
Fusarium proliferatum	3	0.93	1	4.35
Fusarium solani	28	8.64	9	39.13
Fusarium verticillioides	2	0.62	1	4.35
Paecilomyces formosus	4	1.23	2	8.70
Penicillium cf. *verruculosum*	1	0.31	1	4.35
Penicillium citrinum	1	0.31	1	4.35
Penicillium pinophilum	1	0.31	1	4.35
Penicillium purpurogenum	3	0.93	3	13.04
Penicillium sp. 1	96	29.63	11	47.83
Pestalotiopsis disseminata	4	1.23	2	8.70
Phoma insulana	65	20.06	16	69.57
Purpureocillium lilacinum	4	1.23	3	13.04
Pyrenochaeta sp.	3	0.93	2	8.70
Talaromyces assiutensis	3	0.93	2	8.70
Trichoderma atroviride	8	2.47	5	21.74
Trichoderma hamatum	10	3.09	4	17.39

infected by different kinds of wood-decay fungi with obvious cavities developed on the tree trunk and fungal fruiting bodies growing on the tree. The fungal communities detected associated with these trees were relatively smaller. Urban soil conditions can reduce the biodiversity and alter the community structures of soil fungi through the limited nutrients and living space available for trees and the presence of heavy metals and other pollutants from anthropogenic sources (Perez-de-Mora et al. 2006). The highly populated urban environment of Hong Kong has many factors limiting the growth of the trees, such as reduced space restricting the spreading of tree roots, lower plant species diversity, pollution of both atmosphere and soil by vehicles, which are very likely to reduce the biodiversity of soil fungal community and, in turn, to pose threats to the survival of trees in the cosmopolitan city.

Composition of community and diversity

A total of 24 types of RFLP pattern with *Hae*III and *Hin*fI were observed for the 324 fungal isolates obtained from the 166 wood sticks. By combining culture and microscopic morphological characteristics, 25 species were established from the 324 isolates and 33 unique sequences of ITS rDNA were included in the ML tree constructed. Based on MrModeltest 2.2, model GTR+G was chosen for ML analyses and MrBayes analyses. Phylogenetic analysis indicated 25 taxa, which were further confirmed by microscopic observation as 25 fungal species (Table 1). According to ML tree generated based on ITS (Fig. 3), species of a family were well clustered. Branches were highly supported by bootstrap values and Bayesian PP values. ITS yielded high resolution to most of the taxa, but relatively lower resolution to the genus of *Penicillium*, *Phoma*, and *Fusarium*. β-tubulin, which contains more variable characters than ITS rDNA, was applied in these groups, but improvement was only evident in the resolution of the genus *Fusarium* (Fig. 4) (Myllys et al. 2001).

The 25 species (Table 1) identified in this study belonged to 17 genera from four classes, Eurotiomycetes, Sordariomycetes, Dothideomycetes, and Saccharomycetes, which were all Ascomycota (Ainsworth and Kirk 2008). Among them, the genera *Penicillium*, *Fusarium,* and *Phoma* were 31.5, 20.1, and 20.1 % of all isolates, respectively. *Phoma insulana* was the most widely distributed with an occurrence rate of 69.6 % of the trees sampled, followed by *Penicillium* spp. (69.5 %), *Fusarium* spp. (56.5 %), and *Bionectria* sp. (52.2 %). Despite the overlapping of distribution range of certain fungal species, no specificity was detected for the wood sticks or individual trees, which is in agreement with the previous studies in that saprotrophic fungi usually have low host specificity (Lodge 1997; Zhou and Hyde 2001). Isolate number and species richness obtained from each tree varied, ranging from 1 to 69 and 1 to 12, respectively (Fig. 2).

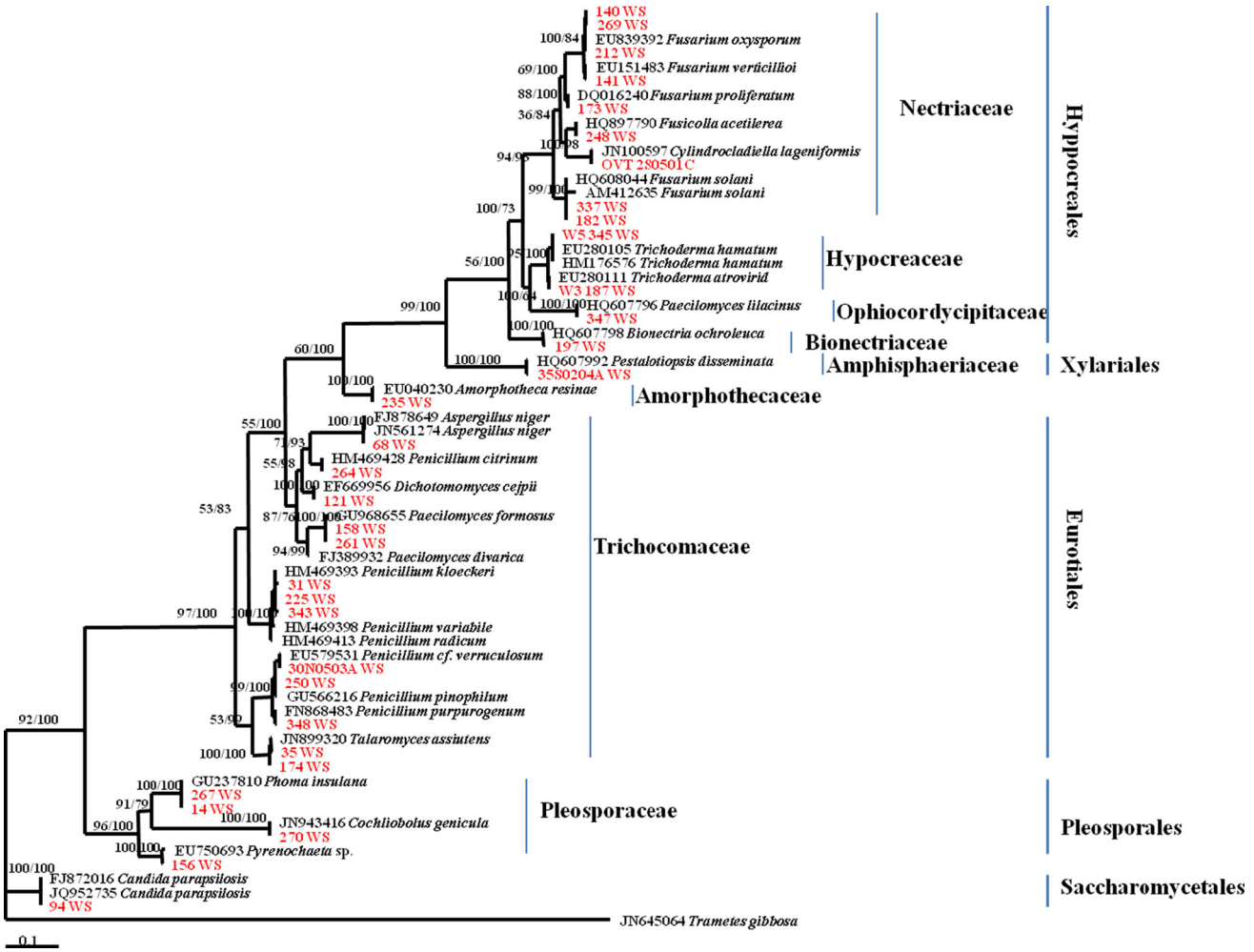

Fig. 3 Phylogenetic relationships among the 25 species obtained in this study. The tree was produced using maximum likelihood (ML) based on ITS region (ITS1, 5.8S, and ITS2). The *numbers before slashes* indicate bootstrap values; the *numbers after slashes* indicate Bayesian PP values. Sequences in *red* were obtained from this study

Generally, with the increasing number of fungi isolated from the incubated wood sticks, the diversity also showed an increase. Sixteen of these trees had less than five isolates obtained, but four (OVT34, 35, 36, 38) had more than 30 isolates. Seventeen of them had less than three fungal species detected, but one had 12 (OVT35).

Among the identified 25 species, *Penicillium* spp., *Fusarium* spp., and *P. insulana* were dominant in the fungal community (Table 1), which is ubiquitous not only in Hong Kong but also elsewhere (Lu 2000; Yanna et al. 2002). A small number of *Aspergillus niger*, *Cochliobolus geniculatus*, *Candida parapsilosis*, *Fusicolla acetilerea,* and other species were also detected in this study, among which *Cylindrocladiella lageniformis*, *Dichotomomyces ceijpii*, *F. acetilerea*, *F. proliferatum*, *F. verticillioides*, *Paecilomyces formosus*, *P. pinophilum*, *Purpureocillium lilacinum*, and *Talaromyces assiutensis* have never been recorded or detected in Hong Kong before. One important reason might be that there are very few relevant studies conducted in Hong Kong.

According to previous reports, most of the fungi detected in this study are very active in plant cell wall degradation (Fukasawa et al. 2011). *Fusarium solani* and *Trichoderma* spp. can cause significant weight loss of wood (Fukasawa et al. 2011). *Penicillium* spp. and *Amorphotheca* spp. isolated from dead beech (*Fagus crenata*) trees were also discovered to have cellulolytic activities (Fujii et al. 2010), which enabled them to degrade wood. *Paecilomyces formosus*, detected in tropical and subtropical soils and wood, suggests that these fungi are capable of degrading plant debris (Grafenhan et al. 2011; Samson et al. 2009). *Pestalotiopsis* spp. can be found in soil, and they can decompose plant debris (Osono and Takeda 1999; Xu et al. 2010). In this study, by the time when the fungal community was examined after the relatively short period of incubation, most of the decays on

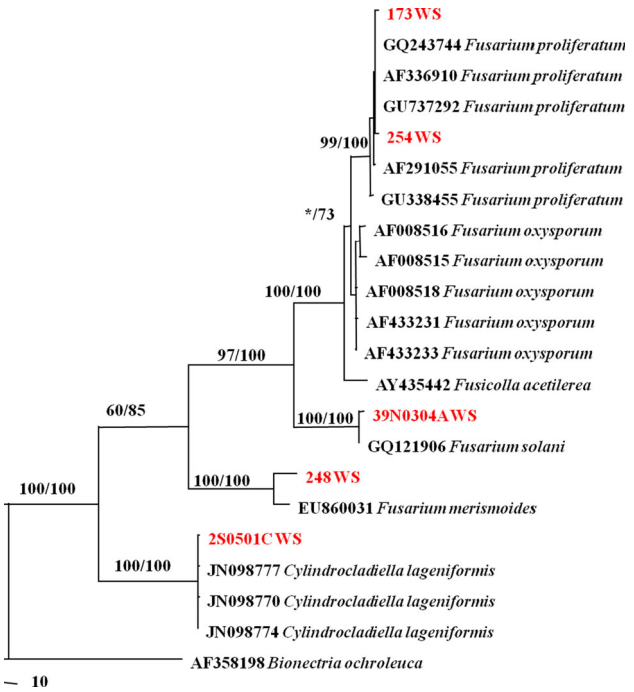

Fig. 4 Phylogenetic relationships among species from the family of Nectriaceae. The tree was produced using MP based on β-tubulin. The *numbers before slashes* indicate bootstrap values; the *numbers after slashes* indicate Bayesian PP values; *asterisk* indicates a supporting rate less than 50 %. Sequences in *red* were obtained from this study

the wood sticks developed were quite superficial, which might be categorized as soft rot. Soft rot, mostly caused by Ascomycetes mainly degrading cellulose and hemicelluloses on the surface layer of the wood, is not as severe as the other two types of rot, white-rot decay and brown-rot decay, which degrade either lignin or cellulose and hemicellulose rapidly and extensively (Bucher et al. 2004). Despite moderate decay by the soft-rot fungi, they are still crucial in the urban ecosystem as a decomposer of plant litter in the soil.

Incubation of wood-stick method could provide a glimpse of the soil fungi, which not only colonize on wood sticks, but also are the main decomposers of wood debris in the soil (Deacon et al. 2006; Kodsueb et al. 2008). Even though a small number of saprotrophic fungi were obtained, a large number of fungi were not isolated through the culturing and incubation methods used inevitably due to the limitations of the medium composition and selectivity as well as the slow-growing characteristics of some fungi. In this study, only 14 % of the wood sticks resulted in successful fungal isolation and more than half of the trees had only less than five isolates. However, the result is not unexpected. Firstly, an exclusion of some fungal species was inevitable given that the strong and large doses of antibiotics applied in the culture media used. Secondly, saprotrophic fungi which can colonize and live on wood sticks are only part of the entire soil fungal community, and among these fungi, only a small portion could grow on artificial culture media in the incubation condition provided. Besides, some fungi may require a longer incubation time to establish colonies on the wood sticks, such as basidiomycetes, which are also important decomposers of wood in natural community and have been reported to account for a significant portion of fungal biomass in the soil (Gams 2007), but none of them was detected in this study. Therefore, in order to thoroughly study and present a more complete community of the wood decomposing fungi in the soil, different length of incubation time and more broad culture media or culture-independent method would be necessary.

Evaluation of molecular detection of taxon and species

Two commonly used restriction enzymes *Hae*III and *Hinf*I were applied to ITS rDNA in detecting species (Viaud et al. 2000; Aanen et al. 2001; Granchi et al. 1999). Different species showed clearly different RFLP patterns for three species in the genus of *Fusarium* and species from different genera (Fig. 5). At the same time, most closely related species according to taxonomic classification (Ainsworth and Kirk 2008) showed similar patterns, such as *Pyrenochaeta* sp. and *P. insulana* showed similar pattern with *Hinf*I, and *Pyrenochaeta* sp. and *C. geniculatus* (lane 11) showed similar patterns with *Hae*III. All of the above three species belonged to the same order. However, there was also an exception, *Trichoderma atroviride* and *Trichoderma hamatum* showed the same RFLP patterns as in Lanes 1–3 for *T. atroviride*, and lanes 4–10 for *T. hamatum* (Fig. 6).

According to the patterns of RFLP, restriction enzymes *Hae*III and *Hinf*I of ITS rDNA could successfully distinguish fungi from different genera and most species from the same genus. However, for some fungal species of the same genus, because of their extremely similar sequences of ITS rDNA, identical RFLP patterns may end up with different species of the same genus, such as *T. atroviride* and *T. hamatum*. In addition, in some cases, different species show the same restriction patterns (Fig. 5, upper, lanes 1 and 2, lanes 10 and 12) or contain no specific recognition site of certain restriction enzyme (Fig. 5, lanes 1, 2, and 12). Therefore, application of more restriction enzymes and better selection of enzymes would lead to better distinction of different species. Results of RFLP in this study agreed with those of Viaud et al. (2000), indicating that identical RFLP patterns show very closely related DNA sequences (Viaud, et al. 2000). It is clear that RFLP is an effective method in detecting the differences of

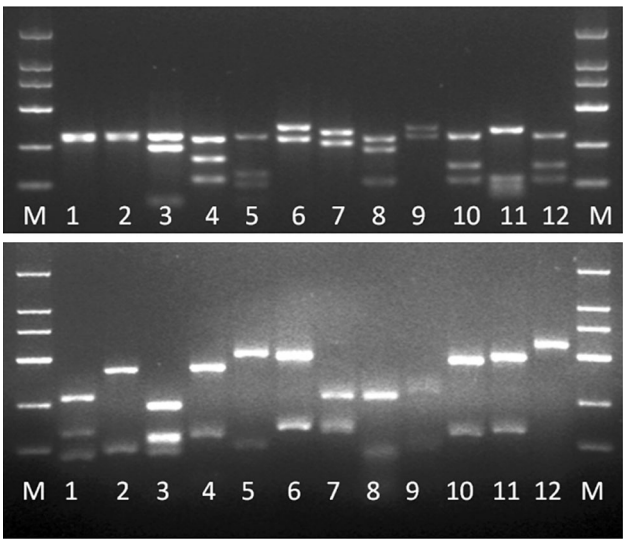

Fig. 5 Restriction patterns of different species. The *upper figure* shows RFLP patterns with restriction enzyme *Hinf*I; the *lower figure* shows RFLP patters with restriction enzyme *Hae*III. *1 F. proliferatum*, *2 F. acetilerea*, *3 F. solani*, *4 Bionectria ochroleuca*, *5 C. lageniformis*, *6 Pestalotiopsis disseminata*, *7 Amorphotheca resinae*, *8 D. ceijpii*, *9 P. formosus*, *10 Pyrenochaeta* sp., *11 C. geniculatus*, and *12 P. insulana*

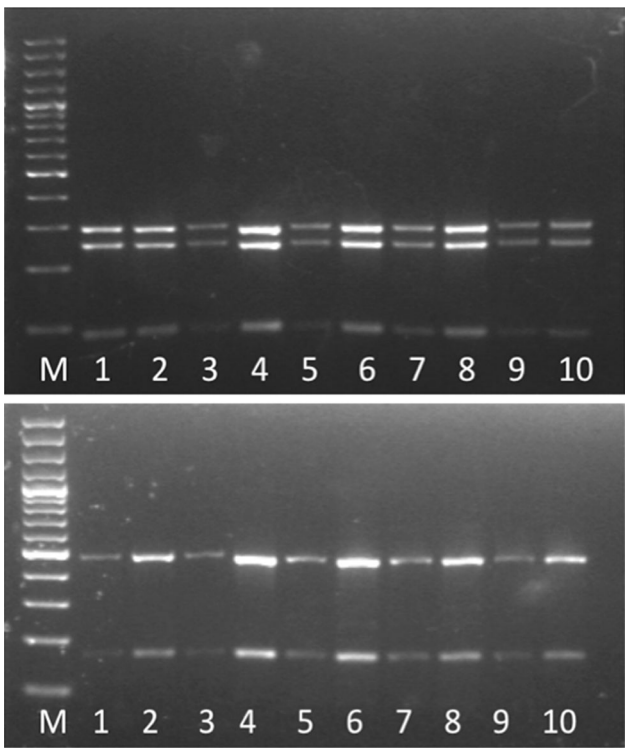

Fig. 6 Restriction patterns of *T. atroviride* (*lanes 1–3*) and *T. hamatum* (*lanes 4–10*). The *upper figure* shows RFLP patterns with restriction enzyme *Hinf*I; the *lower figure* shows RFLP patterns with restriction enzyme *Hae*III

PCR-amplified sequences, whereas identification of the fungal species can be improved when combining with morphological characteristics.

ITS rDNA is widely used in the identification of fungal species because it contains both variable and conserved regions. However, for some genera, such as *Penicillium* and *Phoma*, which contain large numbers of species, ITS cannot yield high resolution in phylogenetic analysis (Aveskamp et al. 2010; Samson et al. 2011). In this study, β-tubulin, which contains more variable regions, was used for more specific species identification of *Penicillium*, *Phoma*, and *Fusarium*. However, the combination of the two genes could not accurately identify some species in *Penicillium* and *Phoma* on molecular scales, and this may be because of the availability of the relevant gene sequences in the GenBank or these groups are better identified with other specific genes (Schoch et al. 2012). Despite this, such multiple gene combination obviously allows a better resolution of the species identification (Rokas and Carroll 2005).

Theoretically, culture-dependent method would inevitably lead to the omission of the unculturable fungi and some slow-growing ones. In particular, culture-dependent method has its own advantages. In this study, the culture morphology provided an important reference to the identification of the fungal species. At the same time, more than 300 of fungal isolations were obtained, among which some could be potentially used as biocontrol agents. *Trichoderma* species were reported to antagonize against wood-decaying fungi such as *Phellinus noxius* and *Ganoderma* spp. (Schwarze et al. 2012), and *P. lilacinum* has long been studied as biocontrol agent to control the growth of destructive root-knot nematodes (Deng et al. 2012; Luangsa-Ard et al. 2011).

Conclusion

In this study, wood sticks allowed isolation and identification of fungi from urban soil. RFLP has successfully detected fungal species from different genera except two species from *Trichoderma*, so combined with culture morphology and microscopic observation, different fungal species can be distinguished. Identification with ITS rDNA and/or ß-tubulin is faster and more accurate than traditional identification methods only based on colony and microscopic characteristics. Most of the fungal species detected with the wood sticks in this study can decompose wood materials and therefore may play an important role in nutrient cycling in the urban ecosystem. Fungal cultures isolated from this study may possess special functions such as antagonistic toward plant pathogens, which could be further investigated.

Acknowledgments This study was supported by project No. DEVB(SS) Q058/2010 from the Tree Management Office, Development Bureau of the Hong Kong SAR Government. The views and opinions expressed here are those of the authors, and no implication should be made to the Hong Kong SAR government. We thank Victor W. Gu for drawing Fig. 1.

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