Journal of Forestry Research (2008) 19(3):219–224
DOI 10.1007/s11676-008-0030-7

RESEARCH PAPER

Identification of mangrove endophytic fungus 1403 (*Fusarium proliferatum*) based on morphological and molecular evidence

CHENG Zhong-shan[1], TANG Wen-cheng[1], SU Zhi-jian[2], CAI Yi[1], SUN Shi-feng[1], CHEN Qi-jin*[1], WANG Fang-hai*[1], LIN Yong-cheng[3], She Zhi-gang[3], LLP Vrijmoed [4]

1School of Life Science, Sun Yat-sen University, Guangzhou 510275, P. R. China
2. School of Pharmaceutical Science, Jinan University, Guangzhou 510632, P. R. China
3. School of Chemistry and Chemical Engineering, Sun Yat-sen University, Guangzhou 510275, P. R. China
4. Department of Biology and Chemistry, City University of Hong Kong, China

Abstract: A mangrove endophytic fungus 1403 isolated from the South China Sea Coast, which is able to produce griseofulvin and anthracenediones under submerged fermentation, was compared with *Fusarium* genus with the similar morphological characters such as elongated, microconidium-producing conidiophores, ovoid microconidia and straight to slightly curved macroconidia. It was found that the fungus 1403 resembles pathogenic *F. verticillioides* (teleomophy *Gibberella moniliforme*) in the production of false head or chains and abundant microconidia on the aerial mycelium, but different in the occasional formation of polyphialides with relatively long as well as short monophialides, in its typical coiled hyphae and mycelia fusion. Through maximum Parsimony and Bayesian analyses, the fungus 1403 was further compared with some similar *Fusarium* species. The results indicated that this endophyte was identified as *Fusarium proliferatum* based on the analyses of partial 18S and 28S rDNA genes, ITS region, and EF-1α gene.
Keywords: *Fusarium*; Mangrove endophyte; morphology; phylogeny

Introduction

Mangrove forests are most distributed in tropical and subtropical regions in the world (Gilbert & Mejia-Chang 2002), and several mangrove species are a valuable source of useful metabolites for medicinal usage (Kathiresan & Bingham 2001). Some of the potency of mangrove plants may be due to mutualistic fungal endophytes associated with host plants (Selosse et al. 2004). In fact fungi from mangroves are the second largest group among the marine fungi (Sheadrer et al. 2007). The practical applications of mangrove endophytic fungi are manifold, as potential biocontrol agents, sources of novel metabolites for therapeutics, plant protection, and other industrial applications (Yuan et al. 2005). In general, the production of secondary metabolites that are potentially useful for pharmaceutical and agricultural applications is widespread among mangrove endophytic fungi (Lin et al. 2002). Our group has recently undertaken several studies on mangrove endophytic fungi from South China Sea Coast and has isolated various bioactive metabolites (Lin et al. 2001).

For any study on endophyte it is important that authors define the understanding of the term. In the present study we define the endophyte as 'fungus capable of beneficial infections in plants, regardless of its systemic nature, and provided the existence of proper host tissues to be colonized' (Guo et al. 2002). However, the majority of the endophytes obtained from mangrove by our group has often no sexual state known and can only be referred to as the Fungi Imperfecti. Among them, for the mangrove endophytic fungus 1403, primarily identified as *Fusarium verticillioides*, its acetate extract of submerging fermentation shows an obvious inhibition effect on yeast and mold. The previous studies on the secondary metabolites of the endophyte revealed that this strain is able to produce a series of antibiotics, including griseofulvin which was originally found in *Penicillium griseofulvum*, and anthracenediones (Jiang et al. 2000). Assembling taxa based primarily on morphological similarities does not necessarily reflect phylogenetic relationships but was rather a convenient scheme, which hinder our exploration of mangrove endophytes that might produce microbial metabolites for using as therapeutic agents, for it necessitates the careful identification and selection of species unique to a particular host before the high-throughput screening of metabolites for desired industrial

Foundation project: This research was supported partly by the Guangzhou Natural Science Foundation (Grant No. 2007Z3-EO581), the Guangdong Provincial Natural Science Foundation (Grant No. 2007A0200300001-7；05003268), the Chinese High-Tech 863 Project (Grant No. 2006AA09Z422), and the National Natural Science Foundation of China (Grant No. 20572136)

Received: 2008-02-29; Accepted: 2008-04-11

The online version is available at http://www.springerlink.com

Biography: CHENG Zhong-shan (1983), male, M.D, College of Life Science, Sun Yat-sen University, Guangzhou 510275, P. R. China

*Correspondence authors: CHEN Qi-jin (E-mail: lsscqj@mail.sysu.edu.cn) and WANG Fang-hai (E-mail: lsswfh@mail.sysu.edu.cn)

Responsible editor: Chai Ruihai

applications. Therefore, the taxonomy of the endophytes is a formidable challenge for most of our application. This inability to identification made us to seek alternative approaches to the identification of these fungi.

Sequence-based approaches allow for a partial solution to the problem inasmuch as they provide an opportunity to identify endophytic fungi, especially for the endophytes of *Fusarium,* however, due to their capacity for rapid change, their identification presents certain problems, some more imaginary than real (O'Donnell et al. 1998). Though several types of molecular techniques have been used to aid in delimiting species within the *Fusarium* genus, such as isozyme methodology and RAPD markers that are used to distinguish the known biological species of *Gibberella fujikuroi* (Huss et al. 1996), these data of RAPD and isozyme variation are too homoplastic to infer phylogenetic relationships (O'Donnell & Cigelnik 1997). Recently, DNA sequences data from the nuclear gene for EF-1α, ITS, partial nuclear 18S rDNA and 28S rDNA have been successfully used to distinguish probable species and phylogenetic subgroups within several groups of *Fusarium* strains (Marasas & Rheeder 2001), and combined molecular phylogenetic and morphological approaches have been shown to be invaluable in the diagnosis of fusaria species (Gams et al. 1999).

The above reputation of complexity and confusion make us to approach the identification of mangrove endophytic fungus 1403 with morphological and molecular technology. In this paper we morphologically compared the endophyte with other similar *Fusarium* strains and assessed its phylogenetic relationships from 18S rDNA, 28S rDNA, ITS and EF-1α gene by equally weighted Parsimony analyses and Bayesian analyses, with special attention to fungi that resemble the strain in morphology or ecology.

Materials AND Methods

Fungal Material

The mangrove endophytic fungus 1403 was isolated from the South China Sea Coast by professor LLP Vrijmoed of City University of Hong Kong, China. A stem segment from Kandelia Wood tissue was surface sterilized by successive submersion in 70% ethanol for 1 min, 10% bleach for 3 min, and then twice in sterile water. After sterilization, the stem was placed on a potato dextrose agar (PDA) plate, and successive subculturing of the outgrowing fungi resulted in a pure culture initially coded 1403. Vouchers of the fungus are deposited in the School of Life Science and School of Chemistry and Chemical Engineering, Sun Yat-sen University, and City University of Hong Kong, China.

Morphological examination

Strains were grown in the dark at 25ºC. Media used were potato-dextrose agar (PDA), malt extract agar (MEA) and glucose-peptone-yeast-extract agar (GPY: glucose 20 g/L, peptone 5 g/L, yeast extract 5 g/L, agar 20 g/L), synthetic nutrient-poor agar (SNA) with and without the addition of a 1 × 3 cm piece of filter paper to the colony surface, using glass Petri dishes of 9-cm diameter. Growth rates and colony diameters of cultures incubated in the dark were measured on PDA and GPY. Characters such as size and shape of conidia and phialides were measured from strains grown on SNA incubated in the dark for 10–14 day. Presence of polyphialides on GPY and PDA also was examined in 3–4 week-old cultures. Water was used as mounting medium in microscopic slides. Images were captured on a Nikon Eclipse TE200-U microscope with bright field and epifluorescence optics and Nikon digital camera DXM1200F using SPOT software. Measurements in the description are given as (i) n1-n3-n2; or (ii) (n1-) n4-n3-n5 (-n2), with n1 = minimum value observed; n2 = maximum value observed; n3 = arithmetical means; n4/n5 = first/third quartile. Macroscopic characters, such as surface texture, odor and colony colors, were described from PDA.

Phylogenetic

A hyphal tip was obtained from fresh culture and was grown on PDA in the dark at 25ºC for seven days. Fresh fungal mycelia (c. 50 mg) were scraped from the surface of the agar plate and grounded in liquid nitrogen. Fungal genomic DNA was prepared using GALEN mini Kit (Catalogue no.69104, Galen biopharm international co. LTD, Beijing, China) according to the manufacturer's protocols. Sequence data were generated from four regions: (1) 18S rDNA bounded by primers NS1 and NS4; (2) 28S rDNA bounded by primers 5.8SOR and LR7; (3) ITS bounded by primers ITS1 and ITS4 (http://www.biology.duke.edu/fungi/mycolab/primers.htm); (4) EF-1α gene bounded by primers EF1-728F and EF1-986R (Ignazi & Linda 1999).

PCR reaction mixes contained 2.5 μl 10×PCR buffer, 5 μM dNTP, 12.5 pM of each PCR primer, and 5 μl DNA in 25 μl. The amplification program included 40 cycles of 94ºC for 30 s, 50ºC for 30 s, and 72ºC for 1 min. To obtain readable sequence data of the 18S, 28S, ITS, and EF-1α gene respectively, the PCR products were cloned into the pMD18-T vector (Takara) and sequenced by the dideoxynucleotide method. Sequences generated in this study were submitted to GenBank (accession number: EU193176, EU193177, EU117221, EU259178). The remainders of the sequences used in this study were obtained from the GenBank database.

Phylogenetic analyses

Sequences were aligned with ClustalX (Thompson et al. 1997) and adjusted by eye in the data editor of PAUP* 4.0b10 (Swofford 2002) with the aid of PAUPUP v1.0.3.1 (Calendini & Martin 2005). All data sets were analyzed in PAUP* 4.0b10 and MrBayes 3.1 (Huelsenbeck & Ronquist 2001), with gaps treated as missing data.

Parsimony analyses were performed using equal weighting of characters and transformations. Heuristic search used the stepwise addition option and was repeated 10 times from different starting points with tree-bisection-reconnection (TBR) branch swapping. Confidence in specific clades from the resulting to-

pology was tested by bootstrap analyses with 100 replicates with a 50% majority rule.

In addition to the maximum parsimony analyses, Bayesian posterior probabilities were computed using the Markov Chain Monte Carlo method (MCMC) in MyBayes 3.1 by running with 1000000 generations and sampled every 100 generations. Posterior nodal probabilities were summarized by generating a majority rule consensus tree by using PAUP.

Results

Morphological examination

Colonies reaching 73-79-86 mm diameter on PDA and 81-84-86 mm diameter on GPY after seven days, with an average radial growth rate of 11.3 mm per day on PDA and 11.9 mm per day on GPY. Aerial mycelium white, on SNA sparsely developed, faint, sometimes almost absent and rarely formed on filter paper, consisting of single hyphae; on PDA and GPY abundantly produced, forming thick white tufts evenly covering the whole colony. Reddish-purple was appeared in the central of colony on the surface and reddish-purple or dark purple on the reverse when incubated on PDA in dark (Fig. 1: A-B). Odor was not detectable. Sporulation started early in aerial mycelium and was abundant after seven days on SNA. Conidiophores formed laterally from hyphae of the aerial mycelium produced microconidia (Fig. 1: F-H). Aerial mycelium bear solitary monophialides (Fig. 1: F-G), occasionally short supporting cells with whorls of up to 3 monophialides (Fig. 1: E), phialides slightly tapering toward the tip or narrowly flask shaped (Fig. 1: E-H), (6.0-) 14.4-20.1-24.0 (-33.6) μm long, (0.96-) 1.20-1.52-1.92 (-2.4) μm wide at base (n = 50). Microconidia formed in false heads on PDA (Fig. 1: D), with longer chain formed on SNA (Fig. 1: C). Aerial conidia mostly 0-septate, ovoid to ellipsoidal, (7.2-) 9.6-11.85-14.4 (-16.8) × (1.44-) 1.80-2.01-2.16 (-2.40) μm (n = 30) (Fig. 1: J, K (arrow b)). Macroconidia borne after 12 days typically 0-septate, straight to slightly curved, measuring (2.4-) 4.8-5.6-6.6 (-8.4) × (1.2-) 1.21-1.81-2.3 (-2.4) μm in the dark (n=30) (Fig. 1: K (arrow a)). Chalmydospores were absent, and teleomophy was unknown.

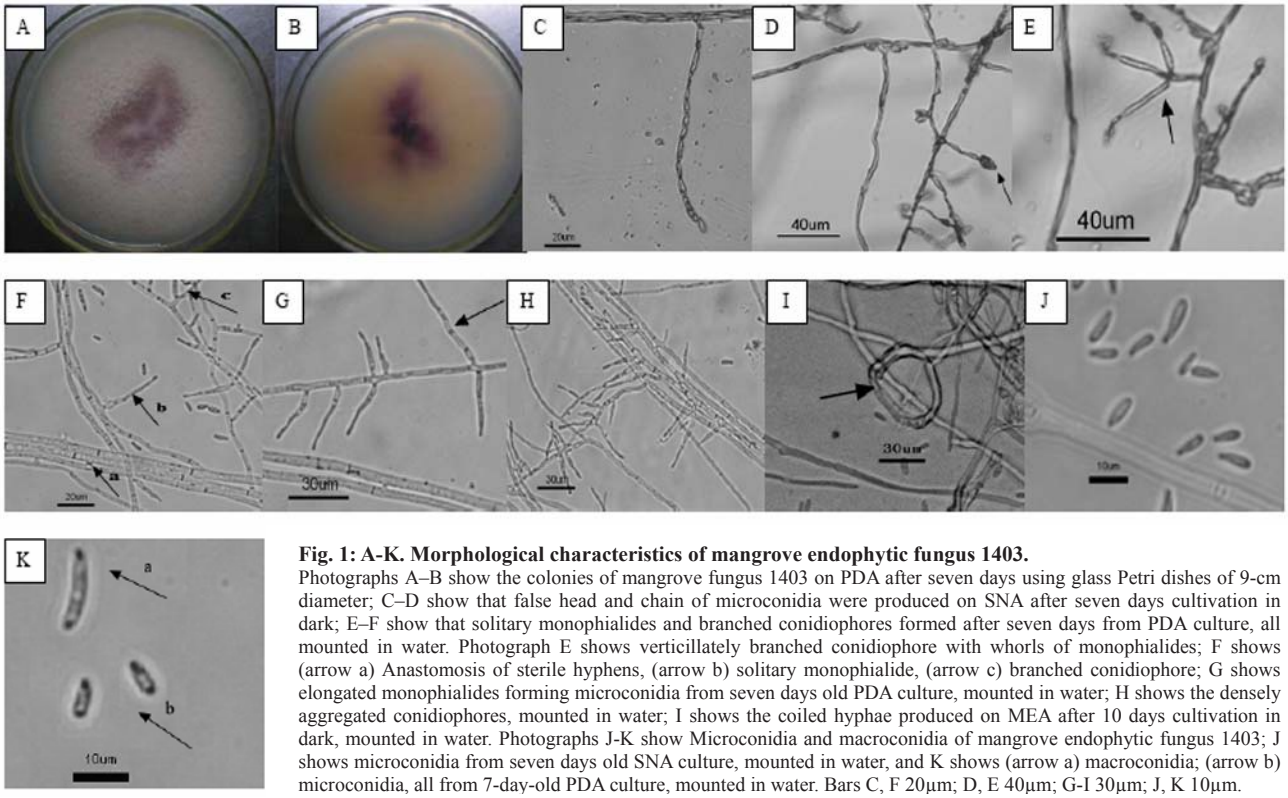

Fig. 1: A-K. Morphological characteristics of mangrove endophytic fungus 1403.
Photographs A–B show the colonies of mangrove fungus 1403 on PDA after seven days using glass Petri dishes of 9-cm diameter; C–D show that false head and chain of microconidia were produced on SNA after seven days cultivation in dark; E–F show that solitary monophialides and branched conidiophores formed after seven days from PDA culture, all mounted in water. Photograph E shows verticillately branched conidiophore with whorls of monophialides; F shows (arrow a) Anastomosis of sterile hyphens, (arrow b) solitary monophialide, (arrow c) branched conidiophore; G shows elongated monophialides forming microconidia from seven days old PDA culture, mounted in water; H shows the densely aggregated conidiophores, mounted in water; I shows the coiled hyphae produced on MEA after 10 days cultivation in dark, mounted in water. Photographs J-K show Microconidia and macroconidia of mangrove endophytic fungus 1403; J shows microconidia from seven days old SNA culture, mounted in water, and K shows (arrow a) macroconidia; (arrow b) microconidia, all from 7-day-old PDA culture, mounted in water. Bars C, F 20μm; D, E 40μm; G-I 30μm; J, K 10μm.

In the light of above characteristics, the endophyte superficially resemble pathogenic *Fusarium verticillioides* (teleomophy *Gibberella moniliforme*) (Kerényi et al. 1999) in the production of false head or chains and abundant microconidia on the aerial mycelium. Initial identification described the fungus as *F. verticillioides*. However, further study found that the endophyte is different phenotypically from *F. verticillioides* and other known *Fusarium* species, in the producing griseofulvin and anthracenediones under submerged cultivation condition (Jiang et al. 2000), which are new products produced by *Fusarium* species (Nelson et al. 1993; Rheeder et al. 2002), in the occasional formation of polyphialides by having relatively long as well as short monophialides (Fig. 1: H), and in its typical coiled hyphae (Fig. 1: I) and mycelia fusion (Fig. 1: F (arrow a)). Phylogenetic analyses is needed to differentiate the endophyte with *F. verticillioides* and identify it to species level.

Phylogenetic analyses

Equally weighted parsimony analyses and Bayesian analyses

based on 18S rDNA genes generated similar tree topologies that only differed in their ability were carried out to resolve certain relationships, and the majority rule consensus tree supported mangrove endophytic fungus 1403 as a member of the *Fusarium* clade (bootstrap=70%, posterior probability=50%, Fig. 2). In line with the analysis, the endophyte can be included in *Fusarium*, however, further analyses were still needed to identify it to species level.

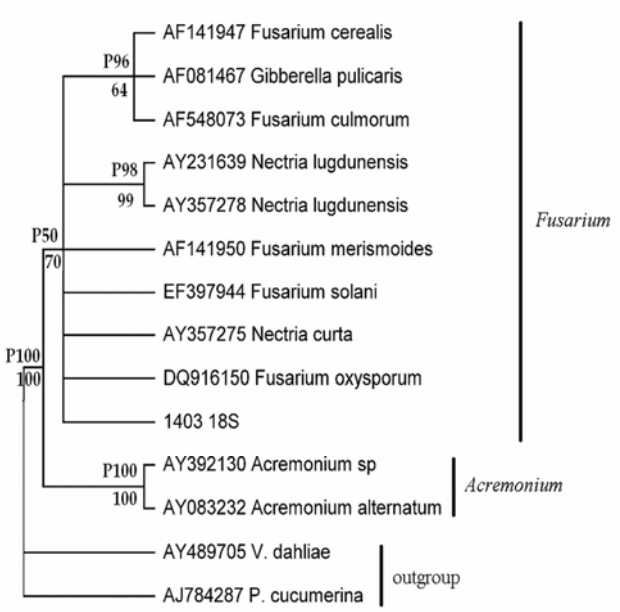

Fig. 2 Phylogenetic analyses of mangrove endophytic fungus 1403 inferred from 18S rDNA.

The majority rule consensus tree (Tree length=165, Consistency index (CI) =0.8970, Retention index (RI) =0.8381) derived by maximum parsimony analyses. Bayesian posterior probabilities and maximum parsimony bootstrap values (>50%) are shown above and below the lines, respectively. *Verticillium dahliae* (AY489705) and *Plectosphaerella cucumerina* (AJ784287) were used as outgroup.

For the partial 28S rDNA, 33 sequences were downloaded from GenBank. We conducted a preliminary parsimony analysis for the 28S rDNA alignment. Out of 1 443 characters, 1 232 were constant, and 163 were parsimony informative. Totally 3 996 equally parsimonious trees were obtained, measuring 326 steps (CI=0.7485, HI=0.2515, RI=0.8713). Two multiple gaps were encountered in the data set; both were caused by sequences of the two related taxa used as outgroup, *Verticillium dahliae* and *Plectosphaerella cucumerina*.

Phylogenetic analyses of 18S and 28S data sets provided great evidence that endophytic fungus 1403 and *Fusarium verticillioides* are different species, which were not included in the same subclade of *Fusarium* section, *Liseola*, *Elegans*, *Sporotrichiella* and others (Fig. 3). However, as what was also obtained by O'Donnell et al. (1998), it did not support the monophyly of either the *Gibberella fujikuroi* complex (section *Liseola*) or the clade comprising the *G. fujikuroi* and *Fusariu oxysporum* complexes (i.e., sections *Liseola-Elegans*), which could be resulted from the poor resolution of 18S and 28S rDNA gene trees. Further analysis is still needed to place our mangrove endophytic fungus 1403 into the *Gibberella fujikuroi* species complex.

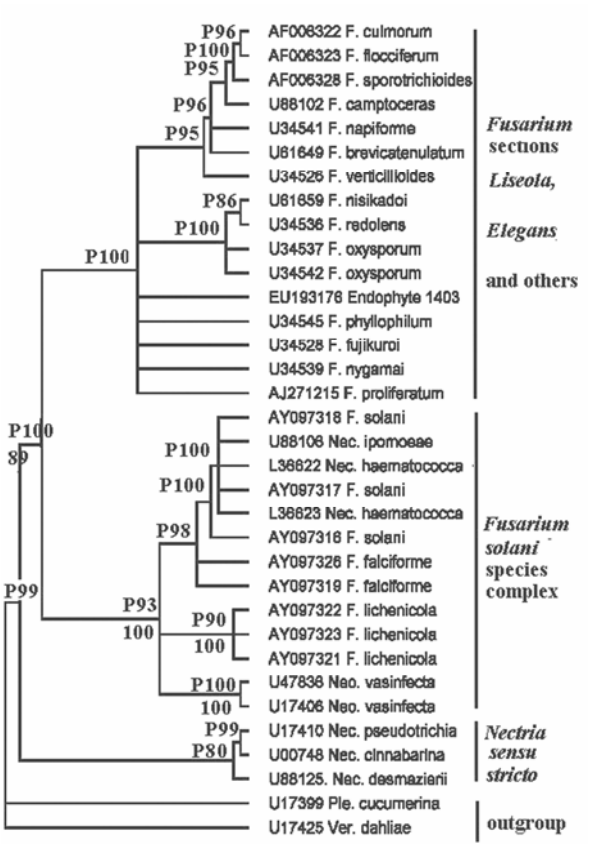

Fig. 3 Phylogenetic analyses of mangrove endophytic fungus 1403 inferred from 28S rDNA.

The majority rule consensus tree derived by Bayesian analyses. Bayesian posterior probabilities and maximum parsimony bootstrap values (>50%) are shown above and below the lines, respectively. Abbreviations for genus names: F., *Fusarium*; Nec., *Nectria*; Neo., *Neocosmospora*; Ple., *Plectosphaerella*; Ver., *Verticillium*.

The endophyte's ITS sequence was compared with species of *Gibberella fujikuroi* complex deposited at GenBank. Result showed that it was 100% equal to *Fusarium proliferatum* (accession number: EU151490, EU151489, EU151486, EU151484, AM162678, AJ810449) and *Gibberella fujikuroi* (accession number: AY898262, AY898261). This result could be explained by considering that for the species *Fusarium proliferatum*, its teleomophy is *Gibberella fujikuroi* var. *intermedia* (*Gibberella fujikuroi*, mating population D) (Stankovic et al. 2007). However, according to Waalwijk's (1996) suggestion, the identical ITS sequences obtained for *G. fujikuroi* and *F. proliferatum* cannot be regarded as proof for their conspecificity in view of other morphological and genetically differences reported in the literature. The other reason is that the ITS based tree was inconsistent with gene trees inferred from the ß-tublin gene exons and introns, 18S rDNA, and the 28S rDNA, due to nonorthologous ITS2 sequences (O'Donnell & Cigelnik 1997). Thus, care should be taken with phylogenetic interpretation of our BLAST results using ITS sequence. To address this problem, other faster evolving parts of the genome must be analyzed. The EF-1α gene,

which encodes an essential part of the protein translation machinery, has high phylogenetic utility because it is highly informative at the species level in *Fusarium* and non-orthologous copies of the gene have not been detected in the genus. This gene appears to be consistently single-copy in *Fusarium*, and it shows a high level of sequence polymorphism among closely related species, even in comparison to the intron–rich protions of protein coding genes such as calmodulin, beta-tubulin and histone H3 (Geiser et al. 2004). Consequently in the present study, we use EF-1α gene, instead of ITS which was used only as BLAST query of the GenBank database to acquire high similarities (99%–100%) species for further analysis, to infer phylogenetic relationships of mangrove endophytic fungus 1403 to other *Gibberella fujikuroi* species by maximum Parsimony and Bayesian analyses. Molecular phylogenetic analyses demonstrated that the endophyte forms a monophyletic group with *F. proliferatum* and its teleomophy *Gibberella intermedia* (Láday et al. 2004) with high Bayesian posterior probabilities (100%) and parsimony bootstrap value (83%)(Fig. 4), which confirmed us to accommodate the endophyte as *Fusarium proliferatum*.

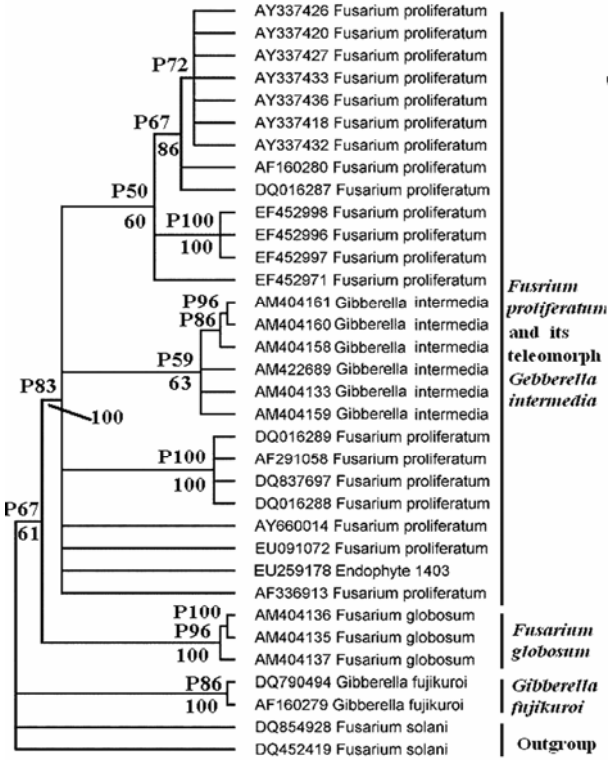

Fig. 4. Phylogenetic analyses of mangrove endophytic fungus 1403 inferred from EF-1a.

The majority rule consensus tree derived by Bayesian analyses. Bayesian posterior probabilities and maximum parsimony bootstrap values (>50%) are shown above and below the lines, respectively.

Discussion

Fusarium has had a confusing and unstable taxonomic history, which is the result of the rampant misapplication and inconsistent application of species names to toxigenic and pathogenic isolates, such as *F. graminearum*, along with *F. verticillioides* and related species with the *Liseola* section, are notorious for the production of mycotoxins on wheat, maize, barley, rice and other cereal grains and foodstuffs (Marasas et al. 1984); this may be part of the reason of the predominant interest in the genus that has been in their role as plant pathogens and fatal toxicoses agents (Voss et al. 2001). However, most *Fusarium* isolates studied by mycotoxicologists and plant pathologists in the first three quarters of the 20th century were initially identified incorrectly using one of several oversimplified morphological systems (Nirenberg & O'Donnell 1998; O'Donnell 2000; O'Donnell et al. 1998). With the recent advent of multilocus phylogenetic methods which allow for the objective identification of species boundaries in the Fungi, relationships among well-defined *Fusarium* species have been inferred, showing a great deal of species diversity that was vastly misidentified by previous morphology-based taxonomic system (O'Donnell 2000; O'Donnell et al. 1998). Furthermore, an exact sequence match with a known isolate in the database can be considered very close to an unambiguous species identification, but even this is not always the case, for example, in our study, the mangrove endophytic fungus 1403 (*Fusarium proliferatum*) has identical ITS sequence with *Gibberella fujikuroi* and its 28s rDNA also has high similarity with other *Gibberella fujikuroi* species. Therefore the correlation reports of mycotoxin production with phylogenetically defined species are needed urgently as the identifying of toxigenic fusaria species still remains an enormous challenge.

Apart from rampant taxa misapplication, the understanding to the problem, what extent species or isolates of this genus are endophytic, is hampered by studies of histological or otherwise that inadequately describe the qualitative and quantitative distribution of *Fusarium* within hosts. None are there studies to indicate any host requirements or benefits derived from such association (Nirenberg & O'Donnell 1998). However, positive physiological interaction recorded for several strains of *F. verticillioides* and biocontrol uses of several *Fusarium* species not only indicate the utility of the genus but also possible mutualistic interactions derived from the associations, including insecticidal, nematocidal, and fungicidal activities (Bacon & Williamson 1992; Hallmann & Sikora 1994; O'Donnell et al. 2000). That's why some of recent studies are highly suggestive that many of these species are endophytic in their associations with plants and certain biotic and abiotic factors may alter *Fusarium* relationships with plants from a symptomless endophytic association to a hemibiotrophic and finally a saprotrohic association. Consequently, many mycotoxins are produced by *Fusarium* species during the pathogenic and saprophytic states (Yates et al. 1997). So the exploration of *Fusarium* species that might produce microbial metabolites for use as therapeutic agents needs much attention as it necessitates careful identification and selection of species before the screening of metabolites for desired industrial applications.

As is the case with any sort of fungal identification, DNA sequence-based identification is a reiterative process that may require further investigation and consideration of other factors, including morphology and ecology. Our mangrove endophytic fungus 1403 was consistent with morphological descriptions of *F.*

proliferatum given by Nirenberg and O'Donnell (1998), but it exhibited some minor differences. A distinctive morphological feature of the endophyte was the production of coiled sterile hyphae, 0-septate sporodochia conidia and hyphal fusion. Another unique feature was its secondary metabolites. Since this strain can produce a series of antibiotics, its acetate extract of submerging fermentation shows an obvious inhibition effect on yeast and mold, including griseofulvin and anthracenediones (Jiang et al. 2000), which are new metabolites for *F. proliferatum* (Nelson et al. 1993; Rheeder et al. 2002). However, Molecular Phylogenetic analyses inferred from EF-1α demonstrated that the endophyte forms a monophyletic group with *F. proliferatum* and its teleomophy *Gibberella intermedia*. Because of the limited recorded EF-1α gene sequences of *F. proliferatum* in GenBank, we can only accommodate the endophyte as *F. proliferatum*; discussion on phylogenic, metabolic and ecological relationships has to wait for future accumulation of data.

Acknowledgements

We thank Yang Jia-hou, Yan Xi and Wang Wen for their excellent technical assistance. We also thank Dr. ZAKA and Dr. Luo Li in South China Agriculture University for technical review of the manuscript.

References

Bacon CW, Williamson JW. 1992. Interactions of *Fusarium moniliforme*, its metabolites and bacteria with maize. *Mycopathologia*, **117**: 65–71.

Calendini F, Martin JF. 2005. PaupUP v1.0.3.1, A free graphical frontend for Paup* Dos software.

Gams W, Klamer M, O'Donnell K. 1999. *Fusarium miscanthi* sp. nov. from *Miscanthus* litter. *Mycologia*, **91**: 263–268.

Geiser D, Del Mar Jiménez-Gasco M, Kang S, et al. 2004. FUSARIUM-ID v.1.0: A DNA sequence database for identifying *Fusarium*. *Eur J Plant Pathol*, **110**: 473–479.

Gilbert GS, Mejía-Chang M. 2002. Fungal diversity and plant disease in mangrove forests: salt excretion as a possible defense mechanism. Oecologia, **132**: 278–285.

Guo LD, Hyde KD, Liew ECY. 2002. Identification of endophytic fungi from *Livistona chinensis* based on morphology and rDNA sequences. *New Phytol*, **147**: 617–630.

Hallmann, J, Sikora RA. 1994. Occurrence of plant parasitic nematodes and endophytic fungi in tomato plants in Kenya and their role as mutualistic synergists for biological control of root-knot nematodes. *Int J Pest Manage*, **40**: 321–325.

Huelsenbeck JP, Ronquist F. 2001. MRBAYES:Bayesian inference of phylogenetic trees. *Bioinformatics*, **17**: 754–755.

Huss MJ, Campbell CL, Jennings DB, et al. 1996. Isozyme variation among biological species in the *Gibberella fujikuroi* species complex (*Fusarium* section *Liseola*). *Appl Environm Microbiol*, **63**: 3750–3756.

Ignazi C, Linda MK. 1999. A method for designing primer sets for speciation studies in Filamentous Ascomycetes. *Mycologia*, **91**(3): 553－556.

Jiang Guangce, Lin Yongcheng, Zhou Shining, Vrijmoed LL, Jones EBG. 2000. Studies on the secondary metabolites of mangrove fungus No. 1403 from the South China Sea. *Acta Sci Nat Univ Sunyatseni*, **39**(6): 68–72.(in Chinese)

Kathiresan K, Bingham BL. 2001. Biology of mangroves and mangrove ecosystems. *Adv Mar Biol*, **40**: 81–251.

Kerényi Z, Zeller KZ, Hornok L, Leslie JF. 1999. Molecular standardization of mating type terminology in the *Gibberella fujikuroi* species complex. *Appl Environ Microbiol*, **65**: 4071–6.

Láday M, Juhász Á, Mulè G, Moretti A, Szécsi Á, Logrieco A. 2004. Mitochondrial DNA variability in *Fusarium proliferatum* (*Gibberella intermedia*). *Eur J Plant Pathol*, **110**: 563–571.

Lin YC, Wu XY, Feng S, Jiang GC, Luo JH, Zhou SN, Vrijmoed LLP, Jones EBG, Krohn K, Steingröver K, Zsila F. 2001. Five unique compounds: xyloketals from mangrove fungus *Xylaria* sp. from the South China Sea coast. *J Org Chem*, **66**: 52–62.

Lin YC, Wu XY, Deng ZJ, Wang J, Zhou SN, Vrijmoed LLP, Jones EBG. 2002. The metabolites of the mangrove fungus *Verruculina enalia* No. 2606 from a salt lake in the Bahamas. *Phytochem*, **59**: 469–471.

Marasas WFO, Nelson PE, Toussoum TA. 1984. *Toxigenic Fusarium species Identification and Mycotoxicology*. University Park, PA: The Pennsylvania State University Press.

Nelson PE, Desiardins AE, Plattner RD. 1993. Fumonisins, mycotoxins produced by *Fusarium* species: biology, chemistry and significance. *Annu Rev Phytopathol*, **31**: 233–251.

Nirenberg HI, O'Donnell K. 1998. New *Fusarium* species and combinations within the *Gibberella fujikuroi* species complex. *Mycologia*, **90**: 434–458.

O'Donnell K. 2000. Molecular phylogeny of the *Nectria haematococca-Fusarium solani* species complex. *Mycologia*, **92**: 919–938.

O'Donnell K, Cigelnik, E. 1997. Two divergent intragenomic rDNA ITS2 types within a monophyletic lineage of the fungus *Fusarium* are nonorthologous. *Mol Phylo Evol*, **1**: 1–14.

O'Donnell K, Cigelnik E, Nirenberg HI. 1998. Molecular systematics and phylogeograhy of the *Gibberella fujikuroi* species complex. *Mycologia*, **90**: 465–493.

O'Donnell K, Nirenberg HI, Aoki T, Cigelnik E. 2000. A multigene phylogeny of the *Gibberella fujikuroi* species complex: Detection of additional phylogenetically distinct species. *Mycoscience*, **41**: 61–78.

Rheeder, JP, Marasas WGO, Vismer HF. 2002. Production of fumonisin analogs by *Fusarium* species. *Appl Environ Microbiol*, **68**(5): 2101–2105.

Selosse MA, Baudoin E, Vandenkoornhuyse P. 2004. Symbiotic microorganisms, a key for ecological success and protection of plants. *C R Biologies*, **327**: 639–648.

Sheadrer CA, Descals E, Kohlmeyer B, Kohlmeyer J, Marvanová L, Padgett D, Porter D, Raja H, Schmit J, Thorton H, Voglymayr H. 2007. Fungal biodiversity in aquatic habitats. *Biodivers Conserv*, **16**: 49–67.

Stankovic S, Levic J, Petrovic T, Logrieco A, Moreti A. 2007. Pathogenicity and mycotoxin production by *Fusarium proliferatum* isolated from onion and garlic in Serbia. *Eur J Plant Pathol*, **118**: 165–172.

Swofford DL. 2002. PAUP*: Phylogenetic Analysis Using Parsimony (* and other methods) Version 4.0b10 (Sinauer Associates, Sunderland, MA).

Thompson JD, Gibson TJ, Plewniak F, Jeanmouqin F, Hiqqins DG. 1997. The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucl Acids Res*, **24**: 4876–4882.

Voss KA, Riley RT, Norred WP, Bacon CW, Meredith FI, Howard PC, Planner RD, Collins TF, Hansen DK, Porter JK. 2001. An overview of Rodent toxicities: Liver and Kidney effects of Fumonisins and Fusarium moniliforme. *Environ Health Persp*, **109**(2): 259–266.

Waalwijk C, De Koning JRA, Baayen RP, Baayen RP, Gams W. 1996. Discordant grouping of *Fusarium* spp. from sections *Elegans, Liseola, Dlaminia* based on ribosomal ITS1 and ITS2 sequences. *Mycologia*, **88**(3): 361–368.

Yuan KP, Vrijmoed LLP, Feng MG. 2005. Survey of coastal mangrove fungi for xylanase production and optimized culture and assay conditions. *Acta Microbiologica Sinica*, **45**(1): 91–96.

Yates IE, Bacon CW, Hinton DM. 1997. Effects of endophytic infection by *Fusarium moniliforme* on corn growth and cellular morphology. *Plant Dis*, **81**: 723–728.