Mycorrhiza (1998) 7:319–321

SHORT NOTE

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Towards a molecular identification of the *Pisonia* mycobiont

Accepted: 22 December 1997

Abstract The internal transcribed spacer (ITS) region of a mycobiont isolated from *Pisonia grandis* R. Br. (Nyctaginaceae) was sequenced and a comparison made with sequences currently available in the GenBank Nucleotide Database. The mycobiont ITS sequence showed a high degree of homology (up to 87% over 637 bases) with a number of Thelephoraceae isolates, strongly implying that it belongs in that taxon. Published descriptions of hyphal morphology also show broad correlation between the *P. grandis* mycobiont and the Thelephoraceae isolates. The data highlight the usefulness of ITS sequence data in mycorrhizal research and the need for researchers to submit to the database ITS sequence data for mycorrhizal fungi with which they are working.

Key words Ectomycorrhiza · Ectomycorrhizal fungus · *Pisonia grandis* · Thelephoraceae · Internal transcribed spacer

Introduction

Pisonia grandis R. Br. (Nyctaginaceae) is a widespread and common tree species throughout the Western Indian and Eastern Pacific oceans (Airy Shaw 1952). Short lateral roots of *P. grandis* frequently possess a distinctive mycorrhizal association wherein the fungus surrounds the root, forming a hyphal mantle, but rarely penetrates between cortical cells to form a Hartig net (Ashford and Allaway 1982, 1985; Allaway et al. 1985; Ashford et al. 1988). The *Pisonia* mycorrhiza is further characterised by the presence of extensive wall ingrowths on the host epidermal cell walls adjacent to the fungus. The ingrowths are regarded as an alternative to the Hartig net characteristic of ectomycorrhizal (ECM) associations (Ashford and Allaway 1982, 1985).

Mycorrhizas of *Pisonia* collected from coral cays in the Great Barrier Reef, Australia and the Seychelles are of two types: bronze smooth and black hairy (Ashford and Allaway 1982, 1985). Extensive morphological similarities between the two strongly suggest that they are separate stages in the development of a single mycorrhizal type and that a single fungal taxon may form mycorrhizas with *P. grandis* across its entire geographical range (Ashford and Allaway 1985; Ashford et al. 1988; Cairney et al. 1994). Isolation of the mycobiont from *P. grandis* roots has proven difficult; however, a single isolate was recently obtained and typical *Pisonia* mycorrhizas synthesised between the isolate and *P. grandis* cuttings (Cairney et al. 1994). The isolated mycobiont, a basidiomycete forming a dark brown mycelium with frequent clamp connections, has been deposited in the following culture collections (accession numbers in parentheses): University of New South Wales (UNSW 014), University of Western Sydney, Nepean (UWSN PIG), American Type Culture Collection (ATCC 200485), Virginia Tech. (VT 3327).

The presence of a sheathing mycorrhiza on roots of *P. grandis* on coral cays is somewhat paradoxical. Natural stands of *P. grandis* are the preferred nesting and roosting sites of large colonies of seabirds which deposit high amounts of nitrogen and phosphorus to the underlying soil annually in the form of guano (Allaway and Ashford 1984). Such observations contrast with the general belief that sheathing mycorrhizal associations are of primary importance in soils of low available nitrogen and phosphorus status (Read 1991). The availability of an isolate of the *P. grandis* mycobiont has led to investigation of the physiological ecology of the association. For example, based on patterns of nitrogen utilisation by the mycobiont in axenic culture, we recently proposed that the primary benefit to the host is the ability of the mycobiont to absorb and utilise organic nitrogen compounds transiently available during degradation of uric acid in the deposited guano (Sharples

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and Cairney 1997). The ecological role of the *Pisonia* mycorrhiza may thus be quite unique. However, comparisons between this fungus and known ECM fungi from other habitats is severely limited by a lack of taxonomic information. Despite repeated isolations from basidiomycete carpophores collected from under *P. grandis* in natural stands on coral cays, we have failed to obtain any isolates with significant similarity to the isolated mycobiont (JWG Cairney, AE Ashford and WG Allaway, unpublished observations). Indeed, comparison of cultural characteristics with descriptions of known basidiomycetes in culture (eg. Watling 1986; Hutchison 1991) has failed to provide any substantive taxonomic indicators. Therefore, we sequenced the internal transcribed spacer (ITS) region from the *P. grandis* mycobiont and conducted a database sequence comparison. We report here a putative taxonomic identification for this isolate.

Materials and methods

DNA was extracted from mycelium of an 8-week-old culture of the *P. grandis* mycobiont using the method of Anderson et al. (1998). Amplification of the ITS region was conducted using 25 pmol of each of the primer pair ITS1-ITS4 (White et al. 1990), approximately 100 ng DNA, 2.5 mM $MgCl_2$, 200 mM of each dATP, dCTP, dGTP and dTTP, 50 mM KCl, 10 mM Tris-HCl (pH 9.0), 0.1% v/v Triton X-100 and 2.5 units Taq DNA polymerase. Cycling parameters and visualisation of PCR products were as described by Anderson et al. (1998). PCR products were cloned using the pGEM-T Easy vector system (Promega) and two clones of the ITS product were sequenced using an automated fluorescent DNA sequencer ABI model 373-A (Applied Biosystems Inc.). The ITS sequence was analysed with the FASTA 3.0 program (Pearson and Lipman 1988) and the *P. grandis* sequence aligned with the closest matches using the Pileup and Pretty programs (Rice 1996).

Results and discussion

A complete ITS sequence (673 nucleotides) was obtained for the *P. grandis* mycobiont (Fig. 1) and submitted to the GenBank Nucleotide Database (accession number AF020770). Comparison with database sequences revealed an 87% homology (over 637 nucleotides) between the *P. grandis* mycobiont ITS sequence and that of an unidentified member of the Thelephoraceae (U83467) and an unidentified *Tomentella* sp. (U83482) (Fig. 1) (Taylor and Bruns 1997). The next closest matches were between the *Pisonia* mycobiont and 21 other Thelephoraceae fungi (homologies ranging from 86% over 640 nucleotides to 77% over 645 nucleotides). The differences between the sequences were dominated by base substitutions in the ITS1 and ITS2 regions. This high degree of homology with the Thelephoraceae indicates strongly that the unidentified mycobiont belongs in that family, and that it represents a *Tomentella* or closely related species. The members of the Thelephoraceae in question have recently been shown to form both ECM and endomycorrhizas with North American tree and orchid species, respectively, and were identified by cultural characteristics and ITS sequence comparisons with known members of the Thelephoraceae (Taylor and Bruns 1997). The fungal isolates were described as slow-growing, thick-walled, brown basidiomycetes with clamp connections (Taylor and Bruns 1997), bearing a strong resemblance to our published descriptions of the *P. grandis* mycobiont

Fig. 1 Alignment of the ITS sequences of the *Pisonia grandis* mycobiont (PIGseq) with Thelephoraceae sp. (u83467) and *Tomentella* sp. (u83482). Identical nucleotides between sequences are indicated in the consensus sequence by *

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           1                                                                                                  110
    PIGseq AAGGATCATT ACCGAACCGT CAACACGAGT TGTTGCTGGT CCCCATACGG GGGCATGTGC ACGCTCTGTT CACATATCCC ACTCACACCT GTGCACCCTC TGTAGTTCTA
u83467.dna AAGGATCATT ACCGAATTGT CAACACGAGT TGTTGCTGGT CCTCAAATGG GGGCATGTGC ACACTCTGTT TGCACATCC. ACTCACACCT GTGCACCCTC CATAGTTCTG
u83482.dna AAGGATCATT ACCGAATTGT CGACACGAGT TGTTGCTGGT CCTCAAACGG GGGCATGTGC ACGCTCTGTT TGCACATCC. ACTCACACAT GTGCACCCTC TGTAGTTCTG
  Consensus ********** ******--** *-******** ********** **-**-*-** ********** **-******* --**-****- ********-* ********** --*******-

           111                                                                                                220
    PIGseq TGGTCNGGGG GGGCATCGCC TTCCTGCCGT GGTTCTATGT CTCTCACACA CACACACCGT GATAGAGTTT TATTGGATGT ATGCCGCGTG TAACGCTATA TAATACAACT
u83467.dna CAGCCTGGGG GCTC.TGTCC CCCCTGCTGT GGCTCTATGT AT.TTACACA CACACACTGT GATAAAGTCT TATGGAATGT ATGCCGCGTT TAACGCAATA CAATACAACT
u83482.dna CAGCCTGGGG GCTC.TGTCC CCCTTGCCGT GGTTCTATGT AT...TTACA TACACACCGT AATAAAGTCT CATGGAATGT GTGCCGCGTT TAACGCAATA CAATACAACT
  Consensus --*-*-**** *--*-*--** --*-***-** **-******* -*-----*** -******-** -***-***-* -**-*-**** -********- ******-*** -*********

           221                                                                                                330
    PIGseq TTCAGCAACG GATCTCTTGG CTCTCGCATC GATGAAAGAA CGCAGCGAAA TGCGATAAG' AATGTGAATT GCAGAATTCA GTGAATCATC GAATCTTTGA ACGCACCTTG
u83467.dna TTCAGCAACG GATCTCTTGG CTCTCGCATC GATGAA.GAA CGCAGCGAAA TGCGATAAGT AATGTGAATT GCAGAATTCA GTGAATCATC GAATCTTTGA ACGCACCTTG
u83482.dna TTCAGCAACG GATCTCTTGG CTCTCGCATC GATGAA.GAA CGCAGCGAAA TGCGATAAGT AATGTGAATT GCAGAATTCA GTGAATCATC GAATCTTTGA ACGCACCTTG
  Consensus ********** ********** ********** ******-*** ********** ********** ********** ********** ********** ********** **********

           331                                                                                                440
    PIGseq CGCCTTCTGG CTATTCCGGA GGGCATGCCT GTTTGAGTAT CATGAACACC TCAACTCCTC ATGGTTTTGC CATGGTGAGC TTGGACTTTG GGGGTTTTGC TGGCCCATGG
u83467.dna CGCCCTTTGG CTATTCCAAA GGGCATGCCT GTTTGAGTAT CATGAACACC TCAACTC.TC ATGGCTT.GC CATGACGAGC TTGGACTTTG GGGGTCTTGC CGGCCTGCGG
u83482.dna CGCCCTTTGG CCATTCCGAA GGGCATGCCT GTTTGAGTAT CATGAACACC TCAACTC.TC ATGGCTT.GC CATGATGAGC TTGGACTTTG GGGGTCTTGC TGGCCTGCGG
  Consensus ****-*-*** *-******--* ********** ********** ********** *******-** ****-**-** ******---- ********** *****-**** -*****---**

           441                                                                                                550
    PIGseq TCGGCTCCTC TGAAACGGAT TAGCTCACCA GCGTCTGGTG GC.TCATGGG TGTGATAACT ATCTACGTCC ATGGGCTTTC CACCGGGTAA CCCTCGCCGA TGGGGGTTCG
u83467.dna TCGGCTCCTC TCAAATGAAT CAGCTTACCA GTGTTTGGTG GCATCACAGG TGTGATAACT ATCTACGCTT GTGG.TTTTC CACCAGGTAA CCTTCAGTGA TGGAGGTTTG
u83482.dna TCGGCTCCTC TCAAATGAAT CAGCTTGCCA GTGTTTGGTG GCATCACAGG TGTGATAACT ATCTACGCTT GTGG.TTTTC CACCAGGTAA CCTTCAGTGA TGGAGGTTCG
  Consensus ********** *-***-*-** -****--*** *-**-***** **-****--** ********** ********--- -****--**** ****-***** **-**---** ***-****-*

           551                                                                  636
    PIGseq CTGGAGCTTA TAGATGTCCC .CCTCTGTGA GGACGGCTCT TTGAATGTTT GATCTCAAAT CAGGTAGGAC TACCCGCTGA ACTTAA
u83467.dna CTGGGGCTCA TAAATGTCTC TCCTCAGTGA GGACAGCTCT TTGAACATTT GATCTCAAAT CAGGTAGGAC TACCCGCTGA ACTTAA
u83482.dna CTGGAGCTCA TAAACGTCTC TCCTCGGTGA GGACAGCTCT TTGAACGTTT GATCTCAAAT CAGGTAG.AC TACCCGCTGA ACTTAA
  Consensus ****-***-* **-*-***-* -****-**** ****-***** ******--*** ********** ********-** ********** ******
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(Ashford and Allaway 1982, 1985; Cairney et al. 1994). Furthermore, Taylor and Bruns (1997) emphasised that the formation by the Thelephoraceae of a classical intercellular ECM structure with tree roots and an intracellular peleton infection with members of the Orchidaceae is an indication that the host can exert control over the type of fungus – root interface formed by this fungal group. This further parallels our observations with the *P. grandis* mycobiont, whereby the fungus forms a sheath but no Hartig net in *P. grandis* roots (rather it induces transfer cell-like ingrowths on host epidermal cell walls), but both a sheath and Hartig net (with no transfer cells) in roots of *Picea sitchensis* (Bong.) Carr (Cairney et al. 1994). Members of the Thelephoraceae produce either flabellate or, more commonly, resupinate basidiocarps, and this may explain why isolates from basidiomycete carpophores collected under *P. grandis* failed to yield isolates resembling the mycobiont.

Morphological descriptions of unidentified ECM types and their assigned binomials (sensu Aegerer 1991) have proven useful in below-ground community studies. ITS-RFLP databases, such as that being compiled by the Uppsala group (Kårén et al. 1997), further permit ECM ecologists to record and compare unidentified fungi from similar or different habitats. ITS sequence data is now relatively straightforward and inexpensive to obtain, particularly for workers already using ITS-RFLP methodologies. Obtaining ITS sequence data for both unidentified and identified ECM fungal taxa and submitting it to the GenBank Nucleotide Database greatly enhances our ability to infer the taxonomic status of unknown isolates along with their relatedness to known and/or unknown isolates. The benefits of such an approach are clear from the present study and, for example, from the recent reviews of relationships within the genera *Suillus* (Kretzer et al. 1996) and *Cortinarius* (Liu et al. 1997).

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