DNA-BASED APPROACHES TO IDENTIFY FOREST FUNGI IN PACIFIC ISLANDS: A PILOT STUDY

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INTRODUCTION

DNA-based diagnostics have been successfully used to characterize diverse forest fungi (e.g., Hoff et al. 2004, Kim et al. 2006, Glaeser & Lindner 2011). DNA sequencing of the internal transcribed spacer (ITS) and large subunit (LSU) regions of nuclear ribosomal DNA (rDNA) has proved especially useful (Sonnenberg et al. 2007, Seifert 2009, Schoch et al. 2012) for identification. Most DNA-based identifications of forest fungi involve taxa that have been previously well-characterized by morphology or mating tests. However, the efficiency of DNA-based identifications of forest fungi in soils or rotting wood, especially in biodiversity hotspots like the Pacific Islands, is largely unexplored. We are conducting a preliminary study with the following objectives: 1) to determine the efficacy of DNA-based identifications of fungi associated with roots and wood rot in the Pacific Islands, from Hawaii to the Philippines; and 2) to evaluate the usefulness of sequences from nuclear rDNA regions, such as the LSU and ITS, for identifying the fungi collected in our surveys.

METHODS

Roots from nine *Pinus merkusii* and *P. kesiya* trees were collected from the forests of Luzon, Philippines (Figure 1). We surface-disinfected the roots and isolated fungal cultures on benomyl-dichloran-streptomycin (BDS) and malt-extract agar (MEA) with streptomycin.

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Sporocarps from Pohnpei, the Federated States of Micronesia (FSM), and Hawaii associated with heart, butt, or root rot were also collected and dried. DNA was extracted from the axenic cultures and dried sporocarps of each sample and PCR was conducted on both the ITS (ITS1-5.8S-ITS2) and LSU (D-domain) regions. PCR products were sequenced and compared to the GenBank® database (http://www.ncbi.nlm.nih.gov/genbank/) using BLAST®. As this project continues, additional fungal isolates associated with sporocarps and root-, butt- and wood-rot will be collected and sequenced from the Federated States of Micronesia, Hawaii, Guam, and other islands in the South Pacific.

PRELIMINARY RESULTS

We used DNA-based identification methods to help identify 25 fungal isolates from the roots of *P. merkusii* and *P. kesiya* collected in the Philippines, and four additional sporocarp samples from Pohnpei, FSM, and Hawaii, U.S. Comparisons of ITS and LSU sequences from the isolates and GenBank® sequences are summarized in Table 1. Of the isolates for which both ITS and LSU sequences were available, 38 percent (11 of 29) produced general agreement between ITS and LSU regions as to the closest species- or genus-level matches in GenBank® (blue and green highlighted rows in Table 1). The remaining isolates (not highlighted in Table 1) either did not show genus-level agreement between their ITS and LSU regions, or sequence data were lacking for one of the regions. Therefore, 62 percent (18 of 29) of these isolates may share ITS or LSU similarities with analogous sequences from identified genera or species in GenBank®, but they cannot yet be definitively assigned to a taxon. The diverse genera identified to date are associated with various ecological roles in forest ecosystems. These roles include mycorrhizal associates, wood decay, plant endophytes, and plant pathogens.

DISCUSSION AND FUTURE STUDIES

LSU and ITS sequences have high utility for fungal species identification because GenBank® contains a large database of fungal LSU and ITS sequences. Most of the LSU and ITS sequences of root-associated fungi from *Pinus merkusii* and *P. kesiya* in the Philippines, showed a reasonably high similarity to some ITS or LSU sequences in GenBank. When both ITS and LSU regions were compared, 38 percent shared general agreement between ITS and LSU as to the closest genus-level match in GenBank® (Table 1). For this reason, we have reasonable confidence in the genus-level identifications for the eight isolates where both ITS and LSU provide GenBank® matches to the same genus. For many isolates, the ITS and LSU each matched different genera in GenBank, which indicates that DNA sequences of other regions are needed to help identify these isolates. In this preliminary study, we have validated the usefulness of DNA sequences for assessing fungal diversity in forest ecosystems. Additional studies are needed, however, to characterize species for which DNA sequence information is unavailable. The DNA sequence database of GenBank is constantly growing, and the capacity for DNA-based identification of fungal taxa continues to improve as more reference sequences become available. Fungal isolates that we could not identify to genus might not be present in GenBank® or they may not have been formally described yet. Only a small proportion of fungal species have been formally described to date (Hawksworth 2012). For this reason, collaborations are needed among mycologists and fungal herbaria to improve the efficacy of DNA-based identification. Our preliminary survey of root-associated fungi in the Philippines was limited to a few pine trees. Most forests in the Philippines and Pacific Islands, however, have high species diversity. Such forest biodiversity is likely associated with a large variety of forest fungi, which remain largely unexplored. We will continue to examine root-, butt-, and heart-rot fungi on hosts in diverse geographic areas of the Pacific. Once baseline data are available for species identification, additional molecular tools such as metagenomics can be used. These tools should provide improved understanding of microbial community interactions within forest ecosystems. These genetic tools may also assist in managing forests with diverse objectives and monitoring the occurrence of invasive species in the Pacific Islands.

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Table 1. Fungal isolates from the Philippines, Federated States of Micronesia, and Hawaii, USA, and their ITS and/or LSU sequence comparisons with GenBank sequences (August 2012). Blue highlighted rows indicate potential genus- or species-level agreement for ITS and LSU sequences, green highlighted rows indicate potential genus-level agreement, and white rows indicate uncertain identifications.

	ITS1+5.8S+ITS2			LSU		
Isolate ID[a]	Closest GenBank Match	GenBank Accession Number	Maximum Identity Score	Closest GenBank Match	GenBank Accession Number	Maximum Identity Score
PI-1C	Uncultured Agaricales clone WAM3	FJ179472.1	99%	*Mycena rubromarginata*	AY207245.1	98%
PI-1E	Fungal sp. ARIZ L157	FJ612709.1	95%	*Rattania setulifera* strain GUFCC15501	HM171322.1	98%
PI-2A	*Fusarium nematophilum* strain BBA70838	HQ897786.1	99%	*Cosmospora glabra*	DQ119556.1	98%
PI-2C	Fungal sp. *Dendrosporium* sp. ICMP14921	AY699695.1 EF029206.1	97% 92%	*Dactylaria hyalotunicata*	EU107298.1	94%
PI-2F	Uncultured Ascomycete clone 2S1.07.F05	EF619849	99%	*Dactylaria zapatensis*	EU107287.1	96%
PI-2G	Uncultured Sordariales type OTV.LH22	GQ268569.1	99%	*Ellisembia calyptrata*	DQ408564.1	96%
PI-4A	*Penicillium inflatum*	AJ608959.1	99%	*Chaetosartorya stromatoides* isolate CBS 500.65	FJ358280.1	96%
PI-4C	*Oidiodendron maius*	HQ608115.1	99%	Uncultured *Oidiodendron* clone	JF519596.1	98%
PI-4D	*Penicillium adametzii*	AF034459.1	99%	*Chromocleista malachitea* isolate CBS 647.95	FJ358281.1	97%
PI-7C	*Diaporthe* sp. XL-C3	EF488448.1	99%	*Diaporthe eucalyptorum*	JX069846.1	97%
PI-7H	Uncultured Hypocreales isolate 6.2 I1	GU056003.1	99%	*Absidia repens* strain SHTH004	JN982937.1	99%
PI-9A	*Scytalidium* sp. 2013	EU334799.1	99%	*Crinula caliciiformis* isolate AFTOL-ID272	AY544680.1	95%
PI-9C	*Scytalidium* sp. 2013	EU334799.1	99%	*Pseudeurotium* sp.	AB470532.1	96%
PI-10A	*Diplogelasinospora inaequalis*	AY681201.1	99%	*Diplogelasinospora inaequalis*	AY681167.1	99%
PI-10B	*Trichoderma spirale* strain DAOM 183974	EU280068.1	100%	*Sphaerodes retispora* var. *retispora* strain CBS 994.72	GU205261.1	99%
PI-10D	*Pleurostomophora richardsiae*	AB364704.1	99%	*Pleurostomophora richardsiae* strain CBS H-7595	AY761080.1	99%
PI-10H	*Umbelopsis isabellina*	JF440625.1	99%	*Umbelopsis isabellina* strain NRRL 1757	JN940879.1	99%
PI-12B	*Nectria mariannaeae* strain JET	HM152982.1	100%	*Mariannaea aquaticola* strain MFU090225	GQ153837.1	100%
PI-12E	*Neonectria radicicola* isolate B	HM214443.1	99%	*Neonectria punicea* strain CBS 124262	HM534901.1	95%
PI-13D	*Phialophora repens*	AF083195.1	98%	*Pleurostomophora repens* strain CBS H-7594	AY761078.1	99%
PI-17C	*Dendrosporium* sp. ICMP14921	EF029206.1	89%	*Hypocrea nigricans* strain NBRC 30611	JN941464.1	99%
PI-17E	*Hypocrea lixii* strain DAOM 234005	EU280091.1	100%	*Hypocrea lutea*	AB027384.1	97%
PI-17J	*Fusarium solani* strain ATCC 56480	FJ345352.1	99%	*Fusarium solani* strain 001AFUS	JN939570.1	100%
PI-17K	*Hypocrea virens* isolate TR039	HQ608709.1	96%	*Sphaerodes retispora* var. *retispora* strain CBS 994.72	GU205261.1	99%
PI-18G	*Biscogniauxia* sp. UFMGCB	JQ327868.1	98%	*Biscogniauxia nummularia*	GQ428318.1	93%
Hawaii-1	*Fuscoporia gilva* strain xsd08128	FJ481039.1	95%	*Fuscoporia gilva*	HQ328525.1	99%
FSM-1	*Fulvifomes* sp., Hymenochaetaceae sp.	AY558633.1	93%	NA		
FSM-2	*Ganoderma* sp.	JN234427.1	94%	NA		
FSM-3	*Fulvifomes* sp., Hymenochaetaceae sp.	AY558615.1	95%	NA		