RESEARCH NOTE

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Filamentous Fungi Isolated from *Platypus koryoensis*, the Insect Vector of Oak Wilt Disease in Korea

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The ambrosia beetle, *Platypus koryoensis*, is a serious pest of oak trees in Korea. In this study we investigated filamentous fungi present in the body of the beetle. Fourteen genera of filamentous fungi belonging to Ascomycota and Basidiomycota were isolated. Among the isolated fungi, some were able to produce wood degrading enzymes. This is first report of fungi associated with *P. koryoensis*.

KEYWORDS : Filamentous fungi, Oak wilt disease, *Platypus koryoensis*, *Raffaelea quercus-mongolicae*

Platypus koryoensis, a wood boring ambrosia beetle, is a pest of oak trees in Korea [1]. Oak tree damage by the attack of the ambrosia beetle has been increasing across Korea. In mythology, "ambrosia" was a term for the food or nectar of the gods. The beetles are named for their close association with ambrosia fungi, which are used as a source of nutrition. Thus, the *P. koryoensis* beetle has been assumed to carry fungal associates to complete its life cycle within the trees it infests. However, there is little actual information on the associated fungi, except for *Raffaelea quercus-mongolicae* that was recently been identified as a new species [2]. This ambrosia fungus, closely associated with and vectored in *P. koryoensis*, is also associated with oak wilt disease in Korea. Both the insect and the pathogen are considered to contribute to oak mortality in Korea. However, the mechanism of tree death has not been clearly elucidated. Therefore, further investigation of fungal associates of the *P. koryoensis* beetle would be helpful to understand the ecology of the beetle and tree damage that accompanies the beetle's infestation.

In recent years, our laboratory has isolated *Raffaelea quercus-mongolicae*, other filamentous fungi, yeast and bacteria from the body of *P. koryoensis* [3]. Our main objective in investigating the associated fungi was to get a general idea of the fungi involved in the life cycle of *P. koryoensis*. They have been identified mainly at the genus level. This is first report of the fungi associated with *P. koryoensis.*

For *P. koryoensis* sampling, beetle-infested Mongolian oak trees (Fig. 1A and 1B) in stands located in Cheonan, Goyang, Paju, Pocheon, and Hanam in Korea were used. From 2007 to 2010, the selected *P. koryoensis*-infested oak trees were cut down and felled into logs, from which small discs were obtained. The discs were brought to the laboratory and maintained in order to capture emerging *P. koryoensis* males and females. Some of the discs were surface sterilized with 0.5% chlorine lax solution and 70% ethanol, washed twice with sterile water and air dried. The dried wood discs were chipped to expose the insect galleries formed inside of the discs and adult beetles of *P. koryoensis* in the gallery were collected (Fig. 1C and 1D). For fungal isolation, some of the collected beetles were directly rolled on potato dextrose agar (PDA) and malt extract agar (MEA) plates, while other beetles were washed with sterile water, which was then spread on PDA and MEA plates. Other collected beetles were ground with a mortar and pestle in sterile water under aseptic conditions and the resulting ground sludge was spread onto PDA and MEA plates. The inoculated PDA and MEA plates were incubated 3~7 days at 25°C. Mycelial tips grown on the culture media were transferred to new media. A total of 189 pure cultures of fungal isolates were obtained and identified to the genus level. Fungal identification was performed based on the microscopic observation of colony properties and characters of micro-structures referring to

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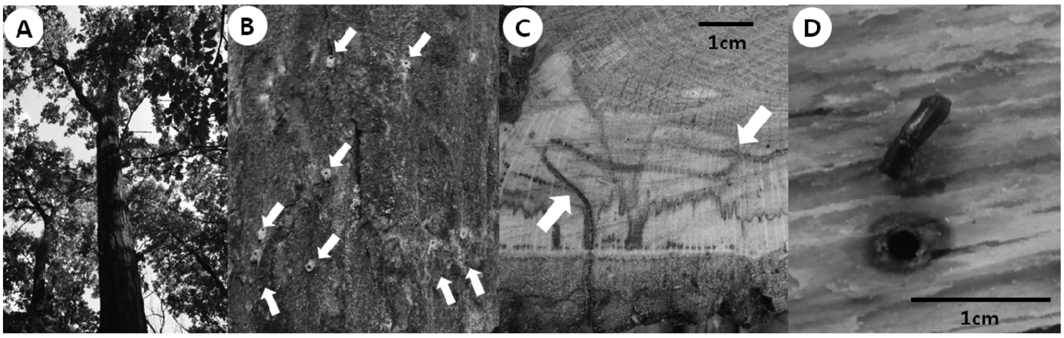

Fig. 1. A *Platypus koryoensis*-infested Mongolian oak tree (*Quercus mongolica* Fisch, ex Ledeb) and the beetle-damaged wood disc. A, An oak tree infested by the beetle; B, An oak stem damaged by mass attack of *P. koryoensis*. Arrows indicate infected holes on the surface of bark; C, Part of the cut surface feature of an oak disc. Arrows indicate insect gallery of the beetle; D, An adult beetle and a hole where the beetle emerged.

books and online tools for fungal taxonomy and identification [4-6] using an Axioskop 40 phase contrast light microscope (Carl Zeiss, Oberkochen, Germany) and a model SZ2-ILST dissecting microscope (Olympus, Tokyo, Japan). When the morphological characteristics were not confirmed, we carried out DNA sequencing analysis of the internal transcribed space (ITS) rDNA region. For ITS rDNA sequencing, genomic DNA was prepared using a method described previously [7]. PCR was performed in a Gene Amp-950 thermal cycler (ABI, Sunnyvale, CA, USA) using ITS1-ITS4 primer pairs for ITS rDNA [8], TEF728-TEF1 for translation elongation factor 1α (*tef-1a*) gene [9], and T10-BT12 for beta-tubulin gene [10]. The PCR products were sequenced by Macrogen (Daejeon, Korea). The determined sequences were searched through BLASTN at the GenBank database and Tricho-BLAST at the website of the International Subcommission on *Trichoderma* and *Hypocrea* Taxonomy (ISTH, http://www.isth.info/).

For the observation of the production of extracellular enzymes, a chromogenic method was used [11]. D-cellobiose (Sigma-Aldrich, St. Louis, MO, USA), polygalactronic acid (MP Biomedicals, Santa Ana, CA, USA), starch (Sigma-Aldrich), xylan (Sigma-Aldrich), CM-cellulose (Sigma-Aldrich), Avicel (Fluka, Cork, Ireland), and skim milk (Fluka) were used as enzymatic carbon sources. Fungal isolates were inoculated on the chromogenic medium with these different substrates and incubated at 25°C for 7 days. The fungus forming a clear zone (plaque) upon growth was judged to be an extracellular enzyme producer.

A total of 14 genera belonging to 11 orders were isolated

Table 1. Filamentous fungi isolated from *Platypus koryoensis* and their ability of producing extracellular enzymes

Isolates			Extracellular enzyme						
Order	Genus	Species	Amy	Avi	CB	CMC	Xyl	Pec	Pro
Agaricales	*Schizophyllum*	*commune* (DUCC7014)	+	+	+	+	–	–	+
Corticiales;	*Rhizochaete*	*filamentosa* (DUCC7013)	+	–	+	–	–	–	+
Eurotiales	*Penicillium*	*paneum* (DUCC7007)	+	+	+	+	+	–	–
		sp. (DUCC7008)	+	+	+	+	+	–	–
Hypocreales	*Fusarium*	sp. (DUCC7003)	–	+	+	+	–	–	–
		solani (DUCC7004)	–	+	+	+	–	–	+
	Hypocrea	*lixii* (DUCC7005)	+	+	+	–	–	–	–
	Trichoderma	*atroviride* (DUCC7016)	+	–	+	–	–	–	–
		sp. (DUCC7017)	+	+	+	+	+	–	+
		viride (DUCC7018)	+	+	+	+	+	–	+
Microascales	*Ceratocystis*	sp. (DUCC7001)	+	+	+	–	–	–	+
Ophiostomatales	*Raffaelea*	*quercus-mongolicae* (DUCC7012)	+	–	+	–	–	–	–
	Sporothrix	sp. (DUCC7015)	+	–	+	–	–	–	+
Pleosporales	*Phoma*	*herbarum* (DUCC7011)	–	–	+	+	–	–	+
Polyporales	*Irpex*	sp. (DUCC7006)	+	+	+	+	+	–	–
Russulales	*Peniophora*	*nuda* (DUCC7009)	+	–	+	–	+	–	+
Sordariales	*Chaetomium*	*globosum* (DUCC7002)	+	+	+	+	+	–	+
Xylariales	*Pestalotiopsis*	sp. (DUCC7010)	+	–	+	–	–	–	–

Amy, amylase; Avi, avicelase; CB, β-glucosidase; CMC, CM-cellulase; Xyl, xylanase; Pec, pectinase; Pro, protease; DUCC, Dankook University Culture Collection; +, production; –, no production.

from *P. koryoensis* (Table 1). All the obtained genera were isolated in the mitosporic state. The identified fungi were classified in 11 distinct orders including the Ascomycota (Eurotiales, Hypocreales, Microascales, Ophiostomatales, *Pleosporales*, and Sordiales) and Basidiomycota (*Agaricales*, *Corticiales*, *Polyporales*, and *Russulales* Xylariales). Within Ascomycota, 13 species were found. Meanwhile five species were found within Basidiomycota. The results showed the presence of diverse fungi in *P. koryoensis*. The diversity of *Platypus* associated fungi has been reported recently [12]. These authors also identified to the genus level and reported 14 genera of fungi associated with *P. cylindrus*, a cork oak mortality agent in Portugal. These genera belonged to eight orders including Eurotiales, Helotiales, Hypocreales, Ophiostomatales, Saccharomycetales, Sordiales, Xylariales, and a genus not assigned to any order. The number of Basidiomycota fungi was less present in *P. cylindrus* than in *P. koryoensis.* Among the genera, *Chaetomium*, *Fusarium*, *Penicillium Raffaelea*, and *Trichoderma* were commonly found both in *P. cylindrus* and *P. koryoensis*. This comparison indicates that diverse filamentous fungi are present in the two *Platypus* beetles and the composition of fungal genera varies depending on the insect species. To date, the variability of filamentous fungal diversity among *Platypus* species has not been extensively examined. More investigation is needed.

Since *Platypus* is a beetle group belonging to Platypodidae, which is among the most successful wood-inhabiting beetles causing damage of economic significance to trees and timber [13], many studies have been performed with the aim of controlling these pests. Given that *Platypus* as an ambrosia beetle, fungal studies have also been performed on the symbiotic relationship of ambrosia fungi with *Platypus* beetles. The symbiotic roles of ambrosia fungi are diverse. They might be involved in insect feeding, assisting the construction of the insect gallery, in the pathogenicity of the insect host, and in the protection of the insect. *Raffaelea* is one of the well-studied fungi as a symbiont of *Platypus* beetles, which has insect species-specific relationships. In general, *Raffaelea* fungi are not aggressive pathogens, but rather are weak pathogens. The pathogen of Japanese oak wilt disease, *R. quercivora*, has been reported in *P. quercivora* [14]. Two *Raffaelea* species, *R. ambrosiae* and *R. montetyi*, were found as the cork oak decline agent in *P. cylindrus* in Portugal and Mediterranean countries [15]. In this study, we also found *R. quercus-mongolicae* in *P. koryoensis.*

Although the names of fungi found in *Platypus* beetles were reported, the properties of these fungi have not been well-studied. Consequently, not much information is available on the properties of filamentous fungi from *Platypus* beetles. Presently, we examined biochemical properties of the fungi (Table 1). Among seven extracellular enzymes, â-glucosidase was produced by all the fungi, but none of the fungi could produce pectinase. Interestingly, several fungi such as *Chaetomium*, *Irpex*, *Peniciilium*, and *Trichoderma* produced extracellular enzymes that could degrade major wood cell components such as cellulose and xylan. This result suggests that these fungi could help the beetle's gallery construction through their colonization of the host. In the end, the *P. koryoensis* infected tree would be decayed in a short time. Meanwhile, the pathogen of oak wilt disease *R. quercus-mongolicae* did not show the ability of producing wood degrading enzymes. It seems this fungus might better serve as a nutrition source for *P. koryoensis* or in other processes in the life cycle of *P. koryoensis.*

In conclusion, we demonstrated the presence of diverse filamentous fungi in the Korean oak wilt disease transmitter *P. koryoensis*. The production of wood degrading extracellular enzymes by some of the identified fungi implies their possible involvement in the life cycle of *P. koryoensis.* We are currently analyzing the fungi isolated from the *P. koryoensis* beetle gallery in oak wood and the yeast species from the beetle body. Together with this study, the information from these analyses should provide insight to understanding the ecological distribution and roles of filamentous fungi in *P. koryoensis.*

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