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

Dong Yeon Suh¹, Min Woo Hyun¹, Seong Hwan Kim¹*, Sang Tae Seo² and Kyung Hee Kim²

¹Department of Microbiology and Institute of Basic Sciences, Dankook University, Cheonan 330-714, Korea
²Department of Forest Diseases and Insect Pests, Korea Forest Research Institute, Seoul 130-712, Korea

(Received August 22, 2011. Revised August 30, 2011. Accepted August 31, 2011)

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. 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. 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.

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

---

*Corresponding author <E-mail : piceae@naver.com>

This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (http://creativecommons.org/licenses/by-nc/3.0/) which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.
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-1α) 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 Hypocreales 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 at the GenBank database and Tricho-BLAST at the website of the International Subcommission on Trichoderma and Hypocreales Taxonomy (ISTH, http://www.isth.info/).

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

| Order           | Genus          | Species                        | Amy | Avi | CB | CMC | Xyl | Pec | Pro |
|-----------------|----------------|--------------------------------|-----|-----|----|-----|-----|-----|-----|
| Agaricales      | Schizophyllum   | commune (DUCC7014)             | +   | +   | +  | +   | −   | −   | +   |
| Corticiales;    | Rhizochaeae     | filamentosa (DUCC7013)         | +   | −   | +  | −   | −   | +   | +   |
| Eurotiales      | Penicillium     | paneum (DUCC7007)              | +   | +   | +  | −   | −   | −   | −   |
| Hypocreales     | Fusarium        | sp. (DUCC7008)                | +   | +   | +  | −   | −   | −   | −   |
| Hypocreales     | Fusarium        | sp. (DUCC7003)                | −   | +   | +  | −   | −   | −   | −   |
| Hypocreales     | Fusarium        | solani (DUCC7004)             | −   | +   | +  | −   | −   | −   | −   |
| Hypocreales     | Hypocrema       | lxxii (DUCC7005)              | +   | +   | +  | −   | −   | −   | −   |
| Hypocreales     | Trichoderma     | atroviride (DUCC7016)         | +   | −   | +  | −   | −   | −   | −   |
| Hypocreales     | Trichoderma     | sp. (DUCC7017)                | +   | +   | +  | −   | −   | −   | −   |
| Hypocreales     | Trichoderma     | viride (DUCC7018)             | +   | +   | +  | −   | −   | −   | −   |
| Microascales    | Ceratocystis    | sp. (DUCC7001)                | +   | +   | −  | −   | −   | −   | +   |
| Ophiostomatales | Raffaeela       | quercus-mongolicae (DUCC7012) | +   | −   | +  | −   | −   | −   | −   |
| Ophiostomatales | Raffaeela       | sp. (DUCC7015)                | +   | +   | −  | −   | −   | −   | −   |
| Pleosporales    | Phoma           | herbarum (DUCC7011)           | −   | −   | +  | −   | −   | −   | −   |
| Pleosporales    | Phoma           | sp. (DUCC7006)                | +   | +   | +  | −   | −   | −   | −   |
| Pleosporales    | Phoma           | nuda (DUCC7009)               | −   | +   | −  | −   | −   | −   | −   |
| Pleosporales    | Phoma           | 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 Sordariales) and Basidiomycota (Agaricales, Corticales, 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, Sordariales, 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. monteti*, 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, α-glucosidase was produced by all the fungi, but none of the fungi could produce pectinase. Interestingly, several fungi such as *Chaetomium*, *Irpex*, *Penicillium*, 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*.

**Acknowledgements**

This research was supported by the Graduate Research Assistantship of Dankook University in 2009 and Korea Forest Research Institute.

**References**

1. Hong KJ, Kwon YD, Park SW, Lyu DP. *Platypus koryoensis* (Murayama) (Platypodidae; Coleoptera), the vector of oak wilt disease. Korean J Appl Entomol 2006;45:113-7.
2. Kim KH, Choi YJ, Seo ST, Shin HD. *Raffaelea quercus-mongolicae* sp. nov. associated with *Platypus koryoensis* on oak in Korea. Mycotaxon 2009;110:189-97.
3. Suh DY, Hyun MW, Choi IJ, Kim SH, Seo ST, Kim KH. Filamentous fungi and yeasts isolated from *Platypus koryoensis* and the beetle infested oak tree in Korea. In: Proceeding of Asian Mycological Congress 2011 & The 12th International Marine and Freshwater Mycology Symposium; 2011 Aug 7-11; Incheon, Korea. Seoul: The Korean Society of Mycology; 2011. p. 196.
4. Dugan FM. The identification of fungi; an illustrated introduction with keys, glossary, and guide to literature. St. Paul: American Phytopathological Society; 2006.
5. Ellis MB. Dematiaceous Hyphomycetes. Surrey: CAB International Mycological Institute; 1971.
6. Jacobs K, Wingfield MJ. *Leptographium* species: tree pathogens, insect associates and agents of blue-stain. St. Paul: American Phytopathological Society; 2001.
7. Kim SH, Uzunovic A, Breuil C. Rapid detection of *Ophiostoma piceae* and *O. quercus* in stained wood by PCR. Appl Environ Microbiol 1999;65:287-90.
8. White TJ, Bruns T, Lee S, Taylor J. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, editors. PCR protocols: a guide to methods and applications. San Diego: Academic Press; 1990. p. 315-22.

9. Evidente A, Ricciardiello G, Andolfi A, Sabatini MA, Ganassi S, Altomare C, Favilla M, Melck D. Citrantifidiene and citrantifidiol: bioactive metabolites produced by *Trichoderma citrinoviride* with potential antifeedant activity toward aphids. J Agric Food Chem 2008;56:3569-73.

10. Schroeder S, Kim SH, Cheung WT, Sterflinger K, Breuil C. Phylogenetic relationship of *Ophiostoma piliferum* to other sapstain fungi based on the nuclear rRNA gene. FEMS Microbiol Lett 2001;195:163-7.

11. Yoon JH, Park JE, Suh DY, Hong SB, Ko SJ, Kim SH. Comparison of dyes for easy detection of extracellular cellulases in fungi. Mycobiology 2007;35:21-4.

12. Henriques J, Inácio ML, Sousa E. Fungi associated to *Platypus cylindrus* Fab. (Coleoptera: Platypodidae) in cork oak. Rev Ciênc Agrár 2009;32:56-66.

13. Cassier P, Lévieux J, Morelet M, Rougon D. The mycangia of *Platypus cylindrus* Fab. and *P. oxyurus* Dufour (Coleoptera: Platypodidae): structure and associated fungi. J Insect Physiol 1996;42:171-9.

14. Murata M, Yamada T, Matsuda Y, Ito S. Discoloured and non-conductive sapwood among six *Fagaceae* species inoculated with *Raffaelea quercivora*. For Pathol 2007;37:73-9.

15. Inácio ML, Henriques J, Lima A, Sousa E. Fungi of *Rafaela* genus (Ascomycota: Ophiostomatales) associated to *Platypus cylindrus* (Coleoptera: Platypodidae) in Portugal. Rev Ciênc Agrár 2008;31:96-104.