

## Determining Fungal Diversity on Mountain Pine Beetle and Its Infested Lodgepole Pine Using Cultural and Molecular Methods

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Lodgepole pine (*Pinus contorta* Dougl. var. *latifolia* Engelm.) is an economically and ecologically important resource of Western Canada and the Northwestern United States. It accounts for 50% of the total growing stock and 25% of the total volume of timber harvested in interior British Columbia, Canada, which is used in construction, for pulpwood, and for railway ties and poles. Although the mountain pine beetle (MPB, *Dendroctonus ponderosae* Hopkins) is natural components of lodgepole pine ecosystems, it is a serious threat for mature lodgepole and several other pine species. Recent moderate winters have caused lower larval mortality, resulting in an epidemic that is currently spreading across British Columbia and causing extensive economic and environmental damage to lodgepole pine forests.

The MPB carry a diversity of fungi on their body surface and symbiotic fungi in mycangia. Within a tree, the MPB remains in the region just under the bark, mining the phloem and reproducing, while the fungi, mainly staining fungi, propagate in beetle galleries in the phloem and into the underlying sapwood. The beetles benefit because the staining fungi lower the wood moisture content and may produce an environment more favorable for the beetle brood and some of the fungal associates are potential nutrient sources for the next beetle generation. In trees that have sustained high levels of attack by MPB, the sapwood is stained by fungi within a few weeks. As there is no appreciable loss in most strength properties, only a small decrease in toughness, stained wood can be used in most applications where appearance is not an important factor. However, MPB-killed trees that are left uncut for extended periods are susceptible to fungal decay that will further reduce their value in markets that are otherwise open to stained wood.

In order to evaluate fungal diversity from the exoskeleton of the MPB and its infested lodgepole pine trees, the cultural and molecular methods were used. All nine fungal taxa were isolated from the MPB. Identification was based on cultural morphology and high sequence similarities of the internal transcribed spacer (ITS) and large subunit ribosomal DNA (LSU rDNA) region to sequences of known fungi. The isolates from the cultural method represented 7 ascomycetes and 2 basidiomycetes. Fungal ITS regions were amplified from DNA directly extracted from the beetle surface. The PCR products were cloned and 125 clones were classified by their restriction pattern with *HaeIII* and *RsaI*. A total of 15 RFLP types were identified and subsequently sequenced. Sequence analysis of the RFLP types showed that 14 ascomycetes and 1 basidiomycetes were represented in the clone libraries. We found

that yeast and non-staining filamentous Euscomycetes fungi were detected efficiently using a molecular approach, while the major sapstaining fungi and decay fungi were best detected using cultural methods. The infested lodgepole pine trees were mainly colonized by *Ophiostoma clavigerum* (Robinson-Jeffrey & Davidson) Harrington, *Ophiostoma montium* (Rumbold) von Arx, *Ophiostoma nigrocarpum* (Davidson) De Hoog, *Ophiostoma minutum* (Olchow. & Reid) Hausner, and unknown *Leptographium* species. Among basidiomycetous fungi, decay fungi were rarely present in green trees but were isolated more frequently in red and grey trees. The frequency and the type of decay fungi isolated varied between harvesting sites.

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Main Scientific Publication	<p>Y.W.Lim, J.S.Lee, K.M.Kim and H.S.Jung. 2005. Wood-rotting fungal flora of Kanghwa Island. <i>Mycobiology</i> 33: 167-172</p> <p>Y.W.Lim, S.A.Lee, S.B.Kim, H.Y.Yong and J.S.Park. 2005 Diversity of denitrifying bacteria isolated from Daejeon sewage treatment plant. <i>J. Microbiol</i> 43: 383-390</p> <p>W.J. Jeong, Y.W.Lim, J.S.Lee and H.S.Jung. 2005. Phylogenetic analysis of <i>Phellinus</i> and related genera inferred from internal transcribed spacer sequences and mitochondrial small subunit ribosomal sequences. <i>Journal of Microbiology and Biotechnology</i> 15: 1028-1038.</p> <p>Y.W.Lim, Y.C.A.Yeung, R.Sturrock, I.Leal and C.Breuil. 2005. Differentiating and evaluating the phylogenetic position of two forms of <i>Phellinus weirii</i>: <i>P. weirii</i> and <i>P. sulphurascens</i>. <i>Forest Pathology</i> 35: 305-314</p> <p>Y.W.Lim, J.-J.Kim, M.Lu, and C.Breuil. 2005. Determining fungal diversity on <i>Dendroctonus ponderosa</i> and <i>Ips pini</i> attacking lodgepole pine using cultural and molecular methods. <i>Fungal Diversity</i> 19: 79-94.</p> <p>Y.W.Lim, J.-J.Kim, R.Chedgy, P.I.Morris and C.Breuil. 2005. Fungal diversity from western redcedar fences and their resistance to-thujaplicin. <i>Antonie van Leeuwenhoek</i> 87: 109-117</p> <p>G.-H.Kim, Y.W.Lim, Y.-S.Song, and J.-J.Kim. 2005. Diversity of decay fungi from playground wood products in service using LSU rDNA sequence analysis. <i>Holzforschung</i> 59: 495-466.</p> <p>G.-H.Kim, J.-J.Kim, Y.W.Lim, and C.Breuil. 2005. Ophiostomatoid fungi isolated from <i>Pinus radiata</i> logs imported from New Zealand to Korea. <i>Canadian Journal of Botany</i> 83: 272-278.</p> <p>J.-J.Kim, Y.W.Lim, K.A.Seifert, S.H.Kim, C.Breuil, and G.-H.Kim. 2005. Taxonomy of <i>Ophiostoma radiaticola</i> sp. nov. (Ophiostomatales, Ascomycetes), the teleomorph of <i>Pesotum pini</i>, isolated from logs of <i>Pinus radiata</i>. <i>Mycotaxon</i> 91: 481-496.</p> <p>J.-J.Kim, Y.W.Lim, C.Breuil, M.J.Wingfield, X.D.Zhou, and G.-H.Kim. 2005. A new <i>Leptographium</i> species associated with <i>Tomicus piniperda</i> L. infesting pine logs in Korea. <i>Mycol. Res.</i> 109: 275-284.</p> <p>Lutzoni F.I. <i>et al.</i> 2004. Assembling the fungal tree of life: progress, classification, and the evolution of subcellular traits. <i>Am. J. Bot.</i> 91(10): 1446-1480.</p> <p>Y.W.Lim, S.Massoumi Alamouti, J.-J.Kim, S.Lee, C.Breuil. 2004. Multigene phylogenies of <i>Ophiostoma clavigerum</i> and closely related species from bark beetle-attacked <i>Pinus</i> in North America. <i>FEMS Microbiology Letters.</i> 237: 89-96.</p> <p>J.-J.Kim, Y.W.Lim, M.J.Wingfield, C.Breuil, and G.-H.Kim. 2004. <i>Leptographium bistatum</i> sp. nov., a new species with a <i>Sporothrix</i> synanamorph from Korea. <i>Mycol. Res.</i> 108(6): 699-706</p> <p>Y.W.Lim, J.S.Lee and H.S.Jung. 2003. Morphological and molecular approach to differentiate <i>Phellinus baumii</i> from <i>Phellinus linteus</i>. <i>Mycotaxon</i> 85: 201-210.</p> <p>Y.W.Lim and H.S.Jung. 2003. A new species of <i>Irpex</i> based on morphological, cultural and molecular data. <i>Mycologia</i> 95: 694-699.</p> <p>Y.W.Lim, K.S.Baik, S.K.Han, S.B.Kim and K.S.Bae. 2003. <i>Burkholderia sordidicola</i> sp. nov., isolated from the white rot fungus, <i>Phanerochaete sordida</i>. <i>IJSEM.</i> 53: 1631-1636.</p> <p>S.I.Yoon, S.Y.Kim, Y.W.Lim, and H.S.Jung. 2003. Phylogenetic evaluation of steroid fungi. <i>Journal of Microbiology and Biotechnology</i> 13: 406-414.</p>
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