学　位　論　文　要　旨

Study on the inter-responses at the gene and protein levels between Japanese birch cultures and a birch canker-rot fungus *Inonotus obliquus* at an early infection stage
（感染初期におけるシラカンバ培養物とカンバ類癌腫病菌カバノアナタケとの遺伝子及びタンパク質レベルでの相互応答に関する研究）

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Canker-rot caused by *Inonotus obliquus* (Fr.) Pilát is the major disease of Japanese birch (*Betula platyphylla* Sukaczev var. *japonica* (Miq.) H. Hara) trees. In order to develop protection strategies, the interactions between Japanese birch and *I. obliquus* should be clarified. Therefore, the objective this study are as follows: 1) to investigate protein expression levels and identify the specific proteins from Japanese birch callus infected with a canker-rot fungus *I. obliquus* at early infection stage, 2) to sequence genome of *I. obliquus* and analyze the draft genome sequence, 3) to clone and characterize cDNA encoding putative versatile peroxidase of *I. obliquus*, 4) to determine the complete mtDNA sequence from genome sequence of *I. obliquus* and analyze its phylogenetic relationship with other basidiomycetes.

The twenty-day-old callii of Japanese birch were artificially wounded and infected with *I. obliquus* strain IO-U1. The protein samples obtained at 2 d post-treatments were subjected for proteome analysis. Furthermore, genome of *I. obliquus* strain IO-B2 was sequenced using Illumina MiSeq, and analyzed by bioinformatics tools. cDNA of gene encoding putative versatile peroxidase was synthesized by RT-PCR. The cDNA products were then cloned and characterized. The phylogenetic analysis was also applied for 56 genes encoding manganese, lignin, and versatile peroxidases. The mitochondrial DNA (mtDNA) was determined by a circular DNA molecule. The mtDNA of *I. obliquus* strain IO-B2 was analyzed. The phylogenetic analysis was also applied among other basidiomycetes.

Twenty-six protein spots significantly responded to wounding, 6 protein spots

were significantly expressed by fungal infection, and 16 protein spots responded to wounding and fungal infection. The identified 10 infection-specific proteins were protochlorophyllide reductase chloroplastic (spot ID 1564), enolase (spot ID 368), mitochondrial-processing peptidase subunit alpha-like (spot ID 441), mitochondrial-processing peptidase subunit alpha isoform B (spot ID 1578), T-complex protein 1 subunit eta (spot ID 356), glutathione *S*-transferase-like protein (spot ID 1121) and glutathione *S*-transferase-like protein, partial (spot ID 1527), protein WALLS ARE THIN 1-like (spot ID 1444), and 1 Sc-3 (spot IDs 1290 and 1322).

Genome of *I. obliquus* generated 42.5 Mbp nucleotides with 47.6% GC content. The genome assembly consisted of two rRNAs, 136 tRNAs, and 21,203 protein coding genes. Among all the predicted genes, 136 genes were potentially involved in degradation of wood chemical components. Based on homology search, 1621 genes had similarity with PHI-base in *I. obliquus* genome.

A cDNA clone encoding putative versatile peroxidase from *I. obliquus* (IO-Px) contained 1078 nucleotides encoding 347 deduced amino acids with a signal peptide consisting of 20 amino acids. Arg43, Phe46, His47, His172, Phe189, and Asp219 were found as deduced heme pocket residues. IO-Px also had three acidic amino acid residues, i.e Glu 36, Glu40, and Asp178, which form Mn-binding site analogous to the active site of manganese peroxidase. However, deduced amino acid sequence of IO-Px did not possess tryptophan residue which is the active site of lignin peroxidase. The phylogenetic analysis showed that IO-Px was not clustered with manganese, lignin, nor even versatile peroxidases.

The mtDNA of *I. obliquus* was a typical circular DNA molecule with a length of 119,110 bp and GC content of 25%. The genome contained two rRNAs, 30 rRNAs, and 58 protein coding genes. Among 58 protein coding genes, 14 genes encoded conserved mitochondrial proteins, one gene rpS3, and 43 genes hypothetical proteins. The phylogenetic analysis showed that this fungus is closely related to *Sanghuangporus sanghuang* and clustered with *Pyrrhoderma noxium*.

Based on the results obtained in the present study, Japanese birch callus is able to induce defense mechanisms as responses to protect itself against *I. obliquus* infection. On the other hand, *I. obliquus* is capable to express the pathogenicity to develop disease in Japanese birch. In addition, *I. obliquus* also possesses a huge variety of enzymes which may be required to complete its parasitic lifestyle. Several enzymes among them might have evolved. It is evidenced by the discovery of a new type of peroxidase from cDNA cloning. The phylogenetic analysis of *I. obliquus* also confirmed that this fungus is a complex species which can act as a pathogen and a medicinal fungus. Achievement of this study will provide new insights into molecular process which may lead to develop protection strategies for Japanese birch against *I. obliquus* infection.