

## 論文の内容の要約

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学位の種類	博士 (学術)
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学位論文名	Study on the inter-responses at the gene and protein levels between Japanese birch cultures and a birch canker-rot fungus <i>Inonotus obliquus</i> at an early infection stage

## 【論文の内容の要約】

Japanese birch (*Betula platyphylla* Sukaczew var. *japonica* (Miq.) H. Hara), called as Shirakanba in Japanese, is a pioneer tree species belonging to the Betulaceae. It is regarded as an important biomass resource in subalpine zone of Japan. However, Japanese birch is infected with a birch pathogenic fungus *Inonotus obliquus* (Fr.) Pilát, and the fungus causes canker-rot disease. In order to develop protection strategies, the interactions between Japanese birch and *I. obliquus* should be clarified as a basic knowledge. Therefore, the objective this study were as follows: 1) to investigate protein expression levels and identify the specific proteins from Japanese birch callus infected with *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 calli 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 to 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.

In the first study, 26 protein spots were significantly expressed by wounding, 6 protein spots by fungal infection, and 16 protein spots by wounding and fungal infection, respectively. The results indicated that the responses of wounding and fungal infection were activated in the callus of Japanese birch. Furthermore, the identified 10 infection-specific proteins were protochlorophyllide reductase chloroplastic, enolase, mitochondrial-processing peptidase subunit alpha-like, mitochondrial-processing peptidase subunit alpha isoform B, T-complex protein 1 subunit eta, glutathione *S*-transferase-like protein, glutathione *S*-transferase-like protein, partial, protein WALLS ARE THIN 1-like, and 1 Sc-3. These results suggest that Japanese birch callus is capable to induce several proteins related to photosynthesis, energy production, protein import machinery in mitochondria, protein folding, detoxification, secondary cell wall formation, and PR-10 protein as responses to defend itself against *I. obliquus* infection.

In the second study, the genome of *I. obliquus* generated 42.5 Mbp nucleotides with 47.6% GC content. The genome assembly consisted of two ribosomal RNAs, 136 transfer RNAs, and 21,203 protein coding genes. An overall view for the annotation of predicted coding genes showed that several genes were involved in medicinal compound biosynthesis and pathogenesis. Among all the predicted genes, 136 genes were potentially involved in degradation of wood chemical components. These genes were typical for white-rot fungi, and included 36 candidate cellulase genes, 35 candidate hemicellulase genes, 16 candidate pectinase genes, 39 candidate lignin modifying enzyme genes, and 10 candidate lignin degrading auxiliary enzyme genes, respectively. These results indicate that *I. obliquus* is able to degrade all chemical components of wood cell wall, reflecting its ability as a white-rot fungus. Possessing huge variety of enzymes in this fungus may be related to its parasitic lifestyle, in which its survival depends on degradation of wood chemical components in the host tree to release the primary carbon source as nutrients for growth during colonization. Based on homology search, 1,621 genes had similarity with PHI-base in *I. obliquus* genome. The number of genes for each level of phenotype showed remarkable variation with the highest number for the phenotype of 'reduced virulence' (718 genes). According to the above results and annotation from PHI-base search, pathogenic mechanisms in *I. obliquus* were considered to include wood degradation, protein and lipid degradations, nutrient uptake and elimination of toxic compounds, fungal cell wall remodelling, secondary metabolite biosynthesis, signal transduction and sclerotial development, and effector secretion and resistant to chemicals. These mechanisms are considered as strategies of *I. obliquus* to cause canker-rot disease in Japanese birch.

In the third study, a cDNA clone encoding putative versatile peroxidase from *I. obliquus* (IO-Px) contained 1,078 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 Glu36, 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. Based on the obtained results, two possibilities were suggested: 1) IO-Px is a new type of manganese peroxidase because of possessing Mn-binding site, and 2) IO-Px is a new member of heme containing peroxidases.

In the fourth study, 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*. These results indicate that the phylogenetic relationship between *I. obliquus* and *S. sanghuang* is related to their abilities to produce medicinal compounds, while the relationship between *I. obliquus* and *P. noxium* may correspond to their abilities as pathogens to cause disease in woody plants. Eventually, the phylogenetic relationship was in agreement with the taxonomic classification among species.

In conclusions 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. A new type of lignin-degrading enzymes was discovered 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, leading to developin protection strategies for Japanese birch against *I. obliquus* infection.