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**DEGRADATION OF PCP BY LACCASES OF
THE WHITE-ROT FUNGUS *TRAMETES* SP.
HR577**

A thesis presented in partial fulfilment of the degree of

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Chemistry**

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ABSTRACT

Pentachlorophenol (PCP) is a biocide used by the NZ forestry industry until 1988. Its use was discontinued due to its toxicity to humans and animals. White-rot fungi have been shown to degrade PCP in laboratory and field trials. New Zealand native white-rot fungi were screened to identify organisms suitable for the clean up of PCP contaminated sites. Four criteria were used for the screening: fungal growth at different temperatures, PCP and creosote resistance and PCP degradation in standard liquid medium. Twenty isolates were identified as potentially useful from over 200 that were screened. One unique isolate, *Trametes* sp. HR577, was chosen for intensive study because it produced the well known laccases previously described from other PCP-degrading white-rot fungi.

The white-rot isolate HR577 was assigned to the genus *Trametes* based on morphological characteristics and gene sequencing studies. The latter showed that the partial laccase gene sequences from *Trametes* sp. HR 577 had high sequence homology to laccases from other *Trametes* species, especially *T. versicolor* and *T. villosa*.

Two laccase isozymes, designated L1c and L2, were purified from *Trametes* sp. HR577. These isozymes had similar biological properties to other *Trametes* species laccase isozymes. Both isozymes had a relatively high temperature optima, however, they were not very stable at elevated temperature. The dependence of laccase on dissolved oxygen for catalysis was demonstrated for isozyme L2. Laccase activity was severely inhibited in the absence of dissolved oxygen. This could be restored by reoxygenation into the assay system.

Whole cultures of *Trametes* sp. HR577 grown in liquid culture removed up to 76% of PCP after 72 hours. PCP removal was mostly due to degradation rather than adsorption of PCP to fungal mycelium. Addition of purified and crude laccase isozymes (100 U mL^{-1}) did not enhance PCP degradation.

6-15% of PCP was removed from solutions containing solely purified isozyme L1c or L2 in acetate buffer over 72 hours. Addition of ethanol or the laccase mediator compound 2,2' azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) resulted in increased PCP disappearance from purified laccase cultures.

These studies show that the white-rot *Trametes* sp. HR577 has potential to be used for the clean up of PCP contaminated sites in NZ.

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ABBREVIATIONS

| | |
|--------------|--|
| 2,3,4,5-TeCP | 2,3,4,5-Tetrachlorophenol |
| 2,3,4,6-TeCP | 2,3,4,6-Tetrachlorophenol |
| 2,4,5-TCP | 2,4,5-Trichlorophenol |
| 2,4-DCP | 2,4-Dichlorophenol |
| 2CIBQ | 2-Chlorobenzoquinone |
| 2-CP | 2-Chlorophenol |
| 4-CP | 4-Chlorophenol |
| ABTS | 2,2'Azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) |
| ATCC | American Type Culture Collection |
| BLAST | Basic Local Alignment Search Tool |
| BLASTN | Basic Local Alignment Search Tool Nucleotide |
| BSA | Bovine Serum Albumin |
| cDNA | complementary Deoxyribonucleic acid |
| CP | Chlorophenol |
| CRI | Crown Research Institute |
| CYS | Cysteine |
| DCP | Dichlorophenol |
| DDT | 1,1,1-Trichloro-2,2-bis(<i>p</i> -chlorophenyl)ethane |
| DEPC | Diethylpyrocarbonate |
| DMP | 2,6-Dimethoxyphenol |
| DNA | Deoxyribonucleic Acid |
| dNTP | Deoxynucleoside Triphosphate |
| DTT | Dithiothreitol |
| ECD | Electron-Capture Detector |
| EDTA | Ethylenediamine Tetraacetic Acid |
| EPR | Electron Paramagnetic Resonance |
| FA | Ferulic Acid |
| FPLC | Fast Protein Liquid Chromatography |
| FRI | Forest Research Institute |
| GC | Gas Chromatography |
| gDNA | genomic Deoxyribonucleic acid |
| GI | GenInfo Identifier |

| | |
|---------------------|--|
| Glu | Glutamic Acid |
| GSH | Glutathione |
| GS-TrCHQ | S-Glutathionyltrichloro-1,4-hydroquinone |
| HAA | 3-Hydroxyanthranilate |
| HBA | 4-Hydroxybenzoic Acid |
| HBT | 1-Hydroxybenztriazole |
| His | Histidine |
| HIV | Human Immunodeficiency Virus |
| HPI | N-Hydroxyphthalimide |
| HPLC | High Performance Liquid Chromatography |
| HRCC | HortResearch Culture Collection |
| HRERM | HortResearch Environment and Risk Management |
| HSNO | Hazardous Substances New Organisms |
| IEF | Isoelectric Focusing |
| IPTG | Isopropyl-1-thio- β -D-galactopyranoside |
| ITS | Internal Transcribed Spacer |
| k_{cat} | Turnover Number |
| K_M | Michaelis-Menton Constant |
| LB | Luria Broth |
| LC50 | Lethal Concentration 50 |
| Leu | Leucine |
| LiP | Lignin Peroxidase |
| MEA | Malt Extract Agar |
| Met | Methionine |
| MnP | Manganese Peroxidase |
| mRNA | messenger RNA |
| MW | Molecular Weight |
| NCBI | National Centre for Biotechnology Information Database |
| NZ | New Zealand |
| <i>o</i> -chloranil | 3,4,5,6-Tetrachloro-3,5-cyclohexadiene-1,2-dione |
| ORF | Open Reading Frame |
| PAH | Polyaromatic Hydrocarbon |
| PCA | Pentachloroanisole |

| | |
|---------------------|--|
| <i>p</i> -chloranil | 2,3,5,6-Tetrachloro-2,5-cyclohexadiene-1,4-dione |
| PCP | Pentachlorophenol |
| PCR | Polymerase Chain Reaction |
| Phe | Phenylalanine |
| pI | Isoelectric Point |
| PNGase F | Peptide: <i>N</i> -glycosidase F |
| psi | Pounds per Square Inch |
| rRNA | ribosomal RNA |
| RNA | Ribosomal Nucleic Acid |
| RT-PCR | Reverse Transcriptase-Polymerase Chain Reaction |
| SCS | Sawdust Cornmeal Starch |
| SDS-PAGE | Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis |
| Ser | Serine |
| T1 | Type 1 |
| T2 | Type 2 |
| T3 | Type 3 |
| TCHD | 2,3,5,6-Tetrachloro-2,5-cyclohexadiene-1,4-dione |
| TCHQ | Tetrachlorobenzohydroquinone |
| TCP | Trichlorophenol |
| TeCP | Tetrachlorophenol |
| TEMPO | 2,2,6,6-Tetramethylpiperidin-1-xyloxy |
| TNT | Trinitrotoluene |
| TrCHQ | Trichlorohydroquinone |
| USEPA | United States Environmental Protection Agency |
| VA | Veratryl Alcohol |
| VP | Versatile Peroxidase |
| XYL | 2,5-Xylidine |