

Development of the Biosystem Useful for Degradation of Polychlorinated Aromatic Hydrocarbons by Using Molecular-genetically Improved Basidiomycete Mushroom

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Research aim

One of the most important problems to be solved in 21st century is the development of the biosystem useful for preservation of environment. Especially, chlorinated dibenzo-*p*-dioxins (CDDs) and pentachlorophenol (PCP) have been of public concern because of their toxicity in animal tests. The aim of this research is the development of the biosystem useful for degradation of CDDs and PCP.

Methods

Extracellular lignin peroxidase (LiP) and manganese peroxidase (MnP) produced by white-rot basidiomycetous mushrooms have been reported to be involved in degradation of various CDDs (and PCP). Mammalian (rat, etc.) cytochrome P450, CYP1A1 has been reported to play an important role in the metabolism (detoxification) of Mono-TriCDDs. So the white-rot mushroom strains producing large amounts of LiP and rat CYP1A1 are produced by molecular-genetical method. The *Coriolus hirsutus* monokaryotic strain is used as a target of molecular breeding.

Results

1) Efficient degradation of 2,7-DCDD by using molecular-genetically bred *C. hirsutus* strains producing large amounts of LiP

The chromosome-integrating vector MIp30 carrying the *C. hirsutus gpd* gene promoter—*Lentinula edodes priA* gene terminator and the selectable marker of *C. hirsutus ARG1* gene was constructed. The *C. hirsutus* LiP gene (*lip*) was fused between the promoter and terminator of MIp30 and the resulting recombinant plasmid MIp30-*lip* was introduced into protoplasts of monokaryotic *C. hirsutus* Arg⁻ Leu⁻ strain (OJ1078), followed by selection of Arg⁺ Lip⁺ colonies. Two Arg⁺ Lip⁺ transformants, namely ChTF6-1(Ch.LiP) and ChTF6-2(Ch.LiP), possessed about five copies of the promoter-*lip*-terminator cassette on their chromosomes and the extracellular LiP activities of the two Arg⁺ Lip⁺ transformants were about five times as high as that of the Arg⁺ control. The supernatants of ChTF6-1(Ch.LiP) and ChTF6-2(Ch.LiP) showed remarkably high 2,7-DCDD degradation activities: at the time when only 33.5% of 2,7-DCDD was degraded by the control Arg⁺

transformant, 73.7% and 63.5% of 2,7-DCDD were degraded by ChTF6-1(Ch.LiP) and ChTF6-2(Ch.LiP), respectively (Fig. 1). These two transformants also showed significantly high PCP conversion activity.

2) Degradation/transformation of 2,7/2,8-DCDDs by using molecular-genetically bred *C. hirsutus* strains producing rat cytochrome P450, CYP1A1

The expression cassette [*C. hirsutus gpd* promoter—*C. hirsutus gpd* 5'-portion (224-bp sequences of 1st exon—8th base of 4th exon)—rat *cyp1a1* cDNA—*L. edodes priA* terminator] was constructed and then inserted into pUCR1 carrying *C. hirsutus ARG1* gene. The resulting recombinant plasmid, MIp5-(*cyp1a1*+*arg1*) was introduced into protoplasts of *C. hirsutus* OJ1078 strain, obtaining three good Arg⁺ transformants. These transformants, namely ChTF5-2(CYP1A1), ChTF5-4(CYP1A1), and ChTF5-6(CYP1A1) were estimated to carry nine, six, and seven copies of the expression cassette on their chromosomes, respectively. Immunoblot analysis revealed that the three transformants produce similar amounts of rat CYP1A1. After 5-day cultivations, ChTF5-2(CYP1A1), ChTF5-4(CYP1A1), and ChTF5-6(CYP1A1) degraded/transformed 71.7%, 69.8%, and 69.4% of 2,7/2,8-DCDDs, respectively, while recipient OJ1078 degraded/transformed only 11.8% of 2,7/2,8-DCDDs (Fig. 2).

Conclusion

We have succeeded in production of the *C. hirsutus* strains producing rat cytochrome P450, CYP1A1 and those

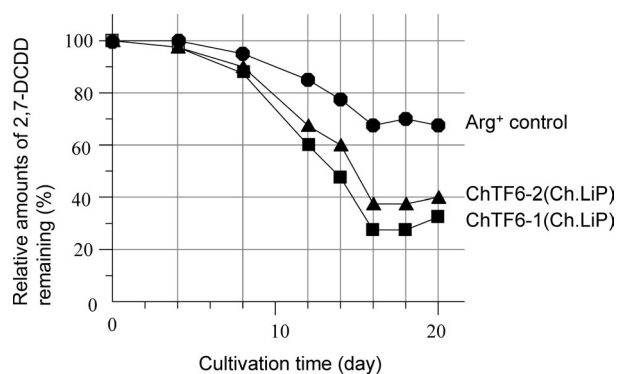


Fig. 1. 2,7-DCDD degradation by the culture supernatants of *C. hirsutus* transformants.

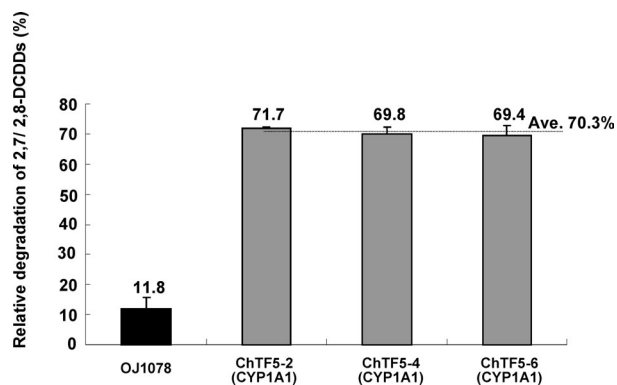


Fig. 2. Degradation (transformation) of 2,7,8-DCDDs during cultivations of the three rat CYP1A1-producing transformants of *C. hirsutus*.

producing large amounts of LiP. A much more efficient degradation/transformation of chlorinated dioxins is thought to be achieved by co-cultivation of these strains. The dioxins are efficiently degraded by both extracellular LiP (and MnP) and intracellular CYP1A1 (refer to Fig. 3).

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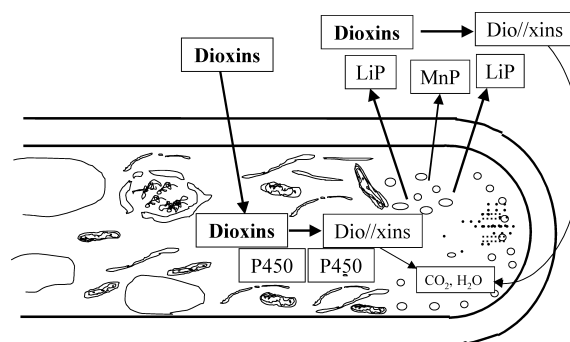


Fig. 3. Degradation of dioxins outside and/or inside mycelial cells of molecular-genetically bred basidiomycetous mushrooms (fungi).

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