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 Mlp30 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 Mlp30 and the resulting recombinant plasmid Mlp30-lip was introduced into protoplasts of LiP and rat CYP1A1 are produced by molecular-genetical method. The Coriolus hirsutus monokaryotic strain is used as a target of molecular breeding.

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, Mlp5-(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.

Conclusion

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

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

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