

F302 Transformation of *Coprinus congregatus* to Phosphinothricin Resistance.

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We have studied laccases which are concerned with the development in *Coprinus congregatus*, the inky cap. In order to research this fungus at the molecular level, the setting of the transformation system must be carried out. *C. congregatus* can not grow on minimal medium with phosphinothricin which inhibits the transamination reaction. We successfully transformed the fungus to phosphinothricin resistance using pBARGEM7-1 which had the resistance gene (*bar* gene) from *Streptomyces hygroscopicus*. The transformants could grow well on Coprinus minimal medium which contained 400 μ g/ml of phosphinothricin. We have confirmed the integrated plasmid into the *C. congregatus* chromosomal DNA by Southern hybridization.

F303 Localization of the Genes Responsible for Dechlorination of 4-Chlorobenzoate in *Pseudomonas* sp. DJ-12

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Dechlorination has been focussed to be one of the critical steps for degradation of 4-chlorobenzoate which is a kind of metabolites of various chlorinated herbicides and PCBs including 4-chlorobiphenyl. *Pseudomonas* sp. DJ-12 is a natural isolate capable of degrading 4CBA via hydrolytic dechlorination. The genes responsible for dechlorination were cloned in *Escherichia coli* from *Pseudomonas* sp. DJ-12. The cloned cell of *E. coli* KC157 produced to accumulate 4-hydroxybenzoate and released chloride ions as 4-chlorobenzoate was degraded. The DNA fragments inserted in pKC157 were hybridized with *pcbA*, *B*, and *C* encoding 4-chlorobenzoate:coenzyme A ligase, 4-chlorobenzoate: coenzyme A dehalogenase, and 4-hydroxybenzoate:coenzyme A thioesterase of *Pseudomonas* sp. CBS3 which were involved in hydrolytic dechlorination of 4CBA. As results of the hybridization, *pcbA*, *B*, and *C* genes responsible for dechlorination in *Pseudomonas* sp. DJ-12 were arranged in the order of *B-A-C* in the 7 kb fragment which was originated from chromosomal DNA.