Transformation vectors utilizing *Ceriporiopsis subvermispora* gene expression signals.

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[Introduction]

White rot fungi are known to degrade plant cell wall lignin and also various aromatic pollutants. Recently, wood biomass conversion systems drew attention as one of solutions for environmental problems, since cyclic utilization of wood is carbon-neutral. Delignification is necessary to convert wood biomass effectively, whereas general white rot fungi degrade not only lignin but also cellulose. A selective lignin-degrading fungus, *Ceriporiopsis subvermispora* is able to degrade lignin selectively without intensive damage of cellulose. To elucidate molecular mechanism for the selective lignin degrading system in this fungus, development of an efficient transformation system would make much contribution. This study reports construction of the transformation vectors containing drug-resistant markers driven by gene expression signals isolated from *C. subvermispora* β-tubulin.

[Materials and Methods]

Degenerated primers for amplification of *C. subvermispora* gene fragment encoding for β-tubulin were designed with CODEHOP method. With these primers, A DNA fragment was amplified by PCR and cloned into pGEM-T vector. Then, a whole gene fragment was cloned by using inverse PCR. Plasmids pCsbtubi-*bar* and pCsbtubi-*hph* were constructed by the ligation of *C. subvermispora* β-tubulin promoter and terminator to the bialaphos resistant gene (*bar*) and hygromycin B resistant gene (*hph*), respectively. These vectors contains the first intron of β-tubulin following to the promoter, since Ma *et al.* reported the presence of a 5' intron accerated the expression of recombinant *egfp* gene in *P. chrysosporium*[1].

To estimate these vectors function in *C. subvermispora*, they were introduced to *P. ostreatus* mediated PEG/$CaCl_2$. Genome DNAs were extracted from resultant drug-resistant isolates and subjected to PCR analysis and Southern blotting. In Southern blotting, DNAs were digested with *Hind* III and *Xba* I and transferred to the nylon membranes (Roche).

[Results and Discussion]

PCR analyses of DNA of the drug-resistant isolates obtained from transformation experiments with pCsbtubi-*bar* and pCsbtubi-*hph* demonstrated specific amplification of the marker fragment in most cases. Drug-resistant isolates without specific amplication of the fragment were suggested to be backgrounds or transient transformants. After five successive cultivation in the absence of drug selection, 7 bialaphos-resistant transformants were subjected to Southern hybridization. Several hybridizing bands were detected in the transformants with stable drug-resistance, whereas no hybridizing signals were observed in the those with transient drug-resistance as well as wild type control. These results confirmed that the introduced *bar* gene functions in these stable transformants. Southern hybridization analysis for hygromycin-resistant isolates, several bands of different sizes were detected in the transformants, suggesting that the probe might be not appropriate and further detailed analysis is required. These results suggested that pCsbtubi-*bar* and pCsbtubi-*hph* functioned in basidiomycete and hereby these vectors will work properly in *C. subvermispora,* from which the gene expression signals were isolated homologously.

[Reference]

1) Ma B., Mayfield M.B., and Gold M.H. (2001) *Appl. Environ. Microbiol.* **67**: 948-955