Development of transformation and promoter assay system in selective lignin-degrading fungus, *Ceriporiopsis subvermispora.*

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Introduction

Recently, wood biomass conversion systems have gained increasing attention as one of the solutions for the environmental problems, since cyclic utilization of wood is carbon-neutral. A white-rot fungus, *Ceriporiopsis subvermsipora* is able to degrade lignin selectively without intensive damage of cellulose. Thus, treatment of wood substrates by this fungus has been expected for a ecofriendly pretreatment in wood biomass conversion. To elucidate molecular mechanism for the selective lignin-degrading system by this fungus, it is essential to develop an efficient genetic transformation system. Furthermore, in basidiomycetes, promoter assay has been done using stable transformants, but they contained different copy numbers of exogenous reporter gene at various loci on the chromosome. So it is very difficult to evaluate accurate activity of a promoter in basidiomycetes. In this context, strongly desired is to develop a new promoter assay system which is completely unaffected by the insertion manner of the reporter construct in the chromosome.

Results and Discussion

In this work, a stable transformation system in *C. subvermispora* was developed for the first time, by modifying experimental conditions of the conventional PEG/$CaCl_2$ method in basidiomycetes. A number of drug resistant colonies were observed on the screening plate: a small portion of them maintained, despite the most remaining isolates (~95%) lost the drug resistance, during successive cultivations on the screening plate. The stable isolates were demonstrated as a integrated transformant in Southern hybridization analysis, whereas the unstable ones were decided to be a transient transformant and not a false positive, taking the fact that no drug resistant colonies were observed in no DNA control experiment. Next, we tried to evaluate activity of a series of promoter sequence which drives the drug resistant marker gene, by measuring the number of the transformants on the first screening plate. With this strategy, a deletion analysis of *C. subvermispora gpd* promoter was performed and, as a result, a 141-bp fragment was determined to be essential for the initiation of transcription. This result indicated that the system developed in this work is useful as a novel promoter assay system in basidiomycetes.