

Development of a novel transformation system in basidiomycetes

Naofumi Sakatoku

Laboratory of Biomass Conversion, RISH, Kyoto University

White rot fungi are known to degrade plant cell lignin and also various aromatic pollutants. Intensive research has been done to make use of their special function for a novel carbon recycle system and also bioremediation of polluted environments, which will contribute for development of a sustainable society. Especially, a selective white rot basidiomycete, *Ceriporiopsis subvermispota* is a promising microorganism which is effective as a biocatalyst to degrade lignin in industrial processes, including pulp and paper manufacture, and conversion of lignocellulosic biomass to various compounds. However, there is no report on development of a transformation system in this fungus. Transformation techniques are valuable tools for not only molecular biological analysis of the selective lignin degradation system but also strain improvement of desired phenotype.

Transforming vector plasmids were designed to contain bialaphos resistant marker gene (*bar*), from *Streptomyces hygroscopicus*, under control of homologous *gpd* promoter and terminator. In pCsG-*bar*, promoter was fused directly with the coding sequence of *bar* gene, whereas in pCsGi-*bar*, the promoter and the first intron of *gpd* gene were inserted at the 5' end of the coding sequence of *bar* gene, since Ma *et al.* reported that an intron insertion enhanced GFP expression [1].

In this research, we tried to develop a transformation system for basidiomycete *C. subvermispota* using the recombinant plasmids including pCsG-*bar* and pCsGi-*bar* by PEG/CaCl₂ protocol and particle bombardment. Moreover, as a control, transformation of *Pleurotus ostreatus* by particle bombardment was performed.

REFERENCE

- [1] Ma B., Mayfield M.B. and Gold M. H. (2001) Appl. Environ. Biotechnol. 67(2): 948-955