특별강연 III-2

Over-expression of lignin degrading enzyme genes using a genetic transformation in white rot fungi

Ryu, Sun-Hwa*, Kim, Bo-Young, Kim, Myungkil, Choi, Hyoungtae[1], Lee, Sung-Suk

Div. Wood Chemistry & Microbiology, Korea Forest Research Institute, [1]Kangwon University

White rot fungi produce extracellular oxidative enzymes that degrade lignin as well as related compounds found in explosive contaminated materials, pesticides, and toxic wastes. In order to over-express useful gene for biotechnological applications, the genetic transformation system has been developed in white rot fungi, *Polyporus brumalis*. We constructed a pHYgpt vector using the modified plasmid of pBARGPE1 which was replaced with the hygromycin resistance gene (hph) instead of the phosphinothricin resistance gene (bar) for the selectable maker, and then introduced the open reading frame sequences of a laccase cDNA, *pblac1* under the control of the gpd promoter for laccase over-expression. Transformation was performed by the REMI(restriction enzyme-mediated integration) method with slight modification. Transformants were selected from minimal medium containing 50 μgml^{-1} hygromycin B and the integration of the constructed DNA was confirmed by PCR using vector *specific primers*. We examined the extracellular laccase activity of transformants by decolorization on dye-plate and oxidation of o-tolidine in liquid culture medium. Compared with the laccase activity of wild type strain, those of transformants were 3~10 times higher. Transformants also showed better decolorizing activity when they were grown on an RBBR (Remazol Brilliant Blue R) plate. Biodegradation of wood chips (*Pinus densiflora* and *Quercus accutisma*) by fungi was investigated. The weight and lignin losses of wood chips after biodegradation by transformants were much higher than those of wild type strain. These results suggest that the over-expression of a laccase gene contribute to the fungal lignin degradation. Transformation system will be biotechnologycally useful for the molecular characterization related to the lignin degradation ability of basidiomycete fungus as well as the degradation of many recalcitrant xenobiotics.