

Heterologous expression of a tannic acid-inducible laccase3 of *Cryphonectria parasitica* in *Saccharomyces cerevisiae*

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The expression and hypoviral regulation of a tannic acid-inducible laccase3 (*lac3*) from the chestnut blight fungus *Cryphonectria parasitica* has been recently characterized but its purification was hampered because of the precipitation of protein product by tannic acid supplementation. Therefore, heterologous expression of the functional laccase3 using a budding yeast *Saccharomyces cerevisiae* was carried out for large-scale production and characterization of laccase3. The cloned gene encoding a matured peptide of laccase3 was placed in a yeast episomal vector and expressed in *S. cerevisiae*. Enzyme activities in the culture filtrate of transformants measured by laccase-specific substrate suggested that the laccase3 gene is successfully expressed and secreted into culture media. In addition, activity staining of a native gel followed by the electrophoresis of culture filtrate indicated that the enzyme activity is co-existed with the protein product of transformed laccase3 gene confirming that the protein product of cloned gene is responsible for the enzyme activity. However, the expressed protein product of laccase3 gene appeared to be unstable and sensitive to the cultured YEPD broth because the enzymatic activity was observed only in the selective culture condition such as *ura*⁻ media but not in the nutrient-rich YEPD media. In addition, a rapid decline of enzymatic activity was observed when the cultured filtrate of *ura*⁻ media was mixed with that of YEPD. Interestingly, the expression of laccase3 may have harmful effect on *S. cerevisiae* based on the fact that the forced expression of laccase3 in the selective *ura*⁻ media resulted in the reduced number of cell compared to that of mock-transformed cell. This study carried out the heterologous expression of *C. parasitica* laccase3 in yeast cell and characterized the enzyme activity of recombinant laccase3.

Key words: *Cryphonectria parasitica*, Laccase3, *Saccharomyces cerevisiae*, Heterologous expression