Expression and functional analysis of a gene encoding dicarboxylic acid methyltransferase from a selective white rot fungus, *Ceriporiopsis subvermispora*

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Selective white rot fungus, *Ceriporiopsis subvermispora* is able to degrade lignin without intensive damage to cellulose. The unique wood decay pattern implies that the extracellular low molecular mass metabolites are principally responsible for the lignin degradation. Alk(en)ylitaconic acids, ceriporic acids have been found as a major extracellular metabolite of *C. subvermispora*. A series of ceriporic acids with different side chain structures have been found and chemically synthesized. Ceriporic acids suppress cellulose degradation by inhibiting reduction of ferric ion. Recently, it was suggested that *C. subvermispora* produces monomethyl ester of ceriporic acids. Purpose of the present study is to identify the enzyme which catalyzes methyl esterification of ceriporic acids.

trans-Aconitate methyltransferase is an enzyme which catalyzes esterification of *trans*-aconitic acid from *S*-adenosylmethionine (SAM). It is known that some *trans*-aconitate methyltransferases exhibited a wide range of substrate specificity including itaconic acid. Therefore, *trans*-aconitate methyltransferase gene (*Cs-tam*) was cloned from *C. subvermispora*, expressed in *Escherichia coli* and substrate specificity of the expressed protein, *Cs*-Tam was analyzed. Purification on a Ni-affinity column and analysis with SDS-PAGE and MALDI-TOF-MS revealed successful expression of *Cs*-Tam in *E. coli*. To analyze the enzyme activity, lysate of recombinant *Cs*-Tam was incubated with itaconic acid in the presence of SAM. GC/MS analysis demonstrated that *Cs*-Tam esterified itaconic acid to its 4- and 6-monomethylester. Substrate specificity of the recombinant *Cs*-Tam to ceriporic acids was also analyzed by LCMS.