Mg^{2+}-dependent *O*-methyltransferase from Streptomyces avermitilis MA-4680: cloning, expression, purification, and enzymecharacterization

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Abstract

Streptomyces avermitilis is one of Streptomyces species to be able to methylate on hydroxyl group of compounds. Based on the sequence homologous search, S. avermitilis have several genes which contained methyltransferase domain. Among them, one gene, namely, saomt5 was cloned into pET-15b expression vector by PCR using sequence-specific oligonucleotide primers. Identification of methylating activities, and expression and purification of enzyme revealed that the SaOMT5 was an S-adenosyl-L-methionine dependent *O*-methyltransferase. The purified SaOMT5 was reacted with several compounds as substrates, so that SaOMT5 catalyzed *O*-methylation of flavonoids such as 6,7-dihydroxyflavone, 3'4'-dihydroxyflavone, quercetin, 7',8'-dihydroxyflavone, and 2',3'-dihydroxyflavone, and caffeic acid. The reaction products were analyzed by TLC, HPLC, and NMR spectrometer. The SaOMT5 could convert phenolic compounds involving ortho-dihydroxy groups into *O*-methoxylated compounds and 6,7-dihydroxyflavone was known to the best substrate. SaOMT5 converted 6,7-diydroxyflavone into 6-hydroxy-7-methoxyflavone and 7-hydroxy-6-methoxyflavone, and caffeic acid into ferulic acid and isoferulic acid, which were determined by HPLC, NMR and LC/MS analysis.[1)]

Reference

1. PALMA P.N., BONIFACIO M.J., LOUREIRO A.I., WRIGHT L.C., LEARMONTH D.A., AND SOARES-DA-SILVA P., Molecular modeling and metabolic studies of the interaction of catechol-O-methyltransferase and a new nitrocatechol inhibitor (2003) Drug Metab Dispos., 31:250-258.