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Cloning, Purification and Characterization of Triphenylmethane reductase from *Citrobacter* sp. MY-5

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A triphenylmethane reductase (TMR) encoding gene from *Citrobacter* sp. MY-5 was isolated using a probe derived from the N-terminal and internal amino acid sequences of the purified enzyme. The triphenylmethane reductase (TMR) gene revealed a single open reading frame of TMR comprised of 849 bp nucleotide base pairs and 282 deduced amino acids with a predicted molecular mass of 30,954 Da. The deduced amino acid sequences showed no overall similarity to any known protein in the public databases. The TMR gene was inserted a expression vector, pET 29-a(+). Recombinant TMR was overproduced in BL21(DE3) in a soluble form and purification was carried out by anion-exchange chromatography with HiPrep Q XL column, Hydrophobic chromatography with HiTrap Phenyl Sepharose column and anion-exchange chromatograph with Mono Q HR column, sequentially.

The purified recombinant enzyme was identified as a single band with a molecular mass of about 31 kDa on SDS-PAGE. In contrast, molecular mass analysis by native PAGE and activity staining gave a molecular mass of about 62kDa, indicating that the recombinant enzyme is composed of two subunits of identical size. The optimal temperature and pH were found to be pH9.0 and 60℃. The metabolic product of triphenylmethane dye crystal violet by the enzyme was idenified to be its leuco-form by UV-visible spectral change and thin-layer chromatography.