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Functional Role of Tyr12 in the Catalytic Activity of Zeta-Like Glutathione S-Transferase from Acidovorax sp. KKS102

Authors : D. Shehu, Z. Alias

Abstract : Glutathione S-transferases (GSTs) are family of enzymes that function in the detoxification of variety of electrophilic substrates. In the present work, we report a novel zeta-like GST (designated as KKSG9) from the biphenyl/polychlorobiphenyl degrading organism Acidovorax sp. KKS102. KKSG9 possessed low sequence similarity but similar biochemical properties to zeta class GSTs. The gene for KKSG9 was cloned, purified and biochemically characterized. Functional analysis showed that the enzyme exhibits wider substrate specificity compared to most zeta class GSTs by reacting with 1-chloro-2,4-dinitrobenzene (CDNB), p-nitrobenzyl chloride (NBC), ethacrynic acid (EA), hydrogen peroxide, and cumene hydroperoxide (CuOOH). The enzyme also displayed dehalogenation function against dichloroacetate (a common substrate for zeta class GSTs) in addition to permethrin, and dieldrin. The functional role of Tyr12 was also investigated by site-directed mutagenesis. The mutant (Y12C) displayed low catalytic activity and dehalogenation function against all the substrates when compared with the wild type. Kinetic analysis using NBC and GSH as substrates showed that the mutant (Y12C) displayed a higher affinity for NBC when compared with the wild type, however, no significant change in GSH affinity was observed. These findings suggest that the presence of tyrosine residue in the motif might represent an evolutionary trend toward improving the catalytic activity of the enzyme. The enzyme as well could be useful in the bioremediation of various types of organochlorine pollutants.

Keywords : Acidovorax sp. KKS102, bioremediation, glutathione s-transferase, site-directed mutagenesis, zeta
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