Abstract

Glutathione-S-transferases (GSTs) are a superfamily of homo- and hetero-dimeric proteins that mediate the catalytic binding of glutathione to an array of endo- and exobiotic compounds as a general detoxification scheme. In this study we have investigated the role of GSTs in two different projects.

(i) In the first project we looked into the putative Glutathione-S-Transferases (GSTs) involved in the conjugation of Isopentenyl pyrophosphate (IPP) to glutathione. In this work we attempted to reconstruct the Felis catus' Felinine biosynthetic pathway in Saccharomyces cerevisiae. Glutathione (GSH) and IPP conjugates to give 3-MBG in cat (Felis catus), the precursor to felinine. The enzyme catalyzing the felinine is exclusive to cats. Through bioinformatics analysis and literature mining, we have shortlisted a putative cat GST candidate GSTM3. The putative GST candidate GSTM3 was cloned and expressed in yeast was shown to conjugate with and IPP through in-vitro studies and the production of felinine by the recombinant yeast was successfully validated using HPLC and HR-MS.

(ii) In the second project study the glutathione-mediated pathway for the detoxification of endogenously derived toxic compounds was investigated. The ade1 / ade2 mutants of Saccharomyces cerevisiae, when grown on adenine-limiting medium, accumulate a characteristic red pigment (ade pigment) in their vacuoles. The precursors of the ade pigments are toxic intermediates that form conjugates with glutathione, followed by their transport inside the vacuole. In this study, putative Glutathione-S-Transferases (GSTs) involved in this conjugation were investigated. We show that the glutaredoxin, GRX4 is the GST responsible for the AIR/CAIR conjugation to GSH. The AIR/CAIR – GSH conjugate is known to be transported into the vacuole through the various GSH conjugate pumps. We also show that, ECM38, a Y-glutamyl transpeptidase that can degrades GSH conjugate, by removal of glutamate is also critical for the red pigmentation.