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Cloning and Expression of Peroxidase Gene from *Polyporaceae.*

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The cDNA library (λcPo10) was constructed starting with poly(A)$^+$ RNA extracted from the PoP producing *Polyporaceae* mycelia by oligo(dT) priming. From this library, two groups of cDNA clones for PoP were isolated by immunoscreening followed by plaque hybridization. The clones cpop 3, 15, 19 having *Bam*HI site around 3' region are included in class I and cpop 18, 21 without *Bam*HI site are in class II. Only the cpop8 clone covers start and stop codons. This clone encodes a protein of 499 amino acid residues preceded by 19-amino acid signal peptide. The amino acid sequence shows little homology to other peroxidases including LiP and MnP. The regions of proximal and distal histidine conserved in LiP, MnP and HRP are not found in the sequence of PoP. Multiple alignment of PoP with other peroxidases revealed that PoP is new type of peroxidase. The genomic DNA library (λgPo) was also constructed and the genomic clones matching to each class of cDNA clones were isolated and characterized. By the comparison of cDNA and genomic DNA, six intevening sequences which have consensus sequences were found. Expression of cDNA in *E. coli* resulted in the production of 52 kDa protein lacking PoP activity. Expression of cDNA in *S. cerevisiae* using pJC78 expression vector resulted in the production of proteins of various size. Recombinant PoP (rPoP) having PoP activity was purified and characterized. Enzyme activity of rPoP was 68% of the native PoP. Molecular mass of rPoP was 200 kDa, 75% of this was N-linked glycan. Heat stabitlity of rPoP was higher than that of the native PoP.

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Induction of Catalases in *Deinococcus radiophilus* Treated by Hydrogen Peroxide and UV

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A UV resistant bacterium, *Deinococcus radiophilus* ATCC 27603 is kwown to possess two of catalase-peroxidase and one of monofunctional catalase. Induction of two of catalase-peroxidase, designated catalase-2 and catalase-3 was investigated during growth phase and in bacterial cells treated by either H_2O_2 or UV. The total catalase activity of *D.radiophilus* varied during growth phases, substantial increase at stationary phase. The activity staining of catalases after polyacrylamide gel electrophoresis (PAGE) of the crude cell-free extracts obtained from each growth phase revealed the uniform appearance of catalase-2 regardless growth phases, and appearance of catalase-3 band from late expoential phase to stationary phase of cell growth. The cells treated with 10mM of H_2O_2 showed 3-4 fold increase of total catalase activity compared with the untreated cells. The activity staining of catalases followed by their densitometry on PAGE of the cell-free extract of the H_2O_2 treated cells showed 7-fold increase of catalase-3 activity with a constant level of catalase-2. A similar result was obtained with cells irradiated with UV (254 nm, 480J/cm^2). These results suggested that the catalase-2 is the constitutive and catalase-3 is an inducible enzyme and different physiological roles of the isocatalases in a UV resistant bacterium, *D.radiophilus.*