PE-51

Molecular cloning and characterization of catalase from disk abalone (*Haliotis discus discus*)

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Introduction

Antioxident enzymes play a major role in protecting organisms from the potentially deleterious effects of ROS. They contribute towards strengthening the defense mechanism in cells, and are studied in detail in several aquatic organism (Nakano *et al.* 1995). Catalase is an antioxidant enzyme in the cellular protection system and catalyzes the decomposition of hydrogen peroxide to oxygen and water (Thuy *et al.* 2004). Presence of catalase is important in the prevention of toxic wastes, which are harmful to cells.

In present study, cDNA sequence of catalase from disk abalone (*Haliotis discus discus*) was cloned, sequenced, expressed in *E. coli* and the expressed protein was examined for its activity. Also it was compared with other known sequences of catalase to identify conserved regions in order to establish its structure and functional relationships.

Materials and methods

A clone with expected function of catalase (aCAT) was selected from disk abalone cDNA library, and determined the full length sequence by three sequencing reactions. After deriving the full length, the sequence was compared against the National Center for Biotechnology Information (NCBI) databases.

The coding region with the signal peptide was amplified with two design primers and ligated into pMAL c2× (New England Biolabs, USA). The recombinant clone was transformed into *E. coli* BL21 (DE3) and produced recombinant protein by IPTG (Isopropyl-D-thiogalactopyranoside). Recombinant protein was analyzed using 12 % SDS polyacrylamide gel electrophoresis. To assesses enzyme activity and further

experiments expressed protein was purified using pMAL proteinTM fusion and purification system. Enzyme activity of aCAT was assayed by the method of Muller (1985). Optimal pH and temperature were also determined.

Results and summary

A gene coding for putative catalase of the disk abalone (*Haliotis discus discus*) was selected from the cDNA library and the full length was determined. The full length of aCAT contained 2,733 bp whilst the coding sequence was 1,503 bp. The recombinant aCAT was expressed soluble form and the molecular weight was showed 56 kDa. The specific activity of expressed aCAT was 30,000 U/mg towards hydrogen peroxide and was stable in a broad range of pH (5.0-10.5). The optimal temperature of aCAT showed at 37 ℃ and was inactivated when it was at 70℃ for 20 min. Based on the phylogenetic analysis, aCAT is most closely related with that of the pacific white shrimp among the known catalases from the animals.

References

Nakano, T., M. Wtanabe, M. Sato and M. Takeuchi. 1995. Characterization of catalase from the seaweed Prophyra yezoensis. Plant Sci. 104, 127-133.

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