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Molecular cloning, characterization and gene expression of an antioxidant enzyme catalase (*Mr*Cat) from *Macrobrachium rosenbergii*

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ABSTRACT

In this study, we reported a full length of catalase gene (designated as *Mr*Cat), identified from the transcriptome database of freshwater prawn *Macrobrachium rosenbergii*. The complete gene sequence of the *Mr*Cat is 2504 base pairs in length, and encodes 516 amino acids. The *Mr*Cat protein contains three domains such as catalase 1 (catalase proximal heme-ligand signature) at 350-358, catalase 2 (catalase proximal active site signature) at 60-76 and catalase 3 (catalase family profile) at 20-499. The mRNA expressions of *Mr*Cat in healthy and the infectious hypodermal and hematopoietic necrosis virus (IHHNV) challenged *M. rosenbergii* were examined using quantitative real time polymerase chain reaction (qRT-PCR). The *Mr*Cat is highly expressed in digestive tract and all the other tissues (walking leg, gills, muscle, hemocyte, hepatopancreas, pleopods, brain and eye stalk) of *M. rosenbergii* taken for analysis. The expression is strongly up-regulated in digestive tract after IHHNV challenge. To understand its biological activity, the recombinant *Mr*Cat gene was constructed and expressed in *Escherichia coli* BL21 (DE3). The recombinant *Mr*Cat existed in high thermal stability and broad spectrum of pH, which showed over 95% enzyme activity between pH 5 and 10.5, and was stable from 40 °C to 70 °C, and exhibited 85–100% enzyme activity from 30 °C to 40 °C.

1. Introduction

Antioxidant enzymes play a major role in protecting organisms from the potentially deleterious effects of oxidative stress and have been implicated in pathophysiological processes such as cancer and aging [1–3]. Oxidative stress causes damage to various organs, and it was recently reported that reactive oxygen species are involved in Alzheimer's disease, Parkinson's disease and amyotrophic lateral sclerosis [4]. Reactive oxygen is not only the cause of such diseases, but it is also used to signal processes such as apoptosis [5], life span determination [6], cell differentiation [7,8] and pathogen defense [9].

The physiological level of reactive oxygen species (ROS) is maintained by an antioxidant defense system. A major component of the antioxidant defense system consists of three types of primary antioxidant enzymes, including the superoxide dismutases (SODs), catalases, and peroxidases. The first line of defense against ROS includes the enzymatic activity of SOD, which catalyzes the disproportionation of superoxide to hydrogen Peroxide (H_2O_2) and water [10,11]. The second involves removal of hydrogen peroxide to water and oxygen, which, in most cells, is normally achieved by catalase and various peroxidases [12,13]. Catalase is a more significant H_2O_2 scavenger at a higher steady-state concentration [14,15].

Hydrogen peroxide, superoxides and hydroxyl radicals are formed unavoidably during aerobic metabolism. All aerobic organisms have enzymatic and non-enzymatic detoxification systems to combat reactive oxygen. Catalase is a key antioxidant enzyme present in virtually all aerobic organisms. Catalase is one of the most potent catalysts known and its function is crucial to life. Catalase catalyzes conversion of H_2O_2, a powerful and potentially harmful oxidizing agent to water and molecular oxygen. Catalase also uses H_2O_2 to oxidize toxins including phenols, formic acid, formaldehyde and alcohols [16]. Catalase has one of the highest turnover numbers of all enzymes; one catalase molecule can convert 40 million molecules of H_2O_2 to water and oxygen each second [17]. Catalases, superoxide dismutases and peroxidases have a central role in enzymatic detoxification [18].

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Catalases are the most important enzymes to degrade H_2O_2, and they are classified into three separate families: Mn-catalases [19], catalase-peroxidases and mono-functional catalases. The mono-functional catalases are the best characterized, and they are homo tetrameric and heme-containing enzymes. As catalases are found in organisms from eubacteria to eukaryotes [9,20–26], they are essential, strongly expressed, and tightly regulated [27]. Catalase comprises four ferriphotophorphyrin groups per molecule, and its enzymatic activity in tissues varies greatly [28,29]. Each monomer harbors a single heme and nicotinamide adenine dinucleotide phosphate (NADPH). The NADPH is bound on the surface of each monomer by 12 amino acid residues [30] and protects the enzyme from oxidation by its H_2O_2 substrate. However, phagocytosis increases the consumption of oxygen and induces the production of ROS [31].

Catalase is a very highly conserved enzyme that has been identified from numerous species including bacteria, fungi, plants and animals. This enzyme is ubiquitous and present in archaea [22], prokaryotes and eukaryotes [32–35]. To date, much information about the structure and regulation of catalase genes and proteins has been accumulated in mammals [36,37], plants [38] and bacteria [39]. Antioxidant related enzymes, including catalase, are known to be involved in crustaceans' innate immune reaction [40–43]. It is reported [41,44] that white spot syndrome virus (WSSV) infection decreased the activity of antioxidant enzymes including catalase in *Fenneropenaeus indicus* and also [42] that the activity of catalase changed in *Penaeus monodon* after WSSV infection. However, the genetic information about catalase in freshwater giant prawn *Macrobrachium rosenbergii* is very limited.

In our earlier findings, we reported [45,46] that freshwater giant prawn *M. rosenbergii* industry is affected all over the World due to various viral and bacterial pathogens. However, infectious diseases mainly, infectious hypodermal and hematopoietic necrosis virus (IHHNV) have affected the *M. rosenbergii* industry enormously. Thus, research into freshwater prawn defense mechanisms is important to develop disease control strategies, but the detailed functions and characterization of immune genes in *M. rosenbergii* are poorly understood. Since the antioxidant related enzymes, including catalase, are known to be involved in crustaceans' innate immune reaction, we obtained a full-length antioxidant enzyme, catalase gene from the constructed *M. rosenbergii* transcriptome unigenes by Illumina's Solexa sequencing technology. In this study we characterized full-length catalase gene from *M. rosenbergii* (designated as *Mr*Cat), at molecular level and investigated the related mRNA expression profile after IHHNV infection and in addition to the functional activities of purified recombinant *Mr*Cat. The results of this article will assist subsequent research on the adaptive responses of *M. rosenbergii* to conditions of oxidative stress and environmental toxicity.

2. Materials and methods

2.1. M. rosenbergii

Healthy prawns (average body weight 10 g) were obtained from the Bandar Sri Sendayan, Negeri Sembilan, Malaysia. Prawns were maintained in flat-bottomed glass tanks (300 L) with aerated and filtered freshwater at 28 ± 1 °C in the laboratory. All prawns were acclimatized for 1 week before challenge to IHHNV. A maximum of 15 prawns per tank were maintained during the experiment.

2.2. Identification of full-length MrCat

A full-length *Mr*Cat gene was identified from the *M. rosenbergii* transcriptome unigenes obtained by Illumina's Solexa sequencing technology. Briefly, unigenes obtained from the assembly of the Illumina Solexa short reads from the RNA sequencing of the muscle, gill and hepatopancreas transcriptomes of *M. rosenbergii* were mined for sequences which had been identified as catalase gene through BLAST homology search against the NCBI database (http://blast.ncbi.nlm.nih.gov/Blast).

2.3. Bioinformatic analysis

The full-length *Mr*Cat sequence was compared with other sequences available in NCBI database and the similarities were analyzed. The open reading frame (ORF) and amino acid sequence of *Mr*Cat was obtained by using DNAssist 2.2. Characteristic domains or motifs were identified using the PROSITE profile database [47]. Identity, similarity and gap percentages were calculated using FASTA program [48]. The N-terminal transmembrane sequence was determined by DAS transmembrane prediction program (http://www.sbc.su.se/~miklos/DAS). Signal peptide analysis was done using the SignalP worldwide P server (http://www.cbs.dtu.dk). Pair-wise and multiple sequence alignment were analyzed using the ClustalW version 2 program [49]. The phylogenetic relationship of *Mr*Cat was determined using the Neighbor-Joining (NJ) Method and PHYLIP (3.69). The presumed tertiary structures were established for *Mr*Cat [50] using the SWISS-MODEL prediction algorithm (http://swissmodel.expasy.org/).

2.4. Gene expression analysis of MrCat mRNA after IHHNV infection

For IHHNV induced mRNA expression analysis, the prawns were injected with IHHNV, as described by Dhar et al. [51]. Briefly, IHHNV infected prawn tail tissue, tested positive by nested PCR was homogenized in sterile 2% NaCl (1:10, w/v) solution and centrifuged in a tabletop centrifuge at 5000 rpm for 5 min at 4 °C. The supernatant was filtered through 0.45 μm filter and used for injecting (100 μl per 10 g prawn) the animals. Samples were collected before (0 h), and after injection (3, 6, 12, 24 and 48 h) and were immediately snap-frozen in liquid nitrogen and stored at −80 °C until the total RNA was isolated. Using a sterilized syringe, the hemolymph (0.2–0.5 mL per prawn) was collected from the prawn heart and immediately centrifuged at 3000× *g* for 10 min at 4 °C to allow hemocyte collection for total RNA extraction. Tissue homogenate prepared from healthy tail muscle served as control. All samples were analyzed in three duplications and the results are expressed as relative fold of one sample as mean ± standard deviation.

2.5. Total RNA isolation and cDNA conversion

Total RNA was isolated from the tissues of each animal using TRI Reagent following manufacturer's protocol (Guangzhou

Table 1
Details of primers used in this study.

Name	Target	Sequence (5′-3′ direction)
*Mr*Cat (F1)	qRT-PCR amplification	ACTACAACCAGGAAAGTGCTCCCA
*Mr*Cat (R2)	qRT-PCR amplification	TGGCGTTCCTCTTCGTTCATGACT
β-actin (F3)	qRT-PCR internal control	ACCACCGAAATTGCTCCATCCTCT
β-actin (R4)	qRT-PCR internal control	ACGGTCACTTGTTCACCATCGGCATT
*Mr*Cat (F5)	ORF amplification	GAGAGAgaattcTCAGAAGAGGAACCC AGCAACACA *EcoRI*
*Mr*Cat (R6)	ORF amplification	GAGAGActgcag ATGGCGATGGGTGTC ATTGTAGGA *PstI*