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Identification and characteristic analysis of the catalase gene from *Locusta migratoria*

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ABSTRACT

Catalase (CAT) is a ubiquitous antioxidant enzyme in almost all living organisms exposed to atmosphere, which involved in decomposing harmful hydrogen peroxide, into oxygen and water. In this study, a full-length cDNA (1524 bp) encoding the catalase gene (*LmCAT*) from *Locusta migratoria* was cloned (accession number KT716445). The open reading frame of the LmCAT gene encoded 507 amino acids and shared 57.8%–97.8% amino acid identities with other insect CATs. The coding region was interrupted by 9 introns, while its promoter region contained 15 putative binding sites for 5 kinds of transcriptional regulation factors. For the stage-specific expression profile, LmCAT was highly expressed in the fourth-instar nymphs. For the tissue-specific expression profile, the LmCAT transcripts were highest in the fat bodies, and relatively abundant in the gastric caecum, Malpighian tubules, ovary and integument. Moreover, the result showed that quercetin could significantly induce the expression level of LmCAT. The expression of LmCAT could be silenced by RNAi, but the moralities were not significantly different between control and RNAi groups. Our results would provide valuable information for further study on the ROS regulation mechanism in insect.

1. Introduction

The oriental migratory locust (*Locusta migratoria*), which widely distributed in the East and South Asia and the Pacific Region, is one of the most important agricultural pest [1]. From ancient to nowadays, the plagues of locusts often caused severe food shortage and considerable economic loss to agricultural production. As a typical herbivorous pest, the *L. migratoria* is constantly challenged with the reactive oxygen species (ROS) (including superoxide ion ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2), and hydroxyl radical ($OH^{\bullet}$) et al.), which can result in the oxidative stress. The sources of the oxidative stress can be divided into 2 classes: exogenous and endogenous. The exogenous sources include pesticides, plant secondary metabolites, heavy metals and environment stress, whereas aerobic respiration, intracellular redox reactions, cell signal transduction and immune response are important endogenous sources [2,3]. ROS have been identified to play roles in immune defense, antibacterial action, vascular tone, and signal transduction et al. [3]. However, excessive ROS can result in cellular damage, oxidative stress, and lipids, proteins, and DNA damage [3]. Fortunately, animals have evolved complex enzymatic and non-enzymatic systems to overcome the oxidative stress. The enzymes system is mainly composed of superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT). The SOD catalyzes the dismutation of $O_2^{\bullet-}$ into O_2 and H_2O_2, while H_2O_2 can be further quenched by GPx and CAT [4].

Due to H_2O_2 reduction-enzyme, the selenium-dependent GPx, is absent in insects, the CATs were consider to be the key scavenger of H_2O_2 in insect, which are response for many important physiological functions [4]. The CAT from *Anopheles gambiae* can regulate the levels of ROS, and can influence the mosquito fecundity and modulate its immunity against bacteria and plasmodium [5]. For the female *Lutzomyia longipalpis*, knockdown of its CAT gene could cause a significant increase of mortality and a reduction in the number of developing oocytes produced after blood feeding [6]. The silence of the CAT gene from *Spodoptera litura* resulted in cell cycle arrest in the SL-1 cells and lower survival rate [7]. As the important roles in maintaining ROS balance of insect, the insect CATs were thought to be a candidate target of insecticide and plant secondary metabolites [7]. It is reported that the LmCAT is play an important role in protecting tissues from the oxidative stress due to altered the metabolic activity during the cellular cryoprotection under cold stress [8]. However, until now it is lack of systematic research on the characterization of LmCAT gene from incomplete metamorphosis insects and that results in a restriction of related research.

In this study, full-length LmCAT gene was cloned and its promoter region sequence was analyzed from the genome fragment of *L. migratoria*, in order to thorough understanding the physiological functions of LmCAT. The RT-qPCR was applied to determine the development and tissue-specific distribution of LmCAT and to analyze the induction of LmCAT by plant secondary metabolites. In addition, we used RNAi method to silence the LmCAT gene, in order to further investigate the consequences of the LmCAT gene knockdown *in vivo*.

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2. Material and methods

2.1. Reagents and assay kits

The RevertAid H minus reverse transcriptase was purchased from Fermentas (MA, USA). The SYBR Green Realtime PCR Master Mix was purchased from Toyobo (Osaka, Japan). The Fastpfu DNA polymerase and pEASY-Blunt Zero cloning kit were obtained from Transgen Biotech Co. Ltd. (Beijing, China). The gel extraction kit, TRIzol and dNTP were obtained from TaKaRa Bio Group (Dalian, China). The T7 RiboMAx Express RNAi System was from Promega Corporation (WI, USA). All other reagents used were of the highest grade commercially available.

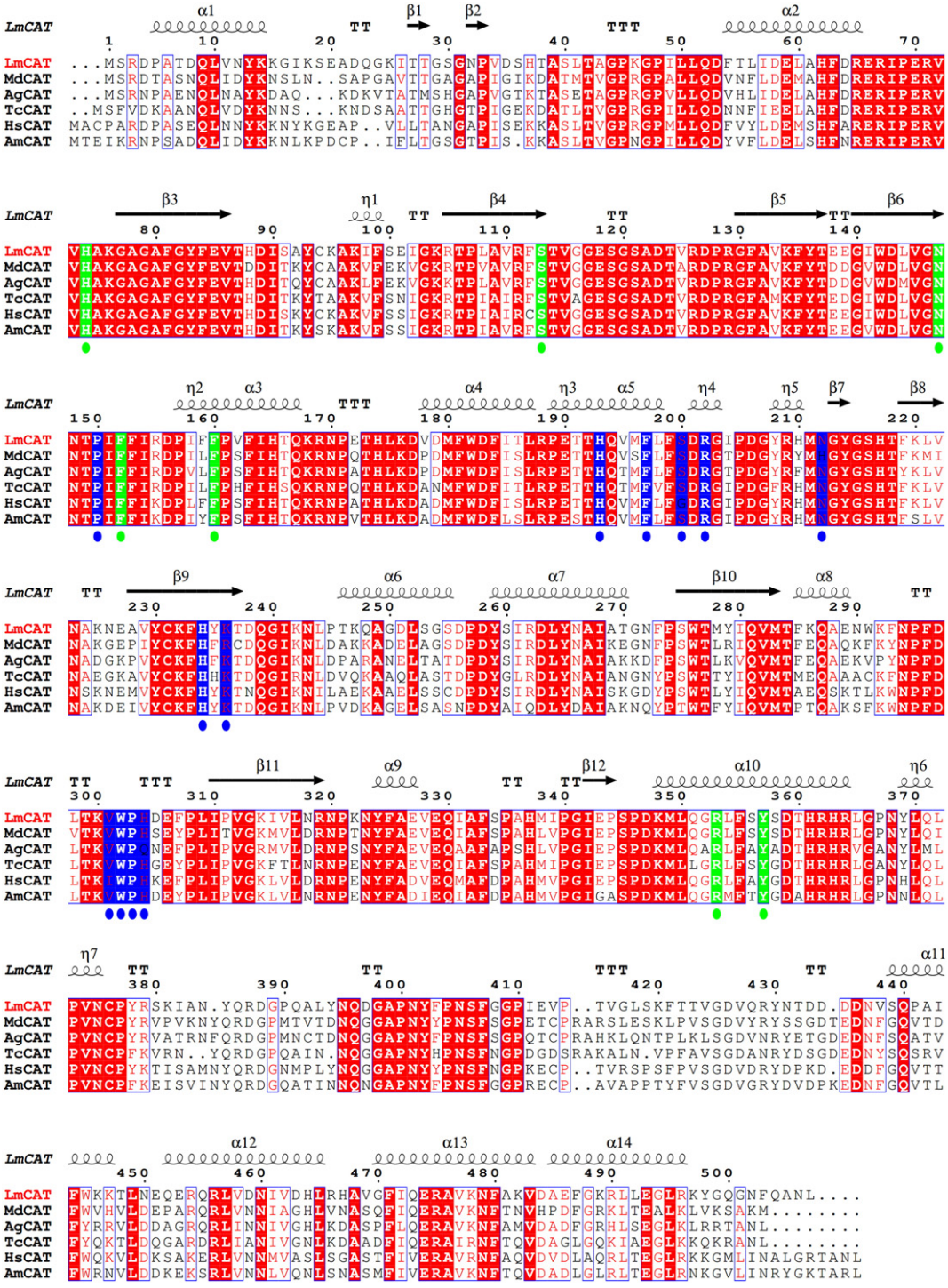

Fig. 1. Multiple sequence alignment of representative insect CATs. The aligned sequences are CAT from *L. migratoria* (LmCAT, accession number KT716445), CAT from *Musca domestica* (MdCAT, accession number XP_005180638.1), CAT from *Anopheles gambiae* (AgCAT, accession number ABL09376.1), CAT from *Tribolium castaneum* (TcCAT, accession number NP_001153712.1), CAT from *Harpegnathos saltator* (HsCAT, accession number XP_011148272.1), and CAT from *Apis mellifera* (AmCAT, accession number NP_001171540.1). The predicted secondary structure elements of LmCAT are presented on top (helices with squiggles, β-strands with arrows and turns with TT letters). The amino acids of Heme and NADPH binding sites are colored with green and blue, respectively.