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The cDNA cloning and mRNA expression of cytoplasmic Cu, Zn superoxide dismutase (SOD) gene in scallop *Chlamys farreri*

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Abstract

Cu, Zn superoxide dismutases (SODs) are metalloenzymes that represent one important line of defence against reactive oxygen species (ROS). A cytoplasmic Cu, Zn SOD cDNA sequence was cloned from scallop *Chlamys farreri* by the homology-based cloning technique. The full-length cDNA of scallop cytoplasmic Cu, Zn SOD (designated CfSOD) was 1022 bp with a 459 bp open reading frame encoding a polypeptide of 153 amino acids. The predicted amino acid sequence of CfSOD shared high identity with cytoplasmic Cu, Zn SOD in molluscs, insects, mammals and other animals, such as cytoplasmic Cu, Zn SOD in oyster *Crassostrea gigas* (CAD42722), mosquito *Aedes aegypti* (ABF18094), and cow *Bos taurus* (XP_584414). A quantitative reverse transcriptase real-time PCR (qRT-PCR) assay was developed to assess the mRNA expression of CfSOD in different tissues and the temporal expression of CfSOD in scallop challenged with *Listonella anguillarum*, *Micrococcus luteus* and *Candida lipolytica* respectively. Higher-level mRNA expression of CfSOD was detected in the tissues of haemocytes, gill filaments and kidney. The expression of CfSOD dropped in the first 8–16 h and then recovered after challenge with *L. anguillarum* and *M. luteus*, but no change was induced by the *C. lipolytica* challenge. The results indicated that CfSOD was a constitutive and inducible acute-phase protein, and could play an important role in the immune responses against *L. anguillarum* and *M. luteus* infection.

Keywords: Scallop; *Chlamys farreri*; Reactive oxygen species (ROS); Superoxide dismutase (SOD); Innate immunity; mRNA expression

1. Introduction

Reactive oxygen species (ROS) such as hydrogen peroxide (H_2O_2) and superoxide anion ($O_2^{\bullet -}$) are constantly generated in all aerobic biological systems. This toxic by-product is responsible for damage to many cell components such as lipids, proteins, and nucleic acids. Organisms have well-developed defence systems against ROS, involving both limiting the formation of ROS as well as instituting its removal. The superoxide dismutases (SOD, superoxide

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oxidoreductase, EC 1.15.1.1) are metalloenzymes that represent one important line of defence against ROS. SOD removes O_2^-, prevents generation of highly toxic OH^-, and catalyses the dismutation of superoxide radical into molecular oxygen and hydrogen peroxide [1]. SOD is ubiquitous and can be found in virtually all oxygen-consuming organisms, aero-tolerant anaerobes, and some obligate anaerobes [2,3]. According to the metal ion cofactor identified in their active site, SODs are classified into three types, Cu, Zn SOD, Mn SOD and Fe SOD.

Cu, Zn SOD is an important type of SOD because of its physiological function and therapeutic potential. This enzyme requires Cu and Zn for its biological activity, and the loss of Cu results in its complete inactivation, and induces many diseases in human and animals [4–8]. There are two types of Cu, Zn SOD, extracellular Cu, Zn SOD with an N-terminal signal cleavage peptide for secretion, and cytoplasmic Cu, Zn SOD without signal peptide [9–12].

Cu, Zn SOD has been found to be expressed in various tissues of animals. In mammals, Cu, Zn SODs are prominent in lung, kidney [9], pancreas [13] and placenta [14]. The distributions of Cu, Zn SODs are related to their various functions. It has been proved that SODs are associated with many acute and chronic pathologies in human, such as ischaemia–reperfusion injury [15] and vascular damage in patients with diabetes mellitus [16], and it can bind peroxinectin and β-1,3-glucan-binding protein in crayfish *Pacifastacus leniusculus* [17]. There are many biological stimulators that can regulate the expression of SOD, such as heat shock [18,19], shear stress [20,21] and heavy metals [22]. In *Bombus ignites*, cytoplasmic Cu, Zn SOD expression was found to be up-regulated after LPS injection [23]. The cytoplasmic Cu, Zn SOD expression in haemocytes of giant freshwater prawn *Macrobrachium rosenbergii* was up-regulated and then recovered after injection with *Lactococcus garvieae*; but in hepatopancreas, the cytoplasmic Cu, Zn SOD expression first decreased and then recovered [24]. A significant decrease in SOD activity occurred when shrimp *Palaemonetes argentinus* was infected with *Probopyrus ringueleti* [25]. The mechanisms by which biological stimulators that either stimulate or suppress Cu, Zn SOD have been intensively investigated in mammals [26–33], but not well studied in invertebrates [23,34,35].

Zhikong scallop *Chlamys farreri* is a commercially important cultured species along the coast of northern China, Korea, Japan, and eastern Russia. The scallop industry has grown rapidly over the last 20 years and has become one of the mainstay mariculture industries in China. After having flourished for several years, the Zhikong scallop culture in China is now suffering from the problem of mortality. Understanding the immunity of scallop may contribute to the development of strategies for the management of diseases and for long-term sustainability of the scallop culture.

Molluscs lack an acquired immune system and their defence mechanisms mainly rely on innate immune responses consisting of both humoral immune responses that employ constitutive and inducible antimicrobial peptides to lyse invading microorganisms [36,37], and cellular responses mediated by haemocytes which can generate ROS through the respiratory burst. Our previous study revealed that the malondialdehyde (MDA) content, an index of lipid peroxidation, in scallop stimulated by *Listonella anguillarum*, *Micrococcus lueus* and *Candida lipolytica* were different, indicating that the microorganisms disrupted the balance of oxidation and reduction in scallop. However, the expression of CfSOD in scallop after challenge with the microorganisms is still not clear. Therefore, the aims of the present study were: (1) to determine the nucleotide sequence of CfSOD from the scallop *C. farreri*; (2) to examine the expression of CfSOD in various tissues; and (3) to evaluate CfSOD expression after the scallop were challenged by the microorganisms *L. anguillarum*, *M. luteus* and *C. lipolytica*.

2. Materials and methods

2.1. Animals, immune challenge and haemolyph collection

Zhikong scallops *C. farreri* (averaging 60 mm in shell length) were collected from a commercial farm, and cultured in filtered seawater at 15 °C for 10 days before proceeding. The seawater was changed 100% daily and the scallops were fed on 0.5% *Isochrysis galbana* twice per day. The tissues, including haemocytes, gonad, kidney, adductor muscle and gill filaments were collected from healthy individuals to investigate the tissue-specificity expression of CfSOD.

The microorganisms were grown separately overnight at 20–25 °C in saline peptone water (peptone 15 g/l; NaCl 15 g/l) for *L. anguillarum* (kindly provided by Prof. Zhaolan Mo), or at 30 °C in Luria–Bertani medium for *M. luteus* (purchased from Microbial Culture Collection Center, Beijing, China), and 2 days at 28 °C in YPD medium for yeast *C. lipolytica* (purchased from Microbial Culture Collection Center, Guangdong, China).