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Original article

Differential expression of catalases in *Vibrio parahaemolyticus* under various stress conditions

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Abstract

Among antioxidant enzymes, catalases protect microorganisms by degrading hydrogen peroxide under oxidative stress. In this study, the activities of at least four *Vibrio parahaemolyticus* catalases (Kat1 to Kat4) were differentially detected during different growth stages and under various stress conditions using zymographic analysis. Our results showed that only Kat2 is stable at 55 °C. Kat1 and Kat2 respond to hydrogen peroxide during the early stationary and exponential growth phases, respectively and the response decreases upon entering the stationary phase. Kat3 and Kat4 are bifunctional, exhibiting both catalase and peroxidase activities and are only expressed during the stationary phase, under starvation or under stress at pH 5.5. Our study also shows that expression of Kat3 and Kat4 depends on RpoS. We confirm that both monofunctional and bifunctional catalases are expressed and function differentially under various stresses to contribute total catalase activities for the survival of *V. parahaemolyticus*. A comparative genomic study among *Vibrio* species revealed that only *V. parahaemolyticus* contains two copies of genes that encode monofunctional and bifunctional catalases. We propose that both types of catalases, whether evolved or acquired horizontally through long-term evolution, may play crucial protective roles in *V. parahaemolyticus* in response to environmental fluctuations.

Keywords: *Vibrio parahaemolyticus*; Catalase activity; Catalase expression; Growth phase; Stress conditions

1. Introduction

Catalases are present in most living organisms and play critical roles in defending oxidative stresses, which reflect an imbalance in the redox status of biological systems. On the basis of enzymatic properties and amino acid sequences, catalases are categorized into three groups: monofunctional, bifunctional and non-heme catalases [1]. Monofunctional catalases are spread extensively in eukaryotes and prokaryotes and mainly exhibit catalase activity. This group is subdivided on the basis of subunit size into small (molecular mass 55–69 kDa) and large subunits (75–84 kDa) [1]. The active forms of these catalases are generally tetrameric, with heme *b* and *d* prosthetic groups present in small and large subunit enzymes, respectively [1,2]. Moreover, bifunctional catalases, which are found in bacteria, archaea, protists and fungi, exhibit both catalase and peroxidase activities. The active forms are typically dimeric and tetrameric (approximately 80 kDa), with the heme *b* prosthetic group. The deduced amino acid sequence of bifunctional catalases does not resemble that of monofunctional catalase, but is similar to that

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of plant peroxidase [1,2]. Compared with these two catalase groups, non-heme catalases are not widespread and are only found in lactic acid and thermophilic bacteria. Moreover, manganese ions are used as cofactors instead of hemes; thus, this catalase group is referred to as manganese catalase. These enzymes are usually hexameric, with the molecular mass of the subunits ranging from 28 to 35 kDa [2].

Catalases are scavenging enzymes that degrade hydrogen peroxide (H_2O_2) into water and oxygen. Hydrogen peroxide, a reactive oxygen species (ROS), is acutely toxic and damages almost all cell components. Thus, catalase is part of the cellular antioxidant machinery. Bacteria encounter endogenous and exogenous H_2O_2 under aerobic metabolism and environmental fluctuations, respectively [3]. Therefore, most bacteria possess one or more catalase to appropriately respond to oxidative stress caused by H_2O_2. For example, *Escherichia coli* has two types of heme-containing catalases, hydroperoxidase I (HPI) and HPII. HPI, encoded by *katG*, is a bifunctional catalase located at the periplasm and is induced during exponential growth in response to sublethal H_2O_2 doses [4]. This induction is dependent on OxyR, which functions as both an H_2O_2 sensor and a transcriptional activator [5]. HPII, encoded by *katE*, is a monofunctional catalase located in the cytoplasm. It is not induced by H_2O_2 and is expressed when *E. coli* enters the stationary phase. The expression is regulated positively by RpoS, which is not only the sigma factor of the stationary phase, but also a general stress response regulator [4,5]. As mentioned earlier, KatG and KatE expression can be independently regulated in *E. coli*. However, the increase in KatG activity during the transition into stationary phase is acetate-mediated and RpoS-dependent [6]. In *katE* mutants, KatG was shown to be induced in an RpoS- and OxyR-independent manner in the stationary phase, demonstrating that an additional pathway for KatG induction is involved in protecting *E. coli* in the absence of KatE during stationary growth under increased oxidative stress [7]. Although catalase is not necessary for *E. coli* cell growth under conventional laboratory conditions [1], the critical role of catalases in several bacteria under stress has been reported [8–16].

Vibrio parahaemolyticus is a curved, rod-shaped, Gram-negative bacterium frequently found in coastal estuarine and marine environments. In aquatic habitats, it is often associated with plankton, zooplankton and other marine organisms such as crustaceans, molluscs and fish [17]. It also causes human gastroenteritis when ingested from contaminated undercooked seafood or shellfish. In many Asian countries, including Taiwan and Japan, and in the United States, *V. parahaemolyticus* is the leading cause of seafood-associated bacterial gastroenteritis; therefore, it is of vital medical importance [18]. Because of its association with such distinct life forms, ROS-induced oxidative stress is a common condition that *V. parahaemolyticus* encounters. In marine environments, the photochemical process (absorption of solar radiation by dissolved organic matter in seawater) generates diverse reactive transients including ROS [19]. Among these ROS, H_2O_2 has the longest lifetime in seawater and the highest steady-state concentration (10^{-7} M) [19]. In addition, the innate immune system of the host also produces ROS to defend against the invading pathogens. Therefore, many pathogens exhibit antioxidant enzymes that detoxify ROS. In this study, the catalase zymogram, a sensitive and convenient staining technique for examining patterns of catalase activity in native polyacryalmide gel, was used [21]. The catalases in *V. parahaemolyticus* were characterized in terms of their enzymatic activity and features under different growth stages and various stress conditions. We also analyzed the genome sequence of *V. parahaemolyticus* [20] for clarifying the possible candidates of *kat* coding genes. Genome comparison among sequenced *Vibrio* species revealed the presence of multiple genes encoding catalases and other antioxidant systems. Differing from the other human *Vibrio* pathogens *V. cholerae* and *V. vulnificus*, only *V. parahaemolyticus* contains two copies of *katG* and *katE* genes; we discuss the evolutionary history of *kat* genes on the basis of a phylogenetic analysis.

2. Materials and methods

2.1. Bacterial strains

V. parahaemolyticus 93 is a clinical isolate with a weak Kanagawa phenomenon phenotype [22]. An *rpoS*-deficient mutant, Vp93ΔrpoS, was derived from *V. parahaemolyticus* 93 by removing a 478-bp region internal to the *rpoS* gene (unpublished). To construct the *rpoS* complemented strain, Vp93ΔrpoS/rpoS, the fragment containing the open reading frame of *rpoS* and its putative promoter region, predicted using the BPROM bioinformatics tool (http://linux1.softberry.com/berry.phtml), was amplified using primer pairs rpoScom-F (5′-CTGGTTAGGATGGGAAACGA-3′) and rpoScom-R (5′-GCCCTTGAAGAGCTGTATCG-3′) and cloned into a pGEM-T easy vector (Promega, Madison, WI, USA). The fragment was subsequently cut from the vector through digestion with *Sph*I and *Kpn*I and ligated into shuttle vector pVSV105 [23]; the recombinant plasmid was introduced into *E. coli* SM10 λpir. The resulting plasmid was transformed into Vp93ΔrpoS through conjugation.

2.2. Culture conditions

V. parahaemolyticus strains were routinely cultured at 37 °C in tryptic soy broth (TSB, Difco) containing 3% NaCl (TSB3S) with shaking, or on tryptic soy agar (TSA) containing 3% NaCl (TSA3S). *E. coli* SM10 λpir was cultivated in Luria-Bertani (LB) medium. For maintaining the shuttle vector in *V. parahaemolyticus* and *E. coli* strains, 6 and 20 μg/mL of chloramphenicol were added to the culture media, respectively. To study stress responses, bacterial cells were cultured to the mid-exponential phase and harvested using a centrifuge. They were then suspended in TSB3S containing 0.1 mM hydrogen peroxide and TSB3S medium acidified to pH 5 by using 1 N HCl and incubated at 37 °C for 30 min for inducing oxidative and acid stress, respectively.