## The regulatory role of CgALDH6A1 in the oxidative stress response of Crassostrea gigas under high-temperature stress

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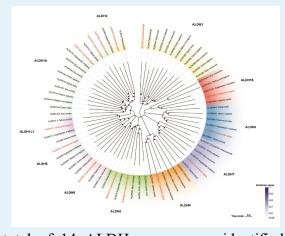
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Abstract: High temperatures induce oxidative stress and the production of a large amount of MDA in the oysters, and even lead to mass mortalities. ALDH degrades MDA and has attracted increasing attention for its role in enhancing antioxidant defense capacity. This study identified 14 ALDH family members in the oyster genome. Among them, CgALDH6A1 harbored a conserved ALDH\_F6\_MMSDH domain and was likely involved in high-temperature stress response through the detoxification of accumulated toxic aldehydes. During the outdoor aquaculture period, the mRNA transcripts of CgALDH6A1 exhibited a significant increase from June to October. Following injection of CgALDH6A1 siRNA, the MDA content increased significantly (1.31-fold), while the activities of SOD (0.93-fold) and CAT (0.45-fold), and T-AOC (0.54-fold) decreased significantly under high-temperature stress. Meanwhile, the gill tissue was observed to be disorganized with obvious filament swelling. Following injection of CgALDH6A1 agonist (Alda-1), the MDA content (0.59-fold) decreased significantly, while the activities of SOD (1.33-fold) and CAT (1.81-fold), as well as T-AOC (1.79-fold) all increased significantly after high-temperature stress. However, no obvious morphological change was observed in the gill. These results demonstrated that CgALDH6A1 played a key role in regulating the oxidative stress response by degrading MDA under high-temperature stress, and occupied a cooperative role with the antioxidant system inalleviating oxidative stress under high-temperature stress.

**Keywords:** CgALDH6A1; MDA; high-temperature stress; Pacific oyster Crassostrea gigas

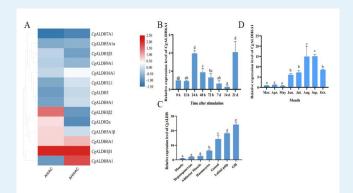
## **Results**

Fig. 1 Phylogenetic tree of ALDH family genes.



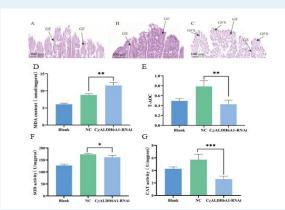
A total of 14 ALDH genes were identified in the *Crassostrea gigas* genome and classified into ten subfamilies. Phylogenetic analysis confirmed the evolutionary conservation and subfamily clustering of thes genes, supported by high bootstrap values.

Fig. 2 The expression profiles of  $\it CgALDHs$  in tissues and under high temperature stress



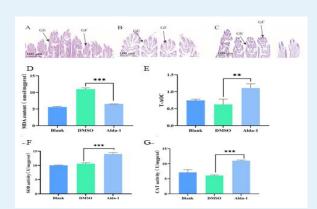
Transcriptome profiling and the expression profiles of *CgALDHs* demonstrated that *CgALDH6* responds to high-temperature stress. *CgALDH6A1* genes were highly expressed in gills, indicating potential roles in environmental sensing and physiological regulation and during the outdoor aquaculture period, the mRNA transcripts of *CgALDH6A1* exhibited a significant increase from June to October.

Fig. 3 The changes of oxidative stress response and histopathological changes in CgALDH6A1-RNAi oysters



Following injection of *Cg*ALDH6A1 siRNA, the MDA content increased significantly, while the activities of SOD and CAT, and T-AOC decreased significantly under high-temperature stress. Meanwhile, the gill tissue was observed to be disorganized with obvious filament swelling. This indicates that inhibiting the expression of *Cg*ALDH6A1 affects the oxidative stress response of *Crassostrea gigas*.

Fig. 4 Changes in the oxidative stress response after the injection of Alda-1 in vivo



Following injection of CgALDH6A1 agonist (Alda-1), the MDA content decreased significantly, while the activities of SOD and CAT, as well as T-AOC all increased significantly after high-temperature stress. However, no obvious morphological change was observed in the gill. These results demonstrated that CgALDH6A1 played a key role in regulating the oxidative stress response by degrading MDA under high-temperature stress.