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Dipartimento di Scienze Fisiche, della Terra e dell'Ambiente

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Molecular bases and evolutionary constraints of
cellular acid tolerance in cyanobacteria and
invertebrates subjected to ocean acidification and
other sources of stress (METASTRESS)

Settore scientifico disciplinare: BIO/07

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Abstract

The ongoing decreasing surface pH that seas and oceans are facing is termed ocean acidification (OA). The primary reason for this phenomenon is the emission of carbon dioxide (CO₂) into the atmosphere from human activities, like the burning of fossil fuels, which has drastically increased since the industrial revolution, reaching higher levels during these decades. This environmental risk is considered among the most hazardous threats to marine ecosystems associated with global change and severely affects marine life worldwide. Responses of marine species to acidified seawaters have been deeply studied and adverse effects at different levels, from single species up to whole communities, have been pointed out. Although OA is clearly posing a threat to marine life, some species have demonstrated the ability to tolerate and thrive in such conditions. Information on the mechanisms driving the tolerance of adapted species to decrease seawater pH is limited, and new knowledge may be obtained from species inhabiting sites with naturally low pH, such as the volcanic CO₂ system off the Castello Aragonese on the Ischia Island (Italy). Understanding the molecular mechanisms of adaptation enabling marine species to tolerate a lowered seawater pH could support predictions of the consequences of future OA scenarios for marine life. Growing evidence of the involvement of ABC transport proteins in resistance towards acid stress in bacteria and tumor cell lines has been demonstrated. Researchers have suggested that the tolerance to this kind of stress is due to the transport of substances that contribute to the maintenance of internal cell homeostasis carried out by the ABC proteins. Here, we aimed at elucidating the involvement of ABC transport proteins in tolerance to low-pH/high-pCO₂ environments, by investigating their gene regulation, in species of marine microorganisms and metazoans considered tolerant to acidified environments. *Halomiconema metazoicum* is a marine filamentous cyanobacterium able to cope with hostile conditions and discovered in association with *Posidonia oceanica* leaves also in areas characterized by low pH. Mattes of this cyanobacterium were exposed in short- and long-term exposure experiments (7 and 30 d) to low seawater pH conditions (7.7, 7.2, 6.5), and the regulation of the ABC-like gene *slr2019* was assessed. At day 7, *slr2019* was up-regulated at pH 7.7 while no changes were observed at lower pH, compared to the controls (pH 8.2). However, after 30 d of exposure, a significant decrease in *slr2019* transcript level was observed in all treatments.

Furthermore, unchanged photosynthetic pigment content indicated that the species can tolerate all three lowered pH conditions. *Platynereis dumerilii* is a marine annelid extensively used as a model in genetics, single-cell genomics, and ecotoxicology. It and its sibling, *P. cfr massiliensis*, were abundant in naturally acidified areas, such as the CO₂ vents off Castello Aragonese. They are adapted to low-pH conditions, making them excellent models for studying acclimation and adaptation mechanisms to OA. Since its genome is completely sequenced, an attempt to identify ABC transport proteins in *P. dumerilii* has been made with the aim to investigate whether these proteins contribute to adaptation/tolerance to low-pH/high-pCO₂ seawater conditions in the two sibling species *P. dumerilii/cfr massiliensis* before and after 30 d of *in-situ* transplant experiment. A total of 81 ABCs were found in *P. dumerilii*, belonging to seven distinct subfamilies (A–G) based on phylogenetic analysis. Furthermore, the distribution within the tissues of a subset of 40 ABC transport proteins from the subfamilies B, C and G was determined. Most of the analyzed ABCs were expressed in the annelid midgut but also found in the other organs and tissues: neurons, body epidermis and ectodermal cells, and somatic and visceral muscle. Based on these findings, we evaluated the expression profile of 7 genes belonging to the ABCB (ABCB_1, ABCB_2, ABCB_3), ABCC (ABCC_1, ABCC_2, ABCC_3) and ABCG (ABCG) subfamilies following a 30-d *in-situ* reciprocal transplant of specimens inhabiting normal-pH marine site (8.18 ± 0.005) and those living in naturally low-pH areas off Castello Aragonese (7.33 ± 0.312). Hypotheses on the involvement of each studied ABC gene in adaptation to low-pH were made based on the basal expression gene level of organisms inhabiting different pH environments and verified through the ABC transcription results of the *in-situ* transplant experiment. Three out of the 7 genes were confirmed to be involved in low-pH resistance. ABCB_1 and ABCG are up-regulated in worms living in low-pH sites compared to those from normal-pH areas while ABCB_3 were lower expressed. The results of transplant experiments supported these findings. ABCB_1 and ABCG gene expression increased in samples transplanted from normal-pH areas to acidified sites while decreased in the reverse transplant. On the contrary, ABCB_3 gene was down-regulated in organisms transferred from normal- to low-pH zones and was up-regulated in specimens subjected to the reverse transplantation. Taken together, our results demonstrate the capacity of the studied species to deal with low-pH environments and confirm previous findings on the involvement of ABC transporters in acid stress adaptation in marine organisms.

These proteins were found to play a role in maintaining cellular homeostasis, buffering the internal cell pH and preventing cell death to guarantee the proper functioning of cellular activities. So, based on our findings, these functions may prevent negative effects following exposure to acidified conditions.

This work will constitute a basis for further research on the physiological functions of ABCs behind the tolerance to low-pH/high- $p\text{CO}_2$ environments to better understand how marine species can cope with OA and to provide vital information on the impact of climate change on marine biodiversity.