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SCIENCE DIRECT
Marine Environmental Research 62 (2006) S118–S121
MARINE ENVIRONMENTAL RESEARCH
www.elsevier.com/locate/marenvrev

Short communication

Cloning and expression of MDR transporters from marine bivalves, and their potential use in biomonitoring

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Abstract

Multidrug resistance transporters (MDRs) are excellent candidates for molecular-level biomonitoring – they function in exporting xenobiotic compounds and their expression is inducible. However, currently available MDR sequence information from aquatic invertebrates is partial and mostly biased towards the conserved ATPase domain. In the present study, two genes belonging to the *MDR/TAP* (*ABCB*) family were cloned and characterized from the bivalve *Brachidontes pharaonis*, which thrives in rocky environments along the Israeli Mediterranean coast. One of these is a complete sequence of a 'half' *ABCB*, probably belonging to the *ABCB10* subfamily, while the second is a 'full' *ABCB1* transporter. A quantitative RT-PCR protocol for biomonitoring was tested in laboratory experiments. Bivalves exposed to diesel showed significant increase in B1 expression levels, while the expression of B10 was suppressed. These results suggest that *B. pharaonis* features an *MDR1* homologue that is induced by pollution and may serve as a sentinel organism for routine biomonitoring programs. However, our findings also exemplify that not all MDRs are equally suitable for this purpose and sequence information must be expanded beyond the ATPase domain for correct classification of cloned genes.

Multidrug resistance transporters (MDRs) serve in cellular detoxification pathways by excluding a wide range of toxic substances from living cells. Therefore, the expression lev-

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doi:10.1016/j.marenvres.2006.04.007

els of MDRs mRNA and protein were considered as biomonitoring tools for marine pollution (Minier et al., 1999; Smital et al., 2003). MDR transporters belong to the ABCB protein family, and posses a highly conserved nucleotide binding domain (specifically, ATPase) that provides the energy for the transport activity. Many researchers have attempted to use conserved sequence motifs within the ATPase domain (Walker A, B and C motifs) in order to clone target *MDR*s (Allikmets and Dean, 1998). Unfortunately, due to the extreme degree of sequence conservation in these sites, one is almost bound to amplify a fragment of *some ABC*, but not necessarily an *ABCB*. In addition, not all ABCB transporters provide the cell with drug resistance. Some (M-ABC) are targeted to the mitochondria, and participate in iron transport. Others (TAP) are antigen presenting proteins. In order to discriminate between the different ABCB subfamilies, there is a need to extend the sequence beyond the conserve ATPase domain, or to use advanced phylogenetic algorithms (Guindon and Gascuel, 2003). We utilized specific identifiers suggested by Anjard and Loomis (2002) between the Walker A&B motifs, to obtain partial clones of two *ABCB* genes from the marine bivalve *Brachidontes pharaonis*.

Brachidontes pharaonis is an indo-pacific bivalve that invaded the Mediterranean sea. It became common along the Mediterranean coast of Israel, and was also reported from Greece and Italy. It is a good candidate for biomonitoring because it is common, easy to collect, found in diverse ecological niches, and in sites that are known to be polluted (Bresler et al., 2003).

Total RNA was isolated from gill tissue using RNeasy (Qiagen) and served as template for cDNA reverse transcription (SuperscriptII, Invitrogene). Degenerate primers were designed according to the ABCB identifiers (Anjard and Loomis, 2002), in the sense V(L)ERFYDP (5′-STNGARMGITTYTAYGAYCC) and antisense AN(H)I(A)HN(D)FI (5′-ATRAARTYRTGIRYRTKIGC) directions. Two different *ABCB* fragments were cloned. In order to obtain the complete coding regions, 5′RACE cDNA was synthesized using the FirstChoise® RLM-RACE (Ambion), and 3′RACE cDNA was prepared with SMART™ RACE cDNA (Clontech). The entire transcript of one *ABCB* gene, and most of a second *ABCB* were cloned (EMBL accessions AJ972911 and AJ972910). Integral stretches of cDNA were amplified for both genes to enable future expression experiments. The translated sequences were aligned against various ABCB proteins (EMBL database) using ClustalX. Phylogenetic trees were reconstructed with the maximum likelihood method using PhyML (http://atgc.lirmm.fr/phyml/) with the JTT substitution model using four rate substitution categories. In the phylogenetic tree, one of the *B. pharaonis* genes clustered with the B10 subfamily of mitochondrial ABCBs and the other with the B1 (=MDR1) subfamily (not shown). The 2 Kb 3′ UTR of the putative B10 gene is also characteristic of this subfamily. The predicted protein sequences encoded by the new genes were then aligned with the partial ABCB sequences available from other molluscs and selected reference sequences. A phylogenetic tree was reconstructed for the conserved ATPase region. It is possible to see that all putative MDRs from molluscs cluster together with the human MDR1 (Fig. 1). They may thus be assumed to serve a similar function in detoxification pathways. However, the sequence from *Urechis caupo*, presumed to be an MDR1 homologue is closest to the *B. pharaonis* B10. Both cluster with the 'half' ABCB transporters, and their function is yet to be revealed. This is the first time that the maximum likelihood method is applied to ABC analysis. Previous analyses of ABC evolution with parsimony and distance methods were not sensitive enough to discriminate between proteins *within* the ABC families because the ATPase domain is highly conserved (Sheps