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Short communication

Cloning and expression pattern of peroxisomal enzymes in the mussel *Mytilus galloprovincialis* and in the thicklip grey mullet *Chelon labrosus*: Generation of new tools to study peroxisome proliferation

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Abstract

Aquatic organisms living in coastal/estuarine areas show peroxisome proliferation after exposure to different environmentally relevant pollutants. In order to generate new tools to assess peroxisome proliferation in aquatic animals, peroxisomal enzymes were cloned using degenerate primers in the mussel *Mytilus galloprovincialis* and in the thicklip grey mullet *Chelon labrosus*. Fragments of catalase (CAT), thiolase (THIO), polyamine oxidase (POX) and xanthine oxidoreductase (XOR) were cloned and their expression pattern studied in different tissues by semi-quantitative RT-PCR. In mussels, CAT, THIO, POX and XOR were expressed in digestive gland, mantle and gills while in mullets CAT, THIO and POX were expressed in liver, spleen, brain, heart, muscle and gills. XOR was mainly expressed in liver and heart. Mature mullets showed the highest expression of peroxisomal enzymes in liver, spleen and brain, while in juveniles expression was mainly found in muscle tissues, liver and gills. Laboratory experiments of exposure to organic pollutants are being performed to study the usefulness of these tools to study peroxisome proliferation in pollution biomonitoring programmes.

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During the last decades, a considerable research effort has been devoted to developing sensitive early warning biomarkers of pollutant exposure and effects. Peroxisome proliferation (PP) is one of these emerging biomarkers of exposure to organic pollutants. Peroxisomes of marine organisms including bivalve molluscs and fish have shown capacity to proliferate under experimental and field exposure to organic xenobiotics (Cancio and Cajaraville, 2000; Cajaraville et al., 2003). PP is accompanied by induction of peroxisomal enzyme activities, especially those involved in lipid homeostasis. This induction occurs at the transcriptional level. In this sense, the aim of this work was to generate new tools to assess PP in aquatic animals by cloning genes that may allow to study changes in expression patterns in animals exposed to peroxisome proliferators (PPs). Mussels (*Mytilus galloprovincialis*) and mullets (*Chelon labrosus*) were selected for this study. Mussels are used worldwide as sentinels of pollution in marine environments and mullets are abundant in Eastern-Atlantic estuaries where they are able to endure highly polluted environments. Cloning of these genes coding for peroxisomal enzymes may be relevant for future applications of PP as biomarker of exposure to organic pollutants in pollution biomonitoring programmes.

Mussels, *M. galloprovincialis* and thicklip grey mullets *C. labrosus* were collected from Arriluze, Biscay Bay (43°20′N, 003°01′W) in winter. Mussels were immediately processed after capture, and mullets were dissected after classification as mature male or female and juveniles (2 individuals per group). Total RNA was isolated using Trizol (Invitrogen) and cDNA synthesis was carried out using random hexamers. Degenerate primers generated aligning known sequences from different species were employed to amplify polyamine oxidase (POX), catalase (CAT), thiolase (THIO) and xanthine oxidoreductase (XOR) fragments in mussels and mullets. For mussel THIO an EST sequence without assigned homology was obtained from the Genbank (AJ624743). PCR amplificates were purified and cloned using a TOPO-TA cloning vector. Sequencing of plasmids was performed by Sanger's method using the Fw-M13 primer. For expression studies specific primers that amplify fragments around 200 bp were designed according to the new sequences obtained. Monoplex-PCR conditions for the amplification of target cDNAs were optimised using *Taq* polymerase (Invitrogen). PCR products were visualized by gel electrophoresis and analysed using a computer-aided gel analyser (Gel-Doc-2000, Bio-Rad). Results were expressed in arbitrary semi-quantitative units, using β-actin and 18S rRNA as housekeeping genes for normalisation.

Peroxisomal genes were partially cloned in mussels and mullets (Table 1). The mullet XOR fragment showed 85% amino acid identity with *Poecilia reticulata* XOR (*e*-value for BlastN algorithm: N $1e^{-54}$) while mussel PCR product showed highest homology with *Strongylocentrotus purpuratus* XOR. XOR is involved in purine metabolism acting either as oxidase or dehydrogenase. Both forms produce superoxide anions but xanthine dehydrogenase has also been implicated in the metabolism of xenobiotics (Kooij, 1994). The dehydrogenase form has been previously found in mussel tissues using enzyme histochemistry and immunohistochemistry (Cancio and Cajaraville, 1999). Cloned POX fragments showed 90% amino acid identity with zebrafish POX (N $4e^{-70}$). POX is a flavin oxidase