Multi-xenobiotic resistance (MXR) transporters and biotransformation enzymes in the blue mussel Mytilus edulis

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In order to clarify the role of MXR and biotransformation proteins in *Mytilus edulis* and their potential use as biomarkers, several gene sequences were partly identified and characterised. Environmental parameters were identified that affect expression levels of these genes and the relevance of the application of expression patterns of these genes was investigated in field studies with respect to habitat-specific predisposition and site-specific responses to point source contamination.

Tissue-specific gene expression of the multidrug resistance protein 1 (P-gp), the major vault protein (MVP, i.e. the lung resistance protein, LRP), glutathione S-transferase pi (GST pi), heat-shock protein 70 (HSP70), and cytochrome P450 4A (CYP4A) was analysed. Additionally, topoisomerase II (TopoII) and actin expression was investigated as indicators for mitotic activity and internal standards. We identified highly-conserved regions in genes in a wide range of species, and synthesised degenerate oligonucleotide primers designed to amplify these regions from *M. edulis* mRNA. PCR-amplified fragments were used as probes for Northern blot hybridisation to identify transcript sizes. Specific oligonucleotide primers were designed from sequences for each gene and used for semi-quantitative multiplex RT-PCR.

Other prominent members of the MXR family are MRPs. MRPs may also provide xenobiotic resistance in aquatic organisms in a polluted environment by binding xenobiotics and excreting them from cells in an energy-dependent manner. We investigated expression of MRPs as part of the multixenobiotic resistance (MXR) system to analyse whether there is a link between P-gp, MVP and MRP expression in *M. edulis*. We isolated and characterised two putative *mrp* cDNAs. The *mrp1* fragment could not be associated with any mRNA in Northern blots whereas the *mrp2* fragment hybridised with a mRNA of approx. 4.6 kb. *Mrp2* showed tissue-specific expression patterns. Highest expression was found in digestive gland and gill in *M. edulis*. Its expression showed to be inducible 2-fold by the model carcinogen 2-acetylaminofluorene (AAF) whereas *mrp1* expression was unaffected by exposure to AAF. The cDNA fragment of the inducible form was then integrated in a multiplex PCR system for the analysis of MXR in the blue mussel in concert with the other detoxification and biotransformation genes.

Marine organisms and especially those living in tidal zones are confronted with dramatic changes in their environment on a daily and/or seasonal basis such as fluctuations in temperature and oxygen availability. An important issue with respect to the use of biomarkers
in monitoring biological effects of pollutants is the interference of natural environmental factors in the expression of biomarkers, that can complicate valid interpretation of data. In order to differentiate between pollution-induced stress and responses to natural environmental variations, we studied the effects of temperature, salinity and oxygen supply (anaerobiosis) on expression of MXR-related genes in gills and digestive gland of the blue mussel *M. edulis*. Changes in expression levels of P-gp, MVP, TopoII, HSP 70 but not of MRP2 were found in laboratory experiments in relation to high but not low temperature, low salinity and anaerobiosis. These effects of environmental factors have to be taken into account in sampling strategies for monitoring programmes to prevent false negative or positive results.

To further study effects of natural environmental factors on gene-expression biomarkers, we examined mussels from habitats of different structure (rocky shore, free water column) to analyse whether differences in ecotypes affect gene-expression responses to hazardous substances (benzo[a]pyrene, cycloheximide, rifampicine and 2-acetylaminofluorene). Furthermore, the value of MXR-related gene expression as biomarkers of pollution was investigated in various fjord sites in Norway that were contaminated in various ways. Expression of P-gp, MVP and MRP2 as phase 0 proteins, HSP 70 as general stress protein and TopoII as indicator of cell proliferation were analysed in gill and digestive gland. Specific inducibility by chemical inducers during experimental exposure was demonstrated in relation to habitat structure. Mussels from the rocky shore appeared to be less responsive as reflected by lack of induction of the MXR transcripts of *p-gp* and *mrp2* genes than mussel attached to buoys living under more constant conditions. During the Norwegian field campaign, site-specific differences in expression of MXR-related genes were detected. It appeared that in non-tidal habitats, contamination with polyaromatic hydrocarbons inhibited P-gp-related protection in digestive glands whereas contamination with copper induced MRP-related mechanisms. Our study indicates that expression of MXR-related genes, either inhibition or induction, can serve as an appropriate biomarker to determine hazardous effects of chemicals in contaminated marine habitats when natural environmental factors are taken into account.

Therefore, sampling strategies for biomonitoring programs must include controls to establish interfering effects of natural environmental variations and differences in sensitivity of mussel populations as part of habitat-specific adaptations to obtain comparable sets of biomarker data.