

Abstract of the PhD thesis of Dr. Letizia Ambrosio

Title: “A chemical-toxicological study of animal models exposed to organohalogen environmental contaminants”

Sensitive effect determination, the understanding of molecular toxicity mechanisms and the discovery of novel biochemical biomarkers are some of the major challenges in ecotoxicology in dealing with chemicals in the environment. Among several ‘omics’ tools, proteomic approaches are used to study the whole proteome of organisms and may provide novel insights into the functional molecular state of a biological system and for discovery of new sensitive biomarkers indicating exposure or effects at low toxicant concentrations.

In this study, a proteomic approach has been used in *Mytilus galloprovincialis* as a screening of changes in protein expression caused by a mixture of polychlorinated biphenyls (PCBs), in order to characterize the effects of these environmental contaminants on protein profile and to develop new molecular biomarkers through identification of more drastically altered proteins.

To achieve this objective, 100 mussels were exposed to PCB 138, 153 and 180 for 3 weeks under controlled conditions at the concentration of 30µg/l. An equal number of mussels was kept under the same conditions, but not treated, as control. The edible parts were homogenized and lyophilized. Extracted proteins were quantified and separated by two-dimensional electrophoresis (2-DE). It has been made a comparative study of two-dimensional electrophoresis gels obtained from proteomic analysis and the changes in protein expression were assessed by image analysis. Image analysis included spot detection, quantification, normalization and matching. On average more than 1000 spots were resolved and altered expression was qualitatively detected. Stained protein spots of interest were excised from preparative gels and their tryptic digests were subjected to protein identification by mass spectrometry. It was used Matrix Science Mascot search engine, database NCBI and for a homology search the program BLAST.

Protein identification using mass spectrometry becomes a challenge when the proteins are from an organism whose genome is not yet sequenced, as is the case of the genus *Mytilus*. However, the identification of 36 proteins of 71 studied proteins was achieved directly with *Mytilus* spp. or with other invertebrate species.

Our results indicate that exposure of the mussel *M. Galloprovincialis* to PCBs had a mainly down regulating effect on protein expression level. Significantly, differentially expressed proteins, identified in the present work, turned out to be related mainly to structure/function of cytoskeleton, which has been proposed as one of the first targets of oxidative stress.

Hence, it was concluded that a toxicoproteomics approach in *Mytilus Galloprovincialis* enables the detection of substance specific effects and more general stress responses in the cellular protein pattern and allows the identification of candidate protein biomarkers.

With this work we demonstrated the high degree of sensitivity of proteomic approach and its utility in toxicological studies, as we have shown that protein expression varies significantly between examined group identifying specific PES in response to pollutants. This proteomics approach can be considered to be a valuable and promising tool for the development of environmental research.