CELL CYCLYING ALTERATIONS IN THE BLUE MUSSEL *MYTILUS GALLOPROVINCIALIS* CAUSED BY ENVIRONMENTAL CONTAMINATION

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Abstract

Flow cytometry (FCM) was employed to determine cell cycling alteration caused by environmental contamination in mussel *Mytilus galloprovincialis* haemocytes and gills. The G_1-arrest and G_2-delay have been detected in haemocytes of mussel in "mussel watch" experiment, when mussels have been transferred from mariculture area to the location under direct influence of industrial and urban runoff near a fish cannery. The cell cycling alterations in haemocytes and gills mussels collected from some polluted sites along the Adriatic coast were observed as well.

Keywords : Ecotoxicology, Bivalves, Pollution, Cell

In recent years, FCM, by virtue of a growing diversity of measurable parameters, has become an important tool in areas of cell biology related to cell proliferation, differentiation and response of cell damage. Use of FCM in identifying the cytological results of exposure to environmental pollutants is still in infancy. Nevertheless, early work has shown that FCM is a powerful tool for identifying and measuring effects of environmental contaminants on the vertebrate genome (1, 2). As far as mussels are concerned, FCM was performed in determination of abnormalities in DNA content distribution and progression in neoplastic disorders (polyploid forms) of mussels from heavily polluted area such as Puget Sound, Washington, USA (3). Therefore, our study has focussed in determination of cell cycling alternations in mussel haemocytes caused by environmental contamination in "mussel watch" experiment as well as from haemocytes and gills of mussels collected from different locations of different polluted areas along the Adriatic coast.

For the mussel watch experiment, mussels (average weight 10 g) were transferred from mariculture area to the selected site and were held in a net anchored at the low neap tide level. After different time intervals, 6 hours and 1,2,3,6,14 and 28 days, mussels were collected and their DNA content in haemocytes was analyzed by FCM. For biomonitoring purposes mussels (average weight 6 g) were collected at different locations so called "hot spots" along the Adriatic coast. Haemolymph was withdrawn from the posterior adductor muscle of individuals, dispersed in DAPI/DMSO (4,6-diaminido-2-phenyl indol/dimethylsulphooxide) solution. A piece of gills were removed from mussels (average 2 mg) and stored as well as haemolymph DAPI/DMSO solution under the liquid nitrogen until FCM analysis was performed. Stained samples were analyzed on PAS II flow cytometer (Partec, Münster, Germany) under the following conditions: excitation - 100 W mercury lamp, UG 1 (290-490 nm, 3mm), chromatic beam splitter (TK 420), emission beam splitter (TK420, TK560), barrier filter (GG 455) for DAPI signals. The data are shown as one parameter frequency histograms or as a percentage of DNA in different cell cycle stages.

Time dependent effect of industrial waste on DNA content distribution in mussel haemocytes are shown in Table 1. During two days of exposure, mussels under direct influence of industrial and urban runoff near a fish cannery showed the increased number of haemocytes in G_2/M cell cycle state (2.9 ± 2.0 up to 13.3 ± 2.9%), while the percentage of DNA in S-phase remained unchanged (3.6±1.3 to 3.3 ± 1.9 %). Further differences between starting conditions of mussels transferred from uncontaminated area and conditions after 6 days of exposure of mussels to contaminated area, could not be detected. The variations in G_0/G_1 and G_2/G_M phases could be attributed to the G_1-arrest and G_2-delay in haemocytes DNA that provide more time for repair of damaged DNA as it was detected after 6 days of exposure. Besides mussels watch experiments we applied the FCM for determination of cell cycle alteration in mussels collected from differently polluted sites along the Adriatic coast. Preliminary results showed the presence of contaminants at one sampling site that altered the cell cycle in mussel haemolymph the (Fig.1c) as well in mussel gills (Fig.1b). Cell cycle alteration of gills DNA content of mussel collected from location 1 showed a reduction of G_0/G_1 phase concomitant with selective loss of G_2/M phase which could be attributed to the apoptotic process (confirmed by electroforetic "ladder-like" DNA pattern, data not shown). The type of alteration in mussels which occurred in mussel collected at location 2 could be due to the occurrence of subpopulations of haemocytes whose whole chromosomes may be lost or gained. This phenomenon has to be elucidated with further investigations (*e.g.* chromosome analysis).

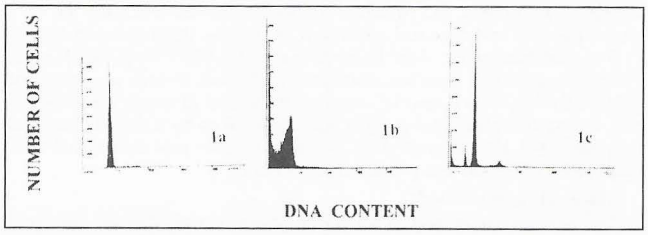

Figure 1. Comparison of DNA histograms of haemocytes or gills of mussels collected from reference site and so called "hot spots" along the Adriatic coast.
1a. Haemocytes of control mussel.
1b. Gills of mussel collected at location 1 along the Adriatic coast.
1c. Haemocytes of mussel collected at location 2 along the Adriatic coast.

Table 1. Cell cycle variation in haemocytes of mussel *Mytilus galloprovincialis* exposed to waste waters of fish cannery.

Time of exposure	G_0/G_1	S	G_2/M
0	93.5 ± 1.7	3.6 ± 1.7	2.9 ± 2.0
2 days	82.6 ± 3.6	3.3 ± 1.9	13.3 ± 2.9
6-28 days	92.3 ± 0.6	3.9 ± 0.5	3.8 ± 0.7

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