

## COLD ADAPTED MARINE ENZYMES

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The world's oceans cover more than 70% of the earth's surface and therefore a large proportion of life on earth is contained in these oceans and includes the largest range of habitats. The marine environment ranges from nutrient rich areas to nutrient sparse areas. In addition high salinity, high pressure, different light conditions and low/high temperature add to the complexity of the marine environment. This may contribute to significant differences between enzymes from marine organisms and homologous enzymes from terrestrial organisms.

Organisms that thrive in cold environments are referred to as psychrophiles or cold-adapted organisms. In order to survive and proliferate in the harsh cold environment, marine organisms must possess a capacity to synthesize cold-adapted enzymes. Cold-adapted enzymes have evolved a range of structural features that are necessary to perform their action at low temperatures and are in general more catalytically efficient and possess usually a lower thermal stability compared to enzymes from organisms adapted to warmer climate. These characteristics make cold-adapted marine enzymes very interesting for biotechnological and industrial purposes.

## LIPID CONTENT OF MUSSELS, *MYTILUS EDULIS*, AS A BIOMARKER OF MARINE ENVIRONMENT HEAVY METAL POLLUTION

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The blue mussel, *Mytilus edulis*, is a unique bioresource of the White Sea (Bioresources of the White Sea, 2008). An important area of applied science use of these bivalves is biomonitoring and biotesting of water quality. *Mytilus edulis* satisfies a variety of criteria of a model object. One of them is the ability to accumulate high pollutant concentrations in tissues (Gudimova, 2002). Changes at the level of lipids are an essential strategy organisms employ to protect themselves against various stress factors (Kurashvili and Vasilkov, 2003). A characteristic response at the lipid composition level to adverse impact of pollutants, including heavy metals, is storage of neutral lipids, primarily triglycerides (Capuzzo and Leavitt, 1988; Chetty and Indira, 1994; Bergen et al., 2001). *Mytilus edulis* from the White Sea have also been shown to undergo a reduction in cholesterol content in response to oil pollution (Bakhmet et al., 2009). Heavy metals are high hazard ecotoxicants (Isidorov, 1999), wherefore optimization of the lipid metabolism and mobilization of its reserves notably promotes the organism's adaptation to the adverse environmental conditions. In this study we investigated the effect of various concentrations of such heavy metals as cadmium and copper on the lipid composition in gill and digestive gland from White Sea mussels, *Mytilus edulis*.

To study how the lipid composition in *Mytilus edulis* L. was modified in response to heavy metal pollution, we carried out an experiment in which the animals were kept for 24 and 72 hours in aquaria with different concentrations of copper and cadmium ions in seawater: 5, 50 and 250 µg/l and 10, 100 and 500 µg/l, respectively. The values of 5 (for copper) and 10 µg/l (for cadmium) are maximum allowable concentrations (MAC). Mussels kept under the same regime in the laboratory, but in unmodified seawater, were used as the control. Lipids were extracted by the chloroform/methanol mixture (2:1 by volume) following Folch et al. (1957). The qualitative and quantitative composition of total lipids was determined by thin-layer chromatography, as well as using spectrophotometric techniques (Sidorov et al., 1972; Endelbrecht et al., 1974).

Cholesterol level in digestive gland of *Mytilus edulis* from the White Sea decreased significantly after 24 h exposure to 10, 100, 500 µg/l and 250 µg/l of cadmium and copper ions, respectively. More prolonged (72 h) exposure of the mussels to water with heavy metals similarly influenced cholesterol content at 10 µg/l of cadmium ions and at 250 µg/l of copper ions. A reduction in cholesterol level in *Mytilus edulis* gills was confidently observed at a cadmium concentration of 10 µg/l (24 h exposure). The reduction in cholesterol content observed in digestive gland and gill of *Mytilus edulis* under the impact of various concentrations of cadmium ions is likely to be related to regulation of the lipid bilayer permeability and the activity of membrane enzymes. Cadmium inhibits the cell's ion transport systems, namely, it can replace calcium in calmodulin thus disturbing the process of cytoplasmic Ca-ATPase activation (WHO Cadmium, 1992; Kutsenko, 2004). The cholesterol decline in the mussels' digestive gland under the impact of 250 µg/l of copper ions may indicate not only the process to maintain optimal microviscosity of the bilayer and activity of membrane enzymes, but also development of a pathological process induced by irreversible degradation of lipids through peroxidation (Burlakova, 1976; Khlusov, 2003). Note however that the impact of copper ions in 5 and 50 µg/l concentrations resulted in opposite changes in cholesterol content in the gills of mussels. The rise in the cholesterol level observed in *Mytilus edulis* gills is presumably an indication protective mechanisms of lipid peroxidation (LP) have been launched, for we know that when LP intensifies the synthesis and re-synthesis of the lipid fractions (mainly cholesterol) enhancing membrane microviscosity in the cell is activated (Burlakova, 1976; Khlusov, 2003).

The digestive gland of mussels exposed for 24 h to 500 µg/l of cadmium ions exhibited a reduced content of storage lipids, mainly triacylglycerols (TAG). Contrastingly, TAG content in *Mytilus edulis* gills under the impact of copper ions in 5 and 50 µg/l concentrations (72 h exposure) rose. The changes in the content of storage lipids (namely TAG) in the tissues assayed presumably indicate a misbalance in the energy metabolism processes. Thus, the reduction in storage lipids in *Mytilus edulis* digestive gland is, in all appearance, related to energy starvation of the cell caused by suppression of the tricarboxylic acid enzyme cycle, electron transport chain, as well as ATP-dependent enzyme systems by cadmium ions (Aksyonova, 2000). The rise in storage lipid content in mussel gills is probably due to a decrease in the rate of TAG metabolism in *Mytilus edulis* as the result of adenylate cyclase inhibition by copper ions (Shpakov and Derkach, 1994). Hence, fluctuations of TAG level in the tissues of *Mytilus edulis* are targeted at mobilization of lipid stores and replenishment of the energy deficit caused by the adverse impact of heavy metals.

The resultant data on modifications of the content of cholesterol and storage lipids (mainly TAG) in *Mytilus edulis* from the White Sea under the impact of various concentrations of cadmium and copper ions suggest that fluctuations of these lipid components, alongside with transformations of the protein and carbohydrate metabolism, reflect the organism's response to the stressful environmental impact, and can be used as a biomarker indicating the degree of to which the environment is contaminated with these pollutants.

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## **STUDIES OF ANTIMICROBIAL PEPTIDES IN THE GREEN SEA URCHIN *STRONGYLOCENTROTUS DROEBACHIENSIS***

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Sea urchins are interesting animals to study as sources for novel compounds that might have promising activities and properties. For the first time from sea urchins, we have recently isolated and characterized two novel families of antimicrobial peptides (AMPs) from the green sea urchin, *Strongylocentrotus droebachiensis*.

The first family of AMPs, named strongylocins, is the cysteine-rich peptides isolated from extracts of the coelomocytes (blood cells). Strongylocins contain 6 cysteines and have a novel cysteine arrangement pattern when compared to other cysteine-rich peptides in the Antimicrobial Peptide Database (<http://aps.unmc.edu/AP/main.php>). Their putative precursor peptides contain a signal peptide, a prosequence and a native region. Their gene sequences indicate that strongylocins (except strongylocin 1b) have three introns and four exons. The mature strongylocins are active against both Gram-positive and Gram-negative bacteria. Similar genes were found in the sister species, *S. purpuratus*. Recombinant products of these genes inhibited growth of bacteria by a nonlytic, presumably intracellular mechanism.

The other family of AMPs, named centrocins, has a heterodimeric structure (a heavy chain and light chain linked by a disulfide bridge). The gene sequences of centrocins code for a signal peptide, two prosequences and a native region. These genes contain one intron and two exons. The native peptides were highly potent against bacteria. A synthetically made heavy chain had anti-fungi and anti-yeast properties in addition to being active against bacteria.

All together, these two families of AMPs from *S. droebachiensis* have promising antibacterial properties for development and future exploitation.