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DISTRIBUTION AND POLYMORPHISM OF ENZYMES INVOLVED IN ANTIOXIDANT PROTECTION AND XENOBIOTICS BIOTRANSFORMATION IN THE MEDITERRANEAN MUSSEL *MYTILUS GALLOPROVINCIALIS*

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The aim of the work was to determine the tissue distribution, activity and polymorphism of 13 enzymes involved in antioxidative protection and xenobiotics biotransformation in a five bivalve mussel organs (hepatopancreas, ctenidia, mantle, leg, adductor muscles). Iso-allozyme analysis was performed by electrophoresis, correlation relationships between the studied enzymes in the whole mussel body were carried out using Spearman's correlation analysis. It was found that all studied enzymes are polymorphic, each organ differed in the level of enzyme activity and a set of multiple forms. The coordinated functioning of protective enzymes in various mussel organs was demonstrated.

K e y w o r d s: iso-allozyme analysis, antioxidant enzymes, enzymes of biotransformation, coordination of protective enzymes, Mytilus galloprovincialis.

The huge role of bivalve mollusks in maintaining a clean environment cannot be neglected. In the Black Sea, the Mediterranean mussel *Mytilus galloprovincialis* (Lamarck, 1819), along with other biofilters, the cerastoderma *Cerastoderma glaucum* (Bruguière, 1789), the anadara *Anadara kagoshimensis* (Tokunaga, 1906) and others is of great importance for ensuring the normal ecology of the sea. Biofilter organisms are of particular importance for the coastal waters of the North-Western part of the Black Sea, which are characterized by significant desalination, natural and anthropogenic pollution, frequent freezing and other unfavorable life-threatening properties.

Marine bivalves are among the most polymorphic of all animal species. A significant level of allozyme diversity in bivalves, in addition to the large population size, can be explained by a high load of non-neutral amino acid polymorphisms [1]. Also, the analysis of various allozyme loci showed a deficiency of heterozygotes in their natural populations. The main reasons for this phenomenon can be: the Valund effect, the presence of null alleles, inbreeding or natural selection [2-5]. It should be noted that information on the genetic and biochemical features of the species *M. galloprovincialis* of the northwestern Black Sea region is limited compared to information on allozyme polymorphism [6-8] and biochemical characteristics of various organs [9, 10] of mussels of the genus *Mytilus* from other regions of the World Ocean.

Knowledge of the protective mechanisms of mussels is important for understanding the biology of these organisms and developing methods of environmental monitoring. A special role in the environmental monitoring system is played by biological monitoring, which is related to two main directions – bioindication and biotesting. The Mediterranean mussel, as a biofilter organism that leads a sedentary lifestyle, is suitable for use not only as an indicator organism in natural conditions [11], but also as a test object in the assessment of the ecological state of the marine environment in clearly defined laboratory conditions [12]. At the cellular level, the stability of the lysosome membranes of the mollusc hemolymph cells is an informative indicator of the "health" of mussels, and the destruction of the lysosomal membrane is an almost universal marker of stress [13].

The list of biological markers for assessing the state of mollusks in the first place are enzymes of biotransformation and oxidative stress [14]. In the genus *Mytilus*, among the enzymes of biotransfor-

mation of xenobiotics, the main attention is paid to the study of esterases [15] and glutathione S-transferases [16].

The choice of the studied enzymes for this work was determined by their significant role in providing protection against tissue damage, changes in the physicochemical properties of cell membranes, deterioration of cell functions and body functions as a whole due to excessive production of reactive oxygen species (ROS) by the body [17], which are formed during aerobic respiration and include peroxides, superoxides, hydroxyl radicals and singlet oxygen. ROS can interact with many biological molecules, including proteins, lipids, and nucleic acids, irreversibly altering the spatial conformation and function of the affected molecule [18]. Being a normal natural by-product of the vital activity of organisms, the level of (ROS) is controlled by various enzyme systems that prevent damage to the structures of living things. Antioxidant enzymes play a paramount role in the adaptation of organisms to any adverse environmental factors, ensuring the preservation of the ROS balance in the norm [19-21]. In addition, antioxidant enzymes are involved in the control of specific ROS-mediated signaling pathways in cells [22, 23].

In this regard, the aim of the work was to determine the tissue distribution and polymorphism of enzymes that protect mussel organisms from various adverse effects.

Materials and Methods

Mollusks were collected at the end of October 2020 in the Odesa Bay near the Hydrobiological station of Odesa National Mechnykov University from a stone ridge at a depth of 6 m at a distance of 300 m from the shore. The samples were frozen in a freezer to -28°C and kept in this condition until analysis.

Individuals with a shell length of 65-85 mm were taken into the experiment. After soft thawing, the soft body of the mollusk was prepared on the surface of the refrigerant to prevent significant heating of the material during processing. The following organs were selected for analysis: hepatopancreas, ctenidia, mantle, leg and adductor muscles. In order to reduce the variation of indicators for the analysis used aggregate material, which consisted of organs of 6-10 individuals.

Enzyme analysis was performed by polyacrylamide gel (PAGE) electrophoresis. Before obtaining extracts from isolated organs, they were repeatedly treated with acetone to discolor the tissues. This procedure significantly reduced the amount of fatty compounds and pigments that prevent electrophoretic separation of proteins and equalized the amount of moisture in various tissues.

Tissues were homogenized in extraction buffer of the following composition: 0.05 M Tris-HCl (pH 6.8), 0.01% dithiothreitol, 0.01% ascorbic acid, 0.01% sodium EDTA, 1% Triton X-100, with the ratio of tissue : buffer (weight, mg : volume, µl) being 1 : 10. Samples triturated directly in centrifuge tubes were subjected to freeze-thawing 3-5 times and then centrifuged in the cold (4°C) for 20 min at 10 000 g. The supernatant obtained was used for electrophoresis [24, 25].

Vertical native electrophoresis was performed at room temperature in gel plates measuring 130×110×1 mm in apparatus VE-4M (Helicon Company). Protein distribution was performed in the Davis system [26] or Tris-borate citrate system [27] with or without the use of a concentrating gel. Depending on the enzyme under study, PAGE with concentrations of 6.5 to 10% were used. Electrophoresis was performed at 15 mA and 110 V until dye front moved from the start to distance of about one third of gel, after which the power and the voltage were increased to 30 mA and 260 V. The total duration of electrophoresis was 4-5 h. Upon completion, the gels were treated to detect certain enzyme activity [25].

Superoxide dismutase (SOD, EC 1.15.1.1), NADH oxidase (OXN, EC 1.6.3.3), NADPH oxidase (NOX, DIA (NADP), 1.6.99.6), glutathione peroxidase (GPx, EC 1.11.1.9), amino oxidase (copper-containing) (AMOX, EC 1.4.3.6), glutathione reductase (GSR, EC 1.6.4.2), glutathione S-transferase (GST, EC 2.5.1.18) were detected by methods using nitrotetrazolium blue and different substrates for each enzymes [27]. Ferroxidase (CP, EC 1.16.3.1) was detected by oxidation of para-phenylenediamine [27]. Nonspecific esterases (EST, EC 3.1.1-) were manifested in the azo coupling reaction of hydrolysis products of naphthylacetate with solid blue [27]. Catalase (CAT, EC 1.11.1.6) was detected after electrophoresis in 6.5% PAGE with the addition of soluble starch to a concentration of 0.5%. The manifestation of the enzyme was carried out on the basis of the reaction of starch with iodine [27]. Carbonic anhydrase (CA, EC 4.2.1.1) was detected by the method with metal salts according to the recommendation [28]. Peroxidase (PER, EC 1.11.1.7) was performed by the method of Lojda [29] in the G-nadoxidase

reaction. Peroxyredoxins (PRX, EC 1.11.1.15) were detected as follows. Separation of proteins was performed in a polyacrylamide gel with the addition of starch (0.4%). Gels after electrophoresis were immersed for 20 min in 1 mM solution of dithiothreitol in sodium acetate buffer pH 7.0. The used solution was drained, and the gel was washed with water. After washing, the gel was kept for 20 min in freshly prepared hydrogen peroxide solution (0.03%). Then, after washing with water, the gel was treated with 0.045 M KJ solution. Zones of the enzyme were found as colorless streaks on a blue background. Documentation of the gel was carried out immediately.

Documentation of electrophoregrams was performed on a Hewlett Packard Scanjet 44c scanner and stored in *.bmp format. The computer program AnaIS (Analyzer of spectrum images) was used for the analysis of electrophoregrams [30]. Enzymatic activity was evaluated by the area of peaks on densitograms of the corresponding multiple forms, and was calculated in conventional units per 1 mg of tissue treated with acetone. Statistical processing of the results was carried out using Microsoft Excel and Statistica. The obtained data were analyzed by two-factor analysis (ANOVA). Spearman's rank correlation criterion [31] was used to determine the coordinated activity between the enzymes under study.

Results and Discussion

Electrophoretic spectra of enzymes that control the level of ROS in tissues are shown in Fig. 1, 2.

Superoxide dismutase (SOD) is the first detoxification enzyme and the most powerful antioxidant in the cell, acting as a component of the first line of defense against ROS. It catalyzes the dismutation of two molecules of superoxide anion radicals ($^{\bullet}O_2$) to hydrogen peroxide (H_2O_2) and molecular oxygen (O_2), making the potentially harmful superoxide anion radical less dangerous. Its danger is related not only to the direct interaction with important organic polymers, but also to the initiation of chain reactions of the formation of various reactive ROS (for example, hydroxyl radical ($^{\bullet}OH$), lipid radical ($^{\bullet}L$), peroxynitrate ($ONOO^{-}$)) [32].

The studied enzyme belongs to the class of oxidoreductases and is a metalloprotein. In eukaryotic cells, this enzyme exists in several isoforms: cytosolic Cu, Zn-containing (Cu/Zu-SOD), mitochondrial Mn-containing (Mn-SOD), and high molecular extracellular (EC-SOD). The content and activity of these isoforms in different tissues differ significantly [33-36].

Up to five forms of SOD were detected in the studied organs of mussels (Fig. 1), that can be products of expression of different genes – SOD1, SOD2, SOD3 and so their protein products Cu/Zu-SOD, Mn-SOD, EC-SOD [32]. The greatest activity per unit mass of tissue and a variety of variants of this enzyme were observed in the hepatopancreas (Fig. 1). Leg muscle tissues also had highly active SOD, but some forms present in the hepatopancreas were absent from their electrophoretic spectrum: the fastest moving and the slowest. Based on the obtained data, it can be assumed that hepatopancreas and leg tissues experience the greatest superoxide radical load, and ctenidia experience the lowest load. The obtained results agree with the data of the literature [37]. Also, among the hydrobionts of the Black Sea, superoxide dismutase activity was investigated in the Black Sea shrimp *Palaemon elegan*s. According to the obtained data [38], the indicators of the activity of this enzyme in the tissues of the Black Sea shrimp were higher compared to the activity in the tissues of the Antarctic krill *Euphausia superba*.

Ferroxidase although has insignificant superoxide dismutase activity. This protein is well studied in mammals, data on its role in mollusks are extremely limited [39]. In contrast to SOD, ferroxidase reduces superoxide to water and oxygen without producing ROS – hydrogen peroxide [40]. This enzyme converts divalent iron to trivalent iron, thereby blocking the Haber-Weiss and Fenton chain reactions [40], which are a source of formation of the hydroxyl radical ($^{\bullet}OH$). Due to this, ferroxidase protects the membranes from the outside. It is known that in mammals ferroxidase acts as an extracellular enzyme that neutralizes superoxide radicals [41, 42], as if supplementing intracellular SOD.

It was shown [43] that four ferritin subunits (PyFer1, PyFer2, PyFer3 and PyFer4) cloned from Yesso scallop, *Patinopecten yessoensis*, have pronounced ferroxidase activity. A novel ferritin subunit gene from the Asian green mussel *Perna viridis* (PvFer) was recently identified [44]. Analysis of the conserved domain showed that PvFer contains a dialysate ferroxidase center.

In all studied organs of mussels 6 forms of protein with ferroxidase activity were found. Its greates activity per unit mass of tissue was observed in the adductor and ctenidia. Mutual complementarity between SOD and ferroxidase is demonstrated by the

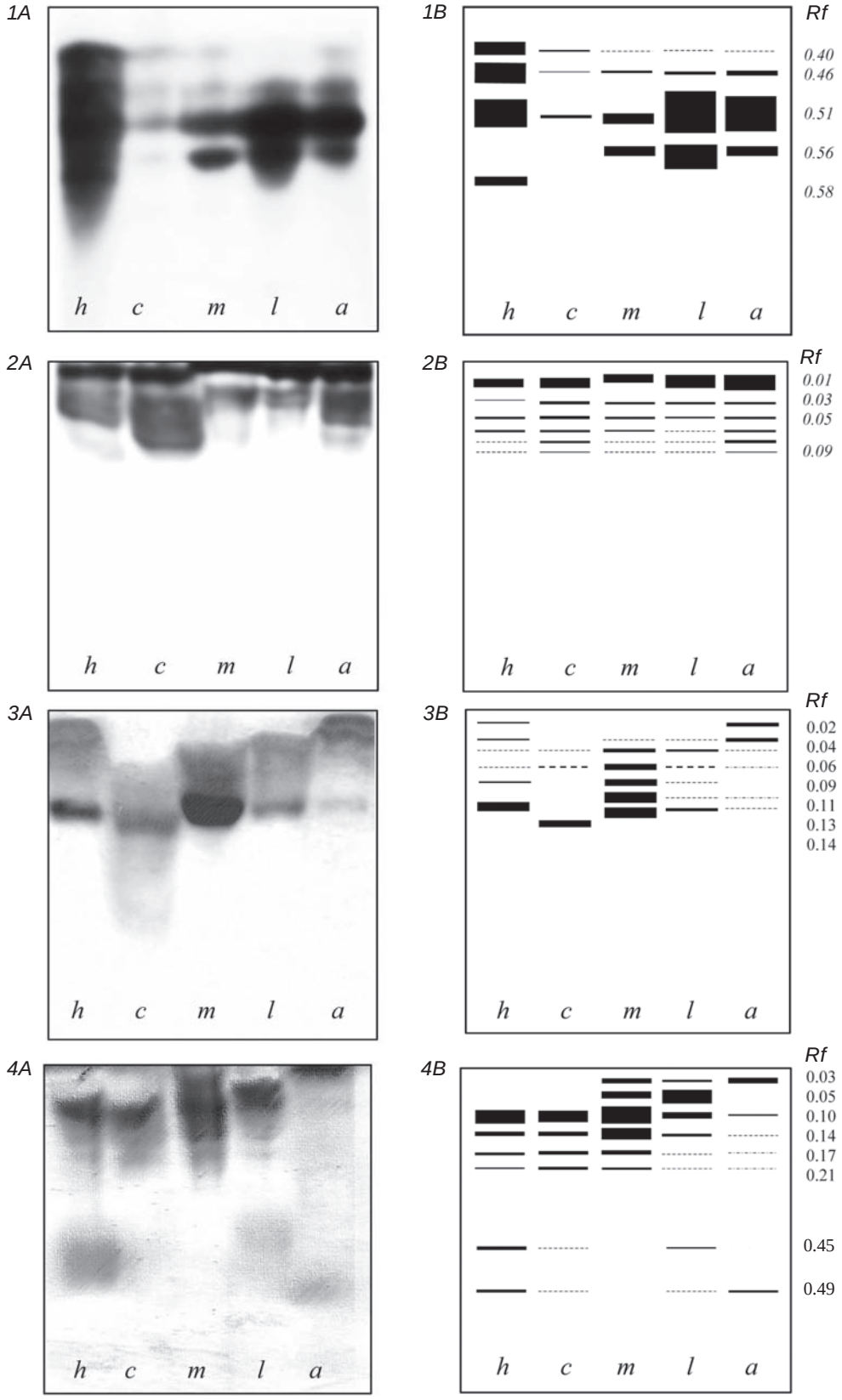

*Fig. 1. Electrophoretic profiles of extracts of various organs of mussels (**A** – electrophoregrams, **B** – schemes of electrophoregrams): 1 – superoxide dismutase; 2 – ferroxidase; 3 – NADH oxidase; 4 – NADPH oxidase. On the tracks: h – hepatopancreas, c – ctenidia, m – mantle, l – leg, a – adductor muscle. Rf – relative electrophoretic mobility of protein*

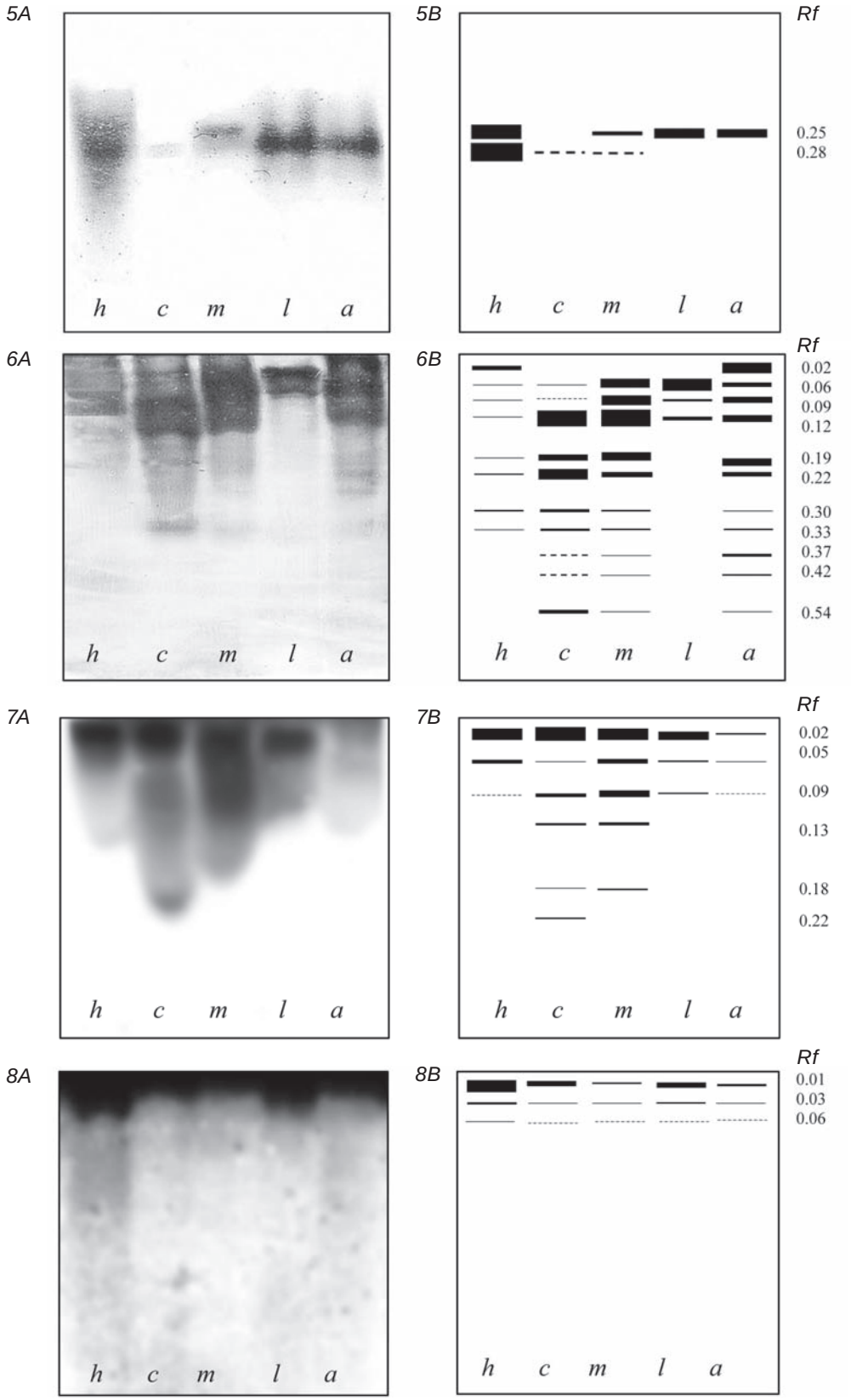

*Fig. 2. Electrophoretic profiles of extracts of various organs of mussels (**A** – electrophoregrams, **B** – schemes of electrophoregrams: 5 – glutathione peroxidase; 6 – peroxidase; 7 – peroxyredoxins; 8 – catalase. On the tracks: h – hepatopancreas, c – ctenidia, m – mantle, l – leg, a – adductor muscle. Rf – relative electrophoretic mobility of protein*

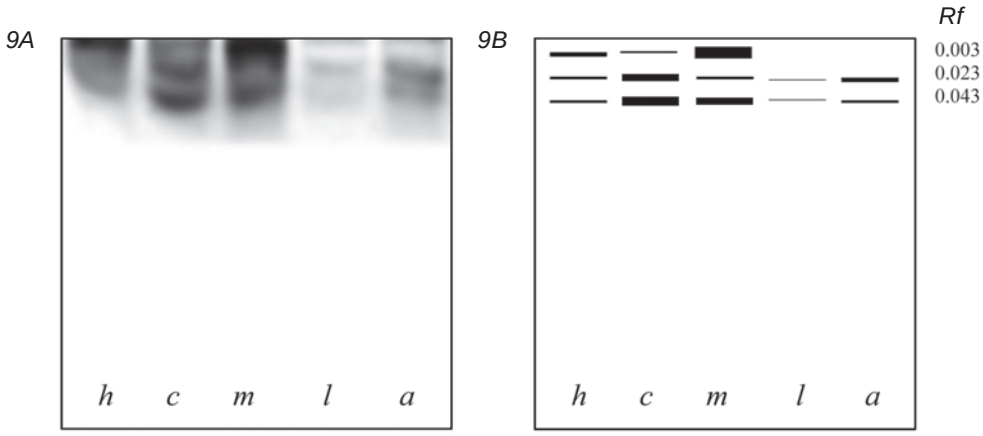

*Fig. 2. Electrophoretic profiles of extracts of various organs of mussels (**A** – electrophoregrams, **B** – schemes of electrophoregrams): 9 – glutathione reductase. On the tracks: h – hepatopancreas, c – ctenidia, m – mantle, l – leg, a – adductor muscle. Rf – relative electrophoretic mobility of protein*

existence of a weak (average degree of relationship on the Chaddock scale) negative relationship between the activity of both enzymes (Spearman's rank correlation coefficient r = -0.50 at n = 5, P = 0.05). It is likely that the existing negative correlation is due to the openness of the circulatory system of mollusks, as a result of which intracellular SOD can enter the location of ferroxidase and compensate its action.

SOD, being a component of the antioxidant system, is also a prooxidant, because as a result of its functioning there is an ROS such as hydrogen peroxide. Other enzymes whose reaction products are peroxide and superoxide radical are NADH oxidase (NADH dehydrogenase) and NADPH oxidase. NADH oxidase is involved in the oxidative phosphorylation of substrates responsible for energy supply to cells. In the spectra of the studied organs of mussels, only up to 6 forms of this enzyme were observed (Fig. 1). The highest activity (by tissue weight) was in the mantle (Fig. 1). Perhaps this is due to the significant energy activity of this organ to perform various energy-intensive processes: synthesis and secretion of substances to build a shell [45], special epithelial cells of the mantle form structures that carry out endocytosis and immune processes are necessary to protection from parasites [46]. In addition, the storage of nutrients (mainly glycogen), the accumulation of metals and organic contaminants that occurs in palliative cells, are also energy-intensive processes [47].

NADH oxidase generates a superoxide radical and hydrogen peroxide inside the cell and NADPH oxidase does so in the extracellular space and outside the plasma membrane. Up to eight forms of this enzyme were detected in mussel organs (Fig. 1). The greatest activity was observed, as for NADH oxidase, in the mantle. There are two possible reasons for this. First, the mantle cells are in direct contact with the environment and transferred signals from the external environment to the other cells. Secondly, as noted above, mantle cells are involved in protection against pathogens, which uses superoxide radicals and hydrogen peroxide.

All known peroxide-destroying enzymes were found in the studied mussel organs (Fig. 1, Fig. 2). The importance of these multifunctional proteins is determined not only in the neutralization of the destructive effects of ROS. By regulating the amount of peroxides, they affect their signaling role [23]. Classical peroxidase neutralizes toxic substances (phenols and others), using them as proton donors to reduce peroxides [48]. In addition, peroxidase can function as myeloperoxidase, creating hypochlorite anions that destroy pathogenic microorganisms. It was shown [49] that the activity of glutathione peroxidase and glutathione reductase is important for predicting the redox state of tissues.

Glutathione peroxidase (GPx) in the hepatopancreas and mantle is represented by two forms. In other organs it is found only in one form. The greatest activity per unit mass of tissue was in the hepatopancreas.

Classical peroxidase (PER) in the studied organs of mussels had up to 11 forms: in the tissues of the adductor – 11, ctenidia and mantle – 10, hepatopancreas – 8. The least forms of PER were observed in the leg (three). According to the activity of peroxi-

dases, the organs were located as follows: mantle > ctenidia ≈ adductor > hepatopancreas ≈ leg.

Peroxyredoxins (PRX) are a diverse group of peroxide-neutralizing enzymes. The largest number of forms is represented in the ctenidia and mantles (six and five forms, respectively). The highest activity were found in the mantle and ctenidia.

The peculiarity of catalase is that, it does not need as proton donors certain additional substances or molecular groups for the destruction of peroxides [50], in contrast to the above characterized enzymes. GPx uses reduced glutathione, PER uses phenolic compounds, and PRX uses its own SH groups. Therefore, these enzymes need to be restored for further work. Catalase uses another peroxide molecule to reduce one peroxide molecule. Thus, catalase is an "economical" enzyme and does not spend valuable substances for life. Three electrophoretic forms of the enzyme were detected in all organs. The highest activity of catalase per unit mass was in the tissues of the hepatopancreas, the lowest – in the mantle and adductor.

It should be noted that in each organ the neutralization of excess peroxides is carried out with the predominant use of certain enzymes. Based on a comparison of the enzymatic activity of peroxide-degrading enzymes in different organs, certain assumptions can be made. Since the activity was calculated in conventional units, the contribution of each enzyme to the peroxidant action was evaluated by the rank of its activity in different organs (Table 1).

Two-factor analysis of variance (ANOVA) showed that the studied factors individually ("enzymes", "organs") and together ("enzymes-organs") have a statistically significant effect on the indicators of activity in mussel organs (F exp. > F tab.), Table 2.

In Fig. 3 a graph of the distribution of the average values of enzymatic activity of the studied enzymes in five organs of mussels, grouped by the influence factor "enzymes-organs", is presented.

In the hepatopancreas peroxides are neutralized primarily by glutathione peroxidase and catalase, in ctenidia – peroxyredoxins. In the mantle tissues, peroxyredoxins and peroxidase perform the main function of peroxide neutralization in comparison with other organs.

In muscle, catalase and glutathione peroxidase (leg) and peroxidase (adductor) play a leading role in protecting against excess peroxides. These assumptions about the functioning of antioxidant enzymes in various organs of mussels are consistent with the data of other authors [37, 51].

As noted above, most peroxide-depleting enzymes require proton donors to recover. An important such donor is reduced glutathione. Its stock is constantly restored with another component of the antioxidant system (AOS) – glutathione reductase (GSR) [52]. Three molecular forms of this enzyme were found in the hepatopancreas, ctenidia, and mantle, and two in the leg and adductor. Its highest activity per unit mass was observed in the mantle and ctenidia, the lowest – in the leg (Fig. 2).

Thus, all mussel organs have a complete set of antioxidant enzymes, and the differences between the organs are clearly related to the functions they perform. Coordination of antioxidant systems of individual organs allows you to successfully resist the effects of the environment on the whole organism. A number of studies also demonstrate the specificity of the location of various antioxidant enzymes in the organs of mollusks and the significant effectiveness of the protective systems of bivalve mollusks-biofilters [37, 51, 53, 54].

The antioxidant enzymes discussed above not only regulate and neutralize ROS formed as a result of their own activities, but also participate in the neutralization of various external organic pollutants (substances with pro-oxidant properties, oxidizing xenobiotics, etc.) [55]. In addition, living organisms have a whole system of neutralization of xenobiotics [56]. This paper presents the results of determination of the following enzymes of biotransformation: glutathione S-transferase (GST), esterase (EST) and amino oxidase (copper-containing) (AMOX).

EST and GST are one of the key enzymes of the I and II phases of xenobiotic biotransformation in living cells [52, 57, 58]. In the hepatopancreas the activity of this enzyme is much higher than in other organs. In addition, the polymorphism of the GST electrophoretic spectrum is higher in the hepatopancreas: both forms of GST were expressed approximately equally in the hepatopancreas, while in the mantle the manifestation of one form predominated (Fig. 4, Table 1).

Esterases are a large family of enzymes that hydrolyze ether bonds and are considered one of the most important in the metabolism of xenobiotics [58]. Due to the large number of forms with different substrate specificity, they neutralize many harmful compounds. Of the studied enzymes, esterase, together with peroxidase and NADPH oxidase, has the largest number of multiple forms (Fig. 4, Table 1). According to the specific esterase activity per unit

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Table 1. Enzymatic activity and number of forms of protective enzymes in the studied organs of mussels

Enzymes	Organs														
	Hepatopancreas			Ctenidia			Mantle			Leg			Adductor muscle		
	Am	R	N	Am	R	N	Am	R	N	Am	R	N	Am	R	N
SOD	23.33 ± 0.38	1	4	1.97 ± 0.05	5	3	7.47 ± 0.14	4	4	23.24 ± 0.20	2	4	16.49 ± 0.13	3	4
CP	4.71 ± 0.06	5	6	7.02 ± 0.15	2	6	4.92 ± 0.04	4	6	5.87 ± 0.04	3	6	8.55 ± 0.06	1	6
GPx	4.91 ± 0.16	1	2	0.04 ± 0.01	5	1	1.12 ± 0.01	4	2	4.27 ± 0.22	2	1	2.67 ± 0.41	3	1
PER	5.89 ± 0.23	5	8	15.71 ± 0.51	3	10	17.69 ± 0.35	1	10	5.97 ± 0.10	4	3	16.06 ± 0.30	2	11
PRX	4.88 ± 0.13	3	3	7.44 ± 0.03	2	6	9.11 ± 0.09	1	5	3.69 ± 0.01	4	3	0.81 ± 0.01	5	3
CAT	3.73 ± 0.03	1	3	1.54 ± 0.02	3	3	0.57 ± 0.01	5	3	1.70 ± 0.03	2	3	0.68 ± 0.01	4	3
GSR	1.84 ± 0.03	3	3	3.72 ± 0.03	2	3	4.73 ± 0.21	1	3	0.47 ± 0.03	5	2	1,78 ± 0.15	4	2
OXN	7.30 ± 0.32	2	6	4.69 ± 0.28	3	3	31.28 ± 0.19	1	6	4.46 ± 0.12	5	6	4.49 ± 0.05	4	6
NOX	15.17 ± 0.13	2	6	12.40 ± 0.13	4	6	24.01 ± 0.14	1	6	13.96 ± 0.06	3	8	5.55 ± 0.08	5	8
CA	5.18 ± 0.06	4	4	8.90 ± 0.08	2	1	6.79 ± 0.08	3	4	2.95 ± 0.03	5	4	11.86 ± 0.05	1	4
AMOX	2.46 ± 0.05	3	5	4.21 ± 0.04	2	5	7.15 ± 0.02	1	5	1.08 ± 0.02	5	5	1.40 ± 0.02	4	5
EST	30.00 ± 0.05	1	7	7.53 ± 0.06	4	7	23.71 ± 0.04	2	8	13.96 ± 0.09	3	8	3.37 ± 0.04	5	6
GST	3.18 ± 0.04	1	2	0.37 ± 0.01	4	1	0.53 ± 0.01	2	2	0.35 ± 0.01	3	1	0.05 ± 0.01	5	1

Note: Am – the total enzymatic activity of all forms of the enzyme (un.act./mg); R – the rank of activity of a certain enzyme in this organ in comparison with other organs; N – the number of molecular forms of the enzyme detected in this organ

Table 2. Analysis of variance (ANOVA) of the results of experimental data on the activity of protective enzymes in the studied mussel organs

Source of variation	SS	df	MS	F	Fcrit	Fate of influence, %
Enzymes	5163.66	12	430.31	9244.39	1.83	48
Organs	746.58	4	186.64	4009.73	2.44	7
Enzymes-Organs	4878.58	48	101.64	2183.51	1.46	45
Within	6.05	130	0.05	–	–	0
Total	10794.87	194	–	–	–	100

Note: SS – sum of squares; df – the value of the degrees of freedom; MS – dispersion values; F – value of F-statistics (Fexp.), F-critical – probability value (Ftab.)

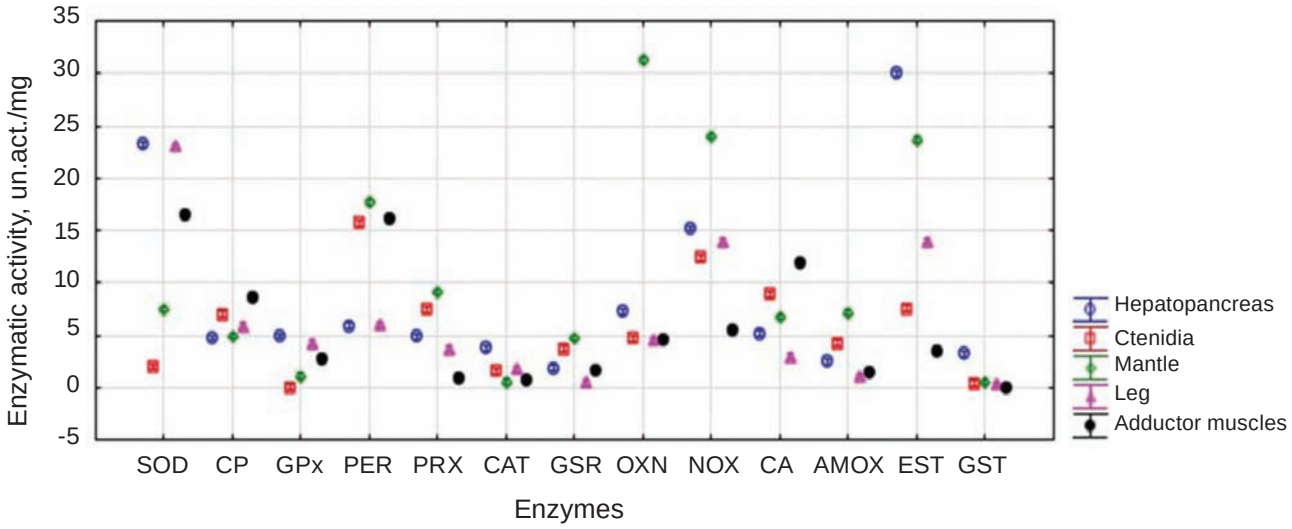

Fig. 3. Distribution of enzymatic activity of enzymes of antioxidant stress and biotransformation of xenobiotics in different organs of mussels

mass, the organs were located as follows: hepatopancreas > mantle >> leg > ctenidia > adductor.

Amine oxidases are involved in the metabolic processing of biogenic amines. These enzymes are classified into two classes based on their prosthetic groups: copper-containing amine oxidases (EC 1.4.3.6) and flavin-containing amine oxidases (EC 1.4.3.4). The main copper-containing amine oxidases are primary amine oxidase and diamine oxidase, which are widely distributed in nature. In marine hydrobionts: *Scylla paramamosain* [59], *Danio rerio* [60], *Cyprinus carpio* [61] flavin-containing amine oxidase, namely monoamine oxidase (MAO), which is found in most living creatures, is studied. In mammals, MAO exists in two isoforms (MAO-A and MAO-B), which are dimers in their membrane-bound forms [62]. It was shown [63] that MAO-A and MAO-B are two different proteins that are encoded by different genes, have an identical exon-intron organization and are regulated by different gene regulators. Despite their similarities, MAO-A and MAO-B differ in tissue distribution and substrate specificity [64]. That is, it performs a dual function, each of which is important. In total, up to 6 forms of the enzyme were observed in the studied organs of mussels (Fig. 4, Table 1). The highest activity was in the mantle and ctenidia, the lowest – in muscle tissue (leg and adductor).

Another enzyme that can be classified as protective is carbonic anhydrase (CA). It is responsible for building the shell needed to protect the soft body of the mollusk from the environment. Also, CA pro-

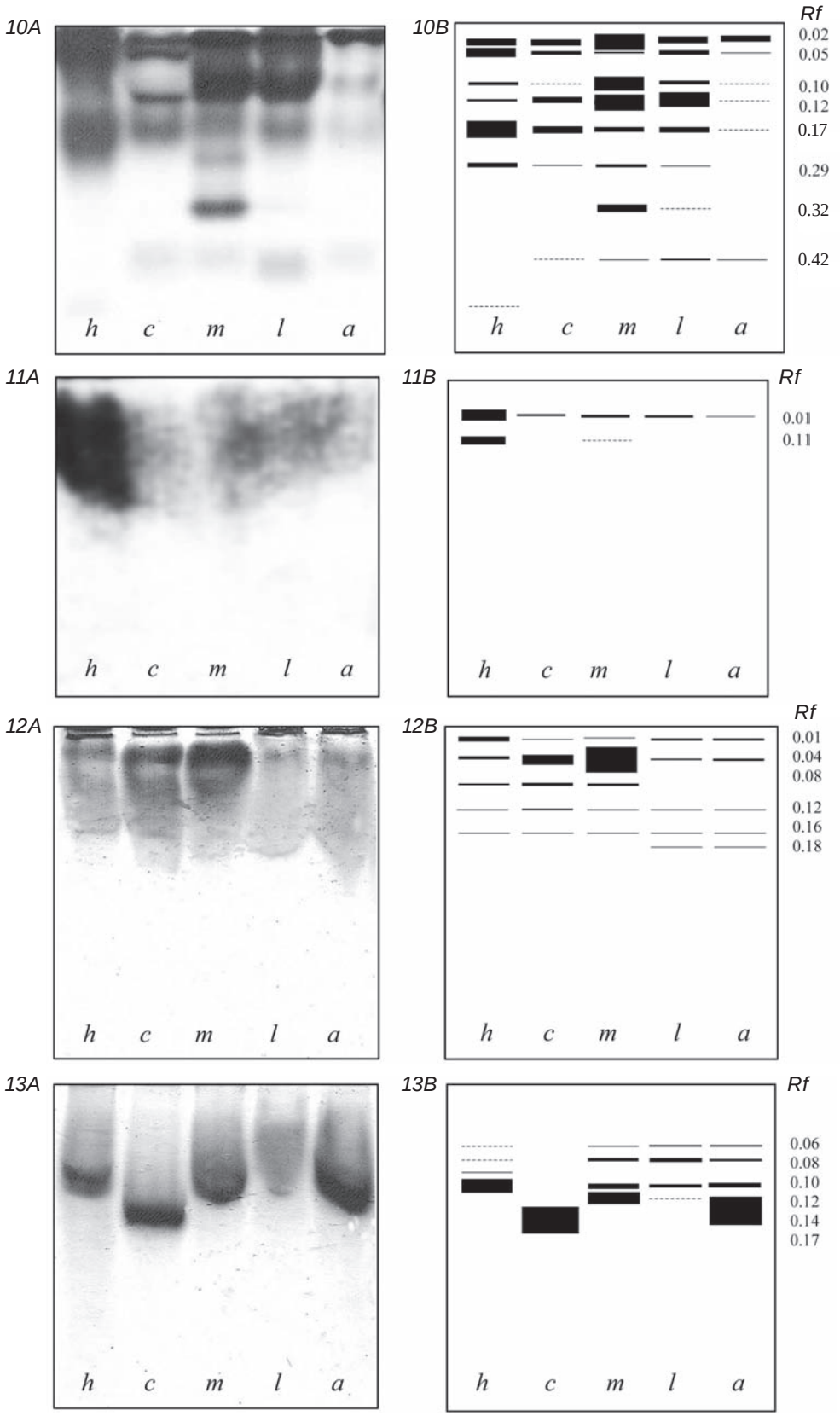

*Fig. 4. Electrophoretic profiles of extracts of various organs of mussels (**A** – electrophoregrams, **B** – schemes of electrophoregrams: 10 – nonspecific esterases; 11 – glutathione S-transferase; 12 – amino oxidase (copper-containing); 13 – carbonic anhydrase. On the tracks: h – hepatopancreas, c – ctenidia, m – mantle, l – leg, a – adductor muscle. Rf – relative electrophoretic mobility of protein*

vides removal from the body of CO_2 caused by cell respiration, and resistance of mollusks to changes in acidity and salinity of water [65, 66].

A total of 6 forms of CA were found in the mussel body, but each organ had a specific composition of enzyme forms (Fig. 4, Table 1). The largest value of the activity of the enzyme per unit mass was in the adductor. In adductor tissues, the set of electrophoretic forms of CA coincided with that in the mantle. A distinctive feature of the CA spectrum of these organs is a significant predominance (from 60 to 80% of the total spectrum) of the fast form. Probably, this form is related to the biocalcification process [67]. The second place in terms of the activity of this enzyme was occupied by ctenidia. They showed a rapidly moving form, which was not in any other organ. It is possible that the main function of this form of CA is related to the removal of CO_2 from the body. Carbonic anhydrase activity was lowest in the hepatopancreas and especially in the leg.

According to the literature [68, 69], the CA superfamily includes seven distinct classes known as α, β, γ, δ, ζ, η, and θ. Perfetto et al. [69] had purified α-CA and biochemically characterized from the mantle tissue of the mollusk *M. galloprovincialis*. In polychaetes, CAs belong mainly to the α-CA family, however, members of the β-CA family have been identified in corals [70]. As in most molluscs, α-CA is involved in the processes of biomineralization, which leads to the precipitation of calcium carbonate in the mussel shell. Purified *M. galloprovincialis* α-CA is either a dimer or similar to the protein identified and described in *Tridacna gigas* CA [71], which may have two different CA domains in its polypeptide chain.

Since any living organism is a whole system, it is important to find out how different protective enzymes interact in all the organs. To do this, a correlation analysis was performed according to the data obtained for all organs, Table 3.

According to the Table 3, catalase (CAT) competes with peroxidase (PER) for the substrate – hydrogen peroxide. In the hepatopancreas, catalase simply destroys peroxide, which is formed due to the high activity of superoxide dismutase (SOD). In ctenidia, mantles and adductors, this substrate is apparently used mainly to control microorganisms.

According to the results of the correlation analysis, glutathione reductase (GSR) activity in the examined tissues of mussels is positively correlated (r = +0.90) with the activity of peroxiredoxins (PRX), which are restored with the participation of glutathione. The high correlation between SOD activity and glutathione peroxidase (GPx) activity indicates that excess peroxides (caused by superoxide dismutase) are eliminated throughout the mussel body first of all by glutathione peroxidase, not catalase, as in hepatopancreas. The results of a positive correlation between these enzymes in different organs have also been shown in studies [72].

The positive correlation between NADN oxidase activity (OXN) and glutathione reductase (GSR) activity can be attributed to the need to recover glutathione, which is spent on neutralizing ROS arising from the functioning of OXN. Similarly, amino oxidase (AMOX), which exhibits prooxidant activity, can be linked to peroxyredoxins (PRX), OXN and GSR. In addition, AMOX can be a source of toxic formaldehyde, one of the ways to neutralize which is the use of reduced glutathione [56]. The latter's intracellular pool is known to support GSR.

Ferroxidase (CP) is characterized by relationships with different function enzymes. It is possible that ferroxidase, as an enzyme responsible for the balance of copper and iron in the body, affects the activity of NOX. Ferroxidase with glutathione S-transferase (GST), NADPH-oxidase (NOX), and esterase (EST) is involved in the neutralization of ROS and xenobiotics. But it is difficult to understand why their coordination is competitive. This may be due to the complex and ambiguous relationship between these enzymes and different intracellular localization. GST, EST and NOX interact closely and in a coordinated manner to neutralize xenobiotics.

Conclusions. This paper presents the results of tissue-specific profiling of a set of radical-scavenging enzymes and several enzymes involved in the biotransformation of xenobiotics in bivalve molluscs. Iso-allozyme tissue distribution of protective enzyme systems and their polymorphism is shown. The coordinated activity of these systems in mussel target organs have been revealed using by correlation analysis using the Spearman's rank correlation method. The significant influence of the studied factors ("enzymes", "organs" and "enzymes-organs") on the enzymatic activity in mussel organs have been detected by factor analysis. The largest number of multiple forms of enzymes was detected using peroxidase activity (up to 11 forms), NADPH-oxidase activity (up to 8 forms) and esterase activity (up to 8 forms). The smallest number of multiple forms of enzymes (up to two forms) were characterized by: glutathione peroxidase and glutathione S-transferase

Table 3. Correlation relationships between the studied protective enzymes

	GSR	GPx	PER	CAT	PRX	SOD	CP	OXN	NOX	GST	EST	AMOX
GSR												
GPx	-0.60											
PER	0.50	-0.70										
CAT	-0.50	0.70	**-1.00**									
PRX	**0.90**	-0.50	0.30	-0.30								
SOD	-0.60	**1.00**	-0.70	0.70	-0.50							
CP	-0.30	-0.50	0.40	-0.40	-0.50	-0.50						
OXN	**0.90**	-0.20	0.30	-0.30	0.80	-0.20	-0.60					
NOX	0.50	0.20	0.00	0.00	0.70	0.20	**-0.90**	0.70				
GST	0.30	0.50	-0.40	0.40	0.50	0.50	**-1.00**	0.60	**0.90**			
EST	0.30	0.50	-0.40	0.40	0.50	0.50	**-1.00**	0.60	**0.90**	**1.00**		
AMOX	**1.00**	-0.60	0.50	-0.50	**0.90**	-0.60	-0.30	**0.90**	0.50	0.30	0.30	
CA	0.30	-0.60	0.60	-0.60	-0.10	-0.60	0.70	0.10	-0.60	-0.70	-0.70	0.30

Note: The critical Spearman's rank correlation coefficient for $n = 5$ is $r = 0.94$ at the confidence level $P = 0.05$. Reliable values of Spearman's rank correlation coefficients are marked in bold

activities. One of the explanations for the presence of multiple forms of enzymes that we have revealed in the work can be the expression of several gene loci, which often changes during the development of the organism and sometimes are tissue specific. Therefore, electrophoretic spectra of enzymes may differ depending on the tissue and stage of development of the organism. The results presented in the work do not exhaust the entire diversity of coordinated activity of different enzyme defense systems of mussels. Establishing such coordinated activities requires further research.

Conflict of interest. Authors have completed the Unified Conflicts of Interest form at http://ukrbiochemjournal.org/wp-content/uploads/2018/12/coi_disclosure.pdf and declare no conflict of interest.

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РОЗПОДІЛ І ПОЛІМОРФІЗМ ЕНЗИМІВ АНТИОКСИДАНТНОГО ЗАХИСТУ ТА БІОТРАНСФОРМАЦІЇ КСЕНОБІОТИКІВ У СЕРЕДЗЕМНОМОРСЬКОЇ МІДІЇ *MYTILUS GALLOPROVINCIALIS*

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Дослідження полягало у визначенні тканинного розподілу, активності і поліморфізму 13 ензимів, що забезпечують антиоксидантний захист та біотрансформацію ксенобіотиків у п'яти органах двостулкових молюсків (гепатопанкреас, ктенідії, мантія, нога, мускул-аддуктор). Ізо-алозимний аналіз проводили методом електрофорезу, кореляційні зв'язки між досліджуваними ензимами у тілі мідії здійснювали за допомогою кореляційного аналізу методом Спірмена. Встановлено, що всі досліджувані ензими є поліморфними, кожен орган відрізняється рівнем активності ензимів та набором множинних форм. Показано скоординоване функціонування захисних ензимних систем у різних органах мідій.

Ключові слова: ізо-алозимний аналіз, антиоксидантні ензими, ензими біотрансформації, скоординованість захисних ензимних систем, *Mytilus galloprovincialis*.

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