RESEARCH ARTICLE

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Study on the relation between some enzyme and protein polymorphism and the zebra mussel organism response to pesticide exposure

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ABSTRACT
In the present study, a biochemical-genetic analysis was performed to determine the relationship between isoenzyme polymorphism and the zebra mussel *Dressena polymorpha* organism response to the harmful effects of the pesticides Cypermethrin and Chlorpyrifos. The probable genetic control of four enzyme and protein groups - malate dehydrogenase, malate enzyme, superoxide dismutase, and soluble proteins - was determined as well. Different intensities of expression of allelic products have been reported in individuals exposed to short- and long-term exposure to the pesticides Cypermethrin and Chlorpyrifos. The observed variability in the expression of superoxide dismutases, malate dehydrogenases, and soluble proteins in control and experimental samples gives us reason to accept these enzyme and protein systems as markers for reporting the negative impact of the tested pesticides on the zebra mussel.

Key words: *Dressena polymorpha*; isoenzyme polymorphism; pesticide exposure

Introduction

Electrophoretic studies of genetically determined biochemical polymorphisms of proteins are used to study the relationship between the level of protein variability and the impact of environmental conditions. Isoenzymes are indicators of gene expression and reveal the characteristic features of gene regulation. The investigation of isoenzyme variability allows the study of genome regulation and contributes to determining the genetic basis of adaptation. Studies show that genetic polymorphism is specific to individual loci and varies between different populations depending on habitat and living conditions (Nevo et al., 1984).

Natural bioindicators are used to assess the anthropogenic impact on the environment and are an important tool for detecting changes in organisms. Mussels are widely used as bioindicators for the pollution of aquatic ecosystems. The present study aims to investigate the relationship between the expressed biochemical-genetic polymorphism of selected enzyme and protein systems and the zebra mussel organism response to the harmful impact of the pesticides Cypermethrin and Chlorpyrifos.

Materials and Methods

In the present study, muscle tissue from 46 individuals of the species *Dressena polymorpha* were examined by biochemical-genetic analysis. Forty of them are exposed to short-term (96 hours) and long-term (30 days) exposure with different concentrations of the broad-spectrum insecticides Cypermethrin ([cyano-(3-phenoxyphenyl) methyl] 3-(2,2-dichloroethenyl)-2,2-dimethylcyclopropane-1-carboxylate) and Chlorpyrifos (O, O-Diethyl O-(3,5,6-trichloropyridin-2-yl) phosphorothioate). The MRLs of the two pesticides are tested according to Directive 2013/39/EU, as well as 50% and 30% of the MRLs, to establish the effect of lower concentrations of pesticides. A zebra mussel sample taken from a clean body of water is used as a control.

Electrophoretic analysis

The method of electrophoresis is widely used in the study of biochemical polymorphism of populations. It is a reliable way to study populations in terms of their homo- and heterozygosity and the manifestation of genetic diseases (Pichot & Pichot, 1980).

In the present study, soluble proteins (SP), malate dehydrogenases (MDH), malate enzymes (ME), and superoxide dismutases (SOD) were studied using native gel electrophoresis in 7.5% polyacrylamide gel by the method of Maurer (1971), with modifications according to Ivanova (1996) to characterize the possible relation with the zebra mussel organism response to the harmful pesticide impact.

Results and Discussion

Malate dehydrogenase (EC 1.1.1.37)

Malate dehydrogenase (MDH) catalyzes the reversible oxidation of malate to oxaloacetate using NAD as a coenzyme. According to biochemical data on the dimeric structure of the enzyme, all forms of malate dehydrogenases consist of two subunits, resulting in a spectrum of three components in heterozygous individuals. Müller et al. (2001) and Gosling et al. (2008) reported polymorphism at malate dehydrogenase loci in *Dressena polymorpha*. In the electrophoretic analysis we have conducted in the spectra of malate dehydrogenases in zebra mussels, a total of four fractions with different electrophoretic mobility were visualized. Their nature of expression gives grounds to assume that the genetic control of these isoenzymes is carried out by three loci – Mdh-1, Mdh-2, and Mdh-3 (Figure 1).

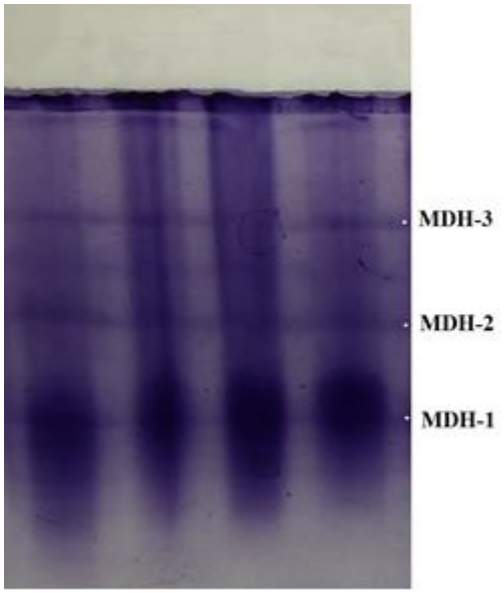

Figure 1. *Spectrum of malate dehydrogenase in individuals of the species Dressena polymorpha (7.5% PAGE).*

There are differences in the intensity of expression of MDH fractions. Weaker expression of the Mdh-1 locus product was observed in the 96-hour sample treated with 30% Chlorpyrifos compared to higher pesticide concentrations.

Malate enzyme (EC 1.1.1.40)

Malate enzyme (ME) catalyzes the reversible oxidation of malate to pyruvate using NADP as a coenzyme. Our analysis showed variability of the ME system in the zebra mussel. The nature of the expression of multiple molecular forms of the malate enzyme in the spectrum of the studied individuals suggests a probable gene control of two polymorphic loci: Me-1, with the presence of the alleles Me-1^{100} and Me-1^{90} and locus Me-2, with the presence of the two codominant alleles – Me-2^{100} and Me-2^{72} (Figure 2). Homozygous and heterozygous combinations of alleles of both loci are detected.

The observed polymorphism gives grounds to consider the malate enzyme as a suitable marker for analyzing the genetic heterogeneity of *Dressena polymorpha* populations.

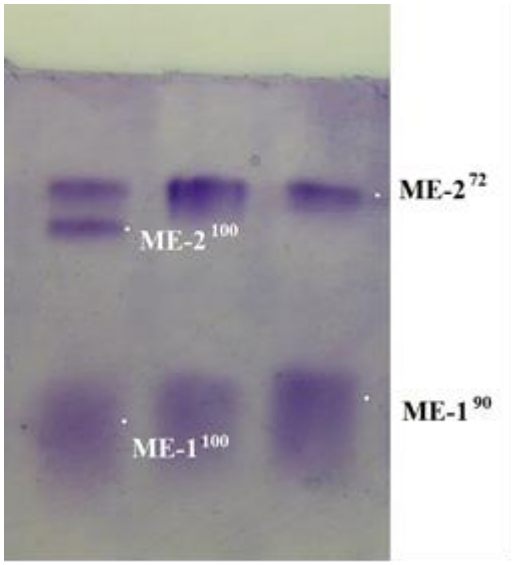

Figure 2. *Spectrum of malate enzyme in individuals of the species Dressena polymorpha (7.5% PAGE).*

Superoxide dismutase (EC 1.6.4.3 SOD)

Superoxide dismutase (SOD) catalyzes the binding of free oxygen radicals to hydrogen peroxide and oxygen. The enzyme acts as a powerful antioxidant and protects cells from the mutagenic action of superoxide radicals. Superoxide dismutase after electrophoresis appears as white areas on a dark blue background. The comparative analysis we conducted showed some differences in the intensity of multiple forms of SOD in individuals treated with different concentrations of the studied pesticides. The pronounced active electrophoretic variants are divided into four zones (SOD-1, SOD-2, SOD-3, and SOD-4) following their decreasing electrophoretic mobility. The expression and combination of the fractions from the four zones suggest that the genetic control of superoxide dismutases is carried out by four loci: Sod-1, Sod-2, Sod-3, and Sod-4 (Figure 3). In the SOD-1 zone of the spectrum of the studied individuals, two fractions with high electrophoretic mobility were visualized. Their nature of expression suggests the action of a polymorphic locus Sod-1, represented by the alleles Sod-1^{100} and Sod-1^{92}. In the SOD-2 zone of the spectrum, the expression of two fractions was found. The expression of SOD-forms from this zone determines a probable two-allele polymorphism at the Sod-2 locus, with the presence of two codominant alleles – Sod-2^{100} and Sod-2^{84}. In the third zone of the SOD spectrum, the expression of two alloforms can be observed, which can be

assumed to be controlled by the codominant alleles Sod-3^{100} and Sod-3^{94} of the Sod-3 locus. In the SOD-4 zone of the spectrum of individuals, an intense fraction is visualized. The lack of variability in the expression of superoxide dismutases in this area determines probable gene control from a monomorphic locus Sod-4.

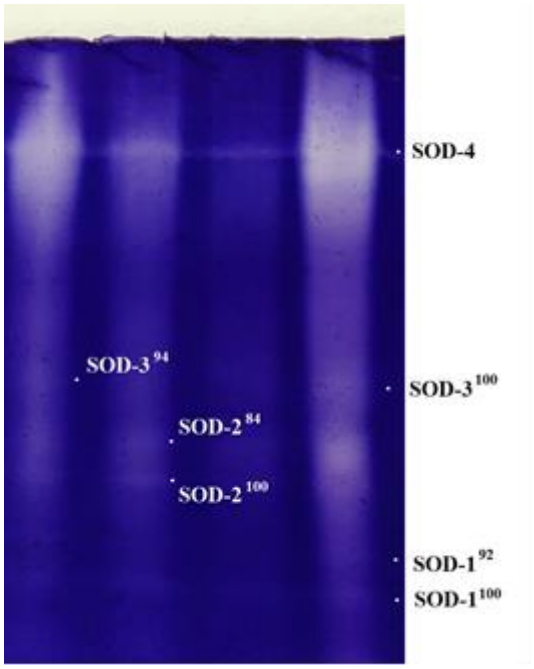

Figure 3. *Spectrum of superoxide dismutases in individuals of the species Dressena polymorpha (7.5% PAGE).*

Differences in terms of intensity of SOD zones between the tested individuals are reported. For samples treated with Chlorpyrifos and Cypermethrin, more intensely pronounced SOD fractions were found compared to the control. We assume that the observed differences in the intensity of expression of the fractions reflect the response of the individuals to the applied chemical effect. This finding is consistent with the dependence found by Johnson et al. (1969) on the variability of enzyme systems on environmental conditions.

Soluble proteins (SP)

The analysis of some genetic and biochemical features of proteins shows that the method of electrophoresis allows for an in-depth interpretation of genetic control, in which cases proteins play the role of markers for the respective locus. The studies we have conducted using polyacrylamide gel electrophoresis allow us to analyze the genetic control of total water-soluble proteins in zebra mussels.

Six zones (SP-1, SP-2, SP-3, SP-4, SP-5, and SP-6) were found in the protein spectrum of the total extract. The results obtained in the present study give grounds to assume that the synthesis of proteins from these zones is controlled by (at least) six loci: Sp-1, Sp-2, Sp-3, Sp-4, Sp-5, and Sp-6 (Figure 4). Polymorphism was found in three of these loci. The expression of only one fraction in the zones SP-1, SP-2, and SP-6 from the spectrum of the analyzed individuals suggests monomorphism at the Sp-1, Sp-2, and Sp-6 loci. There were some differences in the intensity of the SP-2 fraction, which was more pronounced in the samples treated for 96 hours with 30% and 50% Cypermethrin. In the SP-3 zone, three fractions with different electrophoretic mobility were visualized. The intensity and distribution of the three fractions in the studied spectra give grounds to allow monolocus control with three codominant alleles: Sp-3^{100}, Sp-$3^{87,}$ and Sp-3^{83}. The expression of two fractions in the SP-4 region of the spectrum of the studied individuals demonstrates a probable monogenic control of a polymorphic locus represented by the Sp-4^{100} and Sp-4^{94} alleles. In the SP-5 region of the soluble protein spectrum, two bands with low electrophoretic mobility are present. Their expression suggests the presence of an allelic polymorphism at the Sp-5 locus with codominant alleles Sp-5^{100} and Sp-5^{90}. Homozygous and heterozygous individuals at polymorphic loci are reported.

The nature of the expression of soluble proteins in the spectra of the studied individuals *Dressena polymorpha* identifies them as suitable markers for the analysis of genetically determined polymorphism in populations of this species.

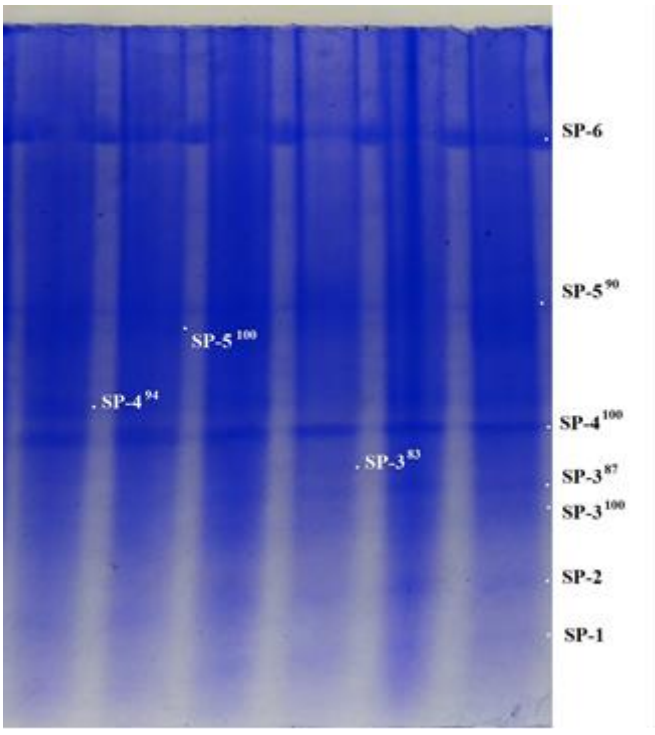

Figure 4. *Spectrum of soluble proteins in individuals of the species Dressena polymorpha (7.5% PAGE).*

Conclusion

The probable genetic control of four enzyme and protein groups – malate dehydrogenase, malate enzyme, superoxide dismutase, and soluble proteins – was determined by the isoenzyme analysis conducted. Nine variable loci have been identified, based on which the degree of genetic heterogeneity in zebra mussel populations can be characterized. Different intensities of the expression of some allelic products have been found in individuals exposed to short- and long-term exposure to the pesticides Cypermethrin and Chlorpyrifos. The observed variability in the expression of superoxide dismutases, malate dehydrogenases, and soluble proteins in control and experimental samples gives us reason to discuss these enzyme and protein systems as markers for reporting the negative impact of the tested pesticides on the zebra mussel. Allelic products with altered expression are probably related to the protection of the organism from harmful effects, which suggests that the increased levels of expression in experimental compared to control samples are associated with anthropogenic stress.

A more detailed comparative analysis of specific population-genetic parameters on the studied enzyme and protein loci in the future would allow the identification of suitable genetic markers for reporting the effects of the studied insecticides on *Dressena polymorpha* species.

Acknowledgements

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References

Gosling E, Astanei J, Was-Barcz A. 2008. Genetic variability in Irish populations of the invasive zebra mussel, *Dreissena polymorpha*: Discordant estimates of population differentiation from allozymes and microsatellites. Freshwater Biology, 53(7): 1303-1315.

Ivanova EN. 1996. Variability in *Apis mellifera* L. - ontogenetic and population-genetic aspects. Author's abstract of the dissertation for the award of educational and scientific degree "Doctor". (in Bulgarian)

Johnson FM, Schaffer HE, Gillaspy JE, Rockwood ES. 1969. Isozyme genotype-environment relationships in natural populations of the harvester ant, *Pogonomyrmex barbatus*, from Texas. Biochemical genetics, 3(5): 429-450.

Maurer G. 1971. Electrophoresis disk. Mir: Moscow, 247 p. (in Russian)

Müller J, Wöll S, Fuchs U, Seitz A. 2001. Genetic interchange of *Dreissena polymorpha* populations across a canal. Heredity, 86(1): 103–109.

Nevo E, Beiles A, Ben-Shlomo R. 1984. The evolutionary significance of genetic diversity: Ecological demographic and life history correlates. Lecture Notes in Biomathematics, 53: 13-213.

Pichot P, Pichot Y. 1980. Electrophoretic polymorphism: A means of studying populations. Review of the Works of the Maritime Fisheries Institute, 44(3): 201-210. (in French).