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T1-P-32 Sensitivity of midgut phosphatases to thermal stress in Gypsy moth (*Lymantria Dispar*) caterpillars

Larisa Ilijin, Anja Grčić, Marija Mrdaković, Milena Vlahović, Dragana Matić, Dajana Todorović, Vesna Perić Mataruga[99]

KEYWORDS: gypsy moth, alkaline phosphatase, total acid phosphatase, increased temperature, thermotolerance

INTRODUCTION: Environmental temperature directly affects the development of phytophagous insects, and indirectly through their host plants. Alkaline phosphatases (ALP) and total acid phosphatases (tot ACP) are midgut enzymes included in metabolic processes. The previous contact of the insect populations with various stressors and their ability to overcome the effects of the raised temperature (thermotolerance) can modify the response of these enzymes to increased environmental temperature.

OBJECTIVES: We aimed to compare the differences in responses of midgut ALP and tot ACP, with the expression of their isoforms, to increased environmental temperature with and without induced thermotolerance, in gypsy moth 5th instar caterpillars from unpolluted and polluted habitats.

METHOD / DESIGN: Caterpillars were hatched from egg masses collected in unpolluted (UP population) and polluted forest (PP population). They were reared at 23°C (PP23 and UP 23) and 28°C (PP28 and UP28) until the 3rd day of the 5th instar. In both populations, a group of individuals was exposed to 28°C for 24 h (induced thermotolerance) at the beginning of the 4th instar. Afterward, they were returned to 23°C until the sacrification (PP23In and UP23In) or exposed to 28°C for 72h before sacrification on the 3rd day of the 5th instar (PP28In and UP28In). The activity of enzymes was measured spectrophotometrically, using p-nitrophenyl phosphate (pNPP) as substrate, under alkaline conditions for ALP and acid conditions for tot ACP. Isoforms of both enzymes were detected on 12% polyacrylamide gel native PAGE.

RESULTS: In the UP groups, midgut ALP showed increased activity upon exposure to 28°C, with and without induced thermotolerance, while in PP caterpillars induced thermotolerance was the only factor that elevated ALP activity. Two way ANOVA analysis revealed that the interaction of temperature treatments and population origin (unpolluted vs polluted forest) was extremely significant ($F_{3,67}$=27.6, p<0.0001) for changes in midgut ALP activity, as well as the individual influence of increased temperature ($F_{3,67}$=30.9, p<0.0001) and the origin of the population ($F_{1,67}$=28.6, p<0.0001). Three ALP isoforms were detected. Isoform 1 was present only in PP groups exposed to 28°C, second is present in all experimental groups, and the third showed lower band density in PP treatments in comparison to UP. In UP23In tot ACP activity was elevated, while in PP treatments it was decreased. The interaction of temperature and population origin was extremely significant for tot ACP activity (two-way ANOVA, $F_{3,72}$=10.48, p<0.0001). Four isoforms of tot ACP were detected on the gel. Isoform 1 was present only in PP groups, isoform 2 has higher density in both populations and all treatments in comparison to controls. High band density of isoform 3 is present in all experimental groups, while induced thermotolerance and increased temperature, in both populations, increased band density of isoform 4.

CONCLUSIONS: Increased environmental temperature and induced thermotolerance have different effects on the activity of both enzymes in caterpillars from unpolluted and polluted habitats. ALP activity was more sensitive to thermal treatments in individuals originating from the unpolluted forests, in comparison to those from the polluted habitats, where on the other

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hand, a completely new isoform was detected upon exposure to increased temperature. Tot ACP activity was decreased in all treatments in caterpillars from polluted habitats and a new isoform band was detected on native gels, while in those from the unpolluted forest, induced thermotolerance affected the activity of tot ACP. Obtained results indicate the differences in sensitivity to an increased environmental temperature between populations with different histories of exposure to pollution and that they must be considered as well.

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T1-P-33 Investigations of the presence of anthropogenic marker for wastewater contamination of the Danube

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KEYWORDS: caffeine; Danube; HPLC

INTRODUCTION:

Caffeine is a purine alkaloid found in more than 60 plant species (coffee seeds, cocoa, and teas). It is mostly used in the production of food (80%), medicines (16%), and cosmetic products (4%). It is an integral part of various drinks (coffee, tea, caffeinated soft drinks), certain food products (chocolate), and medicines where it acts as a cardiac, cerebral, and respiratory stimulant and as a diuretic. Caffeine is found to be a good indicator for human sewage because of its unambiguous anthropogenic origin. The main paths for caffeine to enter the wastewater stream are either from urine or when caffeine-containing products are discharged through household pipelines or sewers.

OBJECTIVES:

The main goal of this study is to determine the presence of caffeine in the Danube samples as an anthropogenic marker for wastewater contamination of the Danube.

METHOD / DESIGN:

Analysis was performed by solid-phase extraction (SPE) followed by reversed-phase high-performance liquid chromatography (HPLC). The chromatography used a Zorbax Eclipse XDB-C8 column (4.6 mm x 150 mm, i.d., 5 µm particle size) at 25°C, with a mobile phase of water/THF (0.1 % THF in water, pH 8) – acetonitrile (85:15, v/v). The flow rate was 0.9 mL/min, and detection by DAD at 273 nm. The samples were collected during September 2019 at ten representative locations of the Danube on the territory of Novi Sad, Serbia, and stored in amber bottles at 4 °C until analysis.

RESULTS:

The caffeine was ubiquitously detected in samples from all ten locations with concentrations ranging from 305,94-375,97 ng/L. Maximum risk indexes (MaxRIs) for resident organisms (fish) in the Danube were calculated for each sampling site and the results showed that all MaxRIs belong to class II (10<MaxRI<100).

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