SENSITIVITY OF MIDGUT PHOSPHATASES TO THERMAL STRESS IN GYSPY MOTH CATERPILLARS

*Larisa Ilijin, Grčić Anja, Mrdaković Marija, Vlahović Milena, Matić Dragana, Filipović Aleksandra and Perić-Mataruga Vesna

Department of insect physiology and biochemistry, Institute for Biological Research „Siniša Stanković“, National Institute of Republic of Serbia, University of Belgrade, Despot Stefan Blv. 142, 11060 Belgrade, Serbia

*E-mail: lararid@ibiss.bg.ac.rs

INTRODUCTION: Environmental temperature has a direct impact on the development of phytophagous insects, and an indirect, through influence on their host plant composition. The negative impact of increased temperature on gypsy moth caterpillar`s survival and growth, magnified with longer exposure time (*Banahene et al, 2018*). In insects, midgut phosphatases activity are sensitive parameters for estimation of stress exposure. Their sensitivity increases with an enlarged stress intensity and prolonged exposure to the stressor. **Alkaline phosphatases (ALP)**, usually located in insect`s midgut microvilli and basal membrane, and **total acid phosphatases (tot ACP)**, present in insect midgut cells, are involved in numerous metabolic processes, like ion transport, water resorption, excretion, growth, etc.; while their primary role is phosphomonoester hydrolysis and transphosphorylation (*Terra and Ferreira, 2012*). Production of phosphate ions is essential in energy metabolism and of immense importance during stress, as well. Previous contact of population with various stressors, but also their ability to overcome the effects of the raised temperature (thermotolerance), can modify the response of these enzymes to increased environmental temperature.

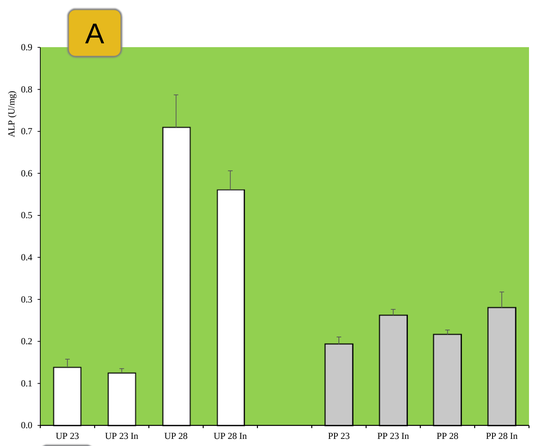
OBJECTIVES: Our aim was to compare the differences in responses of midgut **ALP** and tot **ACP** and expression of their isoforms to increased environmental temperature, with and without induced thermotolerance, in gypsy moth 5th instar caterpillars from unpolluted and polluted forests.

METHOD / DESIGN: Caterpillars were hatched from egg masses collected in unpolluted (mixed oak forests at Kosmaj Mountain, **UP population**) and polluted forest (Lipovica forest, **PP population**). Larvae were fed on an artificial diet designed for *L. dispar* and food was replaced every 48 h.

Each experimental group contained between 50 and 60 larvae	
UP23 and PP23	larvae reared at 23 °C from hatching to sacrifice
UP23In and PP23In	Larvae reared at 23 °C from hatching until the first day of 4th instar, and then exposed to 28 °C for 24 h (induced thermotolerance). Afterwards they were returned to 23 °C until the third day of 5th larval instar.
UP28 and PP28	Larvae reared at 23 °C from hatching until the first day of 5th larval instar, and then exposed to 28 °C for 72 h.
UP28 In and PP28In	Larvae reared at 23 °C from hatching until the first day of 4th instar, and then exposed to 28 °C for 24 h (induced thermotolerance). They were then returned to 23 °C until the first day of 5th larval instar and exposure to 28 °C for 72 h.

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** (a modified method of *Nemec and Socha, 1988*). Isoforms of both enzymes were detected on 12% polyacrylamide gel native PAGE using a modified method of *Allen et al.* (1963). The **ALP** isoform activity was visualize by soaking the gels in the alkaline incubation mixture with 0.1 % Fast Blue B. The **tot ACP** isoforms were visualized in 0.3% Fast Blue B stain dissolved in acetate buffer until bands became visible.

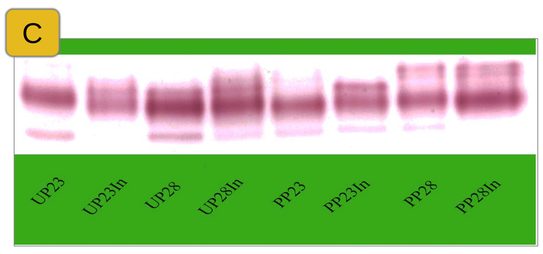
A



B

The interaction of temperature treatment and population origin				
F	DFn	DFd	P	
27.6	3	67	<0.0001	***
The effect of temperature treatment				
F	DFn	DFd	P	
30.3	3	67	<0.0001	***
The effect of population origin				
F	DFn	DFd	P	
28.36	1	67	<0.0001	***

C



D

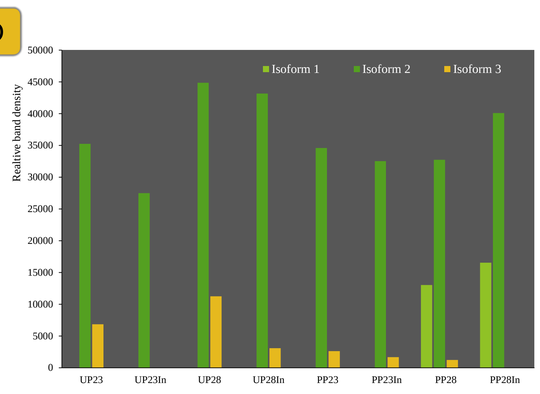
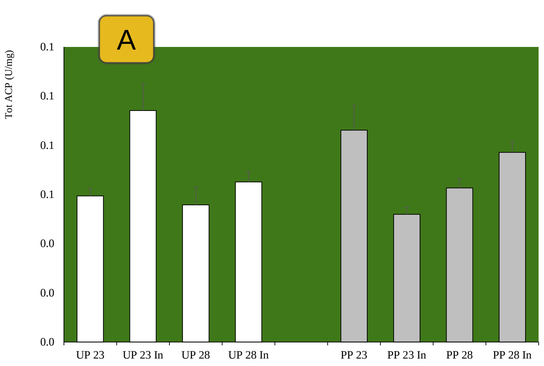
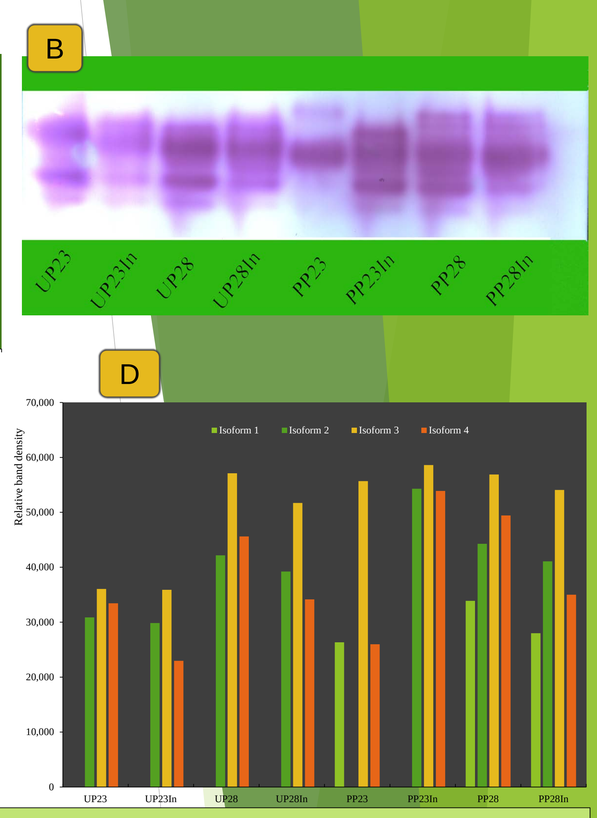


Figure 1. The specific activity of **ALP** (A). Results are presented as means ± S.E.M. (n=11); Two way ANOVA analysis (B); native PAGE gels stained for **ALP** (C) with densitometric analysis of the bands (D)

A



B



C

The interaction of temperature treatment and population origin				
F	DFn	DFd	P	
10.48	3	72	<0.0001	***
The effect of temperature treatment				
F	DFn	DFd	P	
2.05	3	72	0.1151	
2.05				
F	DFn	DFd	P	
0.03	1	72	0.8533	

Figure 2. The specific activity of **tot ACP** (A). Results are presented as means ± S.E.M. (n=11); Two way ANOVA analysis (B); native PAGE gels stained for **tot ACP** (C) with densitometric analysis of the bands (D)

RESULTS AND CONCLUSIONS: Since **ALP** and **ACP** provide phosphate ions from ribonucleoproteins and mononucleotides, they play a vital role in the intoxication and development of resistance to environmental stressors in insects (*Srebrov et al., 2006*). Increased environmental temperature and induced thermotolerance have different effects on the activity of both enzymes in caterpillars from unpolluted and polluted habitats. In 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. **ALP** activity was more sensitive to thermal treatments in individuals originating from unpolluted forests, in comparison to those from polluted habitats, where induced thermotolerance increased the enzyme activity.

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), while their individual influence was not. Four isoforms of **tot ACP** were detected on 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 population, increased band density of isoform 4. Induced thermotolerance had the effect on the activity of **tot ACP** activity in caterpillars originating from unpolluted forest, while in caterpillars from polluted habitats it was decreased in all treatments and a new isoform band was detected on native gels.

Obtained results indicate that **ALP** was more sensitive to elevated temperature regardless induced thermotolerance, in comparison to **tot ACP**, whose activity increased only after induced thermotolerance. In caterpillars from polluted forest both enzymes were less sensitive to increased environmental temperature and induced thermotolerance. Therefore, different sensitivity of analyzed parameters to increased environmental temperature in populations with different histories of exposure to pollution must be considered as well.

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