# A novel function of heat inducible expression of insect antifreeze protein genes from the desert beetle *Microdera punctipesnnis*

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*Microdera punctipennis* (Tenebrionidae) is a special species in the desert region of Central Asia. An antifreeze protein (MpAFP698) from this beetle showed high heat stability. *Mpafps* expression is the lowest, but quite obvious, in summer. In considering the hot and dry desert conditions, we suspect that these expressed MpAFPs may play role in help the beetles adapt to the harsh environment. Thus, we need to check if the expression of *Mpafps* is heat inducible. If so, what other functions do these proteins have?

The adult insects were treated at 42℃. RT-qPCR was employed to detect the mRNA level of *Mpafp*s. *MpAFPS77*, a cDNA cloned from the summer insects, was expressed in *E.coli* as MBP-MpAFPS77. The protective function of *MpAFPS77* was assayed by determining the $OD_{600}$ of *MpAFPS77* protected bacteria and yeast respectively after they were heat treated. The protective function was also indicated by the lactate dehydrogenase (LDH) activity protected by MpAFPS77 at 65 ℃.

RT-qPCR results indicated that the expression of *Mpafps* was up-regulated by heat. MBP-MpAFPS77 displayed higher osmolality than the control protein MBP. The $OD_{600}$ of the bacteria with the MBP-MpAFPS77 protection at -20 ℃ and 50 ℃ were higher than that of MBP control,respectively. For yeast, the $OD_{600}$ of the cells after recovering from 42 ℃ with MpAFPS77 protection was higher than the control. This protein significantly increased LDH activity. The relative conductivity of the bacterial cultures with MpAFPS77 protection at -20 ℃ and 50 ℃ were significantly lower than that of MBP control. Thermogravimetric analysis showed that MpAFPS77 had strong water-holding ability compared to MBP.

The presence of AFP transcript in insects under summer conditions has been proposed to be a protection against a sudden temperature decrease in the autumn or the spring. Another possible role may be involving in water conservation and re-absorption. The TGA results demonstrated that MpAFPS77 has high water-holding capacity. This property verified the structure model that *T.molitor* AFPs form a dimer with a rank of water in between. This is in consistent with the heat inducible expression of *Mpafps* in the present work. The MpAFP may response to heat and dry stress by adsorbing more water so that to protect the beetle cells from the damage, which was confirmed by the thermal protective effect of MpAFPS77 to *E.coli* and yeast. This unusual function of antifreeze protein will expand our knowledge to this wonderful protein.

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