Comparative metabolomic profiling of desiccation tolerant midge

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There are organisms of different complexity, which can survive complete water loss conditions. Among them are well known bacterial and fungal spores, plant seeds, some nematodes, rotifers and tardigrada. The most complex known organism with ability to withstand severe dehydration is larvae of African chironomid *Polypedilum vanderplanki*. During water depletion, larvae cells synthetize trehalose and a number of protective proteins – Lea, Hsp, thioredoxins, etc (Gusev et al., 2014). At the same time larvae pump out water and accumulate trehalose up to 18% of their dry mass (Watanabe et al., 2002). To get more understanding in mechanisms of resistance to complete desiccation we obtained detailed metabolomics profile in larva undergoing dehydration and further rehydration. We also performed such profiling for closely related species *P. nubifer,* which is sensitive to water loss.

Metabolome analysis was performed using CE-TOFMS in two modes for cationic and anionic metabolites. Peaks detected in CE-TOFMS analysis were extracted using automatic integration software (MasterHands ver. 2.16.0.15). The profile of peaks with putative metabolites were represented on metabolic pathway maps using VANTED (Visualization and Analysis of Networks containing Experimental Data) 4) software.

In the current study, a total of 272 metabolites (144 metabolites in Cation mode and 128 metabolites in Anion mode) were identified to provide insights into the specific metabolic pathways essential for the acquisition of desiccation tolerance in sleeping chironomid. The comparative analysis revealed drastic changes in the metabolic profile of both chironomid species upon entering desiccation state. In *P.vanderplanki* we also observed rapid shift

immediately after start of rehydration process. The metabolite composition with altered concentration differed in sister species, suggesting preparation to stress conditions and further exit from ametabolic state in tough species. It is also confirmed by the observation that concentration of key accumulated metabolites is drastically decrease during first hour of rehydration, whereas gene expression of proteins involved in the same pathway does not change much. Surprisingly, in spite of known burst of reactive oxygen scavenging proteins upon desiccation we observed no changes in reduced glutathione level. It suggests that glutathione is not involved or take minimum part in antioxidant protection unlike in other organisms under oxidative stress conditions, which surely accompanies water loss. Moreover, the concentration of gamma-glutathione cysteine – the glutathione precursor is rapidly increasing. From the other side, whole transcriptomic assay showed that Glutathione peroxidase is highly expressed during late stages pf desiccation. Taken together these facts allow us to hypothesize Glutathione peroxidase might use thioredoxin as reducing agent, so that its enzymatic activity is not associated with hydrogen peroxide elimination. Such peculiarity of antioxidant enzyme was shown for fruit fly Drosophila (Missirlis et al., 2003).

From the other side the concentration of glutathione precursors is rising, but glutathione synthase transcription level is not increasing suggesting that gamma-glutathione cysteine is used for phytochelatins synthesys. Thus, there is high chance of the existence of new type of phytochelatin-like ROS-scavenging peptides in this insect.

Another highly abandoned metabolite at full dehydration stage is adenosine. When water become available, it rapidly decrease its concentration suggesting its role in antioxidant defense.

One of the most abandon metabolite during desiccation is L-kynurenine. This compound is precursor of important neuroprotection agent kynurenic acid. The synthesis of kynurenic acid is catalyzed by kynureninase. Interestingly, this enzyme in absent in other insect and genome analysis showed that gene encoding for kynureninase was acquired by horizontal gene transfer event.

We have also noted accumulation of a set of glycogen-associated metabolism, suggesting strong involvement of accumulated trehalose in successful revival, takes place upon rehydration of the larvae.

Thus, metabolic profiling together with whole trascriptomic assay showed involvement of antioxidant defense system in cell protection during complete water loss and the enhancement of oxidative stress response system when compare with other organisms. Obviously, we confirmed the role of trehalose in formation of ametabolic state, but also we found that trehalose is necessary for revival. We also found the increase in metabolites participating in specific protection of neuronal cells, which means that tissue specific mechanisms of protection against severe desiccation were developed in *P. vanderplanki*.

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