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Molecules of Life: Towards New Horizons



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two fold axis into hexamer with 32 point symmetry. Revealed type of PNP hexamer is different from usual hexameric molecules of PNPs which have disc-like shape. The inter-subunit contacts in ThthPNP trimers and hexamer are determined. The peculiarities of polypeptide folding in the protein subunit, the nearest surrounding of phosphate ion located in the active site and positions of amino acid residues responsible for purine base recognition are described.

P-07.5-03

Investigation of the role of the electron bifurcating hydrogenase Hnd in the metabolism of an anaerobic, sulfate-reducing bacterium using NMR-based metabolomics techniques

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The genome of the anaerobic, sulfate-reducing bacterium *Desulfovibrio fructosovorans* encodes for 6 different hydrogenases. The most recently characterized is Hnd, a cytoplasmic heterotrimeric enzyme (HndABCD) that carries out flavin-based electron bifurcation, a newly described mechanism of free energy conservation in anaerobic microorganisms. In the presence of H₂, Hnd couples the exergonic reduction of NAD⁺ to the endergonic reduction of a ferredoxin, previously published in Kpebe A et al. (2018) BBA Bioenergetics 1859, 1302–1312. We have failed to establish the specific metabolic role of Hnd in *D. fructosovorans*, due to a probable compensation mechanism between the other hydrogenases. Deletion mutants of hndC and hndD are non-lethal, and the overall yield during respiratory growth is unaffected. We found that the hnd deletion mutant, when grown on pyruvate with limited sulfate, produces more H₂ than the WT strain, suggesting that Hnd functions as an H₂-consuming hydrogenase in these conditions. We determined that a metabolomics approach would provide a comprehensive view into the metabolic modulations caused by Hnd. The hnd deletion mutant and WT strain were grown in pyruvate/sulfate medium with diminishing concentrations of sulfate, corresponding to respiration, mixed (limited sulfate) and fermentation conditions. Metabolic profiling was carried out using ¹H NMR spectroscopy, and a discrimination between the metabolic profiles of the hnd mutant and the WT strains was obtained across the different growth conditions. Surprisingly, we found that during fermentative growth, the hnd mutant accumulates acetone instead of ethanol in the culture medium, indicating a the presence of a metabolic shunt arising from the deletion. These data demonstrate Hnd's global importance to the energetic metabolism of the cell, but the implicated metabolic pathways still need to be fully elucidated.

P-07.5-04

Co-expression networks and gene expression analysis: different energy supply strategies of Baikal and Holarctic amphipods under temperature stress

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Temperature is one of the most important environmental factors as it exerts a direct effect on all biological processes, from molecular to behavioral. The aim of this study was to assess interspecific differences in energy metabolism in two Baikal endemic amphipods and one species widely distributed in the Holarctic exposed to gradually increasing temperature (0.8°C per day). Thus, the expression of genes involved in key metabolic processes genes was analyzed. All investigated amphipod species are characterized by different energy-supply strategies under temperature increase and we found interspecific differences at the level of co-expression of the studied genes. Gradual temperature increase strongly affected energy metabolism and stress response in the thermosensitive Baikal endemic amphipod *E. verrucosus*; moreover correlation network analysis showed clear disorganization of gene correlation networks. In the more thermotolerant Baikal endemic amphipod *E. cyaneus* there was a switch to anaerobic energy production, insertion of glycolytic transcripts and extinction of ATP turnover-related transcripts in/out correlation network when exposed to increasing temperature. Temperature increase led to an increase in aerobic capacity in *G. lacustris* (ubiquitous Holarctic amphipod) mirrored by rising activity of aerobic capacity indicators. Baikal endemic amphipods showed more pronounced gene expression changes under gradual temperature increase than the Holarctic ubiquitously spread amphipod *G. lacustris*. The study was carried out with the financial support of the Russian Science Foundation and Helmholtz Association grant № 18-44-06201.

P-07.5-05

Effects of vanadate on antioxidant systems in mycelium of fungus *Phycomyces blakesleeanus*

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Fungi represent the main route of vanadium's entrance in ecosystem and are known to tolerate high concentrations of this transition metal. Despite it, its effects on antioxidant systems of fungi are not well-known. The aim of this research was to examine the effects of vanadate on antioxidant enzymes and non-enzymatic antioxidants of filamentous fungus *P. blakesleeanus*. Mycelium in early exponential (20h) and stationary (56h) phase of growth was treated with relatively high concentrations of V⁵⁺ (1, 5 and 10 mM) for 1h or 5h. Activities of peroxidase (POD), glutathione reductase (GR), glutathione transferase (GST), glutathione peroxidase (GPx), concentrations of total phenols and total

glutathione were examined. In the 20h old mycelia, V^{5+} in all concentrations, after 1h treatments, induced decrease in total phenols with the largest decrease ($25 \pm 4\%$) after 10mM V^{5+} treatment, while in 56h old mycelia decrease in total phenols was noticed only after 1 mM V^{5+} treatment ($11 \pm 3\%$). Decrease of total glutathione, in 56h old mycelia, was noticed for all concentrations after 1h treatments, and the largest $38 \pm 7\%$ was induced by 5 mM V^{5+} . The same effect was noticed in 20h old mycelia treated for 5h, with the largest decrease of $29 \pm 7\%$ after 5 mM V^{5+} treatment. In 56h old mycelia, after 1h treatments with 10 mM V^{5+} , activities of GPx and POD increased, while after 5h treatments, concentration dependent increase in activities of GST, GPx and GR was noticed, with the largest increase of $112 \pm 30\%$ for GPx, $74 \pm 39\%$ for GST and $68 \pm 26\%$ for GR. Increase in the activities of GPx and GST was noticed in 20h old mycelia treated for 5h, with 5 mM and 10 mM V^{5+} and also increase of POD in 56h old mycelia, was noticed in same treatments, but the only statistically significant increase was after treatment with 10 mM V^{5+} in 56h old mycelia ($43 \pm 10\%$). The results shown indicate that after 1h treatments, V^{5+} influenced primarily on non-enzymatic antioxidants and after 5h treatments on enzymes.

P-07.5-06

Specialized DNA polymerases of extremophile bacteria of the *Deinococcus phylum*

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Extremophile bacteria of the *Deinococcus phylum* show remarkable resistance to UV and gamma radiation. The molecular pathways ensuring genome stability in these bacteria under stress conditions are only partially understood. Two *Deinococcus* species, the model bacterium *D. radiodurans* and recently characterized *D. gobiensis*, encode specialized DNA polymerases of the X-family (both species) and Y-family (*D. gobiensis*). These polymerases can potentially participate in the replication of damaged DNA containing various lesions, caused by different types of radiation. In this study, we investigated the expression levels of these polymerases in both species under DNA damaging conditions *in vivo*. Utilizing recombinant proteins, we showed *in vitro* that the X-family polymerases from both species surprisingly do not possess the DNA polymerase activity, due to substitutions of key residues in the polymerase active site, but exhibit 3'-5' exonuclease and AP(apurinic site)-endonuclease activities in the presence of Mn^{2+} ions. We localized the active site responsible for these activities in the so-called PHP domain of polymerases. Both of these activities may potentially play a role in DNA repair pathways. Recombinant Y-family polymerase from *D. gobiensis* was also tested for the ability to pass various DNA lesions such as 8-oxoguanine, O-6-methylguanine, AP-sites, thymine dimers, and 1,N6-ethenoadenine. It was shown that this polymerase has an increased ability to replicate damaged DNA in comparison with replicative polymerases. Together, our data provide new insights into the functions of specialized DNA polymerases in DNA repair in DNA-damage resistant bacteria. This project was in part supported by the Russian Foundation for Basic Research and Russian Science Foundation (17-14-01393). *The authors marked with an asterisk equally contributed to the work.

P-07.5-07

Helichrysum italicum: crown jewel of Mediterranean phytomedicine

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Medicinal extremophiles represent an outstanding reservoir of bioactive compounds with unique biological properties. *Helichrysum italicum* (Roth) G. Don is an iconic Mediterranean plant, well adapted to this dry and saline environment, i.e. a xero- and halotolerant plant, respectively. It is better known under the name Immortal or Everlasting, due to its never-fading flower-heads that preserve their yellow colour when dry. The essential oil and organic solvent extracts of *H. italicum* have been studied extensively in the last two decades, but research on traditional extracts is rather scarce. For this purpose, we investigated aqueous whole plant extracts of *H. italicum* grown in the Slovenian coastal region for possible health-promoting properties. Dried plant material was ground and used for hydroalcoholic maceration or water infusion. The extracts were evaluated *in vitro* for their free radical scavenging activity and protective effect against induced oxidative stress. Furthermore, they were tested for cytotoxicity on mammalian enteric cell lines and subjected to gene expression analysis using Human Clariom™ S GeneChip. The whole plant extracts showed high DPPH radical scavenging potential, similar to ascorbic acid. They also managed to protect cells against t-BOOH-induced oxidative stress substantially. At the same dilution, the infusion was toxic to the colorectal adenocarcinoma cell line Caco-2 and non-cytotoxic to primary enteric fibroblasts. Exploratory grouping analysis showed clear clustering into three groups depending on the treatment applied (cells treated with *H. italicum* for 6 h or 24 h and untreated cells). A detailed transcriptome analysis is currently underway. Oxidative stress is an underlying cause of several degenerative diseases that can be prevented by the use of antioxidants. In this context, *H. italicum* can be viewed as the Mediterranean panacea, and its extracts have the potential to be developed as ingredients for dietary supplements.

P-07.5-08

The recorded α -L-fucosidase from *Saccharolobus solfataricus* and its transcript regulation *in vivo*

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The decoding of genetic information is a dynamic mechanism in which, in some genes and in specific physiological conditions, the standard rules can be altered by programmed deviations of the ribosomes, a phenomenon called recoding. Translational recoding has been found in all three domains of life and has crucial roles in the regulation of gene expression (Previously published in: Atkins J.F. et al. (2018) NAR 44(15): 7007–78). Increasing evidences suggests that the flexibility of genetic decoding might have relevant implication in physiology, being a trait selected during evolution that may increase microbial fitness under certain conditions (Previously published in: Ling J. et al. (2015) Nat. Rev.