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Functional characterisation of AnHMGB-A and AnHMGB-B „high mobility group" proteins of *Aspergillus nidulans*

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Aspergillus nidulans is an important modell-organism, many metabolic and regulation pathways and the genome sequence of this organism is known. The two proteins of our interest are members of the „high mobility group B" protein family that are present in both lower and higher eukaryotes.These proteins affect the expression of various genes on chromatin. Due to their DNA and protein binding ability they have an effect on the stability of chromatin-remodelling and transcription-initation complexes on chromatin.

Our main goal was to explore the phsyological role of AnHMGB-A and AnHMGB-B proteins. We deleted the coding sequences of the two proteins from the genome and constructed single and double deleted mutants that were subjected to various experiments to compare their phenotype to the wild type (wt).

The mycelia of Δ*AnhmgB-A* strain showed decrased growth rate, abnormal shape of cell wall and altered osmotic tolerance in comparison to the wt. Abnormal distribution of reactive oxygen species within the mycelia (central location for mutant and peripheric for the wt) was observed by nitro-blue tetrazolium staining that could result the decreased growth rate and compact growing of the mutant. We observed decreased trehalose level of mycelia by thin layer chromatography (TLC) and HPLC analysis that could be accounted for the aberrant cell wall formation and the elevated osmotic sensitivity.

In case of Δ*AnhmgB-B* strain we observed abnormal morphology of the mycelia, drastic decreasement of viability of the asexual spores (52% in wt and 0.35% in mutant at 37°C) and increased sensitivity of the mycelia to oxidative stress. The spores of wild-type and mutant strains were subjected to metabolite analysis by GC-MS analysis that revealed a singinficantly lower level of xylitol and trehalose content in the deleted spores, which could explain the decresed viability. To find out the reason of the oxidative stress sensitivity of mutants, SOD (superoxide-dismutase) content of deleted and wild-type strains were compared, and significant differences in relative ratio of SOD isoenzymes were detected.

The mycelia of double-deleted mutant showed nearly complete inhibition of growth in thermo-stress condition (at 42°C). To find out the cause of the thermosensitivity, a comparative transcriptome sequencing was carried out by „Next Generation Sequencing" (NGS). The analysis process of transcriptom data are in progress. The preliminary results has already revealed that the mutant strain cannot maintain the expression activity of that of wt either at 37 or 42°C. It seems that the transcriptional discrepancies affect the whole chromatin in the mutant. For example, wt strain increases the transcription of 293 genes and decreases the transcription of 540 genes when temperature is shifted from 37 to 42°C. When these data are compared to that of the mutant we observed that out of the normally upregulated 293 genes in wt 52 genes were downregulated and 82 genes were overexpressed. Similarly, transcription activity changed in the downregulated population of 540 genes, where 94 genes were upregulated instead of downregulation and 123 genes were significantly downregulated than that was observed in the wt. As a preliminary result of the NGS analysis the metabolic pathways leading to secondary metabolite production was assumed to be disturbed in the mutant. To prove our finding experimentally, the total metabolite composition of wt and mutant strain was monitored by TLC and we found that certain intermediate metabolic compounds are extremely accumulated in the mutant strain. We expect that several further experiments will be carried out on the basis of the results of the transcriptome analysis. In the future, we would like to purify the two proteins to carry out „pull down" assays and DNA-binding experiments. The identification of the interacting proteins and DNA sequences would give a deeper insight into the function of the proteins at the molecular level.

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Changes in plant antioxidants and photosynthesis in response to abiotic stresses

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As a consequence of their sedentary habit, plants are exposed to changing environmental conditions which may cause stress. A common effect of these abiotic stress factors is the rapid generation of reactive oxygen species (ROS) which are also found at very low concentrations in unstressed plants. Plants have evolved different enzymatic (SOD, POD, etc.) and non-enzymatic (ascorbic acid, phenols, carotenoids, etc.) antioxidants to reduce the amount of these potentially harmful agents.

The elevated amount of ROS in plants is a signal of the antioxidant system being overwhelmed and is thus an important indicator of severe stress. Under these conditions, ROS are detectable directly, by a variety of modern biophysical methods. These, however, are not sensitive enough for detecting the ROS assumed to accompany moderate, acclimatory stress. Under these conditions, measuring changes in activity and quantity of the antioxidants is a widely used stress indicator in plant physiology. The aim of our work was to connect antioxidant

content, specific ROS scavenging capacities and acclimation potential of leaves under various moderate stress conditions.

Methods already exist for measuring total antioxidant capacities and for detecting enzymes specific to a certain ROS (like peroxidases or superoxide dismutases), however some of the ROS are not aimed by specific enzymes and are scavenged by the common effect of different compounds. Therefore we developed new methods for measuring antioxidant capacities of plant samples specific to the hydroxyl radical (Majer et al. 2010b) and the singlet oxygen, (Hideg and Majer 2010). By correlating the ROS specific and total antioxidant parameters we showed the importance of studying the former besides measuring the widely used latter parameters as well (Majer et al. 2010b). In addition, we developed a new method which serves us with an initial screening of photosynthesis in leaves based on digital images (Majer et al. 2010a).

Using the above techniques and chlorophyll-fluorescence-based assessment of leaf photochemistry, we studied contributions of preventive and antioxidant processes to high-light tolerance in linden tree (*Tilia platyphyllos* L.) leaves collected from sun-exposed and shaded parts of the same tree. According to our results, linden sun leaves had 2-times stronger singlet oxygen neutralizing capacities than shade leaves and were able to avoid non-regulated loss of energy under high PAR (Hideg and Majer, 2010). In sun linden leaves significantly higher amounts of flavonoid glycosides were found and contributions of various phenolics to specific ROS capacities are currently investigated.

In an other series of experiments we studied the acclimation potential to UV-B radiation in younger and older grapevine leaves (*Vitis vinifera* L. cv. Chardonnay) and found that younger leaves were able to mobilize screening pigments and antioxidants better and therefore suffered less damage from UV-B as compared to older ones (Majer and Hideg 2011).

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The role of oxidative stress in antibiotic resistance evolution

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The emergence of bacteria that are resistant to multiple antibiotics represents an increasingly significant threat to public health today. There are a number of mechanisms whereby bacteria can develop antibiotic resistance including the physical exchange of genetic material with another organism, the activation of latent mobile genetic elements (transposons or cryptic genes) and the mutagenesis of its own DNA. Chromosomal mutagenesis may arise directly from antibiotic-induced oxidative stress, or indirectly, as a consequence of the interaction between antibiotic and its target molecule or from the activity of bacterium's error-prone DNA polymerases during the repair of DNA lesions.

There are at least three major mutational mechanisms which facilitate gradual evolution of bacterial antibiotics resistance. These mechanisms include mutators (genotypes with increased, constitutive mutation rates), SOS response (a global response that minimizes the lethal and mutagenic consequences of the exposure of cell to DNA damaging agents) and the direct mutagenic effect of reactive oxygen species (ROS). We investigated how far these three above mentioned mechanisms are linked to each other, and what their relative contribution to antibiotics resistance evolution is.

It is known from literature that bactericidal (but not bacteriostatic) antibiotics share a common lethal pathway that involves the generation/accumulation of ROS. In our study we focused on the role of ROS in antibiotic resistance evolution. The effect of ROS has a double edged sword feature. On one hand ROS production contributes significantly to the killing effect of bactericidal antibiotics. On other hand, by directly damaging DNA, ROS accumulation increases the mutation supply of the bacterial cell which promotes the appearance of antibiotic resistant strains.

We employed a series of 10-15 days-long laboratory evolutionary experiments with *E. coli BW25113*. The used strains were initially sensitive to antibiotics and differ only in their respective constitutive genomic mutation rates (wild type *BW25113* versus ΔmutS) and/or activities of the SOS response (wild type versus LexA3 expressing strain or ΔmutSLexA3). 96 independent lineages of each bacterial strain were allowed to evolve in microtiter plates to successively higher antibiotic concentrations by transferring daily 1% of each culture. We employed three antibiotics: ciprofloxacin, ampicillin, tobramycin, representing three major classes of bactericidal antibiotics (quinolones, β-lactams and aminoglycosides respectively) known to stimulate the production of ROS. As a negative control, we also tested the bacteriostatic trimethoprim, a folic acid biosynthesis inhibitor. In order to test the contribution of ROS formation on evolvability we added thiourea to the medium. Thiourea is a hydroxyl radical scavenger which mitigates the damage caused by ROS formation upon antibiotic treatment.