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.

We found that thiourea substantially promotes extinction of evolving bacterial populations for all three cases of bactericid antibiotics treatments both in the case of mutators and non-mutators. Our results indicate that scavenging of reactive oxygen species by thiourea substantially reduces mutational input, and hence the corresponding populations had lower opportunity to evolve resistance against the used antibiotic. Thiourea treatment had no significant impact on extinction rates of the populations adapted to the bacteriostatic drug trimethoprim. We found that the impact of ROS formation on resistance evolution is at least partially independent of the SOS response. Our work suggests that enhancement of ROS formation may be useful for improving antibiotics lethality in the short term, but it also accelerates drug resistance evolution.

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Investigation of a novel stress-induced operon in *Synechocystis* PCC 6803

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Cyanobacteria are photosynthetic prokaryotes that are widely used model organisms of photosynthetic research according to their close relationship to chloroplasts. These prokaryotes possess a variety of adaptation mechanism by which they are able to inhabit different extreme biotops. Since they are autotrophic organisms they are the primary producers of these niches. Among others, arsenic compounds and hydrogen sulfide are widely distributed in nature since the origin of life. Cyanobacteria have evolved mechanisms to cope with the toxicity of these substances. In order to maintain their homeostasis under fluctuating environmental changes cyanobacteria evolved environmentally inducible detoxification systems.

We investigated the expression pattern of different genes upon heavy metal treatment in *Synechocystis* sp. PCC 6803 and discovered a novel gene cluster that responds to As^{3+}. This operon is located on the pSYSM plasmid and contains four genes, termed *artRSCT*. *ArtR* and *artS* are transcribed in the opposite direction of that of *artC* and *artT*, with a common promoter region between these two tandem gene pairs.

The gene product of *artR* shows high homology with DNA-binding proteins of the ArsR family. We have constructed a deletion mutant in order to inactivate the *artR* gene. In this mutant we observed high constitutive expression of the *artS/C/T* genes using quantitative RT-PCR technique. This fact supported our hypothesis that it functions as a common repressor of the genes. We have cloned and expressed this protein in *E. coli* in order to obtain further proof of its function. Using this purified protein we performed an electrophoretic mobility shift assay, which confirmed the specifity of ArtR towards the promoter sequence. Taken all these results into consideration we have proven that the protein product of *artR* is a regulator of the *artRSCT* operon, which binds to the common promoter and dissociates in the presence of As^{3+}.

ArtT shows high homology with membrane-bound heavy metal transporter proteins and because it is induced by As^{3+}, we assumed that it may be part of an arsenic resistance system. In order to test this hypothesis we have constructed an *artT* insertional mutant. The results were contradictory to our hypothesis because the mutant was more resistant to elevated amounts of As^{3+} then the wild type. Thus the function of *artT* may be different from that of the arsenic resistance genes.

The protein product of *artS* gene is homologous to sulfide-quinone oxido-reductases (SQR) and shows a high expression not only to As^{3+} but to Na_2S as well. Moreover *Synechocystis* PCC 6803 was more resistant to high amounts of Na_2S then other cyanobacterial species that lacks the SQR enzyme.

H_2S and As^{3+} can serve as alternative electron donors in many ancient anaerobic photosynthetic systems. The procaryotes capable of this type of electron transport use a single photosystem (PSI) and harbor a SQR enzymes, which oxidize sulfide to sulfur. Other strains can use arsenite oxidase enzyme to grow phototrophically in As^{3+} containing anaerobic environment. The facts that the *artRSCT* genes are induced by As^{3+} and Na_2S and the strain is more tolerant to Na_2S, than those which lack such genes suggest that these genes, especially *artS* may be involved in sulfide tolerance or sulfide metabolism of the cyanobacterium.

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