

and a smaller, heme c binding cytochrome c subunit (FccA). Sulfide quinone oxidoreductases are monomeric membrane-bound flavoproteins which present in all domains of life. Sqr can transfer electrons from sulfide directly into the membrane quinone pool while Fcc reduces periplasmic c-type cytochrome proteins.

*Thiocapsa roseopersicina* is a photosynthetic purple sulfur bacterium. Three genes encoding sulfide oxidizing disulfide oxidoreductases were identified in the genome sequence: *fcc*, *sqr* and *sqn*. The Sqr and Sqn belong to group IV and group VI of the Sqr-type proteins, respectively. A detailed comparative biochemical, structural and functional analysis of these proteins is in the focus of this study.

The FccAB complex, the FccB, the Sqr and the Sqn proteins fused to Strep II affinity tag were expressed in *T. roseopersicina* strains. The recombinant flavocytochrome c variants and the Sqn enzyme could be purified to homogeneity by affinity chromatography. In the absorption spectra of the oxidized and reduced forms of FccB, FccAB and Sqn, characteristic peaks of redox active flavin prosthetic group were identified. The flavin moiety apparently bound covalently to the proteins. The flavocytochrome c had also a redox active heme cofactor non-covalently bound to the FccA subunit. The Fcc variants were subjected to ultrafast fluorescence kinetic measurements in order to determine the interaction between the FAD cofactor and the protein. The affinity purified recombinant FccAB could oxidize sulfide and was able to reduce bovine heart cytochrome c at low sulfide concentrations. The temperature and pH dependences of the activity of the recombinant Fcc complex were determined: the optimal temperature was 45 °C while the optimal pH was 8.0. The FccAB was a moderately thermostable enzyme which had remarkable activity up to 60 °C. The recombinant Sqn and Sqr catalyzed the sulfur-dependent quinone reduction. The temperature and pH optima of quinone reductase activity of the Sqn were the same as determined for FccAB. Kinetic analysis of the Sqn activity at various pH revealed a lag phase preceding the reaction at high pH. This might mean that the enzyme needed activation for being able to reduce quinones at alkaline conditions. Additionally, the macromolecule structure of the Sqn was analyzed to explore the connections between the quaternary structure and the catalytic properties of the protein. Enzyme kinetic parameters of the Sqn disclosed that the enzyme affinity for sulfide was low as compared to other well-known sulfide quinone oxidoreductases. Consequently, Sqn might play role in the sulfide oxidation at high sulfide concentration. In contrast, the FccAB could have important function at low sulfide concentration in the sulfur metabolism in *T. roseopersicina*. The structural and functional analyses of the wild and mutant flavocytochrome c might lead to better understanding of the structure/function relationships of the disulfide oxidoreductase protein family. On the other hand, the biochemical and biophysical characterization of the Sqn should disclose specific properties of the group VI. of the Sqr-type proteins.

Supervisor: Gábor Rákhely, András Tóth  
E-mail: duzs.agnes@gmail.com

## Heavy metal induced nitro-oxidative stress in *Brassica* species

Gábor Feigl

NO-signalling group, Department of Plant Biology, University of Szeged, Szeged, Hungary

Copper (Cu) and zinc (Zn) are essential micronutrients, which can be present in soils naturally or can be accumulated in the environment due to anthropogenic activities. Cu is a redox-active element, directly inducing the formation of reactive oxygen species (ROS) leading to oxidative stress. Zn, on the other hand, is a non-redox-active element, causing oxidative stress indirectly by the modulation of antioxidant capacity. Moreover, in excess, both metal trigger changes in the metabolism of reactive nitrogen species (RNS), such as nitric oxide (NO) and peroxynitrite (ONOO<sup>-</sup>) leading to nitrosative stress. The oxidative and nitrosative signalling interact with each other resulting nitro-oxidative stress during which the cellular functions damage by lipid peroxidation and nitration, protein carbonylation, tyrosine nitration and S-nitrosylation.

The primary goal of my study was to determine the degree of nitro-oxidative stress in two metal tolerant *Brassica* species exposed to Cu or Zn. Furthermore, I wanted to draw conclusions about the Cu- and Zn tolerance and phytoremediation usability of the species.

Nine-days-old hydroponically grown *Brassica juncea* and *Brassica napus* were treated with 0 (control), 10, 25 and 50 µM CuSO<sub>4</sub> or 0 (control), 50, 150 and 300 µM ZnSO<sub>4</sub> in nutrient solution for 7 or 14 days. Changes in microelement contents, formation of different ROS and RNS, cell viability, lipid peroxidation, cell wall alterations and enzymatic- and non-enzymatic antioxidants were examined in the root system.

Most of the Cu and Zn taken up by the plants were retained in the roots; however, the increment of Cu and Zn content within the *Brassica* shoots indicated an efficient translocation. Both metals in excess markedly modified the microelement homeostasis of *Brassica* plants. Both Cu and Zn treatment caused significant morphological alterations in the root system of *Brassica* species, e.g Cu and Zn were able to increase the lateral root number, especially in *B. juncea*, which may be part of a morphological adaptation process. A Cu concentration-dependent decrease of cell viability was also found after both 7 and 14 days of treatment; however in short term *B. juncea* root meristem did not show Zn-induced viability loss. Also, cell wall alterations were notable, since intensified lignification and callose formation were detected in the root system of Cu-stressed plants; however excess Zn caused only increased callose deposition.

Exposure to Cu induced nitric oxide generation in the root tips and this event proved to be dependent on the duration of the exposure and on the plant species. In short- and long-term treatments, *B. juncea* showed more significant activation of superoxide dismutase (SOD), inhibition of ascorbate peroxidase (APX) and oxidation of ascorbate (AsA) than *B. napus*. Moreover, hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>)-dependent lignification was also observed in the Cu-exposed plants. In longer term, significant AsA accumulation and callose deposition were observed,

reflecting serious oxidative stress in *B. juncea*.

Due to the short-term Zn stress, SOD and APX showed higher activities in the roots of *B. juncea* keeping the amount of superoxide anion ( $O_2^-$ ) and  $H_2O_2$  at a control-like or lower level. Contrary, NO and ONOO $^-$  showed significant accumulation as the effect of Zn exposure. Despite the elevation of ONOO $^-$  levels, there was no detectable lipid peroxidation, which may indicate that it has a role in stress tolerance in *B. juncea* roots.

In the background of the serious growth inhibition and the viability loss of *B. napus* roots severe oxidative stress was observed: despite the elevated SOD activity  $O_2^-$  accumulated, while the cells failed to eliminate the formed  $H_2O_2$  because of the reduced APX activity. Moreover, a remarkable lipid peroxidation was visualized in the roots.

Long-term Zn excess caused oxidative and nitrosative stress in both species and despite their higher level in *B. juncea* root tips, it proved to be more tolerant according to the growth parameters.

Based on the morphological and physiological results, I conclude that *B. napus* tolerates Cu excess better than *B. juncea*. In contrast, *B. juncea* possesses elevated Zn tolerance compared to the other species. My results support the species-specificity of metal tolerance.

Supervisors: László Erdei, Zsuzsanna Kolbert  
E-mail: fglgbr@gmail.com

## Study of cuckoo-host relationships on a great reed warbler population in Hungary

Nikoletta Geltsch

Department of Ecology, University of Szeged, Szeged, Hungary

The brood parasitic common cuckoo (*Cuculus canorus*) lays its eggs to nests of other bird species, where the foster parents incubate, hatch and feed the cuckoo. A typical host species is the great reed warbler (*Acrocephalus arundinaceus*), breeds in wetland areas in Hungary, and builds open nest in reed beds. The modal clutch size of great reed warblers is 5 eggs and incubation time is about 11-12 days. We investigated several aspects of ecological relationships between common cuckoos and great reed warblers, including behavioural and evolutionary adaptations. However, we also applied microbiological and molecular methods.

In our first study, we examined bacterial loads on the eggshells of common cuckoos and great reed warblers. During our field work we collected samples from the eggshell surface of both cuckoo and great reed warbler eggs, either from parasitized and non-parasitized clutches to compare bacteria of the eggshells. We hypothesize that cuckoos, as nest visitors, may influence on the hygiene of nests of great reed warblers by changing bacteria loads. Previous studies showed that environmental factors, such as temperature and humidity, may affect bacterial loads on the eggshells in cavity nesting birds. We hypothesized that these environmental factors also affected the hygiene of open nests of great reed warblers. From these factors we measured ambient light conditions, both in the visible and UV spectra.

Keeping eggs dry in avian nests during the incubation period may reduce bacteria load on the eggshells, so it may protect the eggs from bacterial infections. A few previous studies have already showed the antimicrobial effects of incubation in cavity nesting birds, but, in the first time, we studied these effects under more variable environmental conditions, on an open-nesting bird species.

During the co-evolution arms race between common cuckoos and great reed warblers both the brood parasites and hosts developed ecological adaptations. The adaptations developed by the brood parasite help successful parasitism (e.g. "mimetic eggs"), but the adaptations by the hosts are against the brood parasites ("antiparasite adaptations", e.g. egg discrimination). We evaluated the changes of eggshell spottiness of common cuckoos and great reed warblers in time. Previously, we photographed parasitized clutches of host eggs held in museum collections ( Natural History Museum, Tring, Mátra Museum, Gyöngyös, and Hungarian Natural History Museum, Budapest), and we also took digital photos during our field work. All eggs were collected from Hungary. We had four treatments from the years of 1900s, 1930s, 1960s, and 2000s. For analysing images we used ImageJ and Matlab programs. We wanted to reveal how spottiness changed in common cuckoos and great reed warblers. We analysed these changes by statistical pattern analysis on eggs from the last hundred years, focusing on cuckoo egg mimicry to host eggs.

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Supervisor: Csaba Moskát  
E-mail: moskat@nhmus.hu