



Experiment title: Mechanisms of Zn sequestration in the Zn-tolerant ectomycorrhizal fungus *Suillus*

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30-02-808

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Report:

The chemical form of Zn accumulated in fungal mycelium of the ectomycorrhizal fungus *Suillus bovinus* was studied by Zn K-edge EXAFS spectroscopy. Two genotypes of *Suillus bovinus*, a Zn-tolerant (LS1) originating from a Zn-contaminated site and a Zn-sensitive (MG2) from a non-contaminated site were compared. The fungal mycelia were exposed to a Zn concentration range from 20 μ M Zn to 1 mM Zn.

In our previous experiment (EC 121), the spectra for the fungi exposed to the highest Zn concentration (1 mM Zn) were recorded in frozen hydrated state, whereas the spectra for the low concentration exposure (200 μ M) were recorded in freeze-dried state because the signal obtained on frozen hydrated samples was too low. Dehydration may induce some artefacts on Zn speciation, so it was necessary to repeat this experiment in frozen hydrated state (Guiné et al., 2006; Straczek et al., 2008). This is particularly challenging for the tolerant isolate, which accumulates much less Zn than the sensitive one (50 ppm Zn fresh weight compared to 130 ppm for the sensitive). Spectra of reasonable quality were obtained by increasing the thickness of the pellets as much as possible.

We have recorded the spectra for tolerant and sensitive isolates exposed to 1 mM, 500 μ M and 200 μ M Zn, and for the tolerant isolate exposed to 500 μ M after desorption of extracellular Zn to probe specifically intracellular Zn (the desorbed sensitive one had a too low Zn content).

All spectra were dominated by a single frequency typical of Zn bound to oxygen-containing ligands. Three Zn pools were identified including Zn bound to organic acids in solution, Zn bound to polysaccharides of the cell wall and Zn-organic acid complexes in solid state. Other candidate Zn species such as Zn phosphate found in fungi (Fomina et al., 2007; Fomina et al., 2006; Sarret et al., 1998) and in bacteria (Guiné et al., 2006) and Zn-metalllothionein often suggested (Gadd, 2007) but never identified in a fungus were clearly ruled out. Our hypothesis is that the first pool corresponds to vacuolar Zn, the second pool to extracellular Zn, and the localization of the third one is unclear. Organic acid concentrations are being analyzed by capillary electrophoresis.

Our results show that the proportion of Zn bound to organic acids in solution (vacuolar Zn) increased in both isolates with increasing Zn exposure (Fig. 1). For the highest concentration (1 mM Zn), we found almost no difference between the spectra of the two isolates. For the low concentration (500 μ M), the proportion of

vacuolar Zn was higher in the tolerant isolate. This is consistent with the higher proportion of vacuolar Zn as evaluated by kinetic desorption experiments using Zn radiotracer (Adriaensen et al., 2007).

It is not surprising to find comparable speciation, with a slightly higher shift to the cell wall fraction, at the low and high Zn treatment in the sensitive isolates since the internal Zn concentration was in the same order of magnitude.

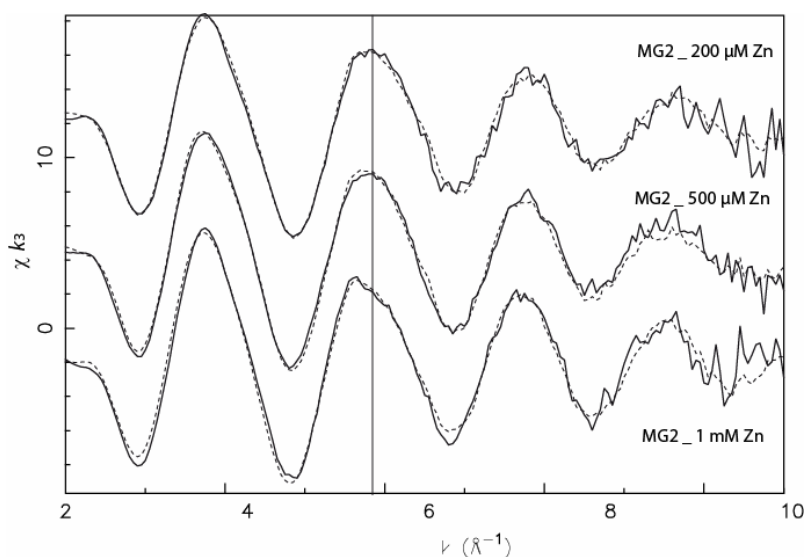


Figure 1. Zn K-edge EXAFS spectra of *S. bovinus* MG2 mycelia exposed for 24h to increasing external Zn concentrations and linear combination fits (LCFs).

In the next experiment we wish to compare roots of pine (host plant) mycorrhized by the tolerant or the sensitive isolate and non mycorrhized roots. We wish to determine the distribution of Zn chemical forms between the two symbiotic organisms. Such information will help to answer to the question: Why does the tolerant fungus confers a higher Zn tolerance to the host plant ? This experiment will be complemented by a micro-EXAFS experiment in April 2008.

References

- Adriaensen K., Van Hees M., Vangronsveld J., and Colpaert J. (2007) Altered Zn fluxes and compartmentation in the ectomycorrhizal fungus *Suillus bovinus* as mechanisms involved in Zn tolerance. *in preparation*.
- Fomina M., Charnock J. M., Bowen A., and Gadd G. M. (2007) X-ray absorption spectroscopy (XAS) of toxic metal mineral transformations by fungi. *Environ. Microbiol.* **9**(2), 308-321
- Fomina M., Charnock J. M., Hillier S., Alexander I. J., and Gadd G. M. (2006) Zinc phosphate transformations by the *Paxillus involutus*/pine ectomycorrhizal association. *Microbial Ecology* **52**(2), 322-333.
- Gadd G. M. (2007) Geomycology: biogeochemical transformations of rocks, minerals, metals and radionuclides by fungi, bioweathering and bioremediation. *Mycological Research* **111**, 3-49.
- Guiné V., Spadini L., Sarret G., Muris M., Delolme C., Gaudet J. P., and Martins J. M. F. (2006) Zinc sorption to three gram-negative bacteria: Combined titration, modeling, and EXAFS study. *Environ. Sci. Technol.* **40**(6), 1806-1813.
- Sarret G., Manceau A., Spadini L., Roux J. C., Hazemann J. L., Soldo Y., Eybert-Bérard L., and Menthonnex J. J. (1998) EXAFS determination of Pb, Zn complexing sites of *Penicillium chrysogenum* cell walls. *Environ. Sci. Technol.* **32**, 1648-1655.
- Straczek A., Sarret G., Manceau A., Hinsinger P., Geoffroy N., and Jaillard B. (2008) Zinc distribution and speciation in roots of various genotypes of tobacco exposed to Zn. *Environmental & Experimental Botany* **63**, 80-90.