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Report:

We studied the Zn speciation by bulk Zn K-edge EXAFS spectroscopy of two genotypes of the mycorrhizal fungus *Suillus bovinus*, a Zn-tolerant (LS1) originating from a Zn-contaminated site and a Zn-sensitive (MG2) from a non-contaminated site. The fungal mycelia were exposed to either a low (200 µM) or a high (1 mM) Zn concentration and different desorption regimes in order to distinct the different cellular compartments (cell walls, cytoplasm and vacuole) involved in Zn compartmentation and detoxification. Spectra were recorded on frozen hydrated samples using a He cryostat.

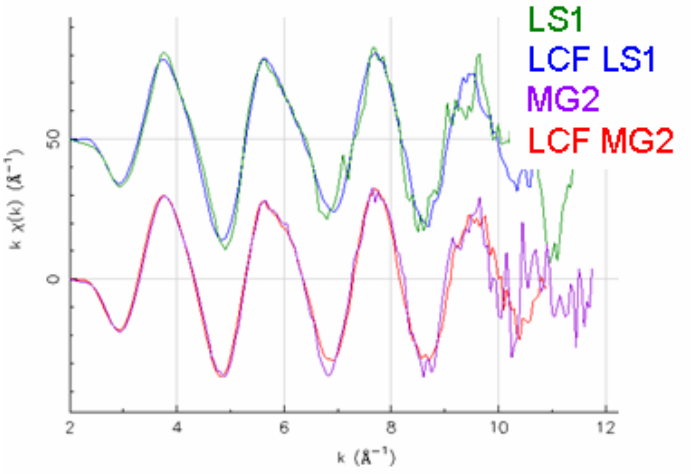

Figure 1. Zn K-edge EXAFS spectra of *Suillus bovinus* mycelia exposed for 24h to 1 mM Zn with their respective lineair combination fits (LCFs).

The spectra contain a dominant single frequency typical of Zn bound to oxygen-containing ligands. They differ by the shape of the second oscillation (figure 1). Linear combination fits of the spectra showed that Zn was present as Zn-organic acid (malate, citrate, succinate, etc…) complexes in solution in LS1, and that MG2 contained an additional species, i.e., Zn bound to the cell wall. 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-metallothionein often suggested (Gadd, 2007) but never identified in a fungus were clearly ruled out.

The local structure of Zn was studied in more detail by FEFF simulations (table 1). The first shell Zn-O distance determined is typical of octahedral coordination. This 6-fold coordination is consistent with Zn-organic acid complexes in solution. The radial structure functions display a second peak, which corresponds to a carbon shell.

Table 1. Structural parameters [a] determined by FEFF simulations of EXAFS spectra for Zn in reference compounds and in *Suillus bovinus* mycelia.

	first shell			second shell		
strain or sample	*CN* and element	*R* (Å)	σ^2 (Å^2)	*CN* and element	*R* (Å)	σ^2 (Å^2)
Zn oxalate	6.6 O	2.09	0.0077	6.04 C	2.82	0.0073
Zn citrate	4.6 O	2.04	0.0099	1.7 C	2.78	0.0076
Aqueous Zn	5.8 O	2.07	0.0075			
S. bovinus tolerant (LS1)	6.5 O	2.08	0.0120	3.1 C	2.85	0.0030
S. bovinus sensitive (MG2)	6.2 O	2.08	0.0080	2.9 C	2.87	0.0090

[a] *CN*: coordination number; *R*: interatomic distance (Å); σ^2: Debye-Waller disorder factor (Å^2). For the first shell simulations, an amplitude reduction factor (S_o^2) of 1.1 was used for all fits. Estimated errors on *R* and *CN* are 0.02 Å and 10%, respectively.

We only recorded slight differences in the spectra of the two isolates when exposed to 1 mM Zn. This can be due to the relatively high Zn concentration to which the mycelia were exposed. At this concentration cell damage was previously recorded, certainly in the sensitive isolate (Adriaensen et al., 2007). Unfortunately the low concentration tested yielded a fairly low signal on frozen hydrated samples, so it was necessary to freeze-dry the samples. However these results should be considered with care since the freeze-drying treatment might modify the geometry of the Zn local environment, although the nature of the Zn binding groups should not be affected (Guiné et al., 2006). We found more or less the same Zn speciation at 200 µM Zn for the Zn sensitive isolate MG2. Zn was also primarily bound to organic acid complexes and partially bound to cell wall components. It is not suprising to find the same speciation at the low and high Zn treatment in the sensitive isolates since the internal Zn concentration was in the same order of magnitude. On the contrary, in the tolerant isolate the differences in internal Zn concentration were much bigger between both Zn treatments, but unfortunately there was no time left to record these samples.

We believe that more data are necessary to draw straightforward conclusions on the possible differences between the two fungal strains. Also this experiment was performed in 16 bunches mode. Working in uniform mode should also improve the detection limit, and allow us the study of more diluted samples.

References

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