	Experiment title: **Cadmium stress response in isolated aquatic fungal cells (*Heliscus lugdunensis*)**	**Experiment number**: EC-712
Beamline: ID21	**Date of experiment**: from: October 20th 2010 to: October 26th 2010	**Date of report**: August 25th 2011
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Report:

Introduction

Some aquatic fungi have developed specific mechanisms to tolerate metals in the environment, but the details of these processes are various and unknown. *Heliscus lugdunensis* is an aquatic fungus isolated from a mining site contaminated with Cd and the aim of our proposal was to investigate its cellular and intracellular response when exposed to cadmium in a potential bioremediation process. Our objectives were to investigate isolated hyphae in order (1) to clarify the location of Cd in the different parts of the hyphae and to particularly determine if the tip cells play an important role in the Cd storage as often mentionned in the literature, and (2), to determine the Cd chemical forms in the various parts of the hyphae, and especially the role of sulfur ligands.

Materials and Methods

Heliscus lugdunensis isolated from a mining environment in Germany (Mansfelder Land) was grown in hydroponic conditions and exposed to 50 µM Cd and 0 µM Cd (control). After 5 and 7 days, hyphae were rinsed, isolated, deposited on ultralene films and frozen in liquid nitrogen to be studied as frozen hydrated samples.

Cd, S and P localization was done by chemical imaging using µ-XRF at 3555 eV (below the K K-edge, which is present in the samples and overlaps Cd L_{III} emission lines) and a beamsize on the sample of 0.7 µm x 0.3 µm. Then, the Cd ligands were studied using µ-XANES at Cd L_{III}-edge with the same lateral resolution, on regions of interest evidenced by µ-XRF. S forms were also investigated using S K-edge µ-XANES. S and

Cd reference compounds were also studied at Cd L_{III}-edge and S K-edge. All measurements were performed using the cryostat (~-170°C) to limit elemental redisribution and speciation change.

Results

To estimate the representativity of the sample, several isolated hyphae were investigated. Chemical maps displayed in Fig. 1 are typical of the observed hyphae after 5 days of Cd exposition. µ-XRF results show that the tip cells of the hyphae are depleted with Cd, and that the metal is rather concentrated in the oldest parts of the hyphae and particularly in the regions surrounding the septa. This is a new result, because the tip cells are often thought to concentrate the metal. Bicolor maps showed that Cd co-localized with S (not shown). After 7 days of Cd exposure, the morphology of hyphae changed and vesicles were observed, suggesting accute toxicity (Fig. 2A). Elemental maps show that these vesicles are highly enriched with Cd in association with S and P (Fig. 2B). Interestingly, control *Heliscus* hyphae are thinner than the Cd metal exposed hyphae, and the elemental distribution is modified. Chemical maps show that P and S do not co-localize (Fig.3). P is present in small vesicles disseminated along the whole hypha whereas S is more homogeneous. We can thus infer that the biological mechanisms of the hyphae are strongly affected by cadmium.

Cd XANES spectra collected on bulk contaminated *Heliscus* show that the main part of Cd is bound to sulfur ligands (Fig.4). The amount of Cd in tip cells of Cd exposed *Heliscus* displayed in Fig.1 was too low to collect Cd µ-XANES spectrum. µ-XANES spectra collected on the most enriched areas surrounding septa have poor quality and show an unexpected broad peak around 3540 ev because these areas were very small and we could not stay on the same spot during the collection of the whole spectrum (Fig.4).

S K-edge measurements show that in *Heliscus* exposed to Cd during 5 days, sulfur in bulk fungi is present under both reduced and oxidized species (Fig.5). However, it was not possible to collect S µ-XANES spectra on (S, Cd)-rich areas of isolated hyphae because spectra were modified under beam exposition, suggesting sample damage although the use of cryostat (Fig.5).

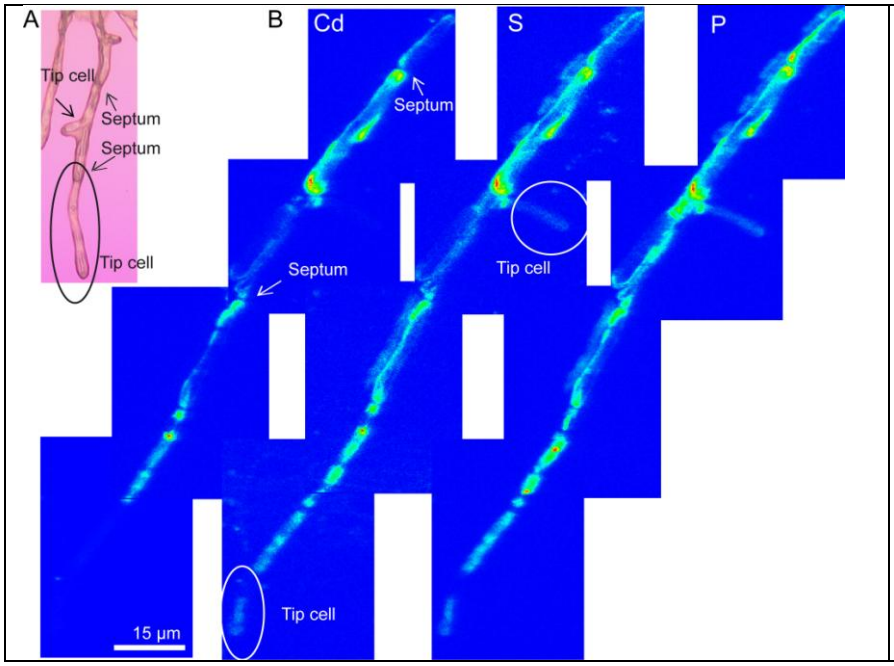

Fig. 1 : Optical microscope image of an isolated hypha of *Heliscus lugdunensis* exposed 5 days to 50µM Cd (A), and Cd, S, and P distribution obtained by µ-XRF at 3555 eV (B). Step = 0.25 µm, Counting time = 400 ms/pt, beamsize = 0.65 µm x 0.3 µm. Chemical imaging shows that Cd is not present in the tip cells but in the oldest parts of the hypha. It is also more concentrated on both sides of the septa.

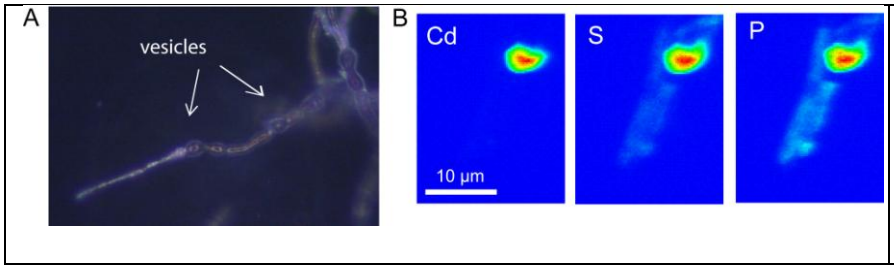

Fig. 2 : Optical microscope image of an isolated hypha of *Heliscus lugdunensis* exposed 7 days to 50µM Cd presenting vesicles (A), and Cd, S, and P distribution by µ-XRF (scans parameters are similar to Fig.1, B). Cd, S, and P are co-localized in vesicles.

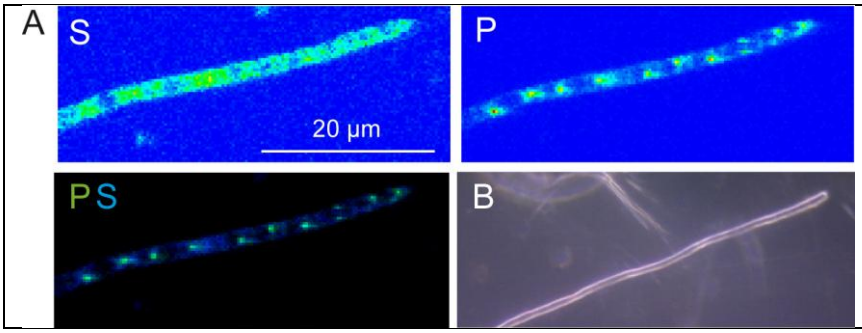

Fig. 3 : S, P distribution and bicolor map obtained by µ-XRF (A) (scans parameters are similar to Fig.1), and optical microscope image of an isolated hypha of *Heliscus lugdunensis* not exposed to Cd (Control).

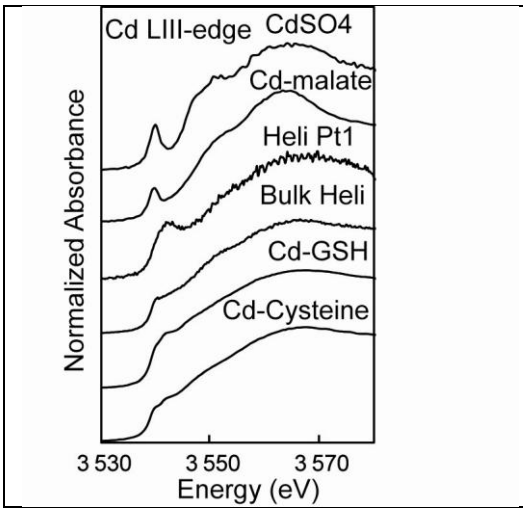

Fig.4 : Cd L_{III}-edge XANES spectra of *Heliscus lugdunensis* exposed to 50 µM Cd during 5 days compared to model compounds. Bulk Heli spectrum was recorded on bulk sample while Heli Pt1 spectrum was recorded on a (Cd,S) rich area of Fig.1. Bulk Heli spectrum shows that Cd ligands are essentially S. The quality of Heli Pt1 spectrum is very poor because it was tricky to stay on this small area during all the spectrum energy range (even with the focus tracking mode on). The large peak on the spectrum does not correspond to O/N ligands.

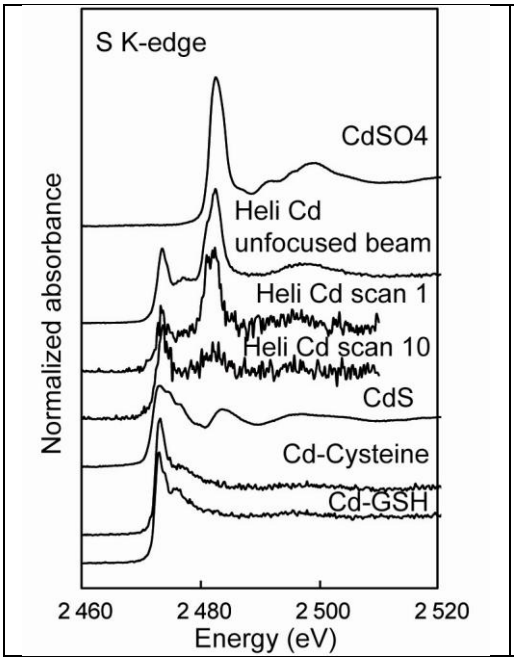

Fig.5 : S K-edge XANES spectra of *Heliscus lugdunensis* exposed to 50 µM Cd during 5 days compared to model compounds. Heli Cd spectrum was recorded on bulk sample with a non-focused beam and spectra did not evolve under beam exposure. Heli Cd scan 1 and 10 spectra were recorded with a focused beam on isolated hyphae and evolve under beam exposure : the proportion of sulfates decreased while sulfides increased, probably due to photoreduction.

Conclusions

This experiment allowed to show that Cd was not localized in the tip cells of *Heliscus* hyphae, but was stored in older areas. This is a new result. Cd was also found to be associated with S ligands. We also found that vesicles were formed after a long metal exposure and that Cd was mainly stored in these vesicles. The role of these vesicles is still unknown. A publication is in progress.