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VARIABILITY OF COORDINATION COMPLEXES OF COPPER ACCUMULATED WITHIN FUNGAL COLONY IN THE PRESENCE OF COPPER-CONTAINING MINERALS

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The aim of work was to elucidate the mechanisms of bioaccumulation of copper leached from minerals by fungus *Aspergillus niger* with great bioremedial potential due to its ability to produce chelating metabolites and transform toxic metals and minerals. The special attention was paid to the chemical speciation of copper bioaccumulated within fungal colony in the process of fungal transformation of copper-containing minerals.

Chemical speciation of copper within different parts of the fungal colony was studied using solid-state chemistry methods such as synchrotron-based X-ray absorption spectroscopy providing information about the oxidation state of the target element, and its coordination environment. The analysis of the obtained X-ray absorption spectroscopy spectra was carried out using Fourier transforms of Extended X-ray Absorption Fine Structure regions, which correspond to the oscillating part of the spectrum to the right of the absorption edge.

Results of this study showed that fungus *A. niger* was involved in the process of solubilization of copper-containing minerals resulted in leaching of mobile copper and its further immobilization by fungal biomass with variable coordination of accumulated copper within fungal colony which depended on the age and physiological/reproductive state of fungal mycelium. X-ray absorption spectroscopy data demonstrated that copper accumulated within outer zone of fungal colony with immature vegetative mycelium was coordinated with sulphur–containing ligands, in contrast to copper coordination with phosphate ligands within mature mycelium with profuse conidia in the central zone of the colony.

The findings of this study not only broaden our understanding of the biogeochemical role of fungi but can also be used in the development of various fungal-based biometallurgy technologies such as bioremediation, bioaccumulation and bioleaching and in the assessment of their reliability.

The main conclusion is that coordination environment of copper bioaccumulated within fungal biomass via the process of transformation of copper minerals is heterogeneous varying from sulphydryl to phosphate.

Key words: bioremediation, metal bioaccumulation, fungi, copper, mineral transformation, chemical speciation, coordination complexes, X-ray absorption spectroscopy.

Toxic impacts of heavy metals in the environment have led to intensive research on various methods of toxic metal bioremediation, and the central thrust of bioremediation depends on microbiology [1–3].

The ability of such microorganisms as fungi to survive and flourish in metal-rich environments, coupled with their capacity to transform a huge variety of metal species, makes them ideal candidates for both bioleaching and bioremedial systems. These metal–resistant characteristics depend upon their intra- and extracellular detoxification strategies, which can be manipulated to ensure either the solubilisation or sequestration of a specific element. Intracellular resistance depends on the immobilization of the metal to prevent damage to essential cellular machinery. This is achieved by the action of metal-binding proteins, or sequestration of the metal in the fungal vacuole [2, 4–6]. Extracellular responses meanwhile, depend upon the action of protons or organic acids that are excreted into the surrounding medium, or bound in the extracellular polysaccharide matrix [4, 7–9].

Determination of metal speciation in such biological systems has been a challenging problem because of the amorphous state or poor crystallinity of metal complexes within biomass and relatively low metal concentrations. There were a few studies for

fungal biomass that mainly clarify the nature of metal adsorption sites on cell walls [10–13]. However, synchrotron–based element–specific X-ray absorption spectroscopy (XAS) provides a means for studying element complexation in environmental samples varying from biological to mineralogical in nature [11–16]. XAS is an element specific technique which gives information about the oxidation state of the target element, and its coordination environment, including the number and identity of neighbouring atoms [17]. It allows studies involving fast data collection, small samples, low concentrations, both crystalline and amorphous solids and even solutions. XAS is also a non-destructive, non-invasive method that could probe metal transformations at the mineral-microbe interface transformations directly studying samples in their natural, often hydrated, states which makes it an ideal approach for investigating metal transformations at the mineral-microbe interface in biogeochemical systems [16, 17].

This work was focused on XAS-studies of the chemical speciation of copper bioaccumulated within fungal colony in the process of fungal transformation of copper-containing minerals.

Materials and methods

The fungal strain *Aspergillus niger* van Tieghem (ATCC 201373) from the collection of microorganisms of the Division of Molecular Microbiology, College of Life Sciences, University of Dundee, UK was used in this study.

A. niger was exposed to model copper-containing minerals azurite and malachite in the Petri-dish microcosms previously designed for studies of fungal ability for mineral transformations [18, 19].

Azurite [$Cu_3(CO_3)_2(OH)_2$] and malachite [$Cu_2(CO_3)(OH)_2$] were crushed and ground using a mortar and pestle and sieved to ensure a final grain size of less than 400 μm. The resulting powder was sterilized by rinsing with deionised water for at least 8 h with intermittent agitation on an orbit shaker. The samples were then immersed in 70% ethanol for at least 24 h, after which the solution was decanted and the samples left to dry in a sterile flow hood. Once dry, they were oven-sterilized at 80 °C for at least 24 h.

Malt extract agar (MEA, Oxoid, UK) was prepared with malachite or azurite by adding sterilized portions of the relevant mineral to create a concentration of 10 mM in the final plates. Dialysis membrane was used to cover the agar and separate the fungus from the medium, but allow the colony easy access to nutrients. Inocula consisted of one 7 mm diameter core cut from a colony of *A. niger* grown overnight on MEA medium. Plates were sealed with parafilm and incubated at 25 °C for the duration of the experiments.

Fungal biomass was harvested after 7 days of growth. Different parts of fungal colony were cut out with sterile razor blade.

Freshly harvested biomass was enclosed into cellotape and quenched in liquid nitrogen, was used for further X-ray absorption spectrometry.

X-ray absorption spectra at the Cu K-edges were collected on Station 7.1 at the CCLRC Daresbury SRS operating at 2 GeV with an average current of 140 mA, using a vertically collimating plane mirror and a sagitally bent focusing Si(111) double crystal monochromator detuned to 80% transmission to minimize harmonic contamination. Sample data were collected with the station operating in fluorescence mode using a 9-element solid state Ge diode detector with high count-rate XPRESS processing electronics; spectra of model compounds were collected in the transmission mode. The monochromator was calibrated using a 5 mm Cu foil. Experiments were performed using a liquid nitrogen cooled cryostat. Single scans were collected for the model compounds, and 3–4 scans were collected and summed for each sample. Model compounds used were Cu-acetate, Cu-gluconate, Cu-malate, Cu-oxalate, $Cu_3(PO_4)_2$ and Cu_2O.

Extended X-ray Absorption Fine Structure (EXAFS) regions of the obtained XAS spectra, which corresponded to the oscillating part of the spectrum to the right of the absorption edge, were used in spectra analysis. Background-subtracted EXAFS spectra were analyzed in EXCURV98 using full curved wave theory [20, 21]. Phaseshifts were derived in the program from *ab initio* calculations using Hedin–Lundqvist potentials and von Barth ground states [22]. Fourier transforms of the EXAFS spectra were used to obtain an approximate radial distribution function around the central copper atom (the absorber atom); the peaks of the Fourier transform can be related to «shells» of surrounding back scattering atoms characterized by atom type, number of atoms

in the shell, the absorber-scatterer distance, and the Debye-Waller factor, $2\sigma^2$ (a measure of both the thermal motion between the absorber and scatterer and of the static disorder or range of absorber-scatterer distances). The data were fitted for each sample by defining a theoretical model and comparing the calculated EXAFS spectrum with the experimental data. Shells of backscatterers were added around the copper and by refining an energy correction Ef (the Fermi energy), the absorber-scatterer distance, and Debye-Waller factor for each shell, a least squares residual (the *R*-factor [22, 23] was minimized). Where appropriate, multiple scattering effects were included in the fits [24].

Results and Discussion

Copper is a trace element essential to life, yet, at high doses it can be toxic. Copper makes a significant contribution to global pollution [25]. The origin of copper pollution is very diverse. In addition to natural origins, mainly from rock weathering and atmospheric deposition, its wide human use in many fields (transportation, manufacturing, currency, construction and agriculture as fungicide and herbicide) generates releases into the environment.

Both processes of metal mobilization (e.g., *in situ* soil washing, extraction and filtration techniques) and immobilization (e.g., *in situ* stabilization techniques) may be applied to remediate contaminated matrices [2, 29]. Many fungi can be highly efficient biogeochemical agents with capability for both metal mobilization and immobilization [4].

Fungi are able to solubilize minerals and weather rocks in the course of «heterotrophic leaching» as a result of protonation (acidolysis), chelation (complexolysis) and metal accumulation by the biomass [7, 30]. Fungal and plant cell walls can act as a cation exchanger due to their negative charge originating from functional groups, e.g. carboxylic, phosphate, amine or sulfhydryl, in different wall components (hemicelluloses, pectin, lignin, chitin, etc.) [4, 11, 13]. Mechanisms for metal immobilization within plant and fungal biomass also include intracellular uptake with complexation to ligands such as S-containing peptides (metallothioneins, phytochelatins), carboxylic acids (citrate, malate, oxalate), and phenolic acids [4, 6, 8, 12, 14, 15, 31].

A ubiquitous fungus *Aspergillus niger* is one of the most efficient transformers of minerals due, first of all, to its ability to over-excrete citric, oxalic and other low molecular weight carboxylic acids [32]. Various strains of *A. niger* are used in the industrial preparation of citric acid (E 330). The ability of this fungus to produce chelating metabolites, combined with its resilience to challenging environment and its uncomplicated and inexpensive nutritional requirements, makes it ideal candidate for bioremedial treatment. Both its solubilizing and metal–immobilization characteristics can be exploited to improve the condition of solid waste, contaminated soil and polluted water. *A. niger* has been previously shown to solubilize various copper–containing minerals [32].

In order to elucidate the mechanisms of bioaccumulation of copper leached from minerals by *A. niger*, we used synchrotron-based element-specific X-ray absorption spectroscopy technique which gives information about the oxidation state of the target element, and its coordination environment, including the number and identity of neighbouring atoms [17]. XAS allows studies involving fast data collection, small samples, low concentrations, both crystalline and amorphous solids and even solutions.

In the experiments with *A. niger*, grown on malt extract agar medium containing azurite or malachite, coordination of bioaccumulated copper by sulphur-containing ligands was found for samples with new vegetative mycelium taken from the outer zone of the fungal colony (Table; Fig. 1, *A*, *C*). A typical example of the best fit to the inner coordination sphere was with 3 sulphurs at 2.26 Å which was significantly improved by the addition of 2 copper scatterers at 2.68 Å clearly indicating the formation of a copper sulphide phase.

In contrast, on both azurite- and malachite-containing media the samples from the central part of *A. niger* colonies with mature aging mycelium and abundant dark-coloured conidiophores with conidia demonstrated phosphate coordination of copper (Table; Fig. 1, *A*, *D*).

Thus, the age and therefore the physiological and reproductive state of fungal mycelium was found to play a crucial role in the formation of coordination environment of metal within the biomass.

The observed variation in coordination complexes of copper in *A. niger* demonstrates that the relative significance of copper resistance mechanisms may vary with the age of the colony. We suggest that the sulphydryl

Cu K-edge EXAFS parameters for copper compounds observed in the biomass of *A. niger* colonies grown on MEA containing 10 mM azurite or malachite

Sample	Scatterers	r/Å	$2\sigma^2/Å^2$	Residual
Fungal colony grown on azurite medium				
Outer zone	3 x S 2 x Cu	2.23 2.60	0.033 0.040	33.6
Inner zone	4 x O 2 x O 2 x P 2 x Cu	1.95 2.53 2.89 3.70	0.022 0.022 0.018 0.018	34.4
Fungal colony grown on malachite medium				
Outer	3 x S 2 x Cu	2.26 2.68	0.017 0.028	37.5
Inner zone	4 x O 2 x O 2 x P	1.94 2.48 3.00	0.032 0.022 0.046	31.9

Notions. Table shows the values obtained from the EXAFS analysis of copper in the biomass of *A. niger* where *r* is the copper-scatterer distance in Angstroms (±0.02 Å inner shells, ±0.05 Å outer shells), $2\sigma^2$ is the Debye–Waller type factor (±15% inner shells, ±30% outer shells), which is a measure of both the thermal motion between the absorber and scatterer and of the static disorder or range of absorber–scatterer distances. The residual is a least squares residual from fitting the spectrum of the model to the experimental data.

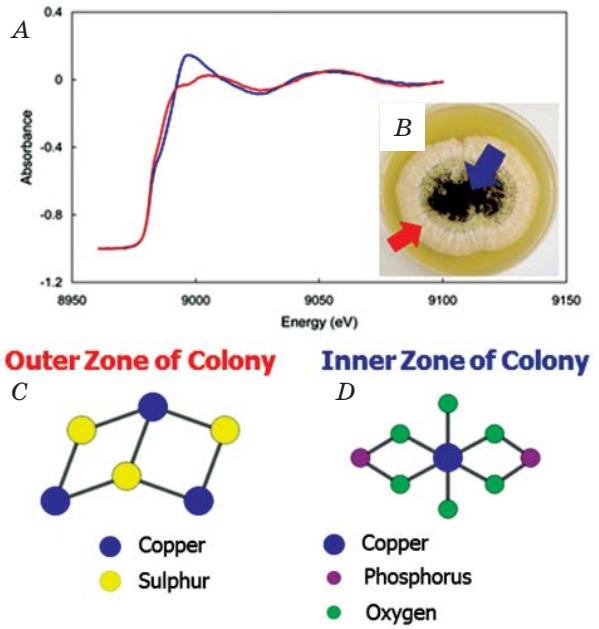

Fig. 1. **Copper coordination in *A. niger* grown on MEA with 10 mM malachite:**
(*A*) XAS spectra displayed entirely different characteristics for (*B*) outer non-sporulating (red arrow) and inner sporulating (blue arrow) areas of the fungal colony. EXAFS analysis suggested that (*C*) copper in the outer zone of the colony was present as copper sulphide, while (*D*) copper in the inner region of the colony was coordinated by phosphate ligands

coordination of copper in the immature outer mycelia originates from the association of copper with metallothioneins. These are small cysteine-rich proteins which are often produced in response to heavy metals introduction and are thought to play an important role in their homeostasis and sequestration [33, 34]. The metallothioneins were first reported in the equine kidney and named because of their extremely high content of sulphur — 4.1% (DW) and cadmium — 2.9% (DW) [35]. These metal–binding proteins have since been found throughout the animal, plant and fungal kingdoms [6]. Metallothioneins have been shown to be particularly important for copper homeostasis in yeast [36], while more recently the presence of copper has been shown to be the controlling factor in metallothionein synthesis in *Neurospora crassa* [34].

There is no current evidence that metallothioneins are found in the extracellular environment so it seems likely that the majority of copper chelation in the young parts of the colony occurs intracellularly. This suggests that the external components of the copper resistance pathway may not be fully developed in immature areas of the fungal colony. Proteins are not ideal for long-term sequestration of copper, as they must be constantly replaced, so efflux or incorporation of the toxic metals into insoluble forms is essential. We propose that it is this shift to a more permanent mode of storage that causes the copper coordination by phosphate ligands in the mature parts of the colony. The activity of acid phosphatases, which have been linked to heavy metal resistance in fungi, increases with increased copper concentration and varies with colony age [37, 38]. These enzymes could mediate the transferal of the polypeptide-bound copper to a more stable form such as polyphosphates.

It has been reported that the different groups of microorganisms accumulate inorganic phosphates intracellularly. Confocal laser scanning microscopy studies revealed single and aggregated cigar-shaped polyphosphate granules present in both vacuoles and cytoplasm of yeast *Saccharomyces cerevisiae* as well as in fungal hyphae and macroconidia of *Fusarium solani* [39]. Polyphosphates are used by ectomycorrhizal fungi to immobilize metals within their vacuoles [40] which would provide the long-term metal storage and protection from the toxic effects.

Thus, these findings can be summarized in the diagram illustrating the overall transformation of copper–containing minerals by *A. niger* where the process of mineral solubilization through heterotrophic leaching of copper from minerals mediated by fungus is followed by the process of immobilization of mobile copper species within fungal biomass via bioaccumulation and copper speciation which varies depending on the age and reproductive state of fungal mycelium (Fig. 2).

The results of this study demonstrated heterogeneity of the toxic metal speciation within microbial biomass and should be taken into account in the development of the effective technologies of fungal-based remediation techniques.

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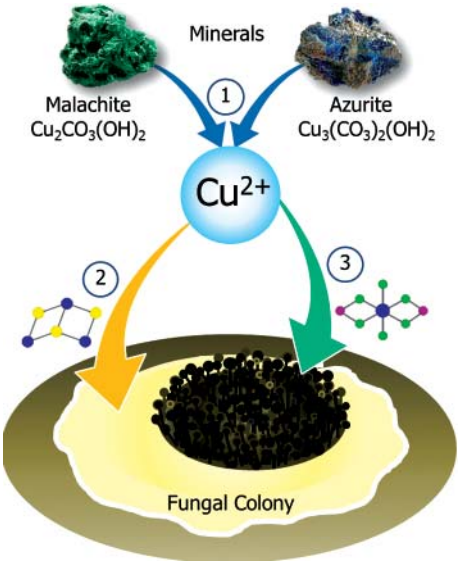

Fig. 2. **Simplified diagram of the transformation of copper–containing minerals by fungus *A. niger*:**
1 — mineral solubilization via the processes of heterotrophic leaching including ligand- and proton-promoted mechanisms. It results in the release of the mobile copper;
2 — mobile copper is bioaccumulated by immature mycelium in the outer part of the fungal colony resulting in the sulphur coordination of copper;
3 — copper bioaccumulation by mature mycelium with abundant conidia results in the copper coordination by phosphate ligands

REFERENCES

1. *Malik A.* Metal bioremediation through growing cells. *Environ. Int*. 2004, V. 30, P. 261–278.
2. *Gadd G. M.* Geomycology: biogeochemical transformations of rocks, minerals, metals and radionuclides by fungi, bioweathering and bioremediation. *Mycol. Res*. 2007, V. 111, — P. 3–49.
3. *Shukla K. P., Singh N. K., Sharma S.* Bioremediation: Developments, Current Practices and Perspectives. *Genet. Engineer. Biotechnol. J*. 2010, V. 3, P. 1–20.
4. *Gadd G. M.* Interactions of fungi with toxic metals. *New Phytol*. 1993, V. 124, P. 25–60.
5. *Blaudez D., Botton B., Chalot M.* Cadmium uptake and subcellular compartmentation in the ectomycorrhizal fungus *Paxillus involutus*. *Microbiology*, 2000, V. 146, P. 1109–1117.
6. *Bellion M., Courbot M., Jacob C., Blaudez D., Chalot M.* Extracellular and cellular mechanisms sustaining metal tolerance in ectomycorrhizal fungi. *FEMS Microbiol. Lett*. 2006, V. 254, P. 173–181.
7. *Burford E. P., Fomina M., Gadd G. M.* Fungal involvement in bioweathering and biotransformation of rocks and minerals. *Mineral. Mag*. 2003, V. 67, P. 1127–1155.
8. *Fomina M. A., Alexander I. J., Hillier S., Gadd G. M.* Zinc phosphate and pyromorphite solubilization by soil plant-symbiotic fungi. *Geomicrobiol. J*. 2004, V. 21, P. 351–366.
9. *Fomina M., Burford E. P., Gadd G. M.* The Fungal Community. Its Organization and Role in the Ecosystem. *Boca Raton: CRC Taylor & Francis* (Dighton J., White J. F., Oudemans P., eds.). 2005. P. 733–758.
10. *Fourest E., Serre A., Roux J. C.* Contribution of carboxylic groups to heavy metal binding sites in fungal cell walls. *Toxicol. Environ. Chem*. 1996, V. 54, P. 1–10.
11. *Sarret G., Manceau A., Spadini L., Roux J.-C., Hazemann J.-L., Soldo Y., Laurent E.-B., Menthonnex J.-J.* Structural determination of Zn and Pb binding sites in Penicillium chrysogenum cell walls by EXAFS spectroscopy. *Environ. Sci. Technol*. 1998, V. 32, P. 1648–1655.
12. *Sarret G., Manceau A., Cuny D., Haluwyn C. V., Déruelle S., Hazemann J.-L., Soldo Y., Laurent E.-B., Menthonnex J.-J.* Mechanisms of lichen resistance to metallic pollution. *Environ. Sci. Technol*. 1998, V. 32, P. 3325–3330.
13. *Sarret G., Manceau A., Spadini L., Jean-Claude Roux, Jean-Louis Hazemann, Yvonne Soldo, Laurent Eybert-Berard, Jean-Jacques Menthonnex.* Structural determination of Pb binding sites in Penicillium chrysogenum cell

walls by EXAFS spectroscopy and solution chemistry. *J. Synch. Radiat*. 1999, V. 6, P. 414–416.
14. *Sarret G., Saumitou-Laprade P., Bert V., Proux O., Hazemann J. L., Traverse A., Marcus M. A., Manceau A.* Forms of zinc accumulated in the hyperaccumulator *Arabidopsis halleri*. *Plant Physiol*. 2002, V. 130, P. 1815–1826.
15. *Sarret G., Schroeder W. H., Marcus M. A., Geoffroy N., Manceau A.* Localization and speciation of Zn in mycorrhized roots by μSXRF and μEXAFS. *J. Phys. IV France*. 2003, V. 107, P. 1193–1196.
16. *Kemner K. M., O'Loughlin E. J., Kelly S. D., Boyanov M. I.* Synchrotron X-ray investigations of mineral-microbe-metal interactions. *Elements*. 2005, V. 1, P. 217–221.
17. *Gardea-Torresdey J. L., de la Rosa G., Peralta-Videa J. R.* Use of phytofiltration technologies in the removal of heavy metals: A review. *Pure Appl. Chem*. 2004, V. 76. — P. 801–813.
18. *Sayer J. A., Raggett S. L., Gadd G. M.* Solubilization of insoluble compounds by soil fungi: development of a screening method for solubilizing ability and metal tolerance. *Mycol. Res*. 1995, V. 99, P. 987–993.
19. *Fomina M. A., Alexander I. J., Colpaert J. V., Gadd G. M.* Solubilization of toxic metal minerals and metal tolerance of mycorrhizal fungi. *Soil Biol. Biochem*. 2005, V. 37, P. 851–866.
20. *Gurman S. J., Binsted N., Ross I.* A rapid, exact, curved-wave theory for EXAFS calculations. *J. Phys. Chem*. 1984, V. 17, P. 143–151.
21. *Binsted N. Daresbury Laboratory EXCURV98 Program*. 1998.
22. *Hedin L., Lundqvist S.* Effects of electron–electron and electron-phonon interactions on the one-electron states of solids. *Sol. State Phys*. 1969, V. 23, P. 1–181.
23. *Binsted N., Strange R. W., Hasnain S. S.* Constrained and restrained refinement in EXAFS data analysis with curved wave theory. *Biochemistry*. 1992, V. 31, P. 12117–12125.
24. *Gurman S. J., Binsted N., Ross I.* A rapid, exact, curved-wave theory for EXAFS calculations. 2. The multiple-scattering contributions. *J. Phys. Chem*. 1986, V. 19, P. 1845–1861.
25. *Nriagu J. O.* A history of global metal pollution. *Science*. 1996, 272(5259), 223–224.
26. *Cotter–Howells J. D., Caporn S.* Remediation of contaminated land by formation of heavy metal phosphates. *Appl. Geochem*. 1996, V. 11, P. 335–342.
27. *Knox A. S., Seaman J. C., Mench M. J., Vangronsveld J.* Remediation of metal- and radionuclides-contaminated soils by in situ stabilization techniques. *Environmental Restoration of Metals–Contaminated Soil. Boca Raton: Lewis Publishers* (Iskander I. K., ed.), 2000, P. 21–61.
28. *Prasad M. N. V., De Oliveira Freitas H. M.* Metal hyperaccumulation in plants — Biodiversity prospecting for phytoremediation technology. *Elect. J. Biotechnol*. 2003, V. 6, P. 285–321.
29. *Brown S., Chaney R., Hallfrisch J., Ryan J. A., Berti W. R.* In situ soil treatments to reduce the phyto– and bioavailability of lead, zinc, and cadmium. *J. Environ. Qual*. 2004, V. 33, P. 522–531.
30. *Burgstaller W., Schinner F.* Leaching of metals with fungi. *J. Biotechnol*. 1993, V. 27, P. 91–116.
31. *Fomina M., Hillier S., Charnock J. M., Melville K., Alexander I. J., Gadd G. M.* Role of oxalic acid over-excretion in toxic metal mineral transformations by *Beauveria caledonica*. *Appl. Environ. Microbiol*. 2005, V. 71, P. 371–381.
32. *Sayer J. A., Gadd G. M.* Solubilisation and transformation of insoluble metal compounds to insoluble metal oxalates by *Aspergillus niger*. *Mycol. Res*. 1997, V. 101, P. 653–661.
33. *Jaeckel P., Krauss G., Menge S., Schierhorn A., Rücknagel P., Krauss G. J.* Cadmium induces a novel metallothionein and phytochelatin 2 in an aquatic fungus. *Biochem. Biophys. Res. Commun*. 2005, V. 333, P. 150–155.
34. *Kumar K. S., Dayananda S., Subramanyam C.* Copper alone, but not oxidative stress, induces copper-metallothionein gene in *Neurospora crassa*. *FEMS Microbiol. Lett*. 2005, V. 242, P. 45–50.
35. *Margoshes M., Vallee B. L.* A cadmium protein from the equine kidney cortex. *J. Am. Chem. Soc*. 1957, V. 79, P. 4813–4814.
36. *Howe R., Evans R. L., Ketteridge S. W.* Copper-binding proteins in ectomycorrhizal fungi. *New Phytol*. 1997, V. 135, P. 123–131.
37. *Tsekova K., Galabova D., Todorova K., Ilieva S.* Phosphatase activity and copper uptake during the growth of *Aspergillus niger*. *Proc. Biochem*. 2002. V. 37, P. 753–758.
38. *Tsekova K., Galabova D.* Phosphatase production and activity in copper (II) accumulating *Rhizopus delemar*. *Enz. Microb. Technol*. 2003, V. 33, P. 926–931.
39. *Gromozova O. M., Voychuk S. I., Kachur T. L., Gorchev V. F., Karakhim S. A.* Structural peculiarities of volutin grains in the cells of the lowest polyphosphate-accumulating eukaryotes. *Biothechnology*. 2010, V. 3, P. 55–61.
40. *Bucking H., Heyser W.* Elemental composition and function of polyphosphates in ectomycorrhizal fungi- and X-ray microanalytical study. *Mycol. Res*. 1999, V. 103, P. 31–39.

ВАРИАБЕЛЬНОСТЬ КООРДИНАЦИОННЫХ СОЕДИНЕНИЙ МЕДИ, АККУМУЛИРОВАННЫХ В ГРИБНЫХ КОЛОНИЯХ В ПРИСУТСТВИИ МЕДЬСОДЕРЖАЩИХ МИНЕРАЛОВ

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Целью работы было выяснение механизмов биоаккумуляции меди, выщелоченной из минералов грибом *Aspergillus niger*, обладающим высоким потенциалом для использования в биоремедиации благодаря способности выделять хелатирующие метаболиты и трансформировать токсичные металлы и минералы. Особое внимание уделяли химическому связыванию меди, биоаккумулированной грибной колонией в процессе трансформации медьсодержащих минералов.

Химическое связывание меди в различных частях грибной колонии изучали с применением методов твердофазной химии, в частности синхротронной рентгеноабсорбционной спектроскопии для получения информации о валентности исследуемого элемента и его координационном окружении. Анализ полученных спектров проводили с использованием преобразований Фурье для данных области спектроскопии протяженной тонкой структуры рентгеновского поглощения, которая соответствует осциллирующей части спектра вправо от края поглощения.

Результаты свидетельствуют о том, что гриб *A. niger* участвовал в процессах растворения медьсодержащих минералов, приводивших к выщелачиванию подвижной меди и ее последующей иммобилизации грибной биомассой с вариабельной координацией меди, аккумулированной грибной колонией, которая зависела от возраста, физиологического и репродуктивного состояния грибного мицелия. Эти данные продемонстрировали, что медь, аккумулированная незрелым вегетативным мицелием во внешней зоне грибной колонии, была координирована серосодержащими лигандами, в отличие от координирования меди фосфатными лигандами внутри зрелого конидиеобразующего мицелия в центральной зоне колонии.

Полученные результаты не только расширяют наши представления о биогеохимической роли грибов, но и могут использоваться в разработках грибных биометаллургических технологий, в частности биоремедиации, биоаккумуляции и биовыщелачивании, и определении их надежности. Координационное окружение меди, биоаккумулированной грибной биомассой в процессе трансформации минералов меди, является гетерогенным, изменяясь от сульфгидрильного до фосфатного.

Ключевые слова: биоремедиация, биоаккумуляция металлов, трансформация минералов, рентгеноабсорбционная спектроскопия.

ВАРІАБЕЛЬНІСТЬ КООРДИНАЦІЙНИХ СПОЛУК МІДІ, ЩО АКУМУЛЬОВАНІ В ГРИБНИХ КОЛОНІЯХ ЗА ПРИСУТНОСТІ МІДЬВМІСНИХ МІНЕРАЛІВ

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Метою роботи було з'ясувати механізми біоакумуляції міді, вилуженої з мінералів грибом *Aspergillus niger*, який має високий потенціал для використання у біоремедіації завдяки здатності виділяти хелатувальні метаболіти і трансформувати токсичні метали та мінерали. Особливу увагу приділено хімічному зв'язуванню міді, що була біоакумульована грибною колонією в процесі трансформації мідьвмісних мінералів.

Хімічне зв'язування міді в різних місцях грибної колонії вивчали із застосуванням методів твердофазної хімії, зокрема синхротронної рентгеноабсорбційної спектроскопії для одержання інформації про валентність досліджуваного елемента та його координаційне середовище. Аналіз отриманих спектрів проводили, використовуючи трансформації Фур'є для даних ділянки спектроскопії протяжної тонкої структури рентгенівського поглинання, що відповідає осцилювальній частині спектра вправо від краю поглинання.

Результати свідчать про те, що гриб *A. niger* брав участь у процесах розчинення мідьвмісних мінералів, що спричинювало вилуження рухливої міді та її подальшу іммобілізацію грибною біомасою з варіабельною координацією міді, акумульованою грибною колонією, яка залежала від віку та фізіологічного й репродуктивного стану грибного міцелію. Ці дані продемонстрували, що акумульована незрілим вегетативним міцелієм у зовнішній зоні грибної колонії мідь була координована сірковмісними лігандами, на відміну від координації міді фосфатними лігандами всередині зрілого конідієпродукувального міцелію в центральній зоні колонії.

Одержані дані не тільки розширюють уявлення про біогеохімічну роль грибів, але й можуть бути використані під час розроблення грибних біометалургійних технологій, зокрема біоочищення, біоакумуляції й біовилуження, та визначенні їхньої надійності. Координаційне середовище міді, біоакумульованої грибною біомасою в процесі трансформації мінералів міді, є гетерогенним, змінюючись від сульфгідрильного до фосфатного.

Ключові слова: біоремедіація, біоакумуляція металів, трансформація мінералів, рентгеноабсорбційна спектроскопія.