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Asbestos Decontamination

Soil Fungal Hyphae Bind and Attack Asbestos Fibers**

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Once a major issue in occupational health and safety, asbestos has now become a general environmental problem.[1] Several mountain areas—from the western Alps in Italy to the Sierra Nevada in the USA—are rich in asbestos and asbestiform minerals, and many defunct asbestos industries and mines have left substantial amounts of asbestos fibers on the abandoned sites. Exposure to airborne asbestos fibrils causes a severe pneumoconiosis (asbestosis) and malignancies such as bronchogenic carcinoma and pleural mesothelioma.[2–4] The decontamination of asbestos fibers dispersed over wide areas of soil and in waters obviously requires a different approach from what was proposed for asbestos localized in buildings.[5] The fibers cannot be removed but have to be inactivated in situ without damaging the environment. New, environmentally friendly techniques are thus required for

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application to a vast territory, whereby the physicochemical properties of the fibers are modified to shut down the pathogenic mechanism.

Iron is known to contribute to asbestos toxicity, and its removal reduces in vitro cell damage caused by asbestos fibers.[2,6] Thus, if iron could be continuously extracted by means of a "natural" process from the fibers dispersed in the environment, consequent modifications at the surface of the fibers could result in a decrease in their carcinogenic potential or even in a full inactivation of the asbestos fibers dispersed in the soil.

Several soil microorganisms are capable of scavenging iron for their own metabolism from recalcitrant substrates[7] by releasing, in the surrounding soil environment, potent chelators with different metal specificity (for example, polycarboxylic acids and other siderophores). Some ericoid mycorrhizal fungi, which are able to colonize roots symbiotically, have been reported to produce several hydroxamate siderophores known to form very stable complexes with iron(III) ions.[8] Fungi are of particular interest because they can extend through extensive volumes of soil thanks to their hyphal structures. We thus investigated the ability of some soil fungi to grow in the presence of crocidolite (fibrous riebeckite or blue asbestos) fibers, release iron chelators that modify the surface of the fibers at the atomic level, and reduce the potential of the generation of free radicals. A number of fungi with different trophic characteristics were selected because of their ability to grow on silicates, produce siderophores, and tolerate different metals.

Crocidolite, one of the most potent fibrous carcinogens and the most studied form of asbestos,[5] contains up to 29% iron in both oxidation states (Fe^{II}, Fe^{III}) in its crystal structure. When at the surface in a low oxidation state, iron ions are highly reactive centers, at which the generation of free radicals,[6,9–11] redox cycling of iron,[12] and coordination of electron-donor molecules[13] may take place. Iron is mobilized from crocidolite by various chelators[14,15] with loss of the potential to generate radicals[16,17] and damage DNA.[6a,18] None of the fungi tested was inhibited in biomass production when grown in liquid culture (9.2 mg mL^{-1} in Czapek glucose medium) when the crocidolite fibers were either in direct contact or in a chamber separated from the fibers by a dialysis membrane. Conversely, crocidolite induced a significant growth increase (>65%) in *Geomyces pannorum* and in three strains of *Oidiodendron maius* (Zn, Cd, and A). In liquid mycelial cultures, crocidolite fibers and fibrils were visibly removed from the suspension in the aqueous phase and tightly bound to the fungal hyphae, so that the supernatant was progressively cleared (Figure 1). At the ultrastructural level the fibrils appear to be in intimate contact with the fungus (Figure 2). After growth of the fungal mycelia with crocidolite (42 days) the culture supernatant contained variable amounts of iron ions. A few fungi (*G. vinaceous*, *O. maius* strains Zn, A, Cd) did not extract iron from asbestos, but the majority of species (*G. pannorum*, *Mortierella hyalina*, sterile mycelium MUT840, *O. griseum*, and *F. oxysporum*) induced the removal of substantial amounts of iron. Of these, *Fusarium oxysporum*, *M. hyalina*, and *O. maius* E were the most active in terms of moles of iron released per gram of fungal biomass (Figure 3). Note that, in the absence of fungi, crocidolite fibers did not release any detectable iron in the medium.

Figure 1. Effects of fungal mycelium on a suspension of asbestos. Fine asbestos fibers (1.5–4 μm mean diameter) form a turbid suspension when dispersed in the Czapek glucose medium (left). When the fungal mycelium, here the sterile mycelium strain MUT840, was grown in the medium containing asbestos fibers, it removed the fibers from the suspension, thus leaving a clear supernatant (right). This phenomenon was observed for all the fungi tested and indicates that the fungal hyphae can bind or entrap the asbestos material.

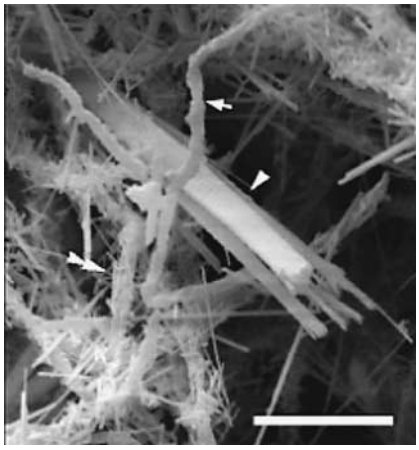

Figure 2. Scanning electron micrograph showing the fungal hyphae of *O. maius* (arrow) intertwined with asbestos fibers of different sizes. A large fiber is clearly visible in the center of the photograph (arrowhead), whereas smaller fibers (double arrowhead) can be found associated with the surface of the fungal hyphae (scale bar corresponds to 10 μm).

All fungi able to induce iron release from crocidolite fibers also secreted siderophores, detected as a clearing halo around the fungal colony on specific plate assays (Figure 4). The halo diameter, which was considered to be proportional to siderophore secretion, appears to parallel iron extraction. Thus, an enhancement of siderophore production might result in the removal of even more iron from the fibers.

In cell-free tests a 1-mM solution of the strong iron chelator desferrioxamine—a hydroxamate siderophore usually employed clinically in the treatment of hemochromatosis and other diseases related to iron-overload—extracted iron from crocidolite fibers. The amount of iron extracted greatly exceeded what was expected to be at the surface based on the crystal structure of crocidolite.[9,19] Both iron and silicon were

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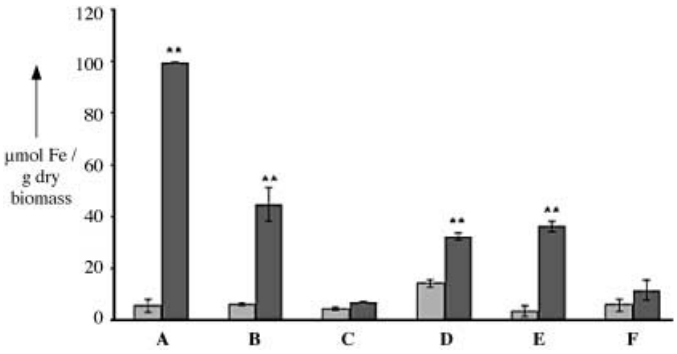

Figure 3. Iron solubilization by soil fungi. Iron was extracted from crocidolite fibers by the various mycelia tested: A = *Fusarium oxysporum*, B = *Mortierella hyalina*, C = *Geomyces pannorum*; D = sterile mycelium MUT 840, E = *Oidiodendron maius* E, F = *Oidiodendron maius* Cd). The diagram shows the amount of iron measured in the culture medium of the control (left-hand bars) and the asbestos-treated samples (right-hand bars). Data are the mean of three independent experiments ± standard deviation. Asterisks indicate significant differences between control and asbestos-containing medium ($P < 0.05$).

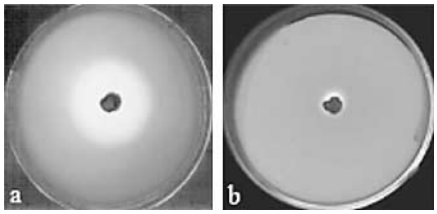

Figure 4. A specific plate assays shows the release of siderophores, detected as a clear halo. Highly solubilizing fungal strains such as sterile mycelium MUT 840 (a) produced significantly higher amounts of siderophores than less solubilizing fungi such as *Oidiodendron maius* Cd (b).

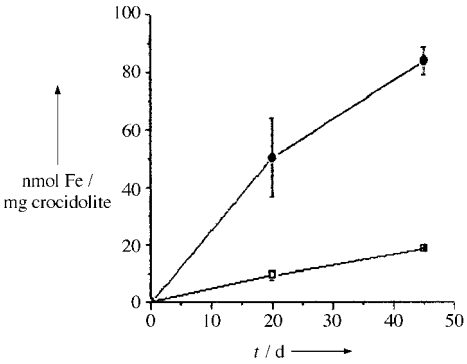

Figure 5. Amount of iron released over time from crocidolite in the presence of *Fusarium oxysporum* (open squares) and *Mortierella hyalina* (filled circles). Data are the mean of three independent experiments ± standard deviation. The amount of iron released progressively increases with the time of incubation, and no plateau values are obtained even after 45 days of culture.

found in the supernatant but with an iron:silicon ratio greater than that in the solid. This suggests that the progressive removal of iron from the bulk of the fiber by desferrioxamine is followed by diffusion of bulk iron ions in the silicate matrix, which eventually leads to collapse of the silicate structure in the outmost layers.[9,20] The newly formed surface is amorphous and is much less reactive in generating free radicals than the original surface.[6a,21] Also the amount of iron extracted by the fungal siderophores was greater than what might have found at the surface. Assuming a maximum of three to four iron atoms per nm^2, which exceeds the average value on the crystal planes of the amphibole structure, the iron mobilized by fungi corresponds to six to seven iron atoms per nm^2, which would correspond to two or more layers below the surface. The trend of iron release in the presence of *F. oxysporum* and *M. hyalina*, which is reported in Figure 5, suggests that iron extraction was far from being exhausted after 45 days of culture but would have proceeded as long as the fibers and the hyphae would have been in contact. Prolonged growth of iron-extracting fungi on the fibers is thus likely to yield transformations at the fiber surface similar to those observed after incubation in desferrioxamine.

The generation of ·OH radicals by the crocidolite fibers in the presence of H_2O_2 was evaluated by EPR spectroscopy and with 5,5-dimethyl-1-pyrroline *N*-oxide (DMPO) as a spin trap. The nitroxide radical formed upon reaction of ·OH with DMPO gives rise to a characteristic four-line EPR signal with the intensity ratio 1:2:2:1 and hyperfine coupling constants $a_N = a_H = 15$ G. The EPR spectra obtained from aqueous suspensions of the original crocidolite and of crocidolite fibers incubated for 45 days with growing *F. oxysporum* are shown in Figure 6. The original fibers, as previously reported,[17] generate substantial ·OH radicals (Figure 6a). Conversely, the fibers that had been treated with *F. oxysporum* and then washed and dried are fully inactivated in their potential to generate ·OH radicals (Figure 6b). Asbestos fibers induce single-strand breaks in DNA, and this effect is lowered by incubation with desferrioxamine.[15,16] Considering that a mild pretreatment of crocidolite fibers with desferrioxamine could prevent DNA mutations at defined loci in Chinese hamster V79 cells,[22] we may expect that, following interaction with the fungi, the biological activity of the fibers might be reduced also.

The extracellular protein profiles for fungi grown in the presence and in the absence of asbestos fibers revealed

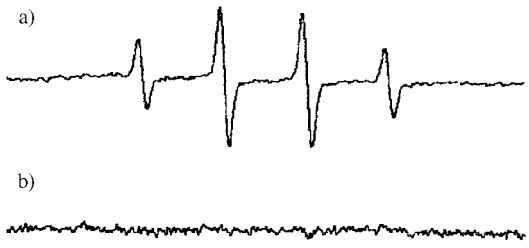

Figure 6. EPR spectra (DMPO as spin trap) obtained from aqueous suspensions of a) crocidolite, which shows the signal of the [HO·–DMPO] adduct, and of b) crocidolite pretreated with *F. oxysporum*.

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substantial changes, with new proteins being induced and other ones repressed following contact with the fibers. Among the induced proteins, a manganese superoxide dismutase (Mn-SOD) was identified in *G. pannorum* by N-terminus sequencing and immuno cross-reactivity. SOD enzymes are potent antioxidant proteins that catalyze the conversion of the superoxide ion in hydrogen peroxide and oxygen. An increase in Mn-SOD enzyme was detected in type II epithelial cells of rat lungs after inhalation of crocidolite asbestos.[10] The expression of Mn-SOD in mesothelioma is also much greater than that in healthy pleural mesothelium,[23] and transfection of a Mn-SOD gene into hamster tracheal epithelial cells ameliorates asbestos-mediated cytotoxicity.[24] The cellular response to asbestos fibers may thus show some similarities in fungi and in mammalian cells.

On the basis of the results described we believe fungi may be possible agents for the decontamination of asbestos-polluted soils. Not only may adhesion of the fibrils on the fungal mycelium limit their dispersal in the specific sites where the fungi grow, but also the action of the potent siderophores could modify the fiber surface, depriving it of the free radical generating sites crucial to triggering of the carcinogenic mechanisms. Soil fungi display a huge functional and genetic biodiversity, and suitable strains with high iron-mobilizing abilities can be selected from natural sources. Alternatively, the identification of genes involved in siderophore production may be the starting point for genetically engineered organisms with increased iron mobilization from asbestos.

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