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Fungal weathering of asbestos in semi arid regions of India

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ABSTRACT

The science of Geomicrobiology, which deals with mineral- microbe interaction in nature contributes effectively to three important processes namely- mineral and metal bioremediation, biomining and soil mineral formation by microbes. Bioremediation one of the important process of the above, degrades or transforms hazardous contaminants to less toxic compounds. Several groups of fungi have proved highly efficient in this aspect, with asbestos being one such toxic entity in the environment on which their activity was studied. The present investigation uses the same tool as a device for detoxifying asbestos, a potent carcinogenic entity; with fungal isolates native to the asbestos mines of Rajasthan, India, being investigated for the first time. The cellular mechanism of asbestos toxicity is mainly attributed to the presence of iron in its chemical composition which catalyzes generation of free radicals leading to oxidation of biomolecules. The two dominant novel species found therein, identified as *Aspergillus tubingenesis* and *Coemansia reversa* have proved capable of actively removing iron from asbestos fibers as studied by scanning electron microscopy- electron diffraction X-ray (SEM–EDX) analysis. This probably could lead to a reduction in toxicity of asbestos, due to reduced iron concentration as reported in related studies. Many fungi are known to release iron chelating compounds, siderophores, which could be instrumental in the study. The findings related to two new fungal species being added to the list of earlier identified fungal bioremediators of asbestos, widens the prospect of using bioremediation as an effective tool for asbestos detoxification

1. Introducton

Intensification of agriculture and manufacturing industries is leading to an unending increase in the content of the wide range of xenobiotic compounds being released to the environment. The global endeavor to sustain and preserve the environment, is at present largely focused on the technique of bioremediation, which capitalizes mainly microorganisms to clean up contaminated soil and water (Strong and Burgess, 2008). The fungi are reported to possess the most varied and efficient system to degrade a huge array of organic molecules including waxes, rubber, feathers, plant polymers, insect cuticles, and animal flesh (Bennet et al., 2002, Rhodes, 2013, 2014). Fungi not only colonize the rock surface but also show effective penetration of their hyphae into the cracks and crevices of the rocks (Caneva et al., 2008; Gueidan et al., 2008), thereby bringing about their biodegradation by various biochemical activities.

One such mineral which has been shown to be a potent hazard for human health is asbestos, being listed as the third most abundant pollutant at the global scale (Air Quality Guidelines for Europe, 1987; Fubini et al., 1990; Wachowski and Domka, 2000). Asbestos fibers have been designated as a Group (I) definite carcinogen by IARC (International Agency for Research on Cancer), a part of WHO (World Health Organization), in the year 1987(Shinya Toyokuni, 2009) causing about half of the deaths from occupational cancer (Concha-Barrientos et al., 2004). Asbestos is a naturally occurring hydrated magnesium phyllosilicate mineral with many of its forms showing a considerable presence of iron. It is broadly divided into two mineralogical groups – the amphibole and the serpentine (Mossman et al., 1996). Internalization of micro to nano sized asbestos fibers suspended in air, leads to their gradual accumulation particularly on lungs and their manifestation later as lung carcinoma, pleural mesothelioma or asbestosis (Kilburn, 2000). Asbestos accumulation in lungs leads to genotoxic effects such as, causing chromosome breaks and deletions (Okayasu et al., 1999) or smaller gene mutations (Rihn et al., 2000, Unfried et al., 2002, Kristina Luus, 2007, Wei et al., 2012, Kim et al., 2013). Reactivity and toxicity of asbestos fibers has been reported to be due to the presence of iron at their surface (Fubini et al.,

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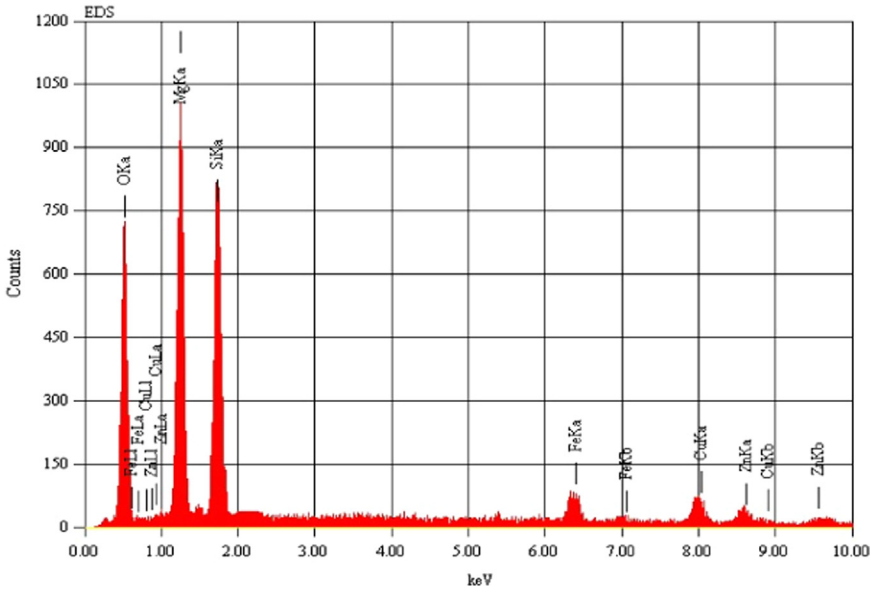

Fig. 2. EDX analysis image of processed asbestos fibers (control).

1995). In vitro experiments have reported that various chelators can extract iron from fibers modify its surface properties (Fubini et al., 1995) and even promote disruption of several sub layers (Prandi et al., 2002). Another important breakthrough was disruption of magnesia–silicate framework of asbestos by the aposymbiotic lichen forming fungus *Xanthoparmelia tinctina* (Favero-Longo et al., 2007). The fungus *Fusarium oxysporum* could effectively extract iron from chrysotile, crocidolite and amosite by siderophore secretion also (Daghino et al., 2005). Fungi contribute to the deterioration of silicate-bearing rocks and iron- and magnesium-bearing minerals (Kumar and Kumar, 1999, Hoffland et al., 2004, Gadd, 2007). While *Verticellum leptobactrum* and *Paecilomyces lilacinus* were able to extract iron from crocidolite (Daghino et al., 2006), *Verticellum leptobactrum* also removed significant amounts of magnesium from chrysotile fibers bulk (Daghino et al., 2009). The results obtained showed a general decrease of reactivity for both chrysotile and crocidolite forms of asbestos (Martino et al., 2003; Daghino et al., 2006). *Fusarium oxysporium* and *Aspergillus fumigatus* (Daghino et al., 2008), have also been shown to be potent bioremediator of asbestos. The mobilization of ferric ions by fungi is attributed to the secretion of low molecular weight iron chelating organic molecules termed as siderophores (Winkelmann, 2007).

The present investigation aims at identifying some novel potential fungal bioremediators of asbestos from the soils of Rajasthan, India which till 2010 had been the leading asbestos producer in the country and still houses a number of defunct asbestos mines, which pose a major health threat in the state. In this context we also propose to study the changes in asbestos elemental composition, in particular iron, upon fungal treatment; using scanning electron microscopy- electron diffraction X-ray analysis. A preliminary analysis of their siderophore synthesizing ability enabling iron extraction would also form a part of the proposed investigation.

2. Materials and method

2.1. Sampling, isolation and identification of fungal isolates

Asbestos rock and asbestos contaminated soil samples were collected from four abandoned mines of Rajasthan, India. Two were from the Rajsamand district and two from the Jodhpur district. As mines have been officially banned by the Government of the country, access to the mines was restricted and thus the small sample size. Fungi were isolated by a modified 'soil dilution plate' technique (Waksman, 1927; Warcup, 1950). The rock samples crushed into small pieces and the soil samples were suspended in sterile water at different concentrations ranging from 1:50 (w/v) to 1:500 (w/v). All suspensions were then vortexed for 10–15 min and plated onto potato dextrose agar plates amended with 4×10^{-5} g mL^{-1} gentamycin and 3×10^{-5} g mL^{-1} streptomycin. Three replicate plates were prepared for each sample. As a part of the preliminary identification of the fungal isolates, colony morphology was studied and colonies transferred to separate culture tubes.

2.2. Asbestos fibers and their fungal treatment

Asbestos fibers for experimental work were obtained from one of the asbestos- cement product manufacturing industry situated in Jaipur, Rajasthan. The fibers were separated from the ore by crushing, air suction, and vibrating screens, and in the process sorted into different lengths, or grades. The processed fibers so obtained, were weighed and a homogenous suspension prepared in sterile water by vortexing so as to maintain a total concentration of 2% (w/v) in the final culture flasks. The fibers were also characterized for structural and elemental composition by Scanning Electron Microscopy-Energy Dispersive X-ray (SEM–EDX) (Figs. 1 and 2).

Based on the nature of growth in asbestos supplemented media six fungal isolates were selected as the dominant species found in the rock and soil isolates and cultured on potato dextrose agar plates. Liquid cultures were then prepared, as reported in literature (Daghino et al., 2005) Culture medium used was Czapek medium supplemented with glucose (composition-glucose 20 mg/mL, NaNO3 3 mg/mL, K2HPO4 1.31 mg/ml, MgSO4 0.5 mg/ml, FeSO4 0.01 mg/ml, KCl 0.5 mg/ml, MES (4-Morpholine ethane sulfonic acid) 3.9 mg/ml) (Daghino et al., 2005).Tubular dialysis membranes bags were filled with suspension of processed asbestos fibers in distilled water, maintaining a final concentration of 2.3% w/v in the culture volume. They were tied at the ends and autoclaved with media before being inoculated with fungi in liquid culture. The fibers were incubated with the fungal isolates for 20