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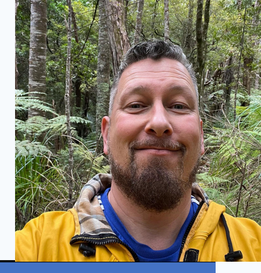

Siderophore production in fungi from asbestos biofilms: the first step towards bioremediation of a carcinogenic mineral

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Introduction

Asbestos refers to six types of naturally-occurring, fibrous, silicate minerals, historically used for a wide range of household and industrial applications. Asbestos exposure is known to cause a range of diseases including asbestosis, pleural mesothelioma and lung cancer, and is responsible for around 170 deaths per year in New Zealand. Asbestos is commonly found in commercial, industrial and residential buildings that were constructed before the mid-1980s. Asbestos-containing materials (ACM) and asbestos-contaminated soil are generally removed and sent to a specialist landfill, which is expensive[1]. Research over the last 20 years has begun to focus on the possibility of using bioremediation to manage asbestos contamination[2].

Inhalation of asbestos fibres can cause inflammation and consequent carcinogenic activity. Active iron at the surface of the fibres induces the production of hydrogen peroxide and other reactive oxygen species from immune cells, leading to DNA damage and carcinogenesis[3]. It has been shown that asbestos fibres can be at least partially degraded by the activity of some fungi, bacteria and lichens. It is thought that this is due to removal of metal ions including iron by siderophores and similar compounds. Production of siderophores can be detected by culturing candidate microbial species on chrome azurol S (CAS)-agar plates. A change in colour of the agar from blue to yellow/orange shows that the microbial colonies have removed iron from the iron (III) complex of the indicator dye (CAS) using a siderophore or similar chelator[4].

This poster reports the fungal diversity found on naturally-occurring asbestos mineral and asbestos containing building materials, and the ability of these isolates to produce siderophores.

Project Aims:

- **Culture fungi from natural asbestos deposits and asbestos-containing materials.**
- **Identify the fungi found using DNA barcoding and morphology.**
- **Screen fungal isolates for siderophore production *in vitro*.**

Methods:

- Two samples of biofilms growing on naturally occurring asbestos rock (Figs. 1, 2) in Kahurangi National Park, Nelson were taken using sterile swabs.
- One swab of a biofilm sample from asbestos cement building material was taken from a pre-identified site in Auckland, New Zealand, by a licensed removalist whose client had agreed to the use of their samples for further testing.
- In a Negative Pressure Unit (NPU), each swab was streaked onto a series of three agar plates (Potato Dextrose Agar (PDA) with chloramphenicol) and incubated at 20°C.
- Morphologically different colonies were subcultured to generate pure cultures and then identified using DNA barcoding of the ITS region and comparison with published sequences on Genbank (Fig. 3).
- Pure cultures were inoculated onto chrome azurol S (CAS)-agar plates to detect siderophore production. The assay was done twice and with replicates (Figs. 4 - 6).

Figure 1: Asbestos mine, Kahurangi National Park, Nelson.

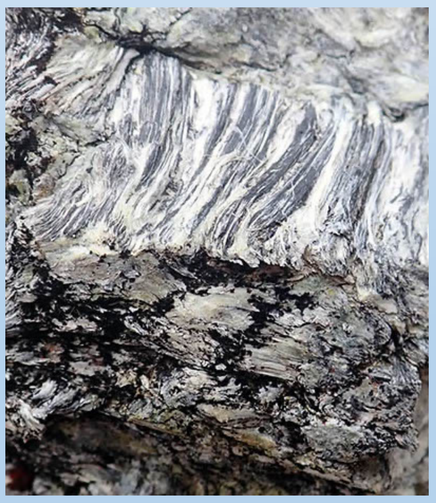

Figure 2: Raw asbestos fibres with biofilm (black)

Figure 3: Isolation of fungal species in NPU

Results and Discussion

- Four different fungal species were isolated from Kahurangi National Park asbestos biofilm samples. These included *Cladosporium cladosporioides*, species of *Biscogniauxia* and *Crustomyces* and an unknown member of the order Pezizales. The three species not given species names did not have 100% matches on Genbank, most likely due to them not previously having been used in a molecular study.
- Three different fungal species were isolated from the Auckland asbestos cement wall biofilm sample. These included *Cladosporium cladosporioides*, the basidiomycete yeast *Leucosporidium scottii* and the common grass saprophyte *Pithomyces chartarum*.
- Three isolates demonstrated clear positive results with the CAS assay (Table 1), while the *Biscogniauxia* isolate filled the agar with a yellow-brown exudate and it was not clear if the blue colour of the plate had been removed or masked.
- The three isolates with evidence of siderophores can now be trialed with asbestos fibres *in vitro*.
- Work is underway to examine the microbial diversity of natural asbestos deposits and asbestos containing materials using high throughput DNA amplicon sequencing. This will be followed by further isolation and culturing of microbes present.

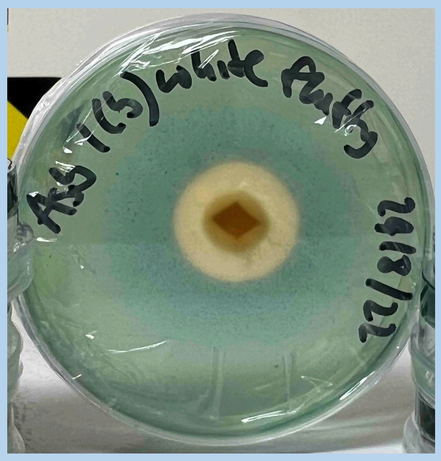

Figure 4: Initial positive CAS assay result with *Crustomyces* sp.

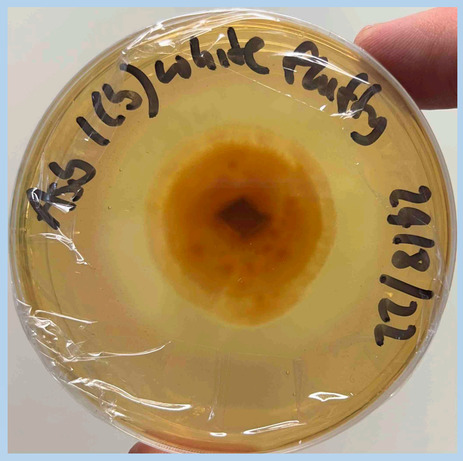

Figure 5: Positive CAS assay result after 8 weeks (same plate as Figure 4)

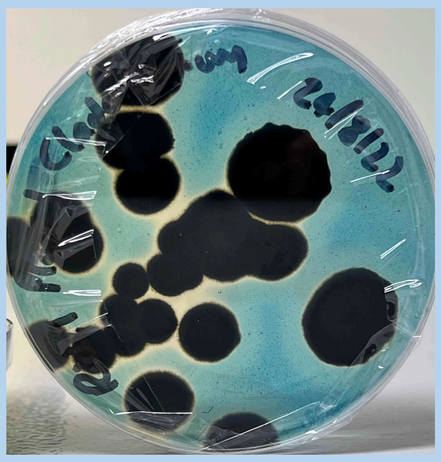

Figure 6: Positive CAS assay result with *Cladosporium cladosporioides*

Fungal isolates	Site	CAS agar result 1	CAS agar result 2
***Biscogniauxia* sp.**	**Kahurangi**	**?**	**?**
Cladosporium cladosporioides	**Kahurangi**	**positive**	**positive**
Cladosporium cladosporioides	**Auckland**	**positive**	**positive**
***Crustomyces* sp.**	**Kahurangi**	**positive**	**positive**
Leucosporidium scottii	**Auckland**	**negative**	**negative**
Pithomyces chartarum	**Auckland**	**Not tested**	**Not tested**
Unknown *Pezizales*	**Kahurangi**	**negative**	**negative**

Table 1. CAS siderophore assay results, including fungal isolate identification, site, and result (negative means the blue agar remained blue, positive means the blue colour disappeared and was replaced with yellow, indicating siderophore production). For *Biscogniauxia* a brown colour was observed.

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