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Contact:
epress@unitec.ac.nz
www.unitec.ac.nz/epress/
Unitec | Te Pūkenga
Private Bag 92025, Victoria Street West
Tāmaki Makaurau Auckland 1142
Aotearoa New Zealand

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

Dan Blanchon[1,2,*], Peter J. de Lange[2], Erin Doyle[2], Tianyi Tang[2], Nick Waipara[3] and Terry-Ann Berry[4]

1. **Auckland War Memorial Museum / Tāmaki Paenga Hira**
2. **Applied Molecular Solutions Research Centre, Unitec | Te Pūkenga**
3. **New Zealand Institute for Plant and Food Research Limited**
4. **Environmental Innovation Centre**

* **Corresponding author: dblanchon@aucklandmuseum.com**

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Abstract

Asbestos refers to six types of fibrous, silicate minerals, historically used for a wide range of household, commercial and industrial applications. Asbestos exposure is known to cause diseases such as asbestosis, pleural mesothelioma and lung cancer, and is responsible for around 220 deaths per year in Aotearoa / New Zealand. Asbestos is disposed of using a designated hazardous landfill facility, an expensive and unsustainable practice. As an alternative, research has focused on bioremediation to manage asbestos contamination. Bioremediation research has shown that asbestos fibres can be partially degraded, and surface reactivity decreased, by the activity of iron-chelating siderophores, produced by some fungi and bacteria. This paper presents initial results of siderophore-detecting chrome azurol S (CAS)-agar plate assays on mycobiota collected from biofilms from asbestos-containing building products in Tāmaki Makaurau / Auckland and natural asbestos mineral deposits in Kahurangi National Park, northwest of Whakatū / Nelson.

Keywords

Asbestos, bioremediation, chrysotile, siderophore

Introduction

Asbestos refers to six types of naturally occurring, fibrous, silicate minerals (Ross et al. 2008), historically used for a wide range of household and industrial applications (LaDou et al. 2010; Skinner 2003). Asbestos exposure is known to cause a range of diseases including asbestosis, pleural mesothelioma and lung cancer, is responsible for around 220 deaths per year in Aotearoa / New Zealand (Worksafe 2023), and potentially affects around 125 million people worldwide (World Health Organization 2020). Asbestos is commonly found in commercial, industrial and residential buildings that were constructed before the mid-2000s (Health and Safety Executive 2023). When identified as being present, asbestos-containing materials (ACM) and asbestos-contaminated soil are generally removed and sent to a specialist landfill, in countries where such a facility exists (Wallis et al. 2020).

Research over the last 20 years has begun to focus on the possibility of using bioremediation to manage asbestos contamination (Favero-Longo et al. 2005). Inhalation of asbestos fibres can cause inflammation and consequent carcinogenic activity, and it is now known that 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 (Hardy & Aust 1995). It has been shown that asbestos fibres can be partially degraded, and the surface reactivity reduced, by the activity of some fungi, bacteria and lichens, and that this is due to removal of metal ions, including iron, by siderophores and similar iron-chelating compounds (Bhattacharya et al. 2015; Daghino et al. 2006; Daghino et al. 2008; Daghino et al. 2010; Favero-Longo et al. 2005; Favero-Longo et al. 2007; Mohanty et al. 2018). Several studies have shown that a range of bacteria, cyanobacteria, filamentous fungi, yeast fungi and lichenised fungi are able to colonise asbestos and related mineral deposits (Bhattacharya et al. 2016; Daghino et al. 2008, Daghino et al. 2009; Doyle et al. 2023).

For microbial bioremediation to be successful, it is necessary to assemble a library of potential siderophore-producing bacteria or fungi that are well suited to the environmental conditions of the local area (Doyle et al. 2023). Production of siderophores in candidate isolates can be detected by culturing them on chrome azurol S (CAS)-agar plates. A change in colour of the agar from blue to orange, yellow-orange, or purple shows that the microbial colonies have removed iron from the iron (III) complex of the indicator dye (CAS) using a siderophore or similar chelator (Schwyn & Neilands 1987; Pérez-Miranda et al. 2007; Kuzyk et al. 2021). Microorganisms that are capable of producing siderophores can then be tested with asbestos fibres in *in vitro* experiments to ascertain their ability to reduce the toxicity of these fibres.

This paper reports on the ability of fungal species isolated from asbestos-containing substrates to produce siderophores *in vitro*.

Methods

Figure 1. Asbestos mine, Kahurangi National Park, Te Waipounamu / South Island, Aotearoa / New Zealand. Photo: P. J. de Lange, February 2022.

Two samples of biofilms growing on naturally occurring asbestos rock (Figures 1, 2) in Kahurangi National Park, Te Wai Pounamu / South Island, Aotearoa / New Zealand (Figure 3) were taken using sterile swabs. One swab of a biofilm sample from asbestos-cement building material was taken from a pre-identified site in Tāmaki Makaurau / Auckland, Aotearoa / 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, WAYSAFE® SA1000 Sample Analysis and Preparation Safety Enclosure, United Kingdom),

Figure 2. Raw asbestos fibres with biofilm (black), Kahurangi National Park, Te Waipounamu / South Island, Aotearoa / New Zealand. Photo: P. J. de Lange, February 2022.

each swab was streaked onto a series of three agar plates (potato dextrose agar with chloramphenicol, Fort Richard, Aotearoa / New Zealand) and incubated at 20 °C. Morphologically different colonies were sub-cultured in the NPU and incubated at 20 °C for two weeks to generate pure cultures. DNA was extracted using a Qiagen DNeasy Plant Mini Kit (Qiagen, Germantown, Maryland, United States of America), following the manufacturer's instructions. The ribosomal DNA internal transcribed spacer region (ITS) was amplified and sequenced using primers ITS1f and ITS4 (Gardes & Bruns 1993; White et al. 1990). Polymerase chain reaction (PCR) products were purified using a Qiagen MinElute PCR purification kit (Qiagen, Germantown, Maryland, United States of America). Sequencing was performed by the Massey Genome Service (Massey University, Papaioea / Palmerston

North, Aotearoa / New Zealand) using an Applied Biosystems model 3730 automated capillary DNA sequencer, using the same primers as for PCR. DNA sequences were assembled, edited and aligned using Geneious Prime® 2023.1.2 software, uploaded to Genbank (Table 1) and a BLAST search was used for comparison with published sequences.

Plugs of pure fungal cultures were inoculated onto chrome azurol S (CAS)-agar plates (Schwyn & Neilands 1987) to detect siderophore production. The assay was completed twice, with three replicates each time, and with uninoculated CAS agar plates to track plate deterioration over time in the absence of fungi (Figures 4, 5, 6). The isolate of *Pithomyces chartarum* (Berk. & M.A. Curtis) M.B. Ellis was not tested further, because this species is a saprophyte of plant material, and was probably present in the samples as spores from nearby grasses.

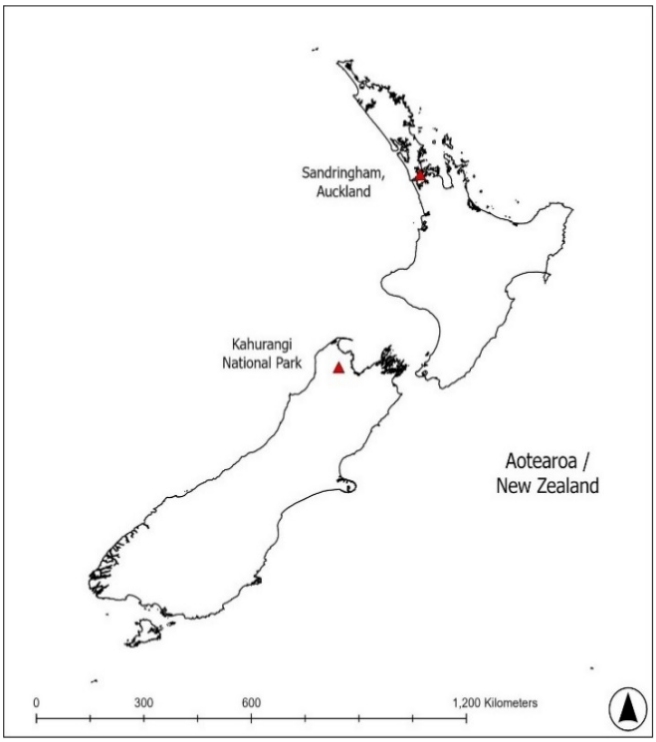

Figure 3. Location of study sites, Tāmaki Makaurau / Auckland and Kahurangi National Park, Te Waipounamu / South Island, Aotearoa / New Zealand. Image: A. J. Marshall.

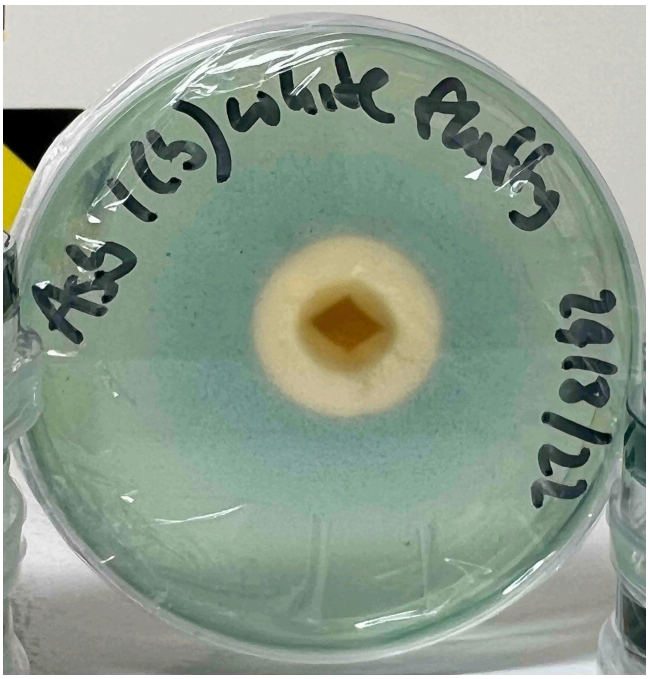

Figure 4. Initial positive CAS assay result with *Crustomyces* sp. Photo: E. Doyle, August 2022.

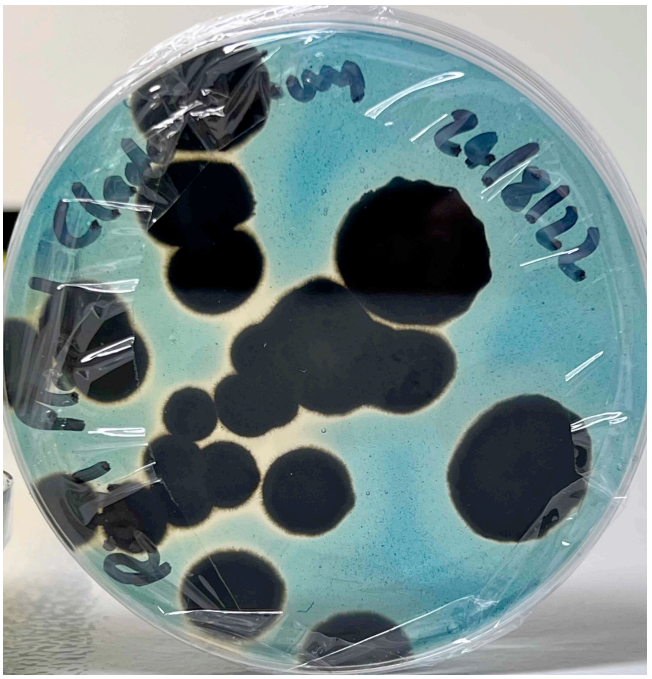

Figure 6. Positive CAS assay result with *Cladosporium cladosporioides*. Photo: E. Doyle, August 2022.

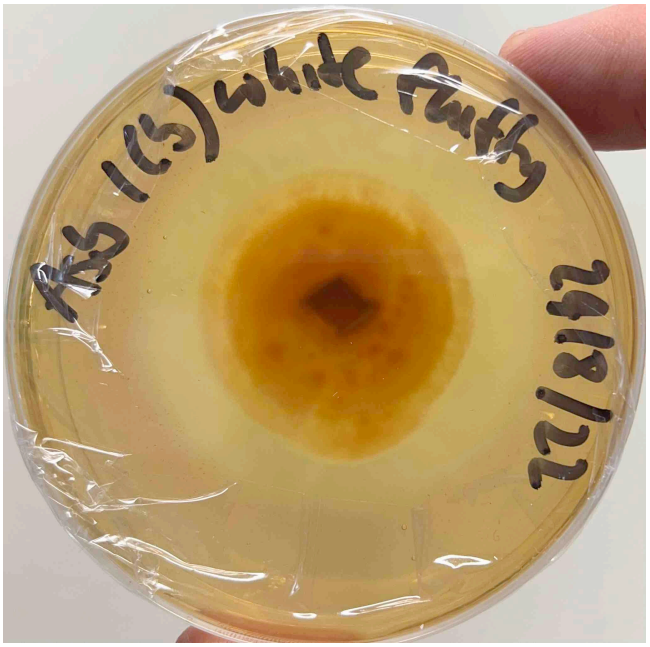

Figure 5. Positive CAS assay result after eight weeks (same plate as Figure 4). Photo: E. Doyle, October 2022.

Table 1. Genbank accession numbers.

Taxon	Isolate	Site	Genbank accession number
Biscogniauxia sp.	Asb10	Kahurangi	OR880384
Cladosporium cladosporioides	Asb6	Kahurangi	OR880385
Cladosporium cladosporioides	Asb1	Auckland	OR880388
Crustomyces sp.	Asb11	Kahurangi	OR880386
Leucosporidium scottii	Asb8	Auckland	OR880389
Pithomyces chartarum	Asb7	Auckland	OR880390
Pezizales	Asb9	Kahurangi	OR880387

Table 2. 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.

Isolate	Site	CAS Agar Result 1	CAS Agar Result 2
Biscogniauxia sp.	Kahurangi	Indeterminate	Indeterminate
Cladosporium cladosporioides	Kahurangi	Positive	Positive
Cladosporium cladosporioides	Auckland	Positive	Positive
Crustomyces sp.	Kahurangi	Positive	Positive
Leucosporidium scottii	Auckland	Positive	Positive
Pithomyces chartarum	Auckland	Not tested	Not tested
Pezizales	Kahurangi	Negative	Negative

Results and Discussion

Four different fungal species were isolated from Kahurangi National Park asbestos biofilm samples. These included *Cladosporium cladosporioides* (Fresen.) G.A. de Vries, an unknown species of *Biscogniauxia* Kuntze and *Crustomyces* Jülich, and an unknown member of the order Pezizales J. Schröt. The three species not given species names did not have 100% matches on Genbank (Table 1), most likely due to their not previously having been sampled for 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* Fell, Statzell, I.L. Hunter & Phaff, and the common grass saprophyte *Pithomyces chartarum*.

Four isolates (*Cladosporium cladosporioides* isolates from both Tāmaki Makaurau / Auckland and Kahurangi National Park, *Crustomyces* sp. and *Leucosporidium scottii*), demonstrated clear positive results (orange or yellow-orange halos and clearing of the blue colouration) with the CAS assay (Table 2), while the *Biscogniauxia* isolate coloured the agar with a yellow-brown exudate and it was not clear if the blue colour of the plate had been removed or masked. While there appear to be no previous reports of *Crustomyces* species producing siderophores, *Leucosporidium scottii* is known to produce them *in vitro* (Vero et al. 2013), and *Cladosporium cladosporioides* has been previously isolated from serpentinite and found to produce the iron-chelating siderophore ferricrocin in culture (Daghino et al. 2008). The three isolates with evidence of siderophores can now be trialed with asbestos fibres *in vitro*.

Data Accessibility Statement

No additional database

Author Contributions

Dan Blanchon: Conceptualisation (lead); methodology (equal); investigation (equal); resources (equal); visualisation (equal); writing – original draft (lead); writing – review and editing (equal); project administration (equal).

Peter de Lange: Methodology (equal); investigation (equal); writing – review and editing (equal).

Erin Doyle: Investigation (equal); resources (equal); writing – review and editing (equal).

Tianyi Tang: Methodology (equal); investigation (equal); writing – review and editing (equal).

Nick Waipara: Writing – review and editing (equal)

Terri-Ann Berry: Conceptualisation (lead); resources (equal); visualisation (lead); writing – review and editing (equal); project administration (lead); funding acquisition (lead).

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Authors

Dr Dan J. Blanchon is Curator of Botany, Auckland War Memorial Museum / Tāmaki Paenga Hira and an Adjunct Associate Professor at Unitec | Te Pūkenga. Dan currently has active research projects on the ecology and management of invasive plants, and the systematics, ecology and conservation of indigenous plants and lichens. Dan has published revisions of *Libertia* (Iridaceae), *Ramalina* (Ramalinaceae) and a range of the lichens of Aotearoa / New Zealand. dblanchon@aucklandmuseum.com

Dr Peter J. de Lange is a Professor teaching at the School of Environmental and Animal Sciences, Unitec | Te Pūkenga. A biosystematist, Peter regularly publishes on plant taxonomy, conservation, threat listing and the flora of South Pacific and outlying Aotearoa / New Zealand islands. pdelange@unitec.ac.nz

Erin J. Doyle is a Molecular Ecology Research Associate in the School of Environmental and Animal Sciences, Unitec | Te Pūkenga. Erin is currently working on a variety of research projects, including assessments of invertebrate diversity, characterisation of the oral biome, and DNA barcoding of Aotearoa / New Zealand plants and lichens. edoyle@unitec.ac.nz

Tianyi Tang holds an MSc degree from the joint graduate programme of The University of Auckland / Waipapa Taumata Rau and the New Zealand Institute of Plant and Food Research. Currently, he serves as a Research Associate at the Applied Molecular Solutions Centre at Unitec | Te Pūkenga, where his research concentrates on various aspects of fungi–plant interactions. His expertise includes the exploration of fungal biocontrol agents, plant protection strategies, and the identification of indoor fungi. ttang@unitec.ac.nzs

Dr Nick Waipara is Science Group Leader at Plant and Food Research / Rangahau Ahumarā Kai, Mount Albert Research Centre, Tāmaki Makaurau / Auckland. Nick is Co-Lead / Kaihautū of Ngā Rākau Taketake, the national research programme on kauri dieback (*Phytophthora agathidicida*) and myrtle rust (*Austropuccinia psidii*), continuing his active project work on plant pathogens and disease management. Nick.Waipara@plantandfood.co.nz

Dr Terri-Ann Berry is a Co-Director of the Environmental Innovation Centre and an Associate Professor at Auckland University of Technology. An environmental engineer, Terri-Ann specialises in sustainable waste-management, indoor air quality and the treatment or remediation of hazardous wastes, such as asbestos. Terri-Ann is a Fellow of the Royal Society of Chemistry. Terri-Ann is also an Adjunct Professor at Southern Cross University, Australia. terri-ann.berry@aut.ac.nz