SUCCESSFUL APPLICATIONS FOR A WALTER FISHER MEMORIAL GRANT 2023

Understanding the molecular landscape and diversity of Queensland's native *Hericium* fungi

Dr Kylie Agnew-Francis, Postdoctoral Research Fellow, The University of Queensland

Background and significance of the topic

Australia is estimated to have 50,000-250,000 species of fungi, the majority of which (~90%) are thought to be endemic. Despite their abundance and the rapidly growing interest in their use, very little research has been conducted into endemic Australian fungi, and our understanding of their diversity, ecological importance, and potential uses remains underdeveloped. One such example is the genus *Hericium,* which are an endemic whiterot genus of mushroom found in temperate rainforests along the eastern and southern coastal regions, and throughout Tasmania. While these are typically assigned as *H. coralloides* based on physical similarities to northern hemisphere varieties, the diversity in Australian *Hericium* and lack of confirming genetic evidence suggests this may be inaccurate. Our interest in *Hericium* species is compelled both by this lack of knowledge, as well as the many interesting medicinal properties, nutritional value, industrial uses, and ecological significance of northern hemisphere species. For example, *H. erinaceus* (lion's mane) is a popular medicinal mushroom known to be rich in bioactive metabolites, and extensively reported as enhancing cognitive function and possessing activity against a variety of neurological conditions (e.g., depressive disorder, anxiety, and Alzheimer's) Despite the clear potential of *Hericium* mushrooms across a wide range of sectors, there have been no reports concerning Australian endemic *Hericium* species, and little is known as to its biological activity, molecular properties, or taxonomic relationships. Over the past year, our research has found that an endemic *Hericium* sp. from Otway National Park differs greatly to North American varieties in its activity against human disease markers, as well as in its protein (determined through proteomics) and chemical composition (determined through metabolomics).

To better understand these differences and the overall diversity and potential uses of Australian Hericium*, this project will complement broader studies being undertaken by the Applicant in these species through the analysis of a small collection of field specimens (approximately five) collected from SEQld national parks.*

Even Fungi Get Stressed Sometimes:
Glutathione and Stress Tolerance in the Amphibian Chytrid

Dr Rebecca Webb, Post-Doctoral Fellow, University of Melbourne

Background and significance of the topic

This cross-disciplinary project will explore the role of glutathione in the amphibian chytrid. I want to understand how glutathione is used to tolerate stress during interactions with the environment and within the host. In particular, I will investigate how the fungus uses its own glutathione to combat environmental heavy metal stress, and how host-produced glutathione might impact fungal pathogenicity. My results will improve understanding of heavy metals as environmental determinants of the fungus and may inform development of targeted treatments to improve frog survival.

The amphibian chytrid, *Batrachochytrium dendrobatidis* (*Bd*) is an unusual fungal pathogen. *Bd* and its salamander-infecting sister species *B. salamandrivorans* (*Bsal*), are the only known examples of chytrids parasitising a vertebrate host. The infective zoospores penetrate the host skin and develop into zoosporangia which release further zoospores. Increased fungal burdens in the epidermis disrupt skin function leading to electrolyte imbalance and eventual death. These ancient fungi originated in Asia, and recently spread to naïve amphibian populations via the amphibian trade. *Bd* has had a dramatic impact on amphibian species worldwide and is considered the worst wildlife disease ever witnessed. It is likely that *Bd* was introduced to Australia via Brisbane in the 1970s, and in the decades since it has driven six Australian frog species to extinction, all of which were from Queensland rainforest. A further two Queensland frog species are at high risk of extinction, and several more have experienced severe population declines due to *Bd*.

Identifying the virulence factors of *Bd* has become a critical area of research. Previously I investigated glutathione biosynthesis as a potential virulence factor. Glutathione is an important antioxidant for many fungal pathogens as it protects them from oxidative stress generated by the hosts' immune system. I have established how to manipulate glutathione levels in *Bd*, and examined the effect on stress tolerance and fungal lifecycle.

My results from this project were unexpected, suggesting the role of glutathione in *Bd* differs from that of other fungi. In particular, there were three main findings that piqued my curiosity for further research:
1. Unlike many other fungi, glutathione is not essential for oxidative stress tolerance, however it is important for **tolerating cadmium**.
2. Low levels of exogenous glutathione **dramatically increased zoospore production**.
3. In contrast, slightly higher levels of glutathione were strongly inhibitory and **decreased tolerance to osmotic stress and heat stress.**

This project aims to expand on these findings to further explore the role of glutathione in Bd during metal exposure, lifecycle progression and host infection.

Investigating the Plant Growth Promotion Potential of Native Seed Fungi to Improve Native Australian Grassland Restoration

Ms Allison Mertin, PhD Candidate, University of Melbourne and Royal Botanic Gardens Sydney

Background and significance of the topic

The need for restoration of degraded grassland ecosystems and the conservation of threatened or endangered grassland ecological communities within Australia is increasing rapidly. As seedling establishment within grassland restoration projects can be as low as 10% it is crucial to investigate novel ways in which rates of plant growth can be increased at these degraded sites. As many grasslands are revegetated using seed rather than seedlings, seed presents an avenue to facilitate this.

Seed fungal endophytes are microscopic fungi that are present within the living tissue of seed. They are dispersed with the plant seed and already are present at the first developmental stages of the new seedling. Seed fungal endophytes therefore form the primary inoculum for the initial plant mycobiome, having the capability to colonise the emerging seedlings with potential impacts on plant fitness. Research on agricultural plants have highlighted important functional roles of seed fungal endophytes but yet the diversity, biology and ecology of fungi of seed are least known of all the plant organs, with studies of natural ecosystem hosts limited. In particular, there is a paucity of studies looking at native Australian host species. A recent meta-analysis of seed microbiomes analysed data from 3190 seed samples of which only 10 samples were from Australia.

The project will contribute to increasing our understanding of seed fungal endophyte biology and raise awareness of the unknown microbial diversity within native Australian seeds and their potential role in ecosystem function and plant survival, with applications to restoration and *ex situ* conservation. Specifically, I will combine mycobiome identity (data already obtained from internal transcribed spacer metabarcoding and Sanger Sequencing of pure cultures) with plant growth promotion potential phenotypes of isolated fungal strains tested using seed inoculation greenhouse experiments to better understand the potential function of seed endophytes for Australian native plants. A synthesised knowledge of seed microbiota will accelerate discovery and help future practices promoting the presence of important seed microorganisms for plant health and productivity.

This project will increase our knowledge of the unexplored diversity of fungal endophytes of native Australian seed.

Structural characterisation of valuable new antibiotics from Queensland rainforest endophytic microfungi

Dr. John Dearnaley, Associate Professor in Plant Microbe Interactions, University of Southern Queensland

Background and significance of the topic

There is a continued need for new antimicrobial compounds in both the medical and agricultural spheres. In the health setting, some bacteria such as multi-resistant *Staphylococcus aureus* (MRSA) are difficult to combat with most standard antibiotics. Options for controlling medically significant fungi such as *Candida albicans* and *Cryptococcus neoformans* are limited and current antimycotics (e.g. amphotericin B) cause significant side effects. In agriculture many commonly used fungicides have begun to lose efficacy (e.g. strobilurans) or have been banned due to significant human health impacts e.g. iprodione).

Fungal endophytes are microfungi that occur in plant tissues without inducing obvious disease symptoms in the host. These fungi appear to improve host fitness by reducing stresses caused by biotic and a biotic factors. Recent studies in our laboratory have shown that Australian plants harbor a diversity of endophytic fungi with the most common taxa represented including *Nigrospora, Alternaria, Xylaria, Phyllosticta* and *Preussia* spp. Endophytic fungi have been shown to produce an array of bioactive compounds including anti-bacterial, anti-fungal, anti-plasmodial and anti-cancer agents. In our laboratory, Mapperson et al. (2014) and Sharma (2021) showed that species of fungal endophytes from dry rainforest plants contained compounds that were bioactive against Gram positive bacteria and *Candida* and *Rhodotorula* yeast fungi. High pressure liquid chromatography (HPLC) and Nuclear Magnetic Resonance (NMR) analysis indicated that some of the bioactive compounds present in the studied endophytes were polyketides of the same structural class to which many important antibiotics such as tetracycline, erythromycin and amphotericin B also belong.

This project is focussed on investigating, for the first time, the diversity and bioactive potential of Queensland subtropical rainforest fungal endophytes. This project seeks to answer four research questions:

1. Do subtropical rainforests harbour novel endophytic microfungi?
2. Do subtropical rainforest microfungi show bioactivity against medically important microbes?
3. What are the compounds responsible for the endophytic bioactivity observed?
4. What is the cellular mode of action of the endophytic bioactive compounds?

The project constitutes a PhD project (2022-2025) at the University of Southern Queensland. Questions 1 and 2 are currently being addressed.

This application is to fund NMR analysis of the isolated bioactive compounds at the Griffith University Institute for Drug Discovery (i.e. question 3). These analyses will assist with the identification and structural characterisation of the isolated bioactive molecules.