Mycobiome of Bondi Beach Sand

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Figure 1: Mixed culture of fungi growing from Bondi beach sand

Introduction

This project is based on the One Health model, acknowledging the concomitance of human, animal and environmental factors influencing health¹. Annually, more than 300 million people suffer from various fungal infections² with an estimated 1.7 million deaths worldwide³. The majority of invasive fungal infections globally are caused by opportunistic pathogens including *Aspergillus*, *Candida*, *Cryptococcus*, *Rhizopus* and *Pneumocystis* species⁴. It is known that human pathogens are often zoonotic in origin with the capacity to survive in the environment⁵ and arid sand is no different. There are many factors influencing the fungal load and multiplicity of beach sand, with the frequency and level of human interaction being a significant contributor⁶. Clinically significant fungi, such as *Trichophyton*, *Microsporum*, *Cladosporium*, *Epidermophyton* and *Candida* have been identified in beach sand around the world⁷.

Aims

1. Characterize mycobiota in Bondi beach sand
2. Identify medically relevant fungi
3. Assess implications to Public Health
4. Find correlations with environmental factors and levels of human traffic

Figure 2: ITS locus with primer binding sites labelled in red

Methods

Traditional culturing methods and Sanger sequencing, together with Diversity Profiling via Next Generation Sequencing (NGS) were used to characterize the mycobiome of Bondi beach sand. Sand was collected from 6 locations on the beach (Figure 4a) at five time points (Figure 3) across peak and shoulder seasons of beach use. From each location, five samples were taken to form a composite sample (Figure 4b) with one gram of sand from each composite placed onto three different media. Fungal growth can be seen in Figure 1. Fungi were sub-cultured as they grew and placed onto fresh media to obtain pure isolates. For Sanger sequencing, DNA from pure fungal cultures was extracted following the conventional phenol-chloroform method, sequenced using the ITS1 and ITS4 primers (Figure 2), with bidirectional sequences assembled and edited using Sequencer™ ver. 5.2.44.9, and finally identified using BLAST database searches. For NGS analysis, total genomic DNA was extracted directly from the sand using PowerMax soil DNA Isolation Kit according to the manufacturer's instructions, genomic DNA was sent to AGRF for ITS diversity profiling. Sequence reads were processed for quality filtering and chimera detection using various bioinformatics tools. The quality filtered paired sequences were clustered to operational taxonomic units (OTUs) with the UPARSE pipeline using the "unoise" algorithm to identify all correct biological sequences in the reads with zero clustering distance. The most abundant sequence of each cluster were selected as a representative for taxonomic assignments using the most recent combined (available on 04.03.2017) UNITE (<https://unite.ut.ee>) full dataset and the ISHAM-ITS (<http://its.mycologylab.org>) database.

Figure 3: Time scale of sample collection points

Figure 4: (A) Sample collection points on Bondi beach (red). (B) Method of sample collection at each site, with 5 samples made into a composite for each location.

Results

Both saprophytic and medically relevant fungi were cultured from sand at Bondi beach (Figure 5). There were 12 saprophytes and 28 medically relevant isolates identified based on culture (Figures 5 and 6). Of the 6 locations sampled (Figure 4A) on Bondi beach, at the 5 time points (Figure 3), no species recovered from the genera *Penicillium* or *Alternaria* were pathogenic. Opportunistic fungal pathogens were recovered from the genera *Aspergillus*, *Cladosporium*, *Fusarium*, *Geotrichum*, *Mucor*, *Rhodotorula* and *Torulaspora*, with genera *Mucor*, *Penicillium* and *Fusarium* being recovered at higher rates. The operational taxonomic units (OTUs) generated from NGS provided 270 identifications at species level, of which 130 were saprophytes and 140 were medically relevant (Figure 6).

References

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Figure 5: Number of isolates identified by traditional culturing methods for each genera

Figure 6: The proportion of fungi identified via next generation sequencing and traditional culture methods, classified as medically relevant or saprophytes

Future Direction

To analyze specific interaction characteristics, data collected both by culture and next generation sequencing will to be further analyzed by categorizing sample locations into low and high traffic areas, as well as categorizing the temporal data into peak and shoulder seasons.