The isolation of environmental *Mycobacterium sp* in Queensland

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Background

In QLD, *M. intracellulare* is the most frequently isolated NTM and has been isolated from soil and tank water, but not municipal water distribution systems. Further investigation of soil and house dust as a source of infection is warranted. Environmental samples such as soil and dust have a wide range of organisms, which overgrow the slowly growing mycobacterial species, making NTM isolation problematic. A review of the methodologies for the isolation of mycobacteria from soil and dust failed to identify a "gold standard" method. This study investigated a range of decontaminating agents to develop a strategy which would yield the highest recovery and diversity of mycobacterial species with the least contamination.

Method

All samples were incubated to encourage spore germination then treated with a decontamination method. Initially, samples were incubated in sterile saline Experiment 1). Later Trypticase Soy Broth with Tween80 (TSB + T80) (Experiment 2) was used to increase the germination of spores. Decontamination included 2% NaOH with 0.3% Malachite Green (MG), 4% NaOH and Cetylpyridinium Chloride (0.005%) (CPC). Decontaminating agents were introduced incrementally. Liquid media (BD Bactec™ MGIT™ (Mycobacterial Growth Indicator Tube), one supplemented with Oxalic acid, Albumin, Dextrose, Catalase (OADC) and a second with PANTA™ (Polymyxin B, Ampicillin, Nalidixic Acid, Trimethoprim and Azlocillin) (Figure 1) and solid media (M7H10 + OADC and M7H11 agars) were included. PANTA supplement was also trialled at double concentration to reduce contamination (Experiment 2). Two incubation temperatures were compared for solid media. Plates were read daily first the first week to monitor contamination. Figure 2 is an example of a culture plate showing a mix of contaminating and mycobacteria. Plates which showed fungal contamination were discarded. Any colonies with the features of mycobacteria underwent Zeihl-Neelsen (ZN) staining (Fig 3) and were sub-cultured before bacterial overgrowth occurred. All ZN positive colonies were stored at -80ºC.

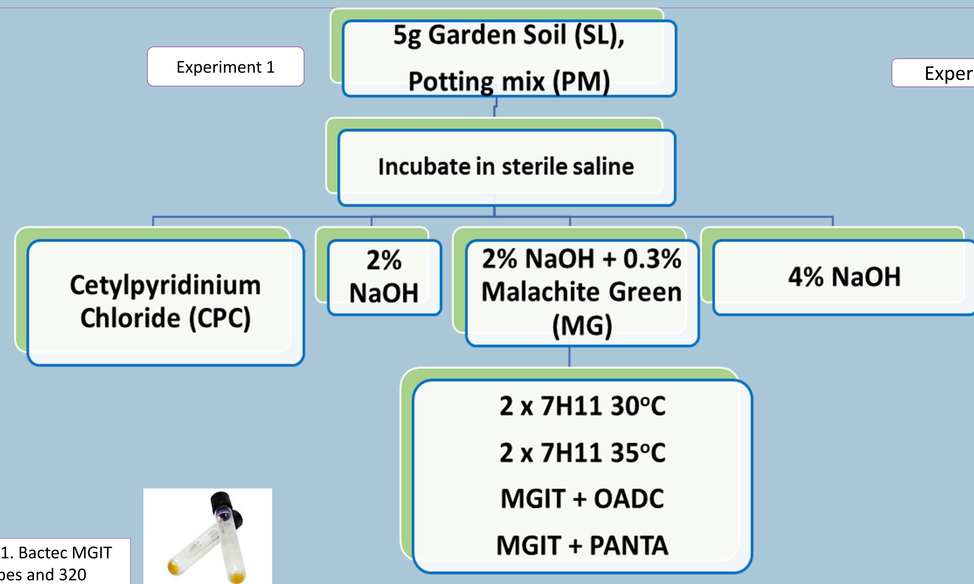

Fig1. Bactec MGIT Tubes and 320 Instrument.

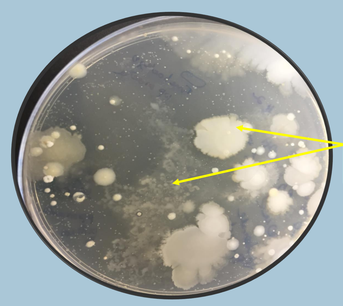

Fig 2. Contamination visible on culture plates

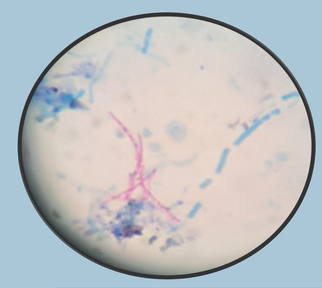

Fig 3. ZN smear showing pink Acid-fast bacilli and blue contaminating organisms

Results and Discussion

The use of TSB +T80 reduced contamination particularly for liquid media. However, malachite green (MG) interfered with the fluorescent indicator in the Bactec™ MGIT™ vial. 4% NaOH (Figure 4) gave the highest recovery of mycobacterial species with a low contamination rate and will provide a basis for future work

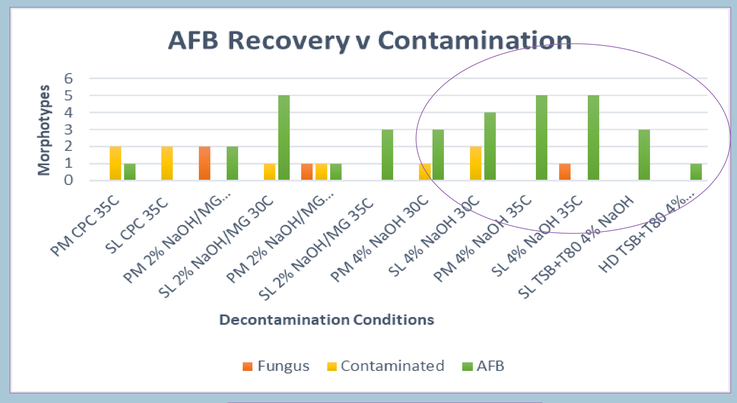

Fig 4. Recovery of mycobacterial sp contamination

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