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Impact of decontaminant and microbiological media on Mycobacterium avium subspecies paratuberculosis

Mycobacterium avium subspecies *paratuberculosis* (Map) has aetiological significance in Crohn's disease of humans, and chronic intestinal disease in domestic and wild ruminants. This opportunistic pathogen can proliferate inside its host, whilst restricted to grow in the environment. Growth restrictions of Map may be due to low ambient temperature, absence of adventitious mycobactin, and the influence of background microflora which may be found in significant numbers with phenotypic and genotypic diversity, thus making it difficult to isolate Map from among many other bacterial species. The slow growing nature of Map – requires up to 18 weeks to grow on primary culture, and no selective medium recognised for isolating the organism calls for use of a range of media for its isolation following preliminary concentration and chemical decontamination procedures in non-sterile samples.

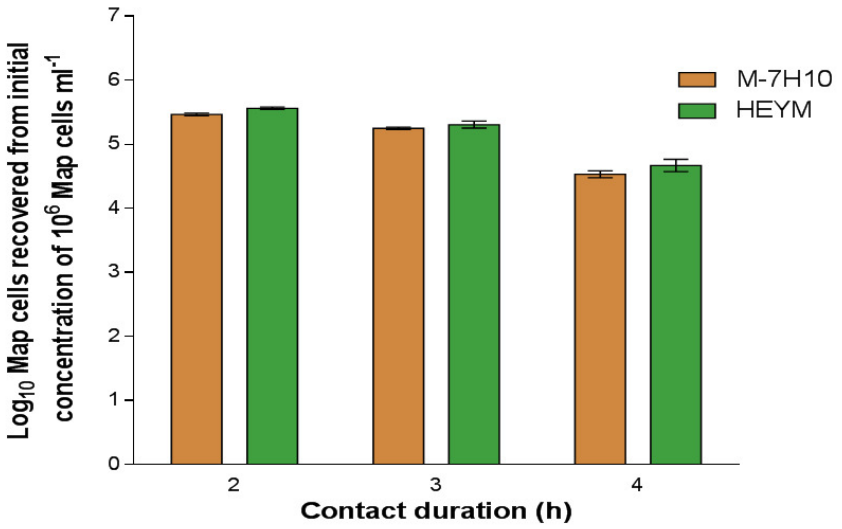

Fig. 1. Effect of decontamination on the recovery of Map from spiked water sediments which represents the mean of 3 replicate runs of decontamination with 0.75% w/v CPC at 2, 3 and 4 h contact durations.

The culture and decontamination practices however, do not always retrieve the original numbers of Map present, as viable non-culturable forms and non-viable or dead cells may be present. Furthermore, the type and concentration of decontaminant as well as contact duration of decontamination could result in complete loss or low yield of Map. The detection of Map from environmental samples presents two major problems viz. the organism is likely to be present in low numbers against a high background microflora which has implications for the sensitivity and specificity of the assay and there are likely to be components in the sample such as humic acids which may render unwanted consequences. In order to overcome these, methods have been devised to both concentrate the target Map cells and remove the non-target cells and possible inhibitory compounds. Such techniques employed include concentrating Map by filtration and centrifugation alongside immunomagnetic separation (IMS) followed by decontamination with 0.75% w/v cetylpyridinium chloride (CPC). Decontamination procedures reported in literature have utilised CPC at a concentration of 0.75% w/v,

at varying contact durations. This method has been successful, and has led to the recovery of Map from samples including milk and raw untreated water. Evaluation of decontamination method at varying intervals (i.e. 2, 3 and 4 hours) instead needed to be carried out empirically to determine which of these time intervals yield significant reduction or complete kill of all background microflora, and promote the recovery of Map from water sediments. For the recovery of Map from water sediments, both solid and liquid media were used viz. conventional culture media HEYM, M-7H10 and Bactec 12B. All media were supplemented with antibiotic cocktail [(PANTA; 40 U/ml polymyxin, 4 µg/ml amphotericin B, 16 µg/ml nalidixic acid, 4 µg/ml trimethoprim, and 4 µg/ml azlocillin) and VAN; vancomycin (10 µg/ml), amphotericin B (4 µg/ml) and nalidixic acid (16 µg/ml) for HEYM and M-7H10; PANTA for Bactec 12B)] and mycobactin J (2 µg l^{-1}). The percentage recovery of Map based on an approximate initial concentration of 10^6 cells ml^{-1} was 91.1%, 87.5% and 75.6% for M-7H10 medium compared with 92.7%, 88.4% and 77.8% for HEYM medium at the respective 2, 3 and 4 h contact durations. Taking into consideration both media individually, there was a significant difference ($P < 0.05$) in recovery at the three contact durations with 2 h giving the greatest recovery which declined as contact duration with CPC increased. There was no significant difference ($P < 0.05$) in recovery between the two media at any of the contact durations investigated – the results, as presented graphically in Figure 1.

Bactec 12B assay also showed a higher growth index with the 2 h than 3 and 4 h following the mean data obtained from their triplicate runs (Fig. 2).

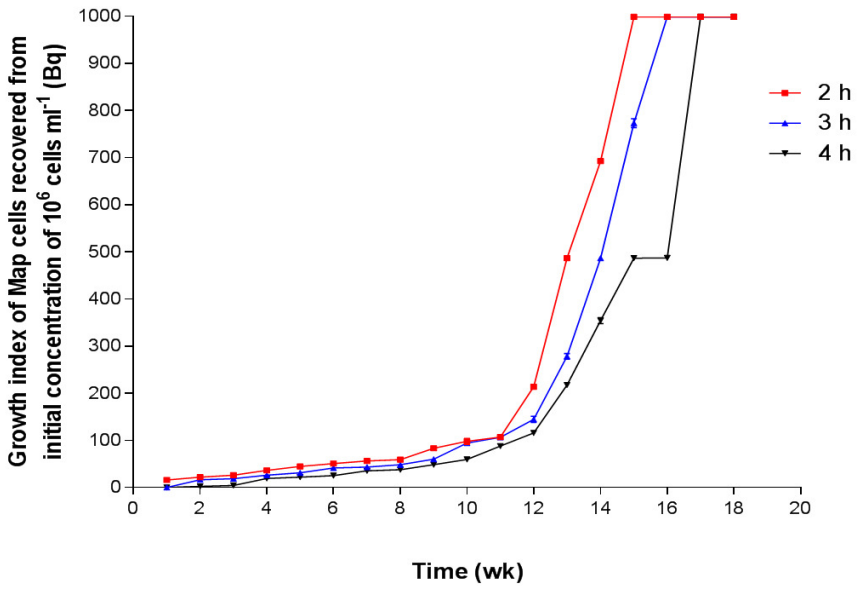

Fig. 2. Mean data of growth index measurement of 10^6 cells ml^{-1} of Map incubated for 18 weeks following decontamination with 0.75% (w/v) CPC at 2, 3 and 4 h contact durations.

There was a prolonged lag phase in the three contact durations up to week 10, with exponential phase ensuing thereafter to 15, 16 and 17 weeks for 2, 3 and 4 h contact durations respectively which stabilised to 18 weeks. Growth index for 2 h was thus higher during the exponential period than 3 and 4 h indicating that increasing

the contact duration was detrimental to the survival of Map during the period of incubation. This result shows a similar recovery pattern to what was shown in Figure 1. Thus using radiometric (Bactec 12B) or non-radiometric (M-7H10 and HEYM) media, a lower recovery was observed as contact duration of the decontaminant increased.

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Publication

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