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Enhanced mycobacterial diagnostics in liquid medium by microaerobic bubble flow in Portable Microbe Enrichment Unit

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Abstract

Portable Microbe Enrichment Unit (PMEU) method with microaerobic bubbling speeded up the growth of otherwise slowly starting and propagating *Mycobacterium* sp. *Mycobacterium fortuitum* growth was detected after 10–11 h and *Mycobacterium marinum* produced clear growth in 4 days. A mycobacterial environmental isolate was verified in 2 days in the PMEU Spectrion® equipped with infrared sensors. In parallel static (without gas bubbling) cultures hardly any growth occurred. In conclusion, PMEU technology provided thus a rapid detection of environmental and clinical mycobacterial isolates. It would also help in the field diagnosis of antibiotic resistant *Mycobacterium tuberculosis*.

Keywords: Mycobacteria; Infection; Early diagnosis; Tuberculosis; PMEU; Rapid microbiology; MDR; XDR; Antibiotic resistance; Field microbiology

1. Introduction

Mycobacteria grow slowly in laboratory and also *in vivo*. Practically all of them are either pathogens or opportunistic pathogens. In global perspective, about 1.4 M lives are lost annually due to *Mycobacterium tuberculosis* infections [1]. In a multi-national study in Estonia, Latvia, Peru, Philippines, Russia, South Africa, South Korea, and Thailand, on average of 6.7% of patients had XDR (extensively drug-resistant) tuberculosis [2]. The range between study sites was 0.8–15.2% and 43.7% of the patients had a strain resistant to at least one of the second-line antibiotics. Clinical confirmation of tuberculosis with antibiotic resistance analysis usually takes several weeks and delays the treatment as well precaution actions [1]. Among new diseases, 4% is caused by a multi-resistant strain (MDR).

M. tuberculosis infections are often associated with lungs, but different mycobacteria cause diseases in different tissues. Both *Mycobacterium fortuitum* and *M. tuberculosis*, for example, can also turn into intracellular pathogens [3].

PMEU technologies with gas bubble flow comprise of different techniques to monitor hygiene in water and other samples. Enhanced microbiological cultivation and detection methods have been developed for clinical, industrial and environmental samples [4,5]. The essential point is the option to boost the microbes with gas bubble flow [6,7]. Various standard media can be used in cultivations. The optimation of gas bubbling in PMEU has improved the recovery rate of the enterobacterial isolates about 2.5 times when compared to the standard cultivation without PMEU acceleration [8].

Regarding the field monitoring of tuberculosis, Kimbrough et al. [9] stated: “Findings suggest the need for early establishment of the tuberculosis services, especially in displaced populations from high-burden areas and for continued innovation and prioritisation of tuberculosis control in crisis setting”.

The aim of this study was to test if the PMEU method and especially its gas flow could enhance the cultivation of *Mycobacterium* sp. As the device is portable these results could also be exploited in field conditions in early detection of *M. tuberculosis* infection and epidemics, and also other pathogenic or opportunistically pathogenic mycobacteria. Their cultivation is at present still time consuming and forms

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the bottle-neck in overall diagnosis of these diseases and hampers the control of the epidemics.

2. Materials and methods

2.1. Bacterial strains and their cultivation

M. tuberculosis is a hazardous study organism. Therefore, *M. fortuitum* (ATCC 6841) and *Mycobacterium marinum* (ATCC 927) were used in this study. They were obtained from the American Type Culture Collection. Our environmental isolate E40 (from river water) was derived from the Finnish National Institute of Health and Welfare.

The cultivation took place at 33–35 °C in PMEU Spectrion® with diminished oxygen concentration (5%) [10] using M7H9 broth with Middlebrook ADC Enrichment supplement (Beckton Dickinson, USA) and used according to the instructions of the manufacturer. Parts of the PMEU cultivation syringes were bubbled with gas flow, while others remained stagnant.

Plate cultures were made on M7H9 broth medium supplemented with 1.25% agar and ChromAgar™.

3. Results and discussion

Enrichment cultivation is crucial in mycobacterial diagnosis. It was remarkably speeded up in case of *M. fortuitum* using PMEU Spectrion®, which is an easily portable cultivation and detection unit. When a heavy inoculum of about 10^5–10^6 cells was used for a broth culture, the onset of growth was recorded within 10–11 h (Fig. 1).

As gas bubbling has been previously shown to promote the onset of growth in the PMEU [6,7]. The gas mixture was obtained from the pressurized bottles and contained 5% O_2, 10% CO_2, and 85% N_2, corresponding to the atmosphere in campylobacterial cultivation in the PMEU [10]. In static mycobacterial reference cultures, hardly any growth occurred in five days. For the colony counts from the static and PMEU cultures, see Table 1.

Strain of *Mycobacterium* sp., isolated from river water in Finland by the National Institute of Health and Welfare, was also tested in the PMEU Spectrion®. The growth started in about 2 days (Fig. 2).

Purity of mycobacterial cultures was tested on Petri dishes (Fig. 3A and B) and by microscopical monitoring (Fig. 4). Final cell concentrations of *Mycobacterium* sp. and *M. marinum* after 3 and 5 days, respectively, are presented in Table 2. The PMEU technique turned out to be a potential approach in developing fast methods to monitor clinical and environmental mycobacteria. It can also be developed for the diagnostics of *M. tuberculosis* in the laboratory and in the field.

Antibiotic resistance monitoring with PMEU Spectrion® has been carried out *e.g.* with *Enterobacter cloaceae* hospital strain No. 165/08 obtained from the Neonatal Intensive Care

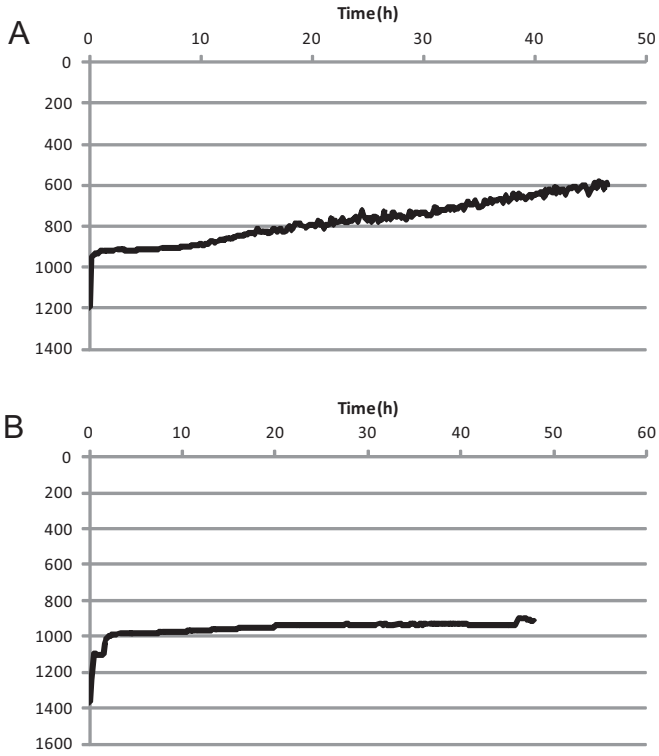

Fig. 1. Growth curves on *M. fortuitum* from PMEU Spectrion® analysis. The culture with gas bubble flow above (A), with static reference culture below (B). In the former one A case, the onset of growth was recorded in less than 12 h.

Unit of Kuopio University Hospital (a gift from Drs. Ulla Sankilampi and Jouni Pesola) [11]. Corresponding to the cultivation of different mycobacteria, the bacterial growth was monitored by the IR sensors also in this case. This strain was shown to be resistant to penicillin G and cefuroxime in 5 and 6 h, respectively.

Versions of the PMEU could be applied widely for the sensitive detection of pathogenic bacteria [5].

PMEU Spectrion® equipment has been validated in the Finnish State Research Centre (VTT) for the analysis of hygiene indicating coliforms [12]. An automated sampling

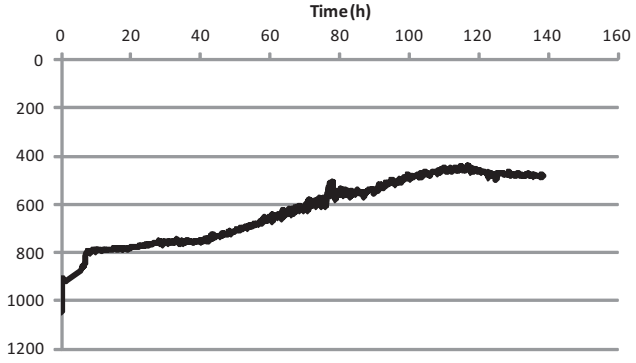

Fig. 2. Development of a *Mycobacterium* sp. culture during 6 days of PMEU Spectrion® cultivation. The early signs of increasing density were seen after 24 h, and clear onset of growth occurred in 48 h.