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Identification of fish isolated mycobacteria using sodium dodecyl sulphate-polyacrylamide gel electrophoresis

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ABTRACT

Mycobacteriosis is a progressive disease of a wide range of wild and captive marine and freshwater fish species. Phenotypical methods for identifying mycobacteria, such as acid fast staining and biochemical tests, are neither specific nor sensitive for identifying mycobacteria to the species level. The aim of this study was to assess the use of sodium dodecyl sulphate- polyacrylamide gel electrophoresis (SDS-PAGE) for the identification of different aquatic mycobacteria species. The SDS-PAGE of whole cell protein of 54 mycobacterial strains was determined. Strains included 3 reference stains and 51 field isolates. All strains had major band patterns between 32 and 90 kDa (kilo Dalton) and few bands were seen above 90 kDA. There were no differences between the band profiles of M. marinum isolates compared with those of the M. marinum reference strain; however, differences in 2 or 3 bands were observed in 6 isolates of M. fortuitum. As for M. chelonae isolates, only 1 isolate showed difference in 3 bands in comparison with that of the reference strain. The results of this study suggest that SDS-PAGE can be a useful method for characterization of both slowly growing and rapidly growing mycobacteria in clinical laboratories. This technique does not require expensive equipment and visual examining of bands patterns is adequate for differentiation of aquatic mycobacteria especially when the reference strains are included in each run.

Keywords: SDS-PAGE, Identification, Fish, Mycobacteria

INTRODUCTION

Mycobacteriosis is a common disease among both wild and captive fish [1]. The *Mycobacterium* species most commonly reported in fish include *M. marinum*, *M. fortuitum* and *M. chelonae*, though some other species of mycobacteria have also been reported [2-7].

The differentiation of *Mycobacterium* species has traditionally been based on growth characteristics and biochemical testing, however, the procedures used in such analyses are complex, laborious and time consuming. A comprehensive rapid detection method, capable of identifying multiple species of mycobacteriain a single procedure, would have a significant impact on the identification of mycobacterial species involved in disease episodes, and in turn lead to faster control of fish disease [8]. Thus, the present study is an attempt to determine whether SDS-PAGE of mycobacterial whole cell protein is a method that can be useful for the correct identification of the most frequently species of mycobacteria that are isolated from aquatic organisms.

MATERIALS AND METHODS

Bacterial isolates

Fifty-four aquatic mycobacterial strains including 3reference strains: *M. fortuitum* (NCIMB 1295), *M. chelonae* (ATCC 19235) and *M. marinum* (NCIMB 1297) and 51 field isolates comprising 17 isolates of *M. fortuitum*, 12 isolates of *M. chelonae* and 22 isolates of *M. marinum* obtained from different geographical locations were used in this study. All strains were grown on Lowenstein Jensen medium (Becton-Dickinson, USA) and incubated at 28°C for 3 to 5 days in case of *M. fortuitum* and *M. chelonae* strains and 3-10 days for *M. marinum* isolates.

Preparation of Whole cell Protein

Cultures of the above mentioned mycobacteria were scraped and suspended in 10 ml phosphate buffered saline (PBS) and centrifuged at 3000 g at 4°C for 15 min. The supernatant were then discarded and centrifugation was repeated and the resulting pellet was suspended in 3 ml PBS and concentration of each sample was adjusted to optical density equivalent 1 at 600 nm on a spectrophotometer, then after, centrifugation was repeated and the final pellet was suspended in 1 ml sample buffer containing Tris-HCl, glycerol, SDS, dithiothreitol, bromophenolblue and distilled water and stored at -20°C until electrophoresis was performed.

Electrophoresis and Staining

The samples were boiled for 5 min and centrifuged at 2300 × g for 2 min from which 15 µl of sample was loaded into the wells of a 4 % SDS-PAGE stacking geland run through a 12 % separating gel according to Laemmli [9]. Broad range molecular weight (MW) protein markers (Biolabs, UK) were run alongside the samples to serve as MW reference. The gels were run at 200 V for 60 min and were then stained overnight in Commassie brilliant blue followed by destaining with 40 % (v/v) methanol and 10 % (v/v) acetic acid. Gels were scanned and the MW of bands was determined visually. Band patterns of field isolates were compared with that of the reference strains of the same species.

RESULTS

A representative selection of the band patterns obtained from SDS-PAGE is shown in Fig. 1. Generally, the electrophoresis bands of the field isolated were similar to those of the reference strains. All had major bands of 91, 87, 85, 69, 59, 50, 35, and 32 kDa. Few bands were seen above 90 kDa, while an increase in the number of bands was observed below 90 kDa. There were no differences between the band profiles of *M. marinum* isolates compared with those of the *M. marinum* reference strain; however, differences in 2 or 3 bands were observed in 6 isolates of *M. fortuitum*. As for *M. chelonae* isolates, only 1 isolate showed difference in 3 bands in comparison with that of the reference strain.

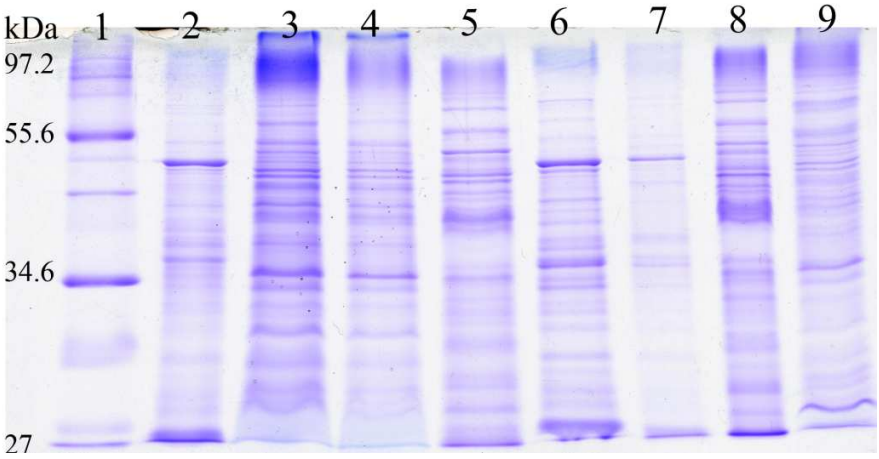

Figure 1: A representative selection of SDS-PAGE results obtained for aquatic *Mycobacterium* spp., stained with Commassie blue
Lanes: 1) Standard marker; 2)*M. fortuitum* (NCIMB 1295); 3) *M. marinum* (NCIMB 1297); 4) *M. marinum* FM-12; 5) *M. marinum* FM-13;6) *M. fortuitum*S89; 7) *M. fortuitum* FM-28; 8) *M. chelonae* (ATCC 19235); 9) *M. chelonae*FM-49

DISCUSSION AND CONCLOSION

The precise species identification of aquatic mycobacteria recovered from infected fish is important from epidemiological and pathogenicity viewpoints [10]. Although there might have been small differences in the minor bands in the SDS-PAGE profiles of whole-cell proteins among the isolates of *M. marinum*, all the strains isolated from the field samples, despite obtained from geographical regions, had an identical pattern; therefore, it was suggested that all the strains came from a common source, i.e., the water.

Mycobacterium chelonae together with *M. fortuitum*are rapidly growing mycobacteria that are widely distributed in soil and aquatic organisms and have been clearly recognised as fish pathogens (11-13). However, their taxonomy at the species level has been subjected to several modifications within recent years (14). These changes have resulted in more complicated species identification, and most often a member of *M. fortuitum* or *M. chelonae* complex is the only identification that could be reported. However, differences in pathological significance, epidemiological aspects and antimicrobial susceptibility of these isolates necessitate precise species identification. In developing countries, identification of mycobacteria is mostly based on microscopy, i.e., acid fast staining and biochemical tests (15). Acid fast staining is relatively rapid, but it is neither specific nor sensitive. Biochemical tests are time consuming and labour intensive and even may not differentiate species of mycobacteria. Other approaches such as polymerase chain reaction followed by DNA sequence analysis are not available in these countries or not accessible for local laboratories.

SDS-PAGE has been previously employed for identification of several bacteria including coryneform bacteria as well as species of mycobacteria (16-17); however, these studies have mainly focused on slowly growing mycobacteria isolated from humans (18-22). The findings of the current research suggest that SDS-PAGE can be a useful method for characterization of both slowly growing and rapidly growing mycobacteria in clinical laboratories within fairly short round time (one day after cultures have grown). This technique does not require expensive equipment and visual examining of band patterns is adequate for differentiation of aquatic mycobacteria especially when the reference strains are run alongside with field isolates. However, more studies, i.e. inclusion of some other species of fish isolated mycobacteria are warranted to confirm the value of this methodfor correct species identification.

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