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CONCISE COMMUNICATION

SDS-PAGE for identification of species belonging to the *Mycobacterium fortuitum* complex

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We performed a study to determine the usefulness of SDS-PAGE of whole cell proteins for the characterization of species of rapidly growing mycobacteria belonging to the *Mycobacterium fortuitum* complex. Strains included 37 *M. fortuitum*, 32 *M. chelonae*, 10 *M. peregrinum*, 5 *M. abscessus*, and 3 *M. mucogenicum*. Eight collection strains (including type strains of the five species) were also included in the study. All strains yielded between 44 and 58 bands in the electrophoretograms. Intraspecies similarity showed Dice coefficients higher than 95%, with only one strain of *M. fortuitum* having a six-band difference (Dice coefficient 87.75%). However, interspecies similarity was always below 75%, the similarity being higher between *M. fortuitum* and *M. peregrinum* (75.51%) and between *M. chelonae* and *M. abscessus* (54.9%). Visual examination of the electrophoretograms was sufficient for species characterization. SDS-PAGE of whole cell proteins is a useful technique for identification of isolates of the *M. fortuitum* complex, and is easy to perform without the need for complex or expensive equipment.

Keywords SDS-PAGE, electrophoresis, rapidly growing mycobacteria

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INTRODUCTION

Infection due to non-tuberculous mycobacteria is a matter of increasing interest. Many of these organisms are currently recognized as human pathogens, and among these, the members of the *Mycobacterium fortuitum* complex are the only rapidly growing mycobacterial species that are frequently isolated as human pathogens [1,2]. Members of this group include *M. fortuitum* and *M. chelonae*, which have been divided classically into several subspecies or biotypes [1]. However, recent taxonomic studies have given a more complex view, by reclassifying some of these subdivisions as new species [3], and even including new members in this group of organisms [4]. These changes represent new difficulties for clinical microbiology laboratories, because traditional identification of these species requires a high number of biochemical tests, and is necessarily slow. Differences in susceptibility, clinical significance and epidemiologic interest make it important to obtain a correct identification of the isolates to the species level. The present study is an attempt to determine whether SDS-PAGE electrophoresis of mycobacterial cell proteins is a technique that can be useful in clinical microbiology laboratories for the correct identification of members of the *M. fortuitum* complex.

MATERIALS AND METHODS

Strains

Ninety-five strains of mycobacteria belonging to the *M. fortuitum* complex were included in this study. These comprised 37 *M. fortuitum*, 32 *M. chelonae*, 10 *M. peregrinum*, five *M. abscessus*, and three *M. mucogenicum*. Type strains of *M. peregrinum* (ATCC 14467), *M. fortuitum* (ATCC 6841), *M. chelonae* (ATCC 35752), *M. abscessus* (ATCC 19977), and *M. mucogenicum* (ATCC 49650), and collection strains of *M. chelonae* (ATCC 19235 and one strain of *M. chelonae* biovar. *niacinogenes* provided by the Centro Nacional de Microbiología (CNM,

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Majadahonda, Spain)) and *M. fortuitum* (ATCC 13756) were also included in the study. All other strains were clinical isolates (one strain/patient) from samples received in the Department of Medical Microbiology of the Fundación Jiménez Díaz for mycobacterial culture and strains sent for identification to the Mycobacteria Laboratory from the CNM. The isolates were identified by using common biochemical tests according to the schemes for identification of the species belonging to the *M. fortuitum* complex [1,3–5]. All clinical isolates from our laboratory were also sent for confirmation of the identification to the Mycobacteria Laboratory of the CNM.

Extraction of cell proteins

All the strains were grown on Middlebrook 7H11 agar plates (BD, Franklin Lakes, NJ, USA) for 3 days at 30 °C. Cultures were then scraped and suspended in 3 mL of 50 mM Tris–HCl (pH 7). Lysozyme (Sigma, St Louis, MO, USA) was added to each tube (final concentration, 200 mg/L) and incubated for 30 min at 37 °C. After incubation, the cells were sonicated for 1.5 min (one cycle, 100 A) in a UP 200 H sonicator (Dr Hielscher GmbH, Teltow, Germany). The samples were then centrifuged at 5000*g* at 4 °C for 10 min, and the pellets were discarded. Supernatants were frozen at −70 °C until electrophoresis was performed. The concentration of proteins in each sample was calculated by using the Bio-Rad DC Protein Assay (Bio-Rad, Hercules, CA, USA), using bovine serum albumin (Bio-Rad) as the control. Samples were diluted with the same buffer to obtain a final protein concentration of 600 mg/L.

Electrophoresis and staining

Polyacrylamide gels were prepared according to a common protocol [6], with a final concentration of 10% acrylamide in the separating gel. Fifty microliters of each sample (30 μg of proteins) was mixed with loading buffer and boiled at 100 °C for 5 min before loading of the gel. Electrophoresis was performed in a Protean II xi Cell apparatus (Bio-Rad) at a constant voltage of 155 V for the stacking gel and 215 V for the separating gel. Electrophoresis was stopped when the loading buffer ran 12 cm of the separating gel. Gels were then stained with Coomasie blue stain [7] and photographed for comparison.

Reading and comparison of the gels

Gel photographs were scanned and then analyzed with the Scion Image for Windows software (Scion Corporation, Frederick, MD, USA). The average similarities between patterns were calculated by using the coefficient of Dice [8] (average percentage similarity = (number of matching bands × 2)/(total number of bands in both strains) × 100). Different clinical isolates were compared with the type strain of the same species.

RESULTS

All strains tested yielded between 44 and 58 bands. Intraspecies similarity analysis showed Dice coefficients higher than 90%, but when the analysis was performed between different species, clear differences appeared. Dice coefficients between type strains of the different species are shown in Table 1. Higher similarities were obtained between *M. fortuitum* and *M. peregrinum* (Dice coefficient 75.51%) and between *M. chelonae* and *M. abscessus* (Dice coefficient 54.9%). Visual inspection of the electrophoretograms was adequate for differentiation between isolates when type strains were included in the run and used for comparison, even before the biochemical characterization was complete (Figure 1 shows the electrophoretograms of

Table 1 Dice coefficients between type strains of the different species of members of the *M. fortuitum* complex

	Values for low-molecular-weight bands (values for all bands)			
	M. abscessus	***M. mucogenicum***	***M. fortuitum***	***M. peregrinum***
M. chelonae	52.50 (54.90)	41.46 (49.01)	27.02 (35.56)	30.13 (38.71)
M. abscessus		45.65 (44.83)	30.95 (33.64)	33.73 (35.51)
M. mucogenicum			46.51 (50.46)	40.00 (44.85)
M. fortuitum				70.12 (75.51)

Values for all bands.

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Figure 1 SDS-PAGE of the type species of members of *M. fortuitum* complex. MW, Molecular weight control. Lane A: *M. abscessus* ATCC 19977. Lane C: *M. chelonae* ATCC 35752. Lane M: *M. mucogenicum* ATCC 49650. Lane F: *M. fortuitum* ATCC 6841. Lane P: *M. peregrinum* ATCC 14467.

type strains); however, densitometric analysis yielded more bands for comparison. Differences between species appeared more clear when the analysis was performed with low-molecular-weight bands (molecular weight lower than 66 000), with lower Dice coefficient values for all comparisons, except between *M. mucogenicum* and *M. abscessus* (Table 1). Intraspecies analysis showed identical profiles compared with the type strain in 32 strains of *M. chelonae*, 29 strains of *M. fortuitum*, eight strains of *M. fortuitum* and all strains of *M. abscessus* and *M. mucogenicum*. Differences in one or two bands appeared in eight strains of *M. fortuitum*, two strains of *M. peregrinum*, and two strains of *M. chelonae*. Only one strain of *M. fortuitum* showed a difference in six bands (Dice coefficient 87.75%).

DISCUSSION

Rapidly growing mycobacteria of the *M. fortuitum* complex are among the commonest species isolated in clinical mycobacteriology laboratories [9–13], and have been clearly recognized as human pathogens [1]. However, their taxonomy has been subject to several modifications, with recent changes in the species status of many of the older members of the genus [3] and descriptions of new species [4]. These changes have made it difficult for clinical laboratories to achieve a proper species identification, and in many cases a report of *M. fortuitum* complex isolate is the only identification provided. However, there are differences in epidemiology, clinical significance and susceptibility that make it desirable to have a more accurate identification of the isolate [14]. Most identification schemes of mycobacteria are based on biochemical tests that give results after several days, or even weeks, of incubation [15]. Other approaches, such as chromatography or DNA analysis, such as DNA hybridization or PCR–restriction fragment length polymorphism (PCR-RFLP) analysis, are not currently available for most clinical laboratories, or are not available for rapidly growing mycobacteria [16,17].

SDS-PAGE of whole cell proteins has been employed previously for identification of several organisms, some of them phylogenetically related to the genus *Mycobacterium* [18,19]. There have also been several reports on the usefulness of this technique in the identification of mycobacteria [20–25]; however, these studies tested mainly slow growers [20–22,25], or evaluated this technique as an aid to taxonomic studies [23,24]. Our work suggests that SDS-PAGE of whole cell proteins can be a useful method for characterization of rapidly growing mycobacteria in clinical laboratories, with results obtained within 3 days after cultures have grown. The technique does not require expensive equipment, and according to our experience, visual inspection of the electrophoretograms is sufficient for differentiation between species belonging to the *M. fortuitum* complex. Among these species, similarity of the electrophoretograms is higher between *M. chelonae*

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and *M. abscessus*, and *M. fortuitum* and *M. peregrinum*, perhaps reflecting a closer phylogenetic relationship between these species, which were previously recognized as subspecies [1]. Similarity percentages for *M. mucogenicum* (formerly *M. chelonae*-like [4]) were also higher when compared with *M. chelonae* and *M. abscessus* than when compared with *M. fortuitum* or *M. peregrinum*. The relatively low numbers of *M. abscessus* and *M. mucogenicum* strains suggest that more studies with these species are desirable to confirm our results. Despite the excellent results, the relative difficulty of interpretation makes the technique useful only for laboratories with the need to identify a high number of isolates of rapidly growing mycobacteria to identify.

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