Identification of *Mycobacterium fortuitum*, *Mycobacterium abscessus*, and *Mycobacterium borstelense* by Polyacrylamide Gel Electrophoresis of Their Cell Proteins

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Strains of *M. fortuitum*, *M. abscessus*, and *M. borstelense* showed different polyacrylamide gel electrophoretic patterns of their cell proteins. *M. fortuitum* strains could easily be distinguished from those of *M. abscessus* and *M. borstelense*, and they appear to belong to a single homogeneous group. *M. abscessus* and *M. borstelense* gels showed similar patterns, thus tending to confirm recent suggestions that they both belong to one and the same species, the correct name of which is *M. chelonae*.

The classification of the rapidly growing mycobacteria *Mycobacterium fortuitum*, *M. abscessus*, and *M. borstelense* has recently received considerable attention. It is generally accepted that *M. fortuitum* is a separate species. Earlier differences of opinion about the status of *M. abscessus* and *M. borstelense* have been resolved. Although Tsukamura (14), on the basis of biochemical and cultural characteristics, initially considered them to be separate species, Jenkins et al. (5), using lipid chromatography and sero-agglutination, concluded that *M. borstelense* is a variety (subspecies) of *M. abscessus*. Stanford and Beck (12) and more recently Stanford et al. (13) and Gruft and Henning (2) regarded them as belonging to one species, for which the correct name appears to be *M. chelonae*. An international cooperative study confirmed this view (6).

Genetic relatedness, as demonstrated by gene compatibility or deoxyribonucleic acid base-sequence analysis, has been suggested as a basis for bacterial taxonomy. Polyacrylamide gel electrophoresis of cell proteins has been shown to reflect such genetic relatedness and to be of taxonomic value in the classification and identification of mycoplasmas (10), of *Enterobacteriaceae* (11), and of mycobacteria (3). We therefore decided to examine the polyacrylamide gel electrophoretic patterns of the cell proteins of strains of *M. fortuitum*, *M. abscessus*, and *M. borstelense*.

MATERIALS AND METHODS

Bacterial strains. The strains used were: *M. fortuitum* TMC 1529 (Trudeau Institute); *M. fortuitum* ATCC 9820; *M. abscessus* TMC 5129 (Trudeau

Institute); *M. borstelense* TMC 1524; eight strains of *M. abscessus* and five strains of *M. borstelense* received from J. L. Stanford, London, England; and three strains of *M. borstelense* received from E. Freerksen, Borstel, Germany. Five strains of mycobacteria isolated locally from clinical material were identified as *M. fortuitum* on the basis of a positive nitrate reduction test. Another strain did not reduce nitrate and was therefore labeled *M. abscessus-M. borstelense*.

Preparation of cell-free protein extract. Cell-free protein was obtained as described previously (3). The strains were cultured in Dubos broth medium (Difco) enriched with Dubos medium albumin (Difco). After incubation for 10 to 14 days, the cultures were centrifuged at 13,000 × *g* for 30 min at 4 °C. The supernatant was discarded, and the cells were washed three times in normal saline. They were then exposed to ultrasonic disruption at an amplitude of 5 μm for 15 min in an MSE 100-W ultrasonic disintegrator. The disrupted cells were centrifuged, and the supernatant fluid was passed through an asbestos filter (Carlson Ford grade EKS). The protein content of the filtrate was determined by the method of Lowry et al. (7). Several extracts were prepared from different cultures of each strain.

Polyacrylamide gel electrophoresis. This was performed according to the methods of Davis (1) and Ornstein (9) in tris(hydroxymethyl)aminomethane-glycine buffer at pH 8.3. The acrylamide concentration of the gels was 7.5%. A 0.1-ml amount of the cell-free extract containing about 400 mg of protein was placed on the top of the gel. The electrophoresis was carried out at room temperature, using a constant current of 4 mA per tube, until the bromophenol blue tracking dye had migrated to within 1 cm of the bottom of the gel. The gels were stained with 1% amido black for 30 min, and the excess dye was then removed by electrophoresis. Several gels were prepared from each extract. Densitometer tracings from the gels were made with a Gilford spectrophotometer (model 240) equipped with a linear transport scanner (2410-S) and a chart recorder (model 6040).

RESULTS

The gel patterns of extracts prepared from different cultures of the same strain were identical, as were those of duplicate gels prepared from the same extract. The method therefore yielded reproducible results. The gel electrophoretic patterns of *M. fortuitum*, *M. abscessus*, and *M. borstelense* were all different (Fig. 1), but the differences between the latter two species were much less marked than those between either of them and *M. fortuitum*.

All seven *M. fortuitum* strains showed an identical pattern, which appeared to be specific for this species. Similarly, the gels of 9 of the 10 strains of *M. abscessus* were identical and clearly different from those of *M. fortuitum*. The tenth strain, which was the one isolated in our clinical laboratory and identified biochemically as *M. abscessus*-*M. borstelense*, had a gel pattern almost identical to that of *M. fortuitum* (Fig. 2).

The gels of the nine strains of *M. borstelense* were all very similar but not identical. The two heavy bands towards the bottom of the gels (Fig. 1) were characteristic and appeared in all the *M. borstelense* strains, but the minor bands

FIG. 1. Left: Polyacrylamide gel electrophoretic patterns of (A) *M. fortuitum* (local strain); (B) *M. borstelense* (Stanford strain 78); and (C) *M. abscessus* (Stanford strain 477). Right: Densitometer tracings from these gels.

FIG. 2. Left: Polyacrylamide gel electrophoretic patterns of (A) *M. fortuitum* (ATCC 9820); (B) a locally isolated strain identified biochemically as *M. abscessus*; and (C) *M. abscessus* (Stanford strain 41). Right: Densitometer tracings from these gels.

were not always identical. In general, there was considerable resemblance between the gels of *M. borstelense* and *M. abscessus*, although the former could be identified by the characteristic two bands referred to above. Figure 3 shows the gels of two strains of *M. borstelense* and one of *M. abscessus*. Both *M. borstelense* strains showed the two characteristic bands, but for the rest, strain TMC 1524 (Fig. 3B) bore a much closer resemblance to *M. abscessus* (Fig. 3C) than to the other *M. borstelense* gel (Fig. 3A).

DISCUSSION

The results of the polyacrylamide gel electrophoretic studies presented here show that *M. fortuitum* strains can be clearly distinguished from those of *M. abscessus* and *M. borstelense*.

The latter two groups can usually be differentiated by means of polyacrylamide gel electrophoresis, but the difference between the individual strains is not always decisive. Some strains of *M. borstelense* bear a closer resemblance to strains of *M. abscessus* than to other strains of *M. borstelense*. These results agree with the conclusions of Stanford (13) that *M. borstelense* and *M. abscessus* are variants (subspecies) of a single species, *M. chelonae*, and of

FIG. 3. Left: Polyacrylamide gel electrophoretic patterns of (A) *M. borstelense* (Stanford strain 78); (B) *M. borstelense* (TMC 1524); and (C) *M. abscessus* (Stanford strain 477). Right: Densitometer tracings from these gels.

Kubica et al. (6) that *M. fortuitum* is a separate, well-defined species.

We have previously reported differences between the susceptibility of these strains to certain antibiotics (4, 8). *M. fortuitum* strains were uniformly susceptible to cephaloridine and vancomycin but resistant to cephalothin. *M. abscessus* and *M. borstelense* were resistant to these antibiotics.

If the results of the polyacrylamide gel electrophoresis truly reflect taxonomic differences between *M. fortuitum* on the one hand and *M. abscessus*-*M. borstelense* on the other, then the same differences appear to be reflected in the susceptibility patterns to the three antibiotics mentioned. It is interesting to note that the locally isolated strain which was identified biochemically as *M. abscessus*-*M. borstelense* but which had a gel pattern identical to that of *M. fortuitum* also showed the antibiotic susceptibility pattern of *M. fortuitum* (susceptibility to cephaloridine and vancomycin and resistance to cephalothin).

Polyacrylamide gel electrophoresis is a relatively simple and reliable method for studying

the genetic relatedness between strains and is therefore a useful tool in classification.

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REPRINT REQUESTS

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