Papers

Rapid identification of mycobacteria from AIDS patients by capillary electrophoretic profiling of amplified SOD gene

T J Bull, D C Shanson, L C Archard

Abstract

Aim—Rapid differentiation of mycobacterial species at the genomic level.

Methods—The manganese superoxide dismutase (SOD) gene (464 bp) and 16SrRNA (353 bp) from 104 isolates (18 species) of mycobacteria were amplified using polymerase chain reaction (PCR). Products were sequenced and a phenogram of SOD sequences derived. PCR products of SOD gene were digested with HaeIII, and restriction fragment profiles visualised using capillary electrophoresis.

Results—Novel SOD sequences were found for *M szulgai*, *M marinum*, *M phlei*, *M smegmatis*, *M chelonae*, *M paratuberculosis*, *M malmoense*, *M intracellulare* serotype 7, *M intracellulare* serotype 18, and *M celatum* types 1, 2, and 3. Phylogenetic analysis indicated that 18 of 19 species studied had 8–29% interspecies and <6% intraspecies sequence diversity in the SOD gene. No consistent differences were detected between AIDS and non-AIDS isolates. *M paratuberculosis* showed a unique SOD sequence with a 1.1% (SD 0.5%) diversity from *M avium*. Capillary electrophoresis profiles were able to differentiate 16 of 18 species within 24 hours.

Conclusions—A phenogram of SOD sequences clearly delineated all mycobacterial species and showed two distinct clusters, fast growing species, and the *M avium* complex (MAC). Within the MAC, *M avium* (five types), *M intracellulare* (five types), *M scrofulaceum* (two types), and *M paratuberculosis* (one type) could be demonstrated. Phylogenetic diversity of *M celatum* from MAC, previously suggested by 16SrRNA data, was confirmed. This simple and rapid method for DNA extraction, in conjunction with capillary electrophoresis of SOD restriction fragments, allows rapid identification of mycobacterial isolates.

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Keywords: Mycobacteria, superoxide dismutase, rapid identification.

Until recently the taxonomic classification and identification of bacteria has been restricted to phenotypic systems based upon biochemical tests.¹ The advent of molecular based techniques, which can rapidly detect and identify bacteria in clinical samples,²⁻⁴ has highlighted the need for a genetically based classification of bacteria. The relatively slow growth of mycobacteria in culture media (1–12 weeks) has emphasised this need, with particular reference to the rapid differential diagnosis of *M tuberculosis* and *M avium* complex (MAC) infection in AIDS patients.⁵⁻⁷ Studies have shown the short variable region of the 16SrRNA gene to be suitable for phylogenetic comparisons⁸ and a detailed taxonomic system has been proposed for the *Mycobacteriaceae* based upon these data.⁹ Confirmation of these relationships in other genes has not been made; however, recent studies of the manganese superoxide dismutase (SOD) gene have indicated a significant sequence diversity,¹⁰ suitable for phylogenetic analysis.

Methods

CULTURES

The 104 isolates used for this study were all identified by conventional biochemical means at the Regional Centre for Tuberculosis Bacteriology, PHLS, Dulwich Hospital, London, and consisted of: (1) 52 isolates of MAC from 36 patients (this group contained 35 isolates of *M avium* from 23 patients; 10 identical isolates from eight patients described here as *M celatum* type 3; four isolates of *M intracellulare*, serotype unknown, from two patients; and three isolates of *M intracellulare* from two patients consistent with serotype 18), 10 isolates of *M kansasii* from eight patients, nine isolates of *M tuberculosis* from nine patients, one isolate of *M scrofulaceum*, and one isolate of *M simiae*, all from patients with AIDS; (2) 10 isolates of MAC from 10 patients, one isolate of *M malmoense*, two isolates of *M kansasii*, all from patients without AIDS; (3) 18 type culture mycobacterial reference strains: *M fortuitum* (NCTC 10394), *M smegmatis* (NCTC 10265), *M phlei* (NCTC 8151), *M*

Medical Microbiology
Department, Charing
Cross and
Westminster Medical
School, London W6
8RF
T J Bull
D C Shanson

Department of
Biochemistry, Charing
Cross Hospital,
London W6 8RF
L C Archard

Correspondence to:
Dr Tim Bull, Medical
Microbiology Department,
Charing Cross Hospital,
London W6 8RF, UK.

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szulgai (NCTC 25932), *M. tuberculosis* H37Rv (NCTC 7416), *M. malmoense* (NCTC 11298), *M. avium* (NCTC 8559), *M. intracellulare* (NCTC 10682), *M. chelonae* (NCTC 0946), *M. xenopi* (NCTC 10042), *M. marinum* (NCTC 2275), *M. gordonae* (NCTC 10267), *M. kansasii* (NCTC 10268), *M. scrofulaceum* (NCTC 10803), *M. paratuberculosis* (NCTC 8578), *M. intracellulare* serotype 7 (ATCC 35847), *M. celatum* type 1 (ATCC 51131), and *M. celatum* type 2 (ATCC 51130).

DNA EXTRACTION

All cultures were grown to purity on Middlebrooks 7H10 for 4–6 weeks. Two or three colonies were emulsified in 500 µl TE buffer (50 mM Tris (pH 8.2), 1 mM EDTA) in a screw capped reaction tube and centrifuged at 13 000 rpm for five minutes. The supernatant was removed and the pellet resuspended in 25 µl TE. This suspension was frozen rapidly for 10 seconds in liquid nitrogen and then sonicated in a water bath (Perkin Elmer) for 30 seconds at room temperature. This procedure was repeated three times and the suspension heated for five minutes at 98°C in a heating block, then rapidly cooled for 10 seconds in liquid nitrogen and sonicated again for one minute. DNA preparations were stored at –20°C before amplification.

PRIMERS

For SOD

Primers were designed from homologous regions of previously published SOD gene sequences from *M. tuberculosis*⁷ and *M. leprae*¹¹ to give a 464 base pair (bp) product. These were as follows:

SF1: ACATCTCGGGTCAGATCAACGACG
SR1: GACGTTCTTGTACTGCAGGTA

For 16SrRNA

Primers were as previously described³ and gave a 353 bp product. These were as follows:

16SpA: AGAGTTTGTATCCTGGCTCAG
16SPC*: CCCACTGCTGCCTCCCGTAG

Primers were synthesised on an Applied Biosystems 380B automated synthesiser and desalted using NAP 10 columns (Pharmacia). Primer quality was assessed following capillary electrophoresis using Microgel capillaries (Applied Biosystems) on an Applied Biosystems 270A capillary electrophoresis unit.

PCR CONDITIONS AND SEQUENCING

PCR reaction mixes (100 µl) for each of the primer pairs were prepared from each of the test isolates using the following conditions: 1 µl of extracted chromosomal DNA was amplified in a Gene Amp 9600 thermocycler (Perkin Elmer) with 10 µl 10X polymerase chain reaction (PCR) buffer (100 mM Tris (pH 8.3), 500 mM KCl, 15 mM MgCl₂, 0.015% gelatin). 16 µl deoxyribonucleotide mix (1.25 µmol per

dNTP), 5 µl of each primer (20 µM), and 5 U Taq polymerase made to 100 µl with water. The cycling profile consisted 30 cycles of one minute at 94°C, one minute at 58°C, and one minute at 72°C, followed by a final incubation at 72°C for five minutes. Relevant products were then isolated by agarose gel electrophoresis of 70 µl amplified reaction product, excised by use of a sterile scalpel, extracted with phenol-chloroform-isoamylalcohol, concentrated by ethanol precipitation, and final concentration estimated for sequencing using a further agarose gel electrophoresis. Automated sequencing was made, with 0.5 µg product DNA, using the PRISM Taq dye termination system (Perkin Elmer) with the original primers. The cycling profile for sequencing was 25 cycles of 96°C for 15 seconds followed by 60°C for five minutes. Sequencing of both strands of product was made and aligned for base verification. Sequences with ambiguities between strands were checked by repeat sequencing from the same DNA preparation.

RESTRICTION DIGESTION AND CAPILLARY ELECTROPHORESIS

The PCR reaction mix (8 µl) was used for restriction digestion by mixing with 1 µl (1 U/µl) HaeIII enzyme (Promega) and 1 µl One-PhorAll buffer (Pharmacia). Digestions were made at 37°C for 1.5 hours. Samples for capillary electrophoresis were prepared by mixing 1 µl sample digest, 18 µl water, and 1 µl (38 µg/ml) standard pBR322 HaeIII digest (Sigma). Samples were electrokinetically loaded for 80 seconds at –5 kV and then electrophoresed for 15 minutes at –13 kV. Capillary electrophoresis profiles were obtained using a DNA fragment analysis kit (Applied Biosystems) according to the manufacturers' instructions on an Applied Biosystems 270HT interfaced with Turbochrom integration software (Perkin Elmer). An estimate of restriction fragment sizes was then calculated by calibrating retention times of the internal standard against sample fragments.

PHYLOGENETIC ANALYSIS

Phylogenetic analyses were made using the PHYLIP package available on SEQNET using the FITCH algorithm and Jukes and Cantor DNADIST method¹² for estimations of phylogenetic diversity. Human SOD sequence¹³ was aligned to mycobacterial sequences and used as an outgroup to root the tree. Values are given as means (SD).

Results

BIOCHEMICAL IDENTIFICATION AND 16SrRNA PROFILES

A 16SrRNA sequence profile was obtained from all 104 isolates. In the majority of isolates the profile corresponded to the biochemical identification and previously reported 16SrRNA profiles³ after sequencing one strand only. Isolates giving irregular sequences were verified from both strands and repeated if

human	193	CACCACAGCAAGCACCACCGCGCTACGTGAACAACCTGAACGTCACCGAGGAGAAGTACCAGGAGCGTGGCCAAGGAGATGTTACAGCCAGACAG
avium type1		CACCACACCAAGCACCACCGCACCTACGTCAAAGGCGTGAACGACGCTCTTGCCAAGCTCGAAGAGGCGCGGCCAACGAGGACACGCTGCG-ATCTTC
avium type2		CACCACACCAAGCACCACCGCACCTACGTCAAAGGCGTGAACGACGCTCTTGCCAAGCTCGAAGAGGCGCGGCCAACGAGGACACGCTGCG-ATCTTC
avium type3		CACCACACCAAGCACCACCGCACCTACGTCAAAGGCGTGAACGACGCTCTTGCCAAGCTCGAAGAGGCGCGGCCAACGAGGACACGCTGCG-ATCTTC
avium type4		CACCACACCAAGCACCACCGCACCTACGTCAAAGGCGTGAACGACGCTCTTGCCAAGCTCGAAGAGGCGCGGCCAACGAGGACACGCTGCG-ATCTTC
avium type5		CACCACACCAAGCACCACCGCACCTACGTCAAAGGCGTGAACGACGCTCTTGCCAAGCTCGAAGAGGCGCGGCCAACGAGGACACGCTGCG-ATCTTC
celatum.t3		CACCACAGCAAAACCATGCGACCTACGTGAAGGGCGCGAACGACGCGCTTGAAAAACTCGAGGAAGCAGCGGCCAAGGACGACGCTGCG-ATCTTC
celatum.t2		CACCACAGCAAAACCATGCGACCTACGTGAAGGGCGCGAACGACGCGCTTGAAAAACTCGAGGAAGCAGCGGCCAAGGACGACGCTGCG-ATCTTC
chelonel		CAACACAGTAAGCACCACGCGGCTACGTGCGGGGTGTAATTCGGCTGTGCGCAAGTTGGAAGAGGCTCGCGGAAGGGCGACGCGCGCG-ATCTTC
fortuitum		CACCACAGCAAGCACCACGCGGCTACGTGAAGGGCGTCAACGACGCGTGGCCAAGCTCGATGAGGCGCGGCCAACGGTGACCACGCGCG-ATCTTC
gordonae		TCACACAGTAAGCACCACGCGACCTACGTCAAAGGGCGTCAACGACGCGTGGCCAAGCTCGAAGAGGCGCGGCCAACGGTGACCACGCGCG-ATCTTC
intra type1		CACCACAGTAAGCACCACGCGACCTACGTCAAAGGGCGTGAACGACGCTCTGTCCAAGCTCGAAGAGGCGCGGCCAACGAGGACGCTGCG-ATCTTC
intra type2		CACCACAGTAAGCACCACGCGACCTACGTCAAAGGGCGTGAACGACGCTCTGTCCAAGCTCGAAGAGGCGCGGCCAACGAGGACGCTGCG-ATCTTC
intra type3		CACCACAGTAAGCACCACGCGACCTACGTCAAAGGGCGTGAACGACGCTCTGTCCAAGCTCGAAGAGGCGCGGCCAACGAGGACGCTGCG-ATCTTC
intra type4		TACCACAGTAAGCACCACGCGACCTACGTCAAAGGGCGTGAATGACGCTCTGTCCAAGCTCGAAGAGGCGCGGCCAACGAGGACGCTGCG-ATCTTC
intra type5		CACCACAGTAAGCACCACGCGACCTACGTCAAAGGGCGTGAACGACGCTCTGTCCAAGCTCGAAGAGGCGCGGCCAACGAGGACGCTGCG-ATCTTC
leprae		CACCACACCAAGCACCACGCGACCTATGTCAAAGGGTGAATGACGCGTGTGCAAACTTGACGAGGCGCGGCCAACGAGGACGCTGCG-ATCTTC
malmoense		CACCACAGCAAGCACCACGCGCTACGTCAAAGGGCGTGAACGACGCGTGGCCAAGCTGGAAGAGGCGCGGCCAACGAGGACGCTGCG-ATCTTC
marinum		TCACACAGTAAGCACCACGCGACCTACGTCAAAGGGCGTGAACGACGCGTGGCCAAGCTCGAAGAGGCGCGGCCAACGAGGACGCTGCG-ATCTTC
kansasii t1		CACCACAGTAAGCACCACGCGACCTACGTCAAAGGGCGCAACGATGCGGTGCGCAAACTCGAAGAGGCGCGGCCAAGGAGACGCTGCG-ATCTTC
kansasii t2		GCACACAGTAAGCACCACGCGACCTACGTCAAAGGGCGCAACGATGCGGTGCGCAAACTCGAAGAGGCGCGGCCAAGGAGACGCTGCG-ATCTTC
paratb		CACCACACCAAGCACCACGCGACCTACGTCAAAGGGCGTGAACGACGCTCTTGCCAAGCTCGAAGAGGCGCGGCCAACGAGGACGCTGCG-ATCTTC
phlei		TCACACAGCAAGCACCACGCGACCTACGTCAAAGGGCGTGAACGACGCGTGGCCAAGCTGGAAGAGGCGCGGCCAACGAGGACGCTGCG-ATCTTC
M.sp.40407		TCACACAGTAAGCACCACGCGACCTACGTCAAAGGGCGTGAACGACGCGTGGCCAAGCTCGAAGAGGCGCGGCCAACGAGGACGCTGCG-ATCTTC
scrof t1		CATCACACCAAGCACCACGCGACCTACGTCAAAGGGCGTGAACGACGCGTGGCCAAGCTCGAAGAGGCGCGGCCAACGAGGACGCTGCG-ATCTTC
scrof t2		CATCACACCAAGCACCACGCGACCTACGTCAAAGGGCGTGAACGACGCGTGGCCAAGCTCGAAGAGGCGCGGCCAACGAGGACGCTGCG-ATCTTC
simiae		CACCACAGCAAGCACCACGCGACCTACGTCAAAGGGTTTGAACGACGCGTGGCCAAGCTGGAAGAGGCGCGGCCAACGAGGACGCTGCG-ATCTTC
smegmatis		CACCACAGCAAGCACCACGCGACCTACGTCAAAGGGTGTGAACGACGCGTGGCCAAGCTCGAAGAGGCGCGGCCAACGAGGACGCTGCG-ATCTTC
szulgai		TCACACAGTAAGCACCACGCGACCTACGTCAAAGGGCGTGAACGACGCTGTGCGCAAACTCGAAGAGGCGCGGCCAACGAGGACGCTGCG-ATCTTC
tb		CACCACAGCAAGCACCACGCGACCTACGTCAAAGGGCGCAATGACGCGTGGCCAAGCTCGAAGAGGCGCGGCCAAGGAGATCACTGCG-ATCTTC
xenopi		CACCACAGCAAGCACCACGCGACGTACGTCAAAGGGCGCAACGACGCGTGGCCAAGCTGGAGAGGCGCGGCCAACGAGATCACTGCG-ATCTTC
human	293	CTCTTCAGCTGCAC-TGAAGTCAATGGTGGTGGTCAATCAATCATAGCATTTCTGGCAAACTCAGCCCTAACGGTGGTGGAGACCCAAAGGGG
avium type1		CTGAACGAAAAGAACCTCGCCTTCCACCTGGGCGGCCACGTCAACCACTCGATCTGGTGGAAAGAACTGTGCGCGGACGGCGGTGACAAAGCCACCGGTG
avium type2		CTGAACGAAAAGAACCTCGCCTTCCACCTGGGCGGCCACGTCAACCACTCGATCTGGTGGAAAGAACTGTGCGCGGACGGCGGTGACAAAGCCACCGGTG
avium type3		CTGAACGAAAAGAACCTCGCCTTCCACCTGGGCGGCCACGTCAACCACTCGATCTGGTGGAAAGAACTGTGCGCGGACGGCGGTGACAAAGCCACCGGTG
avium type4		CTGAACGAAAAGAACCTCGCCTTCCACCTGGGCGGCCACGTCAACCACTCGATCTGGTGGAAAGAACTGTGCGCGGACGGCGGTGACAAAGCCACCGGTG
avium type5		CTGAACGAAAAGAACCTCGCCTTCCACCTGGGCGGCCACGTCAACCACTCGATCTGGTGGAAAGAACTGTGCGCGGACGGCGGTGACAAAGCCACCGGTG
celatum t3		TTGAACGAGAAGAACCTGGCGTTCAACCTGGGCGGCCACGTCAACCACTCGATCTGGTGGAAAGAACTGTGCGCGGACGGCGGTGACAAAGCCACCGGTG
celatum t2		TTGAACGAGAAGAACCTGGCGTTCAACCTGGGCGGCCACGTCAACCACTCGATCTGGTGGAAAGAACTGTGCGCGGACGGCGGTGACAAAGCCACCGGTG
chelonel		CTGAACGAGAAGAACCTGGCGTTCAACCTGGGCGGCCACGTCAACCACTCGATCTGGTGGAAAGAACTGTGCGCGGACGGCGGTGACAAAGCCACCGGTG
fortuitum		CTGAACGAGAAGAACCTGGCGTTCAACCTGGGCGGCCACGTCAACCACTCGATCTGGTGGAAAGAACTGTGCGCGGACGGCGGTGACAAAGCCACCGGTG
gordonae		TTGAACGAGAAGAACCTGGCGTTCAACCTGGGCGGCCACGTCAACCACTCGATCTGGTGGAAAGAACTGTGCGCGGACGGCGGTGACAAAGCCACCGGTG
intra type1		CTGAACGAAAAGAACCTGGCGTTTCACTTGGGCGGCCACGTCAACCACTCGATCTGGTGGAAAGAACTGTGCGCGGACGGCGGTGACAAAGCCACCGGTG
intra type2		CTGAACGAAAAGAACCTGGCGTTTCACTTGGGCGGCCACGTCAACCACTCGATCTGGTGGAAAGAACTGTGCGCGGACGGCGGTGACAAAGCCACCGGTG
intra type3		CTGAACGAAAAGAACCTGGCGTTTCACTTGGGCGGCCACGTCAACCACTCGATCTGGTGGAAAGAACTGTGCGCGGACGGCGGTGACAAAGCCACCGGTG
intra type4		CTGAACGAAAAGAACCTGGCGTTTCACTTGGGCGGCCACGTCAACCACTCGATCTGGTGGAAAGAACTGTGCGCGGACGGCGGTGACAAAGCCACCGGTG
intra type5		CTGAACGAGAAGAACCTGGCGTTTCACTTGGGCGGCCACGTCAACCACTCGATCTGGTGGAAAGAACTGTGCGCGGACGGCGGTGACAAAGCCACCGGTG
leprae		CTGAACGAGAAGAACCTGGCGTTTCACTTGGGCGGCCACGTCAACCACTCGATCTGGTGGAAAGAACTGTGCGCGGACGGCGGTGACAAAGCCACCGGTG
malmoense		CTGAACGAAAAGAACCTGGCGTTTCACTTGGGCGGCCACGTCAACCACTCGATCTGGTGGAAAGAACTGTGCGCGGACGGCGGTGACAAAGCCACCGGTG
marinum		CTGAACGAGAAGAACCTGGCGTTTCACTTGGGCGGCCACGTCAACCACTCGATCTGGTGGAAAGAACTGTGCGCGGACGGCGGTGACAAAGCCACCGGTG
kansasii t1		CTGAACGAGAAGAACCTGGCGTTTCACTTGGGCGGCCACGTCAACCACTCGATCTGGTGGAAAGAACTTCTCCCAACGGAGGGGACAAAGCCACCGGTG
kansasii t2		CTGAACGAGAAGAACCTGGCGTTTCACTTGGGCGGCCACGTCAACCACTCGATCTGGTGGAAAGAACTTCTCCCAACGGAGGGGACAAAGCCACCGGTG
paratb		CTGAACGAGAAGAACCTGGCGTTTCACTTGGGCGGCCACGTCAACCACTCGATCTGGTGGAAAGAACTGTGCGCGGACGGCGGTGACAAAGCCACCGGTG
phlei		CTGAACGAGAAGAACCTGGCGTTTCACTTGGGCGGCCACGTCAACCACTCGATCTGGTGGAAAGAACTGTGCGCGGACGGCGGTGACAAAGCCACCGGTG
M.sp. 40407		CTGAACGAGAAGAACCTGGCGTTTCACTTGGGCGGCCACGTCAACCACTCGATCTGGTGGAAAGAACTGTGCGCGGACGGCGGTGACAAAGCCACCGGTG
scrof t1		CTGAACGAGAAGAACCTGGCGTTTCACTTGGGCGGCCACGTCAACCACTCGATCTGGTGGAAAGAACTGTGCGCGGACGGCGGTGACAAAGCCACCGGTG
scrof t2		CTGAACGAGAAGAACCTGGCGTTTCACTTGGGCGGCCACGTCAACCACTCGATCTGGTGGAAAGAACTGTGCGCGGACGGCGGTGACAAAGCCACCGGTG
simiae		TTGAACGAGAAGAACCTGGCGTTTCACTTGGGCGGCCACGTCAACCACTCGATCTGGTGGAAAGAACTGTGCGCGGACGGCGGTGACAAAGCCACCGGTG
smegmatis		CTGAACGAGAAGAACCTGGCGTTTCACTTGGGCGGCCACGTCAACCACTCGATCTGGTGGAAAGAACTTCTCCCAACGGTGGGACAAAGCCACCGGTG
szulgai		CTGAACGAGAAGAACCTGGCGTTTCACTTGGGCGGCCACGTCAACCACTCGATCTGGTGGAAAGAACTTCTCCCAACGGTGGGACAAAGCCACCGGTG
tb		CTGAACGAGAAGAACCTTACGTTTCACTTGGGCGGCCACGTCAACCACTCGATCTGGTGGAAAGAACTGTGCGCTAACGGTGGTACAAAGCCACCGGTG
xenopi		GGGCTAGAGAAGGCGCTCGCGTTCACTTGGGCGGCCACGTCAACCACTCGATCTGGTGGAAAGAACTGTGCGCTAACGGTGGTACAAAGCCACCGGTG
human	393	AGTTGCTGGAAGCCATCAACCTGACCTTTGGTTCCTTTGACAAGTTTAAAGGAGAAGCTGACGCGTGCATCTGTTGGTGTCAAAGGCTCAGGTTGGGCGT
avium type1		AGCTGGCGCGCGGATCGACGACGCGTTTCGGGTCTTCGACAAGTTCCGAGCGCAATTCAGCGCGCGCGCAACGCGCTCGGTCGGTGGGCGT
avium type2		AGCTGGCGCGCGGATCGACGACGCGTTTCGGGTCTTCGACAAGTTCCGAGCGCAATTCAGCGCGCGCGCAACGCGCTCGGTCGGTGGGCGT
avium type3		AGCTGGCGCGCGGATCGACGACGCGTTTCGGGTCTTCGACAAGTTCCGAGCGCAATTCAGCGCGCGCGCAACGCGCTCGGTCGGTGGGCGT
avium type4		AGCTGGCGCGCGGATCGACGACGCGTTTCGGGTCTTCGACAAGTTCCGAGCGCAATTCAGCGCGCGCGCAACGCGCTCGGTCGGTGGGCGT
avium type5		AGCTGGCGCGCGGATCGACGACGCGTTTCGGGTCTTCGACAAGTTTCGCGCGCAATTCAGCGCGCGCGCAACGCGCTCGGTCGGTGGGCGT
celatum t3		AGCTGGCGCGCGGATCGACGACGCGTTTCGGGTCTTCGACAAGTTTCGCGCGCAATTCAGCGCGCGCGCAACGCGCTCGGTCGGTGGGCGT
celatum t2		AGCTGGCGCGCGGATCGACGACGCGTTTCGGGTCTTCGACAAGTTTCGCGCGCAATTCAGCGCGCGCGCAACGCGCTCGGTCGGTGGGCGT
chelonel		ATCTGGCGCGCGGATCGACGACGCGTTTCGGGTCTTCGACAAGTTTCGAGGCAAGTTTCCGCGCGCAATTCAGCGCGCGCGCAACGCGCTCGGTCGGTGGGCGT
fortuitum		ACCTGGCGCGCGGATCGACGACGCGTTTCGGGTCTTCGACAAGTTTCAGGCGGAGTTTCCGCGCGCAATTCAGCGCGCGCGCAACGCGCTCGGTCGGTGGGCGT
gordonae		ATCTGGCGCGCGGATCGACGACGCGTTTCGGGTCTTCGACAAGTTTCGAGGCAAGTTTCCGCGCGCAATTCAGCGCGCGCGCAACGCGCTCGGTCGGTGGGCGT
intra type1		AATTTGGCGCGCGGATCGACGACGCGTTTCGGATCTTCGACGCGTTTCGCGCGCAATTCAGCGCGCGCGCAACGCGCTCGGTCGGTGGGCGT
intra type2		AATTTGGCGCGCGGATCGACGACGCGTTTCGGATCTTCGACGCGTTTCGCGCGCAATTCAGCGCGCGCGCAACGCGCTCGGTCGGTGGGCGT
intra type3		AATTTGGCGCGCGGATCGACGACGCGTTTCGGATCTTCGACGCGTTTCGCGCGCAATTCAGCGCGCGCGCAACGCGCTCGGTCGGTGGGCGT
intra type4		AATTTGGCTGCTGCGATCGACGACGCGTTTCGGGTCTTCGACGCGTTTCGCGCGCAATTCAGCGCGCGCGCAACGCGCTCGGTCGGTGGGCGT
intra type5		AATTTGGCGCGCGGATCGATGACGCGTTTCGGGTCTTCGACAAGTTTCGCGCGCAATTCAGCGCGCGCGCAACGCGCTCGGTCGGTGGGCGT
leprae		GGCTAGGCACTGACATTGATGAAACGTTTGGGTCTTCGACAAGTTTCGCGCGCAATTCAGCGCGCGCGCAACGCGCTCGGTCGGTGGGCGT
malmoense		ACCTTCGGTTCGGGATCGACGACGCGTTTCGGATCTTCGACAAGTTTCGCGCGCAATTCAGCGCGCGCGCAACGCGCTCGGTCGGTGGGCGT
marinum		AATCTCGCGCGCGGATCGACGACGCGTTTCGGGTCTTCGACAAGTTTCGCGCGCAATTCAGCGCGCGCGCAACGCGCTCGGTCGGTGGGCGT
kansasii t1		AATCTCGCGCGCGGATCGACGACGCGTTTCGGGTCTTCGACAAGTTTCGCGCGCAATTCAGCGCGCGCGCAACGCGCTCGGTCGGTGGGCGT
kansasii t2		AATCTCGCGCGCGGATCGACGACGCGTTTCGGGTCTTCGACAAGTTTCGCGCGCAATTCAGCGCGCGCGCAACGCGCTCGGTCGGTGGGCGT
paratb		AGCTGGCGCGCGGATCGACGACGCGTTTCGGGTCTTCGACAAGTTTCGCGCGCAATTCAGCGCGCGCGCAACGCGCTCGGTCGGTGGGCGT
phlei		ATCTTCGGGTTCGGATCGACGACGCGTTTCGGATCTTCGACAAGTTTCGCGCGCAATTCAGCGCGCGCGCAACGCGCTCGGTCGGTGGGCGT
M.sp.40407		AGCTTCGGCGCGGATCGACGACGCGTTTCGGGTCTTCGACAAGTTTCGCGCGCAATTCAGCGCGCGCGCAACGCGCTCGGTCGGTGGGCGT
scrof t1		AATTTGGCGCGCGGATCGATGACGCGTTTCGGATCTTCGACAAGTTTCGCGCGCAATTCAGCTGCGCGCGCAACGCGCTCGGTCGGTGGGCGT
scrof t2		AGCTGGCGCGCGGATCGACGACGCGTTTCGGATCTTCGACAAGTTTCGCGCGCAATTCAGCTGCGCGCGCAACGCGCTCGGTCGGTGGGCGT
simiae		ATCTTCGGCGCGGATCGACGACGCGTTTCGGTCTTCGACAAGTTTCGCGCGCAATTCAGCGCGCGCGCAACGCGCTCGGTCGGTGGGCGT
smegmatis		AGCTTCGGCGCGGATCGACGACGCGTTTCGGTCTTCGACAAGTTTCGCGCGCAATTCAGCGCGCGCGCAACGCGCTCGGTCGGTGGGCGT
szulgai		AGCTTCGGCGCGGATCGATGACGCGTTTCGGTCTTCGACAAGTTTCGCGCGCAATTCAGCGCGCGCGCAACGCGCTCGGTCGGTGGGCGT
tb		AATCTCGCGCGGATCGCGGACGCGTTTCGGTCTTCGACAAGTTTCGCGCGCAATTCACCGCGCGCGTACCGCTGCAAGGGTTCGGTGGGCGT
xenopi		AATTTGGCGCGGATCGACGACGCGTTTCGGTCTTCGACAAGTTTCGCGCGCAATTCACCGCGCGCGTACCGCTGCAAGGGTTCGGTGGGCGT

necessary. Ten isolates identified originally as MAC gave an identical unique profile which we have reported elsewhere¹⁴ as *M. celatum* type

3. Isolates of *M. scrofulaceum* and *M. kansasii* both had two 16SrRNA profiles, identical across the species specific region, but differing

human	493	GCTTGGTTTCAATAAGGAACGGGGACACTTACAAATGCTGCTGTCCAAATC-AGGATCCACTG--CAAGGAACAAC-AGGCCTTATTCCTGCTGGG
avium type1		GCTGGGCTATGACAC---CCTGGGCA---GCCGGTGTGCTGACCTTCCAGCTCTACGACCAGCAGGCCAACGTCGCCGCTGGGCATCATCCCGCTGCTGCA
avium type2		GCTGGGCTATGACAC---CCTGGGCA---GCCGGTGTGCTGACCTTCCAGCTCTACGACCAGCAGGCCAACGTCGCCGCTGGGCATCATCCCGCTGCTGCA
avium type3		GCTGGGCTATGACAC---CCTGGGCA---GCCGGTGTGCTGACCTTCCAGCTCTACGACCAGCAGGCCAACGTCGCCGCTGGGCATCATCCCGCTGCTGCA
avium type4		GCTGGGTTATGACAC---CCTGGGCA---GCCGGTGTGCTGACCTTCCAGCTCTACGACCAGCAGGCCAACGTCGCCGCTGGGCATCATCCCGCTGCTGCA
avium type5		GCTGGGTTATGACAC---CCTGGGCA---GCCGGTGTGCTGACCTTCCAGCTCTACGACCAGCAGGCCAACGTCGCCGCTGGGCATCATCCCGCTGCTGCA
celatum t3		ACTCGGCTGGGACAA---CCTCGGCG---AGAAGCTGCTGATCTTCCAGGTGTACGACCAGCAGCCAACTTCCCCTCGGGCTGCTCCCGCTGCTGCT
celatum t2		CCTCGGCTGGGACAA---CCTCGGCG---AGAAGCTGCTGATCTTCCAGGTGTACGACCAGCAGCCAACTTCCCCTCGGGCTGCTCCCGCTGCTGCT
chelonel		ACTCGGTACGACAG---CCTGGGCG---AGAAGCTGCTGACCTTCCAGCTGTACGACCAGCAGGCCAACTTCCCCTCGGGCTGCTCCCGCTGCTGCT
fortuitum		GCTCGGCTACGACAG---CCTGGGCG---ATCGGCTGCTGACCTTCCAGCTGTACGACCAGCAGGCCAACGTCGCCGCTGGGCATCATCCCGCTGCTGCT
gordanae		GCTCGGCTACGACAC---TCTGGGCG---GCCGGTGTGCTGACCTTCCAGCTGTACGACCAGCAGGCCAACTTCCCCTCGGGCTGCTCCCGCTGCTGCT
intra type1		GCTGGGCTACGACAC---CCTCGGCA---ACCGGCTGCTGACCTTCCAGCTCTACGACCAGCAGGCCAACGTCGCCGCTGGGCATCATCCCGCTGCTGCA
intra type2		GCTGGGCTACGACAC---CCTCGGCA---ACCGGCTGCTGACCTTCCAGCTCTACGACCAGCAGGCCAACGTCGCCGCTGGGCATCATCCCGCTGCTGCA
intra type3		GCTGGGCTACGACAC---CCTCGGCA---ACCGGCTGCTGACCTTCCAGCTCTACGACCAGCAGGCCAACGTCGCCGCTGGGCATCATCCCGCTGCTGCA
intra type4		GCTGGGCTACGACAC---CCTCGGCA---ACCGGCTGCTGACCTTCCAGCTGTACGACCAGCAGGCCAACGTCGCCGCTGGGCATCATCCCGCTGCTGCA
intra type5		CCTGGGTTACGACAC---CCTGGGCA---GCCGGTGTGCTGACCTTCCAGCTCTACGACCAGCAGGCCAACGTCGCCGCTGGGCATCATCCCGCTGCTGCA
leprae		ACTCGGCTACGACAC---GCTCGGCA---ACAAGCTGCTTACATTCCAGCTGTATGACCAACAGGCCAACGTCCTACTCGGCATCATCCCGCTGCTGCA
malmoense		GCTGGGCTACGACAC---CCTCGGCG---ACAAGCTGCTGACCTTCCAGCTGTACGACCAGCAGGCCAACGTCGCCGCTGGGCATCATCCCGCTGCTGCA
marinum		GCTGGGCTGGGACAC---CCTCGGCA---ACAAGCTGCTGATCTTCCAGGTGTACGACCAGCAGCCAACTTCCCCTCGGGCTGCTCCCGCTGCTGCT
kansasii t1		GCTGGGCTGGGACAC---CCTCGGCA---ACAAGCTGCTGATATTCCAGGTGTACGACCAGCAGCCAACTTCCCCTCGGGCTGCTCCCGCTGCTGCT
kansasii t2		GCTTGGCTGGGACAC---TCTCGGCA---ACAAGCTCTGATATTCCAGGTGTACGACCAGCAGCCAACTTCCCCTCGGGCTGCTCCCGCTGCTGCT
paratb		GCTGGGTTATGACAC---CGTGGGCA---GCCGGTGTGCTGACCTTCCAGCTGTACGACCAGCAGGCCAACGTCGCCGCTGGGCATCATCCCGCTGCTGCA
phlei		GTTGGGCTACGACAC---CCTCGGCG---ACCGGCTGCTGACCTTCCAGTGTACGACCAGCAGGCCAACTGTCCTACTGGGCATCATCCCGCTGCTGCT
M.sp.40407		ACTCGGCTGGGACAG---CCTCGGCG---AGAAGTGTGCTGCTTCCAGGTGTACGACCAGCAGCCAACTTCCCCTCGGGCTGCTCCCGCTGCTGCT
scrof t1		GCTGGGCTACGACAC---GCTCGGCG---GCAAGCTGCTGACCTTCCAGTGTACGACCAGCAGGCCAACGTCGCCGCTGGGCATCATCCCGCTGCTGCA
scrof t2		GCTGGGCTACGACAC---CCTCGGCA---GCAAGCTGCTGACCTTCCAGTGTACGACCAGCAGGCCAACGTCGCCGCTGGGCATCATCCCGCTGCTGCA
simiae		ACTCGGCTACGACAC---CCTCGGCG---ACCGGCTGCTGACCTTCCAGTGTACGACCAGCAGGCCAACGTCGCCGCTGGGCATCATCCCGCTGCTGCA
smegmatis		GCTCGGCTACGACAG---CCTCGGTG---GCCGTGCTGCTGACCTTCCAGTGTACGACCAGCAGGCCAACGTCGCCGCTGGGCATCATCCCGCTGCTGCA
szulgai		ACTGGGATGGGACAC---ACTGGGTA---ACAAGCTGCTGATCTTCCAGGTGTACGACCAGCAGCCAACTTCCCCTCGGGCTGCTCCCGCTGCTGCT
tb		ACTGGGCTGGGACAC---ACTCGGCA---ACAAGCTGCTGATATTCCAGTGTACGACCAGCAGCCAACTTCCCCTAGGCATTTGCTCCGCTGCTGCT
xenopi		ACTCGGCTGGGACAG---CCTGGGTG---GCAAGCTCTGCTGTTCCAGTGTACGACCAGCAGTCCAACTTCCCCTCGGGATCGTCCCGCTGCTGCT
human	593	GATTGATGTGTTGGGAGCACGC
avium type1		GGTCGACATGTGGGAGCACGC
avium type2		GGTCGACATGTGGGAGCACGC
avium type3		GGTCGACATGTGGGAGCACGC
avium type4		GGTCGACATGTGGGAGCACGC
avium type5		GGTCGACATGTGGGAGCACGC
celatum t3		CCTCGACATGTGGGAGCACGC
celatum t2		CCTCGACATGTGGGAGCACGC
chelonel		GGTCGACATGTGGGAGCACGC
fortuitum		GGTCGACATGTGGGAGCACGC
gordanae		GGTCGACATGTGGGAGCACGC
intra t1		GGTCGACATGTGGGAGCACGC
intra t2		GGTCGACATGTGGGAGCACGC
intra t3		GGTCGACATGTGGGAGCACGC
intra t4		GGTCGACATGTGGGAGCACGC
intra t5		GGTCGACATGTGGGAGCACGC
leprae		GGTCGACATGTGGGAACATGC
malmoense		GGTCGACATGTGGGAACACGC
marinum		GCTCGACATGTGGGAGCACGC
kansasii t1		GCTCGACATGTGGGAACACGC
kansasii t2		GCTCGACATGTGGGAACACGC
paratb		GGTCGACATGTGGGAGCACGC
phlei		GGTCGACATGTGGGAACATGC
M.sp.40407		GCTCGACATGTGGGATCACAC
scrof t1		GGTCGACATGTGGGAGCACGC
scrof t2		GGTCGACATGTGGGAGCACGC
simiae		GGTCGACATGTGGGAGCACGC
smegmatis		GGTCGACATGTGGGAGCACGC
szulgai		GCTCGACATGTGGGAACACGC
tb		GCTCGACATGTGGGAACACGC
xenopi		GCTCGACATGTGGGA--ACGC

Figure 1 Alignment of superoxide dismutase gene sequences.

in up to five bases towards the 5' end. One isolate showed a profile very similar to *M. schimoidi* but was identified biochemically as MAC and is reported here as *M. sp.* 40407/94. Four identical isolates identified biochemically as MAC gave a previously unreported unique profile, similar to *M. intracellulare*, referred to here as *M. intracellulare* type 4. These minor changes in 16SrRNA sequences will be reported elsewhere.

SOD SEQUENCES

All 104 isolates gave a product with the primers SF1 and SR1 which were sequenced in both directions at least once (fig 1). Five sequence types of *M. avium* were observed which showed only small variations (fig 2) in base sequence (0.9 (SD 0.4)%). Thirty eight isolates referred to here as *M. avium* type 1 included 33 AIDS, five non-AIDS, and the reference strain NCTC 8559. Four further isolates each had unique sequence variations and are referred to here as *M. avium* types 2 and 3 (AIDS isolates), and *M. avium* types 4 and 5 (non-AIDS isolates).

AIDS isolates of *M. avium* could not be distinguished from non-AIDS isolates on these sequence data. Isolates identified by 16SrRNA sequencing as similar to *M. intracellulare* could be differentiated by minor base variations into five types, referred to as follows: three isolates designated *M. intracellulare* type 1 (one AIDS isolate, one non-AIDS isolate, and reference strain NCTC 10682); one isolate designated *M. intracellulare* type 2 (AIDS isolate); three isolates designated *M. intracellulare* type 3 (AIDS isolates concurrent with *M. intracellulare* serotype 18 16SrRNA profile); three isolates designated *M. intracellulare* type 4 (AIDS isolates with previously unreported 16SrRNA profile); and one isolate designated *M. intracellulare* type 5 (reference strain *M. intracellulare* serotype 7; ATCC 35847). Similarly *M. scrofulaceum* had one isolate designated *M. scrofulaceum* type 1 (reference strain NCTC 10803) and one isolate designated *M. scrofulaceum* type 2 (AIDS isolate). Overall the MAC isolates varied by 7.1 (3.7)% and were distinctly clustered in the calculated phylogenetic tree (fig 3) with a 19.5 (4.1)% diversity of this group from all other

Figure 2 Superoxide dismutase gene variability matrix calculated with Jukes and Cantor distance method.¹²

species. *M. kansasii* had 12 isolates designated *M. kansasii* type 1—including nine AIDS isolates, two non-AIDS isolates, and the reference strain NCTC 10268—and one isolate designated *M. kansasii* type 2 (AIDS isolate with

minor 16SrRNA sequence variation). *M. celatum* type 1 and 10 isolates of *M. celatum* type 3 showed identical sequences but had a 3.5% variation from *M. celatum* type 2 and 21.5 (3.7)% variation from all other species. All 11 isolates of *M. tuberculosis* had identical sequences. Significant variation (range: 10–29) was observed between all the remaining reference strains, with all the rapid growing species also forming a distinct cluster which had 18.6 (4.0)% diversity from all slow growing species. Restriction digest maps produced from each of the 29 sequence variations revealed that HaeIII would be a suitable enzyme for the generation of unique restriction fragment profiles.

CAPILLARY ELECTROPHORESIS PROFILES

Capillary electrophoresis of samples gave chromatograms for the fragment size range 50–264 bp. The inclusion of a standard digest into each capillary electrophoresis sample allowed an estimation of sample fragment sizes (fig 4). Capillary electrophoresis profiles, in the range 70–264 bp, were plotted for at least one representative of all isolates with unique SOD sequences. Twenty unique profiles were determined (table) which could differentiate all the recognised species studied with the exceptions of *M. avium* from *M. paratuberculosis*, and *M. celatum* types 1 and 3 from *M. szulgai*. All internal fragment sizes corresponded to those predicted from the sequence data. The majority of terminal fragments, however, gave estimated sizes of 3–7 bases larger than expected. These increases were constant for each fragment length and were the same for all species and repeat samples tested. In some samples, extra peaks were observed at 57 bp and 60 bp (fig 4). These peaks could not be explained from the sequence data and may have occurred because of non-specific amplification or primer concatenation. Their appearance also varied from sample to sample and did not occur in all repeat samples. These peaks, however, were below the chosen capillary electrophoresis profile range and therefore did not interfere with the differentiation of isolates.

NUCLEOTIDE SEQUENCE ACCESSION NUMBERS

The following sequences have been submitted to EMBL (European Molecular Bank Library) under the accession numbers: Z48208 (*M. marinum*), Z48209 (*M. celatum* type 3), Z48210 (*M. malmoense*), Z48211 (*M. phlei*), Z48212 (*M. paratuberculosis*), Z48213 (*M. szulgai*), Z48214 (*M. smegmatis*), Z48215 (*M. celatum* type 3), Z48216 (*M. chelonae*), Z48217 (*M. intracellulare* serotype 18), and Z48218 (*M. intracellulare* serotype 7). Other sequences in this work were found to be identical to the following existing sequences: X52861 (*M. tuberculosis*), X16453 (*M. leprae*), X81384 (*M. avium*), X81387 (*M. intracellulare*), X81385 (*M. fortuitum*), X81386 (*M. gordonae*), X81388 (*M. kansasii*), and X81390 (*M. simiae*).

Figure 3 Phylogenetic tree of mycobacteria derived from superoxide dismutase (SOD) gene by FITCH algorithm using *H. sapiens* SOD¹³ as outgroup.

Discussion

The diversity shown by the sequences presented in this study indicates that the SOD gene is suitable as an alternative to genes such as 16SrRNA for the genomic identification of the *Mycobacteriaceae*. AIDS isolates of *M. avium* could not be distinguished from non-AIDS isolates; however, a phenogram derived from SOD sequences (fig 3) clearly delineates fast and slow growing species and MAC. *M. avium*, *M. intracellulare*, and *M. scrofulaceum* are seen to form a cluster representative of MAC which does not include *M. celatum* as suggested by biochemical identifications alone.¹⁵ The phylogenetic diversity of the *M. celatum* group from MAC (23.3 (1.3)%) which had been previously suggested using 16SrRNA data is thus confirmed, with *M. celatum* being more related to *M. xenopi* than MAC. All recognised species used in this study were differentiated by 8–29% base variation with the exception of *M. paratuberculosis*, which differed from the observed five *M. avium* types by up to four bases (1.1 (0.5)%). This difference could be significant

considering the close genetic homology already recognised in studies of other genes from these two species, and that the variation at base No 513 (fig 1) predicts a change in amino acid sequence (valine to leucine). These data concur with 16SrRNA studies in that *M. paratuberculosis* should be regarded as a subtype of *M. avium*. We believe this report is the first to be able to differentiate *M. paratuberculosis* from *M. avium* by gene sequence data alone. Minor intra-species sequence differences were also observed between clinical isolates and reference strains within the species of *M. scrofulaceum* (3.9%), *M. kansasii* (1.9%), and *M. celatum* (3.5%). In the *M. intracellulare* samples a larger variation of 5.4 (2.7)% was observed, illustrating the high diversity within this loosely defined species.

We show here that sequence diversity in SOD can be used to predict unique capillary electrophoresis profiles and have rapidly differentiated 16 of 18 species tested with this method. We have only used a single restriction enzyme digest in this study; however, in combination with further restriction digests it would be possible to differentiate all the species described. The advantage of using a single low cut restriction digest can be seen with the results from *M. avium* and *M. kansasii*, where intraspecies base variation did not occur across restriction sites and thus only one unique profile was seen for all the species types (table).

Terminal restriction digest fragment sizes observed by capillary electrophoresis in the majority of species were constantly larger than predicted from the sequence data. Similar migration retardation anomalies have been reported,¹⁶ possibly due to DNA fragment secondary structure and the concentration and type of polymer used in the capillary. The reproducibility of the technique described here suggests, however, that the observed larger fragment sizes will not present a problem in the identification of species in unknown samples, providing adequate control profiles are used.

A recently described method¹⁷ also uses the SOD gene to detect mycobacterial species-specific sequences. This method presupposes that intraspecies diversity is low enough to allow relatively high stringency hybridisation of short species-specific DNA probes homologous to amplified SOD product. This study shows that four of 42 (10%) clinical isolates of *M. avium* contained sequence variations. If variation occurs within the probe sites it could result in failure to hybridise, thereby reducing the specificity of the test. Our approach using identification by low cut restriction analysis circumvents this potential problem by producing capillary electrophoresis profiles for all isolates, regardless of sequence variation. The use of a low cut enzyme guarantees a low probability of variation across restriction sites which would produce profiles dissimilar to the control profile bank. Even in this event, profiles can be extrapolated to give the most likely identification from the nearest fit. This study shows that the high diversity between SOD sequences in mycobacteria ensures that profiles of one species will not be transformed by minor

Figure 4 Capillary electrophoresis profiles of amplified superoxide dismutase gene *HaeIII* restriction digests from *mycobacterium* species.

Restriction fragment sizes of *HaeIII* digests of superoxide dismutase from mycobacteria measured by capillary electrophoresis

Taxon	Restriction fragment size (bp)																									
	70	71	74	77	83	84	87	90	93	98	104	131	134	138	144	145	147	156	160	167	175	179	191	224	227	
<i>M avium</i> type 1-5 & <i>M paratuberculosis</i>																										
<i>M celatum</i> type 1 & 3 <i>M szulgai</i>																										
<i>M celatum</i> type 2																										
<i>M tuberculosis</i>																										
<i>M intracellulare</i> type 1,2 & 3																										
<i>M intracellulare</i> type 4																										
<i>M intracellulare</i> type 5																										
<i>M scrofulaceum</i> type 1																										
<i>M scrofulaceum</i> type 2																										
<i>M gordonae</i>																										
<i>M chelonae</i>																										
<i>M malmoense</i>																										
<i>M marinum</i>																										
<i>M kansasii</i> type 1 & 2																										
<i>M phlei</i>																										
<i>M sp.</i> 40407/94																										
<i>M simiae</i>																										
<i>M fortuitum</i>																										
<i>M smegmatis</i>																										
<i>M xenopi</i>																										

sequence variation into those corresponding to another species.

The need for rapid detection of small numbers of bacteria present in clinical samples requires an efficient DNA extraction technique to maintain sensitivity. The nature of the mycobacterial cell wall in species such as *M intracellulare* and *M avium* makes it difficult to achieve lysis efficiently without the use of long and technically demanding procedures.¹⁸ Rapid detection techniques have therefore tended to be concentrated on the more easily lysed *M tuberculosis*. We have developed a novel, simple, and rapid method for DNA extraction which, in conjunction with capillary electrophoresis profile analysis of the amplified SOD gene, enables identifications to be carried out on all mycobacterial species within 24 hours. Preliminary work on clinical samples using extraction techniques and PCR conditions described here indicate that our SOD primers are genus specific. We therefore believe that this method will be useful for rapid differential diagnosis of mycobacteria when applied directly to clinical samples.

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