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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 chelonei, 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 Mcelatum 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

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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 chelonei (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 tuberculosis⁷ and M leprae¹¹ 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: AGAGTTTGATCCTGGCTCAG 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 μ l) for each of the primer pairs were prepared from each of the test isolates using the following conditions: 1 μ l of extracted chromosomal DNA was amplified in a Gene Amp 9600 thermocycler (Perkin Elmer) with 10 μ l 10X polymerase chain reaction (PCR) buffer (100 mM Tris (pH 8·3), 500 mM KCl, 15 mM MgCl₂, 0·015% gelatin). 16 μ l deoxyribonucleotide mix (1·25 μ mol per

dNTP), 5 μ l of each primer (20 μ M), 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 $0.5 \,\mu g$ 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 $1 \mu l$ (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 $\mu g/$ 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

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human	CACCACAGCAAGCACCACG	GGCCTACGTC	SAACAACCTGAACG	TCACCGAGGAGAAGT7		GCCAAGGGAG	
avium type1	CACCACACCAAGCACCACG	CACCTACGIC	TAAAGGCGTGAACG	ACCCTCTTGCCAAGC	CGAAGAGGCCCG	CCCAACGAGG	ACCACGCIGCG-AICIIC
avium type2	CACCACACCAAGCACCACG	CACCTACGIC	TAAAGGCGTGAACG	ACCOUNTRACTANCE	CCARCACCCCC	CCCAACGAGG	
avium type4	CACCACACCAAGCACCACG	CACCTACGTO	CAAAGGCGTGAACG	ACGCTCTTGCCAAGC	CGAAGAGGCCCG	CGCCAACGAGG	ACCACGCTGCG-ATCTTC
avium type5	CACCACACCAAGCACCACG	CACCTACGT	CAAAGGCGTGAACG	ACGCTCTTGCCAAGC	CGAAGAGGCCCG	CGCCAACGAGG	ACCACGCTGCG-ATCTTC
celatum.t3	CACCACAGCAAACACCATGO	GACCTACGTO	GAAGGGCGCCAACG	ACGCGCTCGAAAAAC	ICGAGGAAGCACG	CGCCAAGGACG	ACCAGTCGACC-ATCCTG
celatum.t2	CACCACAGCAAACACCATGO	GACCTACGTO	GAAGGGCGCGAACG	ACGCGCTTGAAAAAC	rcgaggaagcacg	CGCCAAGGACG	ACCAGTCGACC-GTCCTG
chelonei	CAACACAGTAAGCACCACG	GGCCTACGT	CGCGGGTGTCAATT	CCGCTGTCGCCAAGT	IGGAAGAGGCTCG	CGAGAAGGGCG	ACCACGCCGCG-ATCTTC
fortuitum	CACCACAGCAAGCACCACG	GGCGTACGT	CAAGGGCGTCAACG	ACGCCGTGGCCAAGC	ICGATGAGGCGCG	GCCAACGGTG	ACCACGCGGCG-ATCTTC
gordonae	TCACACAGTAAGCACCACG	CACCTACGT	CAAAGGCGTCAACG	ACGCGGTCGCCAAGC	I'GGAAGAAGCGCG	CGCCAAAGGCG	ACCACTCGGCC-ATCTTT
intra typei	CACCACAGIAAGCACCACG	CACCIACGI	CARAGGEGIGAAEG	ACGUIUIGIUUAAGU.		TCCCAACGAAG	
intra type2	TCACACAGTAAGCACCACG	CACCTACGI	TAAAGGCGTGAACG	ACCOUTCIGICCAAGC	TCGAAGAGGCCCCG	TGCCAACGAAG	
intra type4	TACCACAGTAAGCACCACG	CACATACGT	CAAAGGCGTGAATG	ACGCTCTGTCCAAGC'	FCGAAGAGGCCCG	CGCCAACGAGG	ACCACGCTGCG-ATCTTC
intra type5	CACCACACTAAGCACCACG	CACGTACGT	CAAAGGCGTGAACG	ACGCTCTGTCCAAGC	TCGAAGAGGCCCG	CGCCAACGAGG	ACCACGCTGCG-ATCTTC
leprae	CACCACACCAAGCACCACGO	CGCATATGTO	CAAAGGTGTCAATG	ACGCGCTTGCCAAAC	TTGACGAGGCACG	CGCCAAAGACG	ACCACTCCGCG-ATTTTT
malmoense	CACCACAGCAAGCACCACG	CGCCTACGT	CAAGGGCGTGAACG	ACGCCGTCGCCAAGC	FTGAAGAGGCGCG	GGCCAAGGACG	ACCACTCGGCG-ATCTTC
marinum	TCACACAGTAAGCACCACG	CACCTACGT	CAAGGGTGCCAATG	ACGCCGTCACCAAAC	TCGAGGAAGCGCG	CGCTAAGGAAG	ACCACTCGACG-ATCCTG
kansasii tl	CACCACAGTAAGCACCACG	CACCTACGT	CAAGGGCGCCAACG	ATGCGGTCGCCAAAC	TCGAAGAGGCGCG	CGCCAAGGAAG	ACCACTCGGCG-ATCTTG
kansasii t2	GCACACAGTAAGCACCACG	CACCTACGT		ATGCGGTCGCCAAAC	ICGAAGAGGCGCG	CGCCAAGGAAG	ACCACTCGGCG-ATCTTG
parato				ACGUTUTTGCCAAGU	TCGAAGAGGCCCCG		ACCACGCTGCG-ATCTTC
M sp 40407	TCACACAGCAAGCACCACG	CACCTACGI	TAAGGGCGICAACG	ACGCGATCGCCAAGC	TCAAGAAGCGCG		
scrof t1	CATCACACCAAGCACCACG	CACGTACGT	CAAGGGCGTGAACG	ACGCCGTCGCCAAAC	TCCAAGAGGCGCG	CCCCARCORIG	ACCACCCCCCCC-ATCTTC
scrof t2	CATCACACCAAGCACCACG	CACCTACGT	CAAGGGCGTGAACG	ACGCGGTCGCCAAAC'	TCCAAGAGGCGCG	CGCCAACGACG	ACCACGCCGCG-ATCTTC
simiae	CACCACAGCAAGCACCATG	GACGTACGT	CAAGGGTTTGAACG	ACGCCATTGCCAAGC	TTGAAGAGGCACG	GGCCAACGACG	ACCACGCCGCG-ATCTTC
smegmatis	CACCACAGCAAGCACCACG	GACCTACGT	CAAGGGTGTGAACG	ACGCGATTGCCAAGC	TCGAGGAGGCACG	GGCCAACGGTG	ACCACGCGGCC-ATCTTC
szulgai	TCACACAGCAAGCACCACG	CACCTACGT	CAAGGGCGCCAATG	ACGCTGTCGCCAAAC	TCGAGGAGGCGCG	CGCTCAGGAGG	ACTTTTCGTCG-ATCTTG
tb	CACCACAGCAAGCACCACG	CACCTACGT	AAAGGGCGCCAATG	ACGCCGTCGCCAAAC	TCGAAGAGGCGCG	CGCCAAGGAAG	ATCACTCAGCG-ATCTTG
xenopi		GACGTACGT	CAAAGGCGCCAACG	ACGCGCTCGCCAAGC	IGGAGGAGGCGCG	CGCCAAAGACG	ATCATTCCGCG-ATCGTC
human		CTTC AATCCI	PCGTCGTCATATCA	aTCaTaCCaTTTCT	COCADAACCTCAC	CCCTAACCCTC	TTCCACAACCCAAACCCC
avium typel	CTGAACGAAAAGAACCTCG	CTTCCACCT	GGCGGCCACGTCA	ACCACTCGATCTGGT	CGABGABCCTCTC	CCCGGACGCCG	TGACA ACCCCARAGGGG
avium type2	CTGAACGAAAAGAACCTCG	CTTCCACCT	GGCCGCCACGTCA	ACCACTCGATCTGGT	GGAAGAACCTGTC	GCCGGACGGCG	GTGACAAGCCCACCGGTG
avium type3	CTGAACGAAAAGAACCTCG	CTTCCAGCTO	GGGCGGCCACGTCA	ACCACTCGATCTGGT	GGAAGAACCTGTC	GCCGGACGGCG	GTGACAAGCCCACCGGTG
avium type4	CTGAACGAAAAGAACCTCG	CTTCCACCTO	GGGCGGCCACGTCA	ACCACTCGATCTGGT	GGAAGAACCTGTC	GCCGGACGGCG	GTGACAAGCCCACCGGCG
avium type5	CTGAACGAAAAGAACCTCG	CTTCCACCTO	GGGCGGCCACGTCA	ACCACTCGATCTGGT	GGAAGAACCTGTC	GCCGGACGGCG	GTGACAAGCCCACCGGCG
celatum t3	TTGAACGAGAAGAACCTGG	GTTCAACCT	GGCCGGCCACGTGA	ACCACACCATCTGGT	GGAAGAACCTGTC	GCCTAACGGCG	GTGACAAGCCGACCGGTG
celatum t2	TTGAACGAGAAGAACTTGG	GTTCAACCTC	GCCCGCCACGTCA	ACCACACCATCTGGT	GGAAGAACCTGTC	GCCCAACGGCG	GTGACAAGCCGACCGGGG
fortuitum	CTCAACGAGAAGAACCIGG	CITCCACCI	CCCCCCCCCACGIGA	ACCACICCATCIGGI	GGAAGAACCTGTC	GCCCAACGGCG	GUGACAAGUUUAUUGGUG
gordonae	TTGAACGAGAAGAACCTGG	CTTCCACCT	SGCCGGCCACGIGA	ACCACTCGATCTGGT	GGAAGAACCIGIC	CCCCAACGGIG	CCACAAGCCGACGGGCG
intra typel	CTGAACGAAAAGAACCTGG	CTTTCACCT	GGCCGCCACGTCA	ACCACTCCATCTGGT	GGAAGAACCTGTC	GCCGGACGGCG	GCGACAAGCCGACCGGCG
intra type2	CTGAACGAAAAGAACCTGG	CTTTCACTTC	GGGCGGCCACGTCA	ACCACTCCATCTGGT	GGAAGAACCTGTC	GCCGGACGGCG	GCGACAAGCCGACCGGCG
intra type3	CTGAACGAAAAGAACCTGGG	CTTTCACCTO	GGGCGGCCACGTCA	ACCACTCCATCTGGT	GGAAGAACCTGTC	GCCGGACGGCG	GCGACAAGCCGACCGGCG
intra type4	CTGAACGAAAAGAACCTCG	CATTCCACCTO	CGGTGGCCACGTCA	ACCACTCGATCTGGT	GGAAGAACCTGTC	GCCGGACGGCG	GCGACAAGCCGACCGGCG
intra type5	CTGAACGAAAAGAACCTCG	CTTCCACTTC	GGGCGGCCACGTCA	ACCACTCCATCTGGT	GGAAGAACCTGTC	GCCGGACGGCG	GCGACAAGCCGACCGGCG
reprae	CTGAACGABABGAACCTGG	CTITCAITIC	GGGGGGGGGCACGTCA	ACCACTCCATCTGGT	GGAAGAACCTCTC	ICCGAACGGIG	GUGATAAGUUGAUUGGUG
marinum	CTGAACGAGAGAAGAACCTCG	TTTCAACCT	CCCCGCCACGTCA	ACCACACCATCTGGT	GGAAGAACCIGIC	CCCCAALGGCG	CCACAAGCCCACCGCCG
kansasii tl	CTGAACGAGAAGAACTTGG	CTTCAACCT	CGCCGGCCACGTCA	ACCACACGATCTGGT	GGAAGAACCTTTC	TCCCAACGGAG	GEGACAAGCCGACCGGIG
kansasii t2	CTGAACGAGAAGAACTTGG	CTTCAACCT	CGCCGGCCACGTCA	ACCACACGATCTGGT	GGAAGAACCTTTC	TCCCAACGGAG	GCGACAAGCCGACCGGCG
paratb	CTGAACGAAAAGAACCTCG	CTTCCACCTO	GGGCGGCCACGTCA	ACCACTCGATCTGGT	GGAAGAACCTGTC	GCCGGACGGCG	GTGACAAGCCCACCGGCG
phlei	CTGCACGAGAAGAACCTTG	GTTCCACCTO	CGGCGGGGCACGTCA	ACCACACGATCTGGT	GGAAGAACCTGTC	CCCGCACGGTG	GCGACAAGCCGACCGGGG
M.sp. 40407	CTGAACGAAAAGAACTTGG	GTTCAACCT	GGCGGGGCCACGTCA	ACCACAGCCTGTGGT	GGAAGAACCTGTC	GCCCGACGGTG	GCGACAAGCCGACCGGCG
scrof ti	CTGAACGAAAAGAACCTGG	GTTCCACCT	CGGCGGCCACGTGA	ACCACTCGATCTGGT	GGAAGAACCTCTC	ccccc h ccccc	~~~``
scrol tz						GCCGGACGGCG	GCGACAAGCCGACCGGAG
SIMIAE	TTCAACCACAACAACCTCC	GTTCCACCT	CGGCGGCCACGTGA	ACCATTCCATCTGGT	GGAAGAACCTGTC	GCCGGACGGCG	GCGACAAGCCGACCGGAG GCGACAAGCCGACCGGAG
smeanatis	TTGAACGAGAAGAACCTGG	CATTCCACCTO	CGGCGGCCACGTGA CGGTGGCCACGTCA	ACCATTCCATCTGGT	GGAAGAACCTGTC GGAAAAAACCTGTC	GCCGGACGGCG GCCGGACGGCG CCCGAACGGCG	GCGACAAGCCGACCGGAG GCGACAAGCCGACCGGAG GAGACAAGCCGACCGGAG
smegmatis szulgai	TTGAACGAGAAGAACCTGG CTGAACGAGAAGAACCTCG CTGAGCGAAAAGAACCTCG	CGTTCCACCTO CATTCCACCTO CGTTCCATCTO	CGGCGGCCACGTGA CGGTGGCCACGTCA CGGTGGCCACATCA CGCCGGCCACGTCA	ACCATTCCATCTGGT ACCACTCCATCTGGT ACCACTCGATCTGGT ACCACTCGATCTGGT	GGAAGAACCTGTC GGAAAAAACCTGTC GGAAGAACCTCTC GGAAGAACCTCTC	GCCGGACGGCG GCCGGACGGCG CCCGAACGGCG CCCCAACGGTG	GCGACAAGCCGACCGGAG GCGACAAGCCGACCGGAG GAGACAAGCCGACCGGAG GCGACAAGCCCACCGGCG GCGACAAGCCCACCGGCG
smegmatis szulgai tb	TTGAACGAGAAGAACCTGG CTGAACGAGAAGAACCTGG CTGACGAGAAGAACCTGG CTGACGAAAAGAACCTGG CTGAACGAAAAGAACCTGG	CGTTCCACCTO CATTCCACCTO CGTTCCATCTO CGTTCAACTTO CTTTCAACCTO	CGGCGGCCACGTGA CGGTGGCCACGTCA CGGTGGCCACATCA GGCCGGCCACGTCA CGCCGGCCACGTCA	ACCATTCCATCTGGT ACCACTCCATCTGGT ACCACTCGATCTGGT ATCACACCATCTGGT ATCACACCATCTGGT	GGAAGAACCTGTC GGAAAAAACCTGTC GGAAGAACCTCTC GGAAGAATCTGTC GGAAGAACCTGTC	GCCGGACGGCG GCCGGACGGCG CCCCAACGGTG TCCCAACGGTG GCCTAACGGTG	GCGACAAGCCGACCGGAG GCGACAAGCCGACCGGAG GAGACAAGCCGACCGGAG GCGACAAGCCCACCGGCG GTGACAAGCCCAACGGGCG GTGACAAGCCCACCGGCG
smegmatis szulgai tb xenopi	TTGAACGAGAAGAACCTGG CTGAACGAGAAGAACCTCG CTGAGCGAAAAGAACCTGG CTGAACGAAAAGAACCTGG GGGCATGAGAAAGAATCTAG GGGCATGAGAAAGCCCTCG	CGTTCCACCT CATTCCACCT CGTTCCATCT CGTTCAACTT CTTTCAACCT CGTTCAACCT	CGGCGGCCACGTGA CGGTGGCCACGTCA CGGTGGCCACATCA GGCCGGCCACGTCA CGCCGGCCACGTCA GGCCGGCCATGTCA	ACCATTCCATCTGGT ACCACTCCATCTGGT ACCACTCGATCTGGT ATCACACCATCTGGT ATCACACCATCTGGT ATCACCCCTGTGGT	GGAAGAACCTGTC GGAAGAACCTGTC GGAAGAACCTCTC GGAAGAATCTGTC GGAAGAACCTGTC GGAAGAACCTGTC	GCCGGACGGCG GCCGGACGGCG CCCCAACGGTG TCCCAACGGTG GCCTAACGGTG CCCCAACGGCG	GCGACAAGCCGACCGGAG GCGACAAGCCGACCGGAG GAGACAAGCCGACCGGAG GCGACAAGCCCAACGGCG GTGACAAGCCAACGGGCG GTGACAAGCCCACCGGCG GTGACAAGCCGACCGGCG
smegmatis szulgai tb xenopi	TTGAACGAGAAGAACCTGG CTGAACGAGAAGAACCTGG CTGAACGAGAAGAACCTGG CTGAACGAAAAGAACCTGG CTGAACGAAAAGAACCTGG GGCCATGAGAAAGACCTCG 393	CGTTCCACCTC CATTCCACCTC CGTTCCATCTC CGTTCAACTTC CTTTCAACCTC CGTTCAACCTC	CGGCGGCCACGTGA CGGTGGCCACGTCA CGGTGGCCACATCA GGCCGGCCACGTCA CGCCGGCCACGTCA GGCCGGCCATGTCA	ACCATTCCATCTGGT ACCACTCCATCTGGT ACCACTCGATCTGGT ATCACACCATCTGGT ATCACACCATCTGGT ATCACTGCCTGTGGT	GGAAGAACCTGTC GGAAGAACCTGTC GGAAGAACCTCTC GGAAGAACCTCTC GGAAGAACCTGTC GGAAGAACCTGTC	GCCGGACGGCG CCCGAACGGCG CCCCAACGGTG TCCCAACGGTG GCCTAACGGTG GCCTAACGGTG	GCGACAAGCCGACCGGAG GCGACAAGCCGACCGGAG GAGACAAGCCGACCGGAG GCGACAAGCCCACCGGCG GTGACAAGCCAACGGGCG GTGACAAGCCCACCGGCG GTGACAAGCCGACCGGCG
smegmatis szulgai tb xenopi human	TTGAACGAGAAGAACCTGG CTGAACGAGAAGAACCTGG CTGAACGACAAAGAACCTGG CTGAACGAAAAGAATCTAG GGGCATGAGAAGGCCCTCG 393 AGTTGCTGGAAGCCATCAAJ	CGTTCCACCTC CATTCCACCTC CGTTCCATCTC CGTTCAACTTC CTTTCAACCTC CGTTCAACCTC	CGGCGGCCACGTGA CGGTGGCCACGTCA CGGTGGCCACGTCA CGCTGGCCACGTCA CGCCGGCCACGTCA CGCCGGCCACGTCA GGCCGGCCATGTCA	IACCATTCCATCTGGT IACCACTCCATCTGGT IACCACTCCATCTGGT IATCACACCATCTGGT IATCACACCATCTGGT IATCACACCATCTGGT IATCACTGCCTGTGGT IGTTTAAGGAGAAGCT	GGAAGAACCTGTC GGAAGAACCTGTC GGAAGAACCTCTC GGAAGAACCTGTC GGAAGAACCTGTC GGAAGAACCTGTC GAAGGACCTGCATCT	GCCGGACGGCG CCCGAACGGCG CCCCAACGGTG TCCCAACGGTG GCCTAACGGTG GCCTAACGGTG CCCCAACGGCG	GCGACAAGCCGACCGGAG GCGACAAGCCGACCGGAG GAGACAAGCCGACCGGAG GCGACAAGCCCACCGGCG GTGACAAGCCCACCGGCG GTGACAAGCCCACCGGCG GTGACAAGCCCACCGGCG
smegmatis szulgai tb xenopi human avium typel	TTGAACGAGAAGAACCTGG CTGAACGAGAAGAACCTGG CTGAACGAGAAAGAACCTGG CTGAACGAAAAGAACTGG GGCATGAGAAAGGAATCTAG GGCATGAGAAAGGCCCTCG 393 AGTTGCTGGAAGCCATCAAA AGCTGGCCGCCGCGATCGAA	CTTCCACCTO CATTCCACCTO CGTTCCATCTO CGTTCAACCTO CGTTCAACCTO CGTTCAACCTO ACGTGACTTTO CGACGCGTTCO	CGGCGGCCACGTGA CGTGGCCACATCA GGCGGCCACATCA GGCCGGCCACGTCA CGCCGGCCACGTCA GGCCGGCCATGTCA GGTTCCTTTGACAA GGTTCCTTTGACAA	ACCATTCCATCTGGT ACCACTCCATCTGGT ACCACTCCATCTGGT ATCACACCATCTGGT ATCACACCATCTGGT ATCACACCATCTGGT ATCACTGCCTGTGGT GTTTAAGGAGAAGCT GTTTCCGAGGGCAATT	GGAAGAACCTGTC GGAAGAACCTGTC GGAAGAACTCTCC GGAAGAACCTGTC GGAAGAACCTGTC GGAAGAACCTGTC GAAGGACCGCTGCATCT CAGCGCCGCCGCCGCC	GCCGGACGCGGG GCCGGACGGCG CCCCAACGGTG TCCCAACGGTG GCCTAACGGTG GCCCAACGGCG GTTGGTGTCCA AACGGCCTGCA	GCGACAAGCCGACCGGAG GCGACAAGCCGACCGGAG GACAAGCCGACCGGAG GCGACAAGCCCACCGGCG GTGACAAGCCCACCGGCG GTGACAAGCCCACCGGCG GTGACAAGCCCACCGGCG AGGCTCAGGTTGGGGTTG GGGCTCCGGCTGGGCGGT
smegmatis szulgai tb xenopi human avium type1 avium type2	TTGAACGAGAAGAACCTGG CTGAACGAGAAGAACCTGG CTGAACGACAAAGAACCTGG CTGAACGAAAAGAACTGG GGGCATGAGAAAGGACCTGG 393 AGTTGCTGGAAGCCATCAA AGCTGGCCGCCGCGATCGAA AGCTGGCCGCCGCGATCGAA	CGTTCCACCTIC CGTTCCACTTC CGTTCCACCTC CTTTCAACCTC CGTTCAACCTC CGTCCAACCTC CGCGCGCCTCC CGACGCGCTTCC	CGGCGGCCACGTGA CGTGGCCACATCA CGGTGGCCACATCA CGCCGGCCACGTCA CGCCGGCCACGTCA CGCCGGCCATGTCA CGCCGGCCATGTCA CGTTCCTTTGACAA CGTCCTTCGACAA CGCTCCTTCGACAA	ACCATTCCATCTGGT ACCACTCCATCTGGT ACCACTCATCTGGT ATCACACCATCTGGT ATCACACCATCTGGT ATCACTGCCTGTGGT GTTCAGGAGAGAGCT GTTCCGAGGGCAATT GTTCCGAGGGCCAATT	GGANGAACCTGTC GGANGAACCTGTC GGANGAACCTGTC GGANGAACCTGTC GGANGAACCTGTC GGANGAACCTGTC GAACGACCTGCATCT CAGCGCCGCCGCCCCC CAGCGCCCCCCCCCC	GCCGGACGGCG GCCGAACGGCG CCCCAACGGTG GCCTAACGGTG GCCTAACGGTG GCCTAACGGCG GTTGGTGTCCA AACGGCCTGCA AACGGCCTGCA	GCGACAAGCCGACCGGAG GCGACAAGCCGACCGGAG GAACAAGCCGACCGGAG GCGACAAGCCCACCGGCG GTGACAAGCCCACCGGCG GTGACAAGCCCACCGGCG GTGACAAGCCCACCGGCG GGGCTCAGGTTGGGGCTG GGGCTCCGGCTGGGCGGT
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<pre>smegmatis szulgai tb xenopi human avium type1 avium type2 avium type3 avium type4 avium type5 celatum t2 celatum t2 celatum t2 celatum t2 celatum t2 chelonei fortuitum gordonae intra type1 intra type3 intra type4 intra type4 intra type4 intra type4 intra type4 intra type5 leprae malmoense marinum kansasii t1 kansasii t2 paratb phlei M.sp.40407 scrof t2 simiae</pre>	TTGAACGAGAAGAACTTGG CTGAACGAGAAGAACTTGG CTGAACGAGAAAGAACTTGG CTGAACGACAAAGAACTTGG GGCATGACGAAAGGAACTTGG 393 AGTTGCTGGAAGCCATCAA AGCTGGCCGCCGCGATCGAA AGCTGGCCGCCGCGATCGAA AGCTGGCCGCCGCGATCGAA AGCTGGCCGCCGCGATCGAA AGCTGGCCGCCGCGCATCGAA AGCTGGCCGCCGCGCATCGAA ATTGGCCGCCGCGCATCGAA ATTGGCCGCCGCGCATCGAA AATTGGCCGCCGCGCATCGAA AATTGGCCGCCGCGCATCGAA AATTGGCCGCCGCGCATCGAA AATTGGCCGCCGCGCATCGAA AATTGGCCGCCGCGCATCGAA AATTGGCCGCCGCGATCGAA AATTGGCCGCCGCGATCGAA AATTGGCCGCCGCGATCGAA AATTGGCCGCCGCGATCGAA AATTGGCCGCCGCGATCGAA AATTGGCCGCCGCGCATCGAA AATTGGCCGCCGCGATCGAA AATTGGCCGCCGCGATCGAA AATTGGCCGCCGCGCATCGAA AACTGGCCGCCGCGATCGAA AACTGGCCGCCGCGATCGAA AACTGGCCGCCGCGATCGAA AACTGGCCGCCGCGATCGAA AACTGGCCGCCGCGATCGAA AACTGGCCGCCGCCATCGAA ACCTGCCCGCCGCCATCGAA ACCTGGCCGCCGCCATCGAA ACCTGCCCGCCGCCATCGAA ACCTGCCCGCCGCCATCGAA ACCTGCCCCCCCCCC	GTTCCACCTI GTTCCACCTI GTTCAACTT CTTCAACTT CGTTCAACTT CGTCAACCTI GGTCGACGGTTCC GACGCGTTCC GACGCGTTCC GACGCGTTCC GACGCGTTCC GACGCGTTCC GACGCGTTCC GACGCCTTCC GACCGCTTCC GACGCCTTCC	CGCGGCCACGTCA CGTGGCCACGTCA CGCTGGCCACGTCA CGCCGGCCACGTCA CGCCGGCCACGTCA CGCCGGCCACGTCA CGCCGGCCACGTCA CGCCGTCCTTCGACAA CGTCCTTCGACAA CGTCCTTCGACAA CGTCCTTCGACAA CGTCGTTCGACAA CGCTCGTTCGACAA CGCTCGTTCGACAA CGTCGTTCGACAA CGTCCTTCGACCA CGTCCTTCGACCA CGTCCTTCGACCA CGTCCTTCGACCA CGTCCTTCGACCA CGTCCTTCGACAA	ACCATTCCATCTGGT ACCACTCCATCTGGT ACCACTCCATCTGGT ATCACACCATCTGGT ATCACACCATCTGGT GTTCAGACGCATCTGGT GTTCCGACGCGCAATT GTTCCGACGCGCAATT GTTCCGACGCGCAATT GTTCCGGCGCGCAATT GTTCCGGCGCGCAATT GTTCCGGCGCCACATT GTTCCGGCGCCACATT GTTCCCGCGCCCACT GTTCCCGCGCCCACT GTTCCGCGCCCCACT GTTCCGCGCCCCACT GTTCCGCGCCCCACT GTTCCGCGCCCCACT GTTCCGCGCCCCACT GTTCCGCGCCCCACT GTTCCGCGCCCCACT GTTCCGCGCCCCACT GTTCCGCGCCCCACT GTTCCGCGCCCCACT GTTCCGCGCCCCACT GTTCCGCGCCCCCCT	GGAAGAACCTGTC GGAAGAACCTGTC GGAAGAACCTGTC GGAAGAACCTGTC GGAAGAACCTGTC GGAAGAACCTGTC GGAAGAACCTGTC GGAAGAACCTGTC GACGCCGCCGCCGC CAGCGCCGCCGCC CAGCGCCGCCGCC CAGCGCCGCCGCC CAGCGCCGCCGCC CACCGCCGCCGCC CACCGCCGCCGCC CACCGCCGCCGCC CAGCGCGGCCGCC CAGCGCGGCCGCC CAGCGCGGCCGCC CAGCGCGGCCGCC CACGCCGCCGCCCCC CAGCGCGCCGCCCCC CAGCGCGCCGCCCCC CAGCGCGCCCCCC CAGCGCGCCCCCC CAGCGCGCCCCCC CAGCGCGCCCCCC CAGCGCGCCCCCC CAGCGCGCCCCCC CAGCGCCGCCCCCC CAGCGCGCCCCCCC CAGCGCCGCCCCCC CAGCGCGCCCCCCCC	CCCGAACGGCG CCCCAACGGCG CCCCAACGGTG GCCTAACGGTG GCCTAACGGTG GCCTAACGGTG GCCCAACGGTGCA AACGGCTGCA AACGGCCTGCA AACGGCCTGCA AACGGCTGCA	GGACAAGCCGACCGGAC GGCACAAGCCCACCGGAG GGCACAAGCCCACCGGAG GGCACAAGCCCACCGGCG GTGACAAGCCCACCGGCG GTGACAAGCCCACCGGCG GTGACAAGCCCACCGGCG GGCTCCGGCTGGCCGGT GGGCTCCGGCTGGCGGGT GGGCTCCGGCTGGCGGGT GGGCTCGGCTGGCCGGT GGGCTCGGCTGGCCGGT GGGCTCGGCTGGCCGGT GGGCTCGGCTGGCCGGT GGGCTCGGCTGGCCGGT GGGCTCGGCTGGCCGGT GGGCTCGGCTGGCCGGT GGGCTCGGCTGGGCGGT GGGCTCGGCTGGGCGGT GGGCTCGGCTGGGCGGT GGGCTCGGCTGGGCGGT GGGCTCGGCTGGGCGGT GGGTCGGCTGGGCGGT GGGTCGGCTGGGCGGT GGGTCGGCTGGGCGGT GGGTCGGCTGGGCGGT GGGTCGGCTGGGCGGC GGGTCGGCTGGGCGGC GGGCTCGGCTGGCCGGT GGGTCCGGCTGGCCGGT GGGTCCGGCTGGCCGGC GGGTCCGGCTGGCCGC GGGTCCGGCTGGCCGC GGGTCCGGCTGGCCGC GGGTCCGGCTGGCCGC GGGTCCGGCTGGCCGC GGGTCGGCTGGCCGC GGGTCCGGCTGGCCGC GGGTCCGGCTGGCCGC GGGTCGGCTGGCCGC GGGTCGGCTGGCCGC GGGTCGGCTGGCCGC GGGTCGGCTGGCCGC GGGTCGGCTGGCCGC GGGTCGGCTGGCCGC GGGTCGGCCGCC GGGTCGGCCGCC GGGTCGGCCGCC GGGTCGGCTGGCCGC GGGTCGGCCGC GGGTCGGCCGCC GGGTCGGCCGC GGGTCGGCCGC GGCTCGGCTGGCCGCC GGGTCGGCCGCC GGGTCGGCCGCC GGGTCGGCCGCC GGCTCGGCTGGCCGCC GGGTCGGCCGCC GGGTCGGCCGCC GGCCCGCCGCC GGGTCGGCCGCC GGGTCGGCCGCC GGGTCGGCCGCC GGGTCGGCCGCC GGCTCGGCCGCC GGGTCGGCCGCC GGCCCGCCGCC GGCTCGGCCGCC GGCCCGCCGCC GGCCCGCCGCCGCC GGCCCGCCCGCC GGCCCGCCG
smegmatis szulgai tb xenopi human avium type1 avium type2 avium type3 avium type5 celatum t3 celatum t2 celatum t2 chelonei fortuitum gordonae intra type1 intra type2 intra type3 intra type4 intra type4 intra type4 intra type4 intra type4 intra type4 intra type4 intra type5 leprae malmoense marinum kansasii t1 paratb phlei M.sp.40407 scrof t2 simiae smegmatis szulgai	TTGAACGAGAAGAACCTCGG CTGAACGAGAAGAACCTCGG CTGAACGAGAAAGAACCTGGG CTGAACGACAAAGAACCTGGG CTGAACGACAAAGAACCTGGG GGCATGACAGAAGGCCCTCGA 393 AGTTGCTGGAAGCCATCAAA AGCTGGCCGCCGCGATCGAA AGCTGGCCGCCGCGATCGAA AGCTGGCCGCCGCGATCGAA AGCTGGCCGCCGCGATCGAA ACTGGCCGCGCGCCATCGAA ATCTGGCCGCCGCGCATCGAA AATTGGCCGCCGCGCATCGAA AATTGGCCGCCGCGCATCGAA AATTGGCCGCCGCGCATCGAA AATTGGCCGCCGCGCATCGAA AATTGGCCGCCGCGCATCGAA AATTGGCCGCCGCGCATCGAA AATTGGCCGCCGCGATCGAA AATTGGCCGCCGCGATCGAA AATTGGCCGCCGCGATCGAA AATTGGCCGCCGCCATCGAA AATTGGCCGCCCGCGATCGAA AACTGGCCGCCGCCATCGAA AACTGGCCGCCGCCATCGAA AACTGGCCGCCGCCATCGAA AACTGGCCGCCGCCATCGAA AACTGGCCGCCGCCATCGAA AACTGGCCGCCGCCATCGAA AACTGGCCGCCGCCATCGAA AACTGGCCGCCGCCATCGAA AACTGGCCGCCGCCATCGAA AACTGGCCGCCGCCATCGAA AACTGGCCGCCGCCATCGAA AACTGGCCGCCGCCATCGAA AACTGGCCGCCGCCATCGAA AACTGGCCGCCGCCGCATCGAA AACTGGCCGCCGCCGCATCGAA AACTGGCCGCCGCCGCATCGAA ACTCGCCCCCCGCCATCGAA ACTCGCCCCCCGCCATCGAA ACTCGCCCCCCGCCATCGAA ACTCGCCCCCCCGCCATCGAA ACTCGCCCCCCCGCATCGAA ACTCGCCCCCCCGCATCGAA ACTCGCCCCCCCCGCATCGAA ACTCGCCCCCCCGCATCGAA ACTCGCCCCCCCCCC	GTTCCACCTI GTTCCACCT GTTCCAACTT GTTCAACTT GTTCAACTT GTTCAACCTI GACGCGTTC GACGCGTTC GACGCGTTC GACGCGTTC GACGCGTTC GACGCGTTC GACCAGTTC GACCAGTTC GACCAGTTC GACGCCTTC GACGCCTTC GACGCCTTC GACGCGTTC		ACCATTCCATTIGGT ACCATTCCATTIGGT ACCATCCATCTIGGT ATCACACCATCTIGGT ATCACACCATCTIGGT GTTCAGCACATCTIGGT GTTCCGACGCATTG GTTCCGACGCGCAATT GTTCCGACGCGCAATT GTTCCGGCGCGCAATT GTTCCGGCGCCAATT GTTCCGGCGCCAATT GTTCCGGCGCCACATT GTTCCAGCGCCCACATT GTTCCGCGCCGCACATT GTTCCGCGCCGCACTT GTTCCGCGCCCCACTT GTTCCGCGCCCCACTT GTTCCGCGCCCCACTT GTTCCGGCGCCCACTT GTTCCGGCGCCCACTT GTTCCGGCGCCCACTT GTTCCGGCGCCCACTT GTTCCGGCGCCCACTT GTTCCGGCGCCCACTT GTTCCGGCCCCACTT GTTCCGGCCCCACTT GTTCCGGCCCCACTT GTTCCGGCCCCACTT GTTCCGGCCCCACTT GTTCCGGCGCCCACTT GTTCCGGCCCCCCTT	GGAAGAACTTATC GGAAGAACCTGTC GGAAGAACCTGTC GGAAGAACCTGTC GGAAGAACCTGTC GGAAGAACCTGTC GGAAGAACCTGTC GGAAGAACCTGTC GGAGGACCGCCCCC CAGCGCCGCCGCCCC CAGCGCCGCCGCC CAGCGCCGCCGCC CAGCGCCGCCGCC CACGCCGCCGCCCC CACGCCGCCGCCCCC CAGCGCGCCGCCCCC CAGCGCGCCGCCCCC CAGCGCGCCCCCC CAGCGCCCCCCCC	SCCGGACGGCG GCCGAACGGCG CCCCAACGGTG GCCTAACGGTG GCCTAACGGTG GCCTAACGGTG GCCCAACGGTGCA AACGGCTGCA	GGGACAAGCCGACCGGAC GGCACAAGCCCACCGGAG GAGACAAGCCCACCGGAG GGCACAAGCCCACCGGCG GTGACAAGCCCACCGGCG GTGACAAGCCCACCGGCG GTGACAAGCCCACCGGCG GGCTCGGCTGGGCGGT GGGCTCGGCTGGGCGGT GGGCTCGGCTGGGCGGT GGGCTCGGCTGGGCGGT GGGCTCGGGCTGGGCGGT GGGCTCGGGCTGGGCGGT GGGCTCGGGCTGGGCGGT GGGCTCGGGCTGGGCGGT GGGCTCGGCTGGGCGGT GGGCTCGGCTGGGCGGT GGGCTCGGCTGGGCGGT GGGCTCGGCTGGGCGGT GGGCTCGGCTGGGCGGT GGGCTCGGCTGGGCGGT GGGCTCGGCTGGGCGGT GGGCTCGGCTGGGCGGT GGGCTCGGCTGGGCGGT GGGCTCGGCTGGGCGGT GGGCTCGGCTGGGCGGT GGGCTCGGCTGGGCGGT GGGCTCGGCTGGGCGGT GGGCTCGGCTGGGCGGT GGGCTCGGCTGGGCGGT GGGCTCGGCTGGGCGGT GGGCTCGGCTGGGCGGT GGGCTCGGCTGGGCGGT GGGTCCGGCTGGGCGGT GGGTCCGGCTGGGCGGT GGGTCCGGCTGGGCGGT GGGTCCGGCTGGGCGGT GGGTCCGGCTGGGCGGT GGGTCCGGCTGGGCGGT GGGTCCGGCTGGGCGGT GGGTCCGGCTGGCCGCC GGTCCGGCTGGCCGCC GGTCCGGCTGGCCGCC GGTCCGGCTGGCCGCC GGTCCGGCTGGCCGCC GGTCCGGCTGGCCGCC GGTCCGGCTGGCCGCC GGTCCGGCTGGCCGCC GGTCCGGCTGGCCCC GGTCCGGCTGGCCGCC GGTCCGCCGCCCCCCCC
smegmatis szulgai tb xenopi human avium type1 avium type2 avium type3 avium type5 celatum type5 celatum t3 celatum t2 chelonei fortuitum gordonae intra type1 intra type2 intra type3 intra type4 intra type5 leprae malmoense marinum kansasii t1 kansasii t2 paratb phlei M.sp.40407 scrof t1 scrof t2 simiae smegmatis szulgai tb	TTGAACGAGAAGAACCTGG CTGAACGAGAAGAACCTGG CTGAACGAGAAAGAACCTGG CTGAACGACAAAGAACCTGG CTGAACGAAAAGAACCTGG GGCATGACGAAAGCAACTGAG AGCTGCCGCGCGCGATCGAA AGCTGGCCGCCGCGGATCGAA AGCTGGCCGCCGCGGATCGAA AGCTGGCCGCCGCGCATCGAA AGCTGGCCGCCGCGCATCGAA AGCTGGCCGCCGCGCATCGAA ACCTGGCCGCGCGCCATCGAA ATCTGGCCGCCGCGCATCGAA ATCTGGCCGCCGCGCATCGAA ATTGGCCGCCGCGCATCGAA AATTGGCCGCCGCGCATCGAA AATTGGCCGCCGCGCATCGAA AATTGGCCGCCGCGCATCGAA AATTGGCCGCCGCGCATCGAA AATTGGCCGCCGCGCATCGAA AATTGGCCGCCGCGCATCGAA AATTGGCCGCCGCGCATCGAA AATTGGCCGCCGCGCATCGAA AATTGGCCGCCGCGCATCGAA AACTGGCCGCCGCGATCGAA AACTGGCCGCCGCGATCGAA AACTGGCCGCCGCGATCGAA AACTGGCCGCCGCGATCGAA AACTGGCCGCCGCCATCGAA AACTGGCCGCCGCGATCGAA AACTGGCCGCCGCCATCGAA AACTGGCCGCCGCCATCGAA AACTGGCCGCCGCCATCGAA ACTCGCCGCCGCCATCGAA ACTCGCCGCCGCCATCGAA ACTCGCCGCCGCCATCGAA ACTCGCCGCCGCCATCGAA ACTCGCCGCCGCCATCGAA ACTCGCCGCCGCCATCGAA ACTCGCCGCCGCCATCGAA ACTCGCCGCCGCCATCGAA ACTCGCCGCCGCCATCGAA ACTCGCCGCCGCCATCGAA ACTCGCCGCCGCCATCGAA ACTCGCCGCCGCCATCGAA ACTCGCCGCCGCCATCGAA ACTCGCCGCCGCCATCGAA ACTCGCCGCCGCCATCGAA ACTCGCCGCCCCGCATCGAA ACTCGCCGCCCCCGCATCGAA ACTCGCCGCCCCCGCATCGAA ACTCGCCGCCCCCCGCATCGAA ACTCGCCGCCCCCGCATCGAA ACTCGCCGCCCCCCGCATCGAA ACTCGCCGCCCCCGCATCGAA ACTCGCCGCCCCCCGCATCGAA ACTCGCCGCCCCCCCCCC	GTTCCACCTI GTTCCACCTI GTTCAACTT GTTCAACTT GTTCAACTT GTTCAACTT GACGCGTTC GACGCGTTC GACGCGTTC GACGCGTTC GACGCGTTC GACGCGTTC GACCAGTTC GACCAGTTC GACCAGTTC GACGCCTTC GACGCCTTC GACGCCTTC GACGCCTTC GACGCGTTC		ACCATTCCATCTGGT ACCATCCATCTGGT ACCACTCATCTGGT ATCACCCATCTGGT ATCACCCATCTGGT GTTCAGCACCATCTGGT GTTCCGACGCAATT GTTCCGACGCAATT GTTCCGACGCCAATT GTTCCGGCGCGCAATT GTTCCGGCGCGCAATT GTTCCGGCGCGCAATT GTTCCGGCGCCACATT GTTCCCGCGCCCACATT GTTCCCAGCCCCACATT GTTCCCGCGCGCACTT GTTCCGCGCGCCACTT GTTCCGCGCCCCACTT GTTCCGCGCCCCACTT GTTCCGCGCCCCACTT GTTCCGCGCCCCACTT GTTCCGCGCCCCACTT GTTCCGCGCCCCACTT GTTCCGCGCCCCACTT GTTCCGCGCCCCACTT GTTCCGCGCCCCACTT GTTCCGCGCCCCCCCT GTTCCGCGCCCCCCCCT GTTCCGCGCCCCCCCT GTTCCGCGCCCCCCTCATT GTTCCGCGCCCCCCCCCC	GGAAGAACTTGTC GGAAGAACCTGTC GGAAGAACCTGTC GGAAGAACCTGTC GGAAGAACCTGTC GGAAGAACCTGTC GGAAGAACCTGTC GGAGGACCCCCCC CAGCGCCGCCGCCCC CAGCGCCGCCGCCCC CAGCGCCGCCGCC CAGCGCCGCCGCC CACCGCCGCCGCC CACCGCCGCCGCC CACCGCCGCCCCC CAGCGCGGCCGCC CAGCGCGGCCCCC CAGCGCGGCCGCC CAGCGCGGCCCCC CAGCGCGGCCCCC CAGCGCGCCCCCC CAGCGCGCCCCCC CAGCGCGCCCCCC CAGCGCGCCCCCC CACGCCGCCCCCC CACGCCGCCCCCCC CACGCCGCCCCCCC CACGCCGCCCCCCCC	CCCGAACGGCG CCCGAACGGCG CCCCAACGGTG GCCTAACGGTG GCCTAACGGTG GCCCAACGGTG GCCCAACGGTG CCCCAACGGTG AACGGCTGCA	GGACAAGCCGACCGAC GCGACAAGCCCACCGGAG GAGACAAGCCCACCGGAG GCGACAAGCCCACCGGCG GTGACAAGCCCACCGGCG GTGACAAGCCCACCGGCG GTGACAAGCCCACCGGCG GGGCTCGGCTGGGCGGT GGGCTCCGGCTGGGCGGT GGGCTCGGCTGGGCGGT GGGCTCGGCTGGGCGGT GGGCTCGGCTGGGCGGT GGGCTCGGCTGGGCGGT GGGCTCGGCTGGGCGGT GGGCTCGGCTGGGCGGT GGGCTCGGCTGGGCGGT GGGCTCGGCTGGGCGGT GGGCTCGGCTGGGCGGT GGGCTCGGCTGGGCGGT GGGCTCGGCTGGGCGGT GGGCTCGGCTGGGCGGT GGGCTCGGCTGGGCGGT GGGCTCGGCTGGGCGGT GGGCTCGGCTGGGCGGT GGGCTCGGCTGGGCGGT GGGCTCGGCTGGCCGGT GGGCTCGGCTGGCCGGT GGGCTCGGCTGGCCGGT GGGCTCGGCTGGCCGGT GGGCTCGGCTGGCCGGT GGGCTCGGCTGGCCGGT GGGCTCGGCTGGCCGGT GGGCTCGGCTGGCCGGT GGGTCGGCTGGCCGGT GGGTCGGCTGGCCGGT GGGTCGGCTGGCCGGT GGGTCGGCTGGCCGGT GGGTCGGCTGGCCGGT GGGTCGGCTGGCCGGT GGGTCGGCTGGCCGGT GGGTCGGCTGGCCGCT GGGTCGGCTGGCCGCT GGGTCGGCTGGCCGCC GGGTCGGCTGGCCGCC GGGTCGGCTGGCCGCC GGGTCGGCTGGCCGCC GGGTCGGCTGGCCGCC GGGTCGGCTGGCCGCCCC GGGTCGGCTGGCCGCCCC GGGTCGGCTGGCCGCCCCCCCC

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

	493	
human	GCTTGGTTTCAATAAGGAACGGGGACACTTACAA	ATTGCTGCTTGTCCAAATC-AGGATCCACTGCAAGGAACAAC-AGGCCTTATTCCACTGCTGGG
avium typel	GCTGGGCTATGACACCCTGGGCAGCCG	GTTGCTGACCTTCCAGCTCTACGACCAGCAGGCCAACGTCCCGCTGGGCATCATCCCGCTGCTGCA
avium type2	GCTGGGCTATGACACCCTGGACAGCCG	GTTGCTGACCTTCCAGCTCTACGACCAGCAGGCCAACGTCCCGCTGGGCATCATCCCGCTGCCA
avium type3	GCTGGGCTATGACACCCTGGGCAGCCG	GTTGCTGACCTTCCACCTTCCACCACCCCACCCCCCCCCC
avium typed	CCTCCCTTATCACACCCTCCCCCACCCC	
avium types	CCTCCCTTATCACAC CCTCCCCACCCC	
avium types		GITECTEATECTACTIC IACEACCACACAGECAAGECTACETECTECTECTECTECTECTECTECT
celatum t3		Generican Chickson Construction
celatum t2		Genericanenteereeneeree
chelonei	ACTCGGTTACGACAGCCTGGGGCAGAA	GCTGCTGACCTTCCAGCTGTACGACCAGCAGGCCAATGTCCCGCTGGGCATCATCCCGCTGCTCCA
fortuitum	GCTCGGCTACGACAGCCTGGGCGATCG	GCTGCTGACCTTCCAGCTCTACGACCAGCAGGCCAACGTGCCGCTCGGCATCATCCCGCTGCTCCA
gordonae	GCTCGGCTACGACACTCTGGGCGGCCG	GTTGCTCACCTTCCAGCTCTACGACCAGCAGGCCAATGTCCCGCTCGGTGTCATTCCGCTGTTGCA
intra typel	GCTGGGCTACGACACCCTCGGCAACCG	GCTGCTGACCTTCCAGCTCTACGACCAGCAGGCCAACGTGCCGCTGGGCATCATTCCGCTGCTGCA
intra type2	GTTGGGCTACGACACCCTCGGCAGCCG	GCTGCTGACCTTCCAGCTCTACGACCAGCAGGCCAACGTGCCGCTGGGCATCATTCCGCTGCTGCA
intra type3	GCTGGGCTACGACACCCTCGGCAACCG	GCTGCTGACCTTCCAGCTCTACGACCAGCAGGCCAACGTGCCGCTGGGCATCATTCCGCTGCTGCA
intra type4	CCTGGGCTACGACACCCTCGGCAACCG	GCTGCTGACCTTCCAGCTGTACGACCAGCAGGCCAACGTTCCGCCATCATCCCCTTGCTGCA
intra type5	CCTGGGTTACGACACCCTGGGCAGCCG	GCTGCTGACCTTCCAGCTCTACGACCAGCAGGCCAACGTTCCGCTTGGCATCATCCCCCTTGCTGCA
leprae	ACTCGGCTACGACACGCTCGGCAACAA	GCTGCTTACATTCCAGCTGTATGACCAACAGGCCAACGTCTCACTCGGCATCATTCCCTTGTTGCA
malmoense	CCTCCCCTATCACTCGCTCCCCCCACAA	GCTGCTGACGTTCCAGCTCTACGACCAACGCCAACGTCCCCTCCGCATCATTCCCCTCCA
marinum	CCTCCCCTCCCACACCCTCCCCCAACAA	
kengegii ti	COTCCCCTCCCACAC COTCCCCCA ACAA	
Kansasii tz	GUTTGGUTGGGACACTUTUGGCAACAA	GCTCCTGATATTCCAGGTCTACGACCACCAGACGAACTTTCCGCTCGGAATCATTCCGTTACTGCT
parato	GCTGGGTTATGACACCGTGGGCAGCCG	GTTGCTGACCTTCCAGCTCTACGACCAGCAGGCCAACGTCCCGCTGGGCATCATCCCGCTGCTGCA
phlei	GTTGGGCTACGACACCCTCGGTGACCG	GCTGCTGACCTTCCAGTTGTACGACCAGCAAGCAAATGTCCCACTGGGGATCATCCCGCTGTTGCT
M.sp.40407	ACTCGGCTGGGACAGCCTCGGCGAGAA	GTTGCTGGTGTTCCAGGTGTACGACCACCAGACGAACTTCCCGGCTCGGCATCGTCCCGTTGCTGGT
scrof tl	GCTGGGCTATGACACGCTCGGCAGCAG	GCTGCTCACCTTCCAGCTTTACGACCAGCAGGCCAACGTCCCGCTCGGCATCATTCCGCTGCTGCA
scrof t2	GCTGGGCTACGACACCCTCGGCAGCAG	GCTGCTCACCTTCCAGCTTTACGACCAGCAGGCGAACGTCCCCCTCGGCATCATTCCGCTGCTGCA
simiae	ACTCGGCTACGACACCCTCGGCGACCG	ACTGCTGACCTTCCAGCTTTACGACCAGCAGGCCAACGTCCCGCTGGGCATCATCCCCGCTGCTGCA
smegmatis	GCTCGGATACGACAGCCTCGGTGGCCG	TCTGCTGACCTTCCAGCTCTACGACCAGCCAGCCAACGTGCCGCTCGGCATCATCCCCGCTGCTCCA
szulgai	ACTGGGATGGGACACACTGGGTAACAA	GCTGCTGATCTTCCAGGTCTACGACCACCAGACCAACTTCCCCCTCGGCATCGTTCCGTTACTGCT
+ h		
	ACTGGGCTGGGACACACTCGGCAACAA	GCTGCTGATATTCC\CCTTTACCACCACCACACCACCTCCCCCTACCCATTCTCCCCCTCCT
venoni	ACTGGGCTGGGACACACTCGGCAACAA ACTCGCTGGGACACCCTGGGTGGCAA	GCTGCTGATATTCCAGGTTTACGACCACCAGACGAACTTCCCGCTAGGCATTGTTCCGCTGCTGCT GCTCCTGGTGTTCCAGGTCTACGACCACCAGTCCAACTTCCCCGCTCGCGCATCGTCCCCCCTGCTGGT
xenopi	ACTGGGCTGGGACACACTCGGCAACAA ACTCGGCTGGGACAGCCTGGGTGGCAA 5 03	GCTGCTGATATTCCAGGTTTACGACCACCAGACGAACTTCCCGCTAGGCATTGTTCCGCTGCTGCT GCTCCTGGTGTTCCAGGTCTACGACCACCAGTCCAACTTCCCGCTCGGGATCGTCCCCCTGCTGGT
xenopi	ACTGGGCTGGGACACACTCGGCAACAA ACTCGGCTGGGACAGCCTGGGTGGCAA 593	GCTGCTGATATTCCAGGTTTACGACCACCAGACGAACTTCCCGCTAGGCATTGTTCCGCTGCTGCT GCTCCTGGTGTTCCAGGTCTACGACCACCAGTCCAACTTCCCGCTCGGGATCGTCCCCCTGCTGGT
kenopi human	ACTEGGETEGGACACACTEGGEAACAA ACTEGGETGGGACAGCCTGGGTGGCAA 593 GATTGATGTGTGGGAGCACGC CCTCGAATGTGCGACGACGACGC	GCTGCTGATATTCCAGGTTTACGACCACCAGACGAACTTCCCGCTAGGCATTGTTCCGCTGCTGCT GCTCCTGGTGTTCCAGGTCTACGACCACCAGTCCAACTTCCCGCTCGGGATCGTCCCCCTGCTGGT
xenopi human avium typel	ACTEGGETEGGACACACTEGGEAACAA ACTEGGETGGGACAGCETGGGTGGCAA 593 GATTGATGTGTGGGGAGCACGC GGTCGACATGTGGGGAGCACGC GGTCGACATGTGGGAGCACGC	GCTGCTGATATTCCAGGTTTACGACCACCAGACGAACTTCCCCCTAGGCATTGTTCCGCTGCTGCT GCTCCTGGTGTTCCAGGTCTACGACCACCAGTCCAACTTCCCGCTCGGGATCGTCCCCCTGCTGGT
kuman avium type1 avium type2	ACTEGGETEGGACACACTEGGEAACAA ACTEGGETGGGACAGCETGGGTGGCAA 593 GATTGATGTGTGGGGAGCACGC GGTCGACATGTGGGAGCACGC GGTCGACATGTGGGAGCACGC	GCTGCTGATATTCCAGGTTTACGACCACCAGACGAACTTCCCGCTAGGCATTGTTCCGCTGCTGCT GCTCCTGGTGTTCCAGGTCTACGACCACCAGTCCAACTTCCCGCTCGGGATCGTCCCCCTGCTGGT
xenopi human avium typel avium type3 avium type3	ACTEGGETEGGACACACTEGGEAACAA ACTEGGETGGGACAGCCTGGGTGGCAA 593 GATTGATGTGTGGGAGCACGC GGTCGACATGTGGGAGCACGC GGTCGACATGTGGGAGCACGC GGTCGACATGTGGGAGCACGC	GCTGCTGATATTCCAGGTTTACGACCACCAGACGAACTTCCCGCTAGGCATTGTTCCGCTGCTGCT GCTCCTGGTGTTCCAGGTCTACGACCACCAGTCCAACTTCCCGCTCGGGATCGTCCCCCTGCTGGT
xenopi human avium type1 avium type2 avium type3 avium type4	ACTEGGETEGGACACACTEGGEAACAA ACTEGGETEGGACAGCETGGGTGGCAA 593 GATTGATGTGTGGGAGCACGC GGTCGACATGTGGGAGCACGC GGTCGACATGTGGGAGCACGC GGTCGACATGTGGGAGCACGC GGTCGACATGTGGGAGCACGC	GCTGCTGATATTCCAGGTTTACGACCACCAGACGAACTTCCCGCTAGGCATTGTTCCGCTGCTGCT GCTCCTGGTGTTCCAGGTCTACGACCACCAGTCCAACTTCCCGCTCGGGATCGTCCCCCTGCTGGT
kuman avium typel avium type2 avium type3 avium type4 avium type5	ACTEGGETEGGACACACTEGGEAACAA ACTEGGETEGGACAGCETEGGTGGCAA 593 GATTGATGTGTGGGGAGCACGC GGTCGACATGTGGGAGCACGC GGTCGACATGTGGGAGCACGC GGTCGACATGTGGGAGCACGC GGTCGACATGTGGGAGCACGC GGTCGACATGTGGGAGCACGC	GCTGCTGATATTCCAGGTTTACGACCACCAGACGAACTTCCCGCTAGGCATTGTTCCGCTGCTGCT GCTCCTGGTGTTCCAGGTCTACGACCACCAGTCCAACTTCCCGCTCGGGATCGTCCCCCTGCTGGT
kenopi human avium type1 avium type2 avium type3 avium type5 celatum t3	ACTEGGETEGGACACACTEGGEAACAA ACTEGGETGGGACAGCCTGGGTGGCAA 593 GATTGATGTGTGGGAGGACGCC GGTCGACATGTGGGAGGACGCC GGTCGACATGTGGGAGGACGCC GGTCGACATGTGGGAGGACGCC GGTCGACATGTGGGAGCACGC CCTCGACATGTGGGAGCACGC	GCTGCTGATATTCCAGGTTTACGACCACCAGACGAACTTCCCGCTAGGCATTGTTCCGCTGCTGCT GCTCCTGGTGTTCCAGGTCTACGACCACCAGTCCAACTTCCCGCTCGGGATCGTCCCCCTGCTGGT
kenopi human avium type1 avium type2 avium type3 avium type3 avium type5 celatum type5 celatum t2	ACTEGGETEGGACACACTEGGEAACAA ACTEGGETEGGACAGCCTGGGTGGCAA 593 GATTGATGTGTGGGAGGACGC GGTCGACATGTGGGAGGACGACGC GGTCGACATGTGGGAGGACGACGC GGTCGACATGTGGGAGGACGACGC GGTCGACATGTGGGAGCACGC CCTCGACATGTGGGAGCACGC CCTCGACATGTGGGAGCACGC	GCTGCTGATATTCCAGGTTTACGACCACCAGACGAACTTCCCGCTAGGCATTGTTCCGCTGCTGCT GCTCCTGGTGTTCCAGGTCTACGACCACCAGTCCAACTTCCCGCTCGGGATCGTCCCCCTGCTGGT
kenopi human avium type1 avium type2 avium type3 avium type4 avium type5 celatum t3 celatum t2 chelonei	ACTEGGCTEGGACACACTEGGCAACAA ACTEGGCTGGGACAGCCTGGGTGGCAA 593 GATTGATGATGTGGGAGCACGC GGTCGACATGTGGGAGCACGC GGTCGACATGTGGGAGCACGC GGTCGACATGTGGGAGCACGC GGTCGACATGTGGGAGCACGC CCTCGACATGTGGGAGCACGC CCTCGACATGTGGGAGCACGC GGTCGACATGTGGGAGCACGC	GCTGCTGATATTCCAGGTTTACGACCACCAGACGAACTTCCCGCTAGGCATTGTTCCGCTGGTGCT GCTCCTGGTGTTCCAGGTCTACGACCACCAGTCCAACTTCCCGCTCGGGATCGTCCCCCTGCTGGT
kenopi human avium type1 avium type2 avium type3 avium type4 avium type5 celatum t3 celatum t2 chelonei fortuitum	ACTEGGETEGGACACACTEGGEAACAA ACTEGGETGGGACAGCCTGGETGGCAA 593 GATTGATGTGTGGGAGGACGCG GGTCGACATGTGGGAGCACGC GGTCGACATGTGGGAGCACGC GGTCGACATGTGGGAGCACGC GGTCGACATGTGGGAGCACGC CCTCGACATGTGGGAGCACGC GGTCGACATGTGGGAGCACGC GGTCGACATGTGGGAGCACGC GGTCGACATGTGGGAGCACGC GGTCGACATGTGGGAGCACGC	GCTGCTGATATTCCAGGTTTACGACCACCAGACGAACTTCCCGCTAGGCATTGTTCCGCTGCTGCT GCTCCTGGTGTTCCAGGTCTACGACCACCAGTCCAACTTCCCGCTCGGGATCGTCCCCCTGCTGGT
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kenopi human avium type1 avium type2 avium type3 avium type5 celatum t3 celatum t2 chelone1 fortuitum gordonae intra t1 intra t2 intra t3	ACTIGGCTIGGGACACACTIGGCAACAA ACTIGGCTIGGGACACACTIGGGTGACAA S533 GATTGATGTGTGGGAGGACGACGC GGTCGACATGTGGGAGGACGACGC GGTCGACATGTGGGAGGACGACGC GGTCGACATGTGGGAGGACGACGC CCTCGACATGTGGGAGGACGACGC GGTCGACATGTGGGAGCACGC GGTCGACATGTGGGAGCACGC GGTCGACATGTGGGAGCACGC GGTCGACATGTGGGAGCACGC GGTCGACATGTGGGAGCACGC GGTCGACATGTGGGAGCACGC GGTCGACATGTGGGAGCACGC GGTCGACATGTGGGAGCACGC	GCTGCTGATATTCCAGGTTTACGACCACCAGACGAACTTCCCGCTAGGCATTGTTCCGCTGGTGCT GCTCCTGGTGTTCCAGGTCTACGACCACCAGTCCAACTTCCCGCTCGGGATCGTCCCCCTGCTGGT
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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 schimoidei 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

GCTCGACATGTGGGAACACGC

GCTCGACATGTGGGAACACGC GCTCGACATGTGGGA--ACGC

szulgai

xenopi

tb

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 chelonei), 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 SOD^{13} 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 $(23\cdot3(1\cdot3)\%)$ 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 $(1 \cdot 1)$ (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 intraspecies 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 speciesspecific 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 Mavium 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

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

										L CC	estrict	ion fra	gment	size (b	(d									
Taxon	70	11	74	77	83	84	87	06	6 3	. 86	104	131 1	34 1:	38 1,	44 14	15 14	7 15	6 16	0 167	175	179	191	224	227
M avium type 1–5 & M paratuberculosis																								
M celatum type 1 & 3 M szulgai																								
M celatum type 2																								
M tuberculosis																								
M intracellulare type 1,2 & 3																								
M intracellulare type 4														-							 			
M intracellulare type 5																			-					
M scrofulaceum type 1																								
M scrofulaceum type 2																		- 23. -						
M gordonae																								
M chelonae																								
M malmoense																								
M marinum																								
M kansasii type 1 & 2																								
M phlei																						1.100		
M sp. 40407/94																								
M simiae									1272.5	(1454) (1454)														
M fortuitum																								
M smegmatis																								
M xenopi																								

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

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 Mtuberculosis. 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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