Mycobacterium parakoreense sp. nov., a slowly growing non-chromogenic species related to Mycobacterium koreense, isolated from a human clinical specimen

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A previously undescribed, slowly growing, non-chromogenic Mycobacterium strain (299T) was isolated from the sputum sample of a patient with a symptomatic pulmonary infection. Phenotypically, strain 299T was generally similar to Mycobacterium koreense DSM 45576T and Mycobacterium triviale ATCC 23292T. The 16S rRNA gene sequence of strain 299T was similar to that of M. koreense DSM 45576T (GenBank accession no. AY734996, 99.5 % similarity); however, it differed substantially from that of M. triviale ATCC 23292T (X88924, 98.2 %). Phylogenetic analysis based on 16S rRNA gene sequences showed that strain 299T clustered together with M. koreense DSM 45576T and M. triviale ATCC 23292T, supported by high bootstrapping values (99 %). Unique mycolic acid profiles and phylogenetic analysis based on two different chronometer molecules, the hsp65 and rpoB genes, strongly supported the taxonomic status of this strain as representing a distinct species. These data support the conclusion that strain 299T represents a novel mycobacterial species, for which the name Mycobacterium parakoreense sp. nov. is proposed. The type strain is 299T (=DSM 45575T=KCTC 19818T).

Together with conventional biochemical tests, several molecular analyses have been applied to differentiate and identify Mycobacterium species. For example, PCR-related methods based on housekeeping genes such as the 16S rRNA gene (Rogall et al., 1990; Springer et al., 1996), RNA polymerase β-subunit (rpoB) gene (Adékambi et al., 2003; Kim et al., 1999) and heat-shock protein 65 (hsp65) gene (Kim et al., 2005, 2006) have been used for mycobacterial species identification.

Abbreviations: EMB, ethambutol; PNB, p-nitrobenzoate; TCH, thio-phene-2-carboxylic acid hydrazide.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA, hsp65 and rpoB gene sequences of strain 299T are JF271823, JF271824 and JF271825, respectively.

A supplementary figure and three supplementary tables are available with the online version of this paper.

Mycobacterium triviale, a non-chromogenic slow-growing Mycobacterium species, is an independent taxonomic entity distinct from the Mycobacterium terrae subclade. M. triviale is genetically distantly related to two members of the M. terrae clade, M. terrae and Mycobacterium nonchromogenicum (Magee & Ward, 2011). Phylogenetic analysis based on 16S rRNA gene sequences supports the genetic disparity of M. triviale from them. Recently, we have introduced a novel species, Mycobacterium koreense, which is phylogenetically closely related to M. triviale (Kim et al., 2012).

In the present study, we describe a novel clinical isolate closely related to M. koreense based on data from a polyphasic taxonomic approach via a combination of biochemical tests and molecular analyses targeting 16S rRNA (Rogall et al., 1990; Springer et al., 1996), hsp65 (Kim et al., 2005, 2006) and rpoB (Kim et al., 1999) gene sequences.
The strain used in this study (299T) was a clinical isolate from the sputum sample of a patient with a symptomatic pulmonary infection. It was submitted by the mycobacteriology laboratory of the Korean Institute of Tuberculosis during 2001. To isolate mycobacteria from the sputum specimen, the sputa was treated with 1% NaOH to liquefy it and to eliminate other contaminants. The treated sample was sedimented by centrifugation (at 13,000 g for 15 min), and resuspended in a phosphate buffer (pH 6.8) to culture in 7H9 broth with ADC (Albumin Dextrose Catalase) and a 7H10 agar plate with OADC (Oleic Albumin Dextrose Catalase) for about four or more weeks. The strain was stored at −70°C in the deep-freezer and recovered for further analysis by subculturing from stocks in Middlebrook 7H9 broth media and incubated at 37°C.

The phenotypic characteristics of strain 299T and closely related species M. koreense and M. triviale were analysed by biochemical study and then compared (Table 1). The biochemical traits were investigated as described by Kent & Kubica (1985). Colony morphology, patterns of pigment production in the dark, photo-induction and the ability to grow at different temperatures ranging from 25 to 45°C were examined by cultivation on a 7H10 agar plate with OADC over 6 weeks. Acid-alcohol-fastness was determined by Ziehl–Neelsen and auramine O staining. Biochemical tests including niacin accumulation, nitrate reductase, arylsulfatase on days 3 and 14, heat-stable catalase (pH 7, 68°C), tellurite reductase, Tween 80 hydrolysis, urease and pyrazinamidase were investigated. Inhibition tests included tolerance to thiophene-2-carboxylic acid hydrazide (TCH), p-nitrobenzoate (PNB), 5% sodium chloride, ethambutol (EMB) and picric acid and ability to grow on MacConkey agar without crystal violet. In addition, antimicrobial susceptibility was determined by the agar proportion method on 7H10 medium (Kent & Kubica, 1985). The production of alkaline phosphatase, esterase (C4), esterase lipase (C8), lipase (C14), leucine arylamidase, valine arylamidase and cystine arylamidase was also analysed with the API ZYM kit (bioMérieux) following the recommendations of the manufacturer.

Microscopic examination showed generally rod-shaped acid-fast bacilli, with frequent curved shapes. Spores and filaments were not present. The optimal growth temperature was 37°C. There was no growth at either 25 or 45°C. On Middlebrook 7H10 agar medium, mature colonies developed in about four or more weeks. This organism grew more slowly than M. koreense and M. triviale, which generally developed colonies in about 7–10 days. Colonies grown on Middlebrook 7H10 agar medium were usually rough and white- or yellow-pigmented forms under both dark and photo-induced conditions. No growth was observed with 500 mg PNB or 5 mg picric acid ml⁻¹ added to the MacConkey agar medium. However, strain 299T was tolerant of 5% NaCl, EMB at 5 mg ml⁻¹ and TCH at 10 mg ml⁻¹. The strain was negative for arylsulfatase, nisin

### Table 1. Cultural and biochemical characteristics among strain 299T, M. koreense DSM 45576T and M. triviale ATCC 23292T

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Strain 299T</th>
<th>M. koreense DSM 45576T</th>
<th>M. triviale ATCC 23292T</th>
</tr>
</thead>
<tbody>
<tr>
<td>Growth at:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>25°C</td>
<td>−</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>37°C</td>
<td>++</td>
<td>++</td>
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<tr>
<td>45°C</td>
<td>−</td>
<td>−</td>
<td>−</td>
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<tr>
<td>Growth detectable after:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;7 days</td>
<td>−</td>
<td>+</td>
<td>±</td>
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<tr>
<td>&gt;7 days</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Morphology*</td>
<td>RWY</td>
<td>RWY</td>
<td>RWY</td>
</tr>
<tr>
<td>Pigmentation†</td>
<td>N</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>Nitrate reductase</td>
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<td>−</td>
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<tr>
<td>Arylsulfatase</td>
<td>14 days</td>
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<td>+</td>
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<tr>
<td>Tween hydrolysis</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>&lt;5 days</td>
<td>±</td>
<td>+</td>
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<td>Growth with:</td>
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<td>10 mg TCH ml⁻¹</td>
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<td>500 mg PNB ml⁻¹</td>
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<td>5% NaCl</td>
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<td>Growth on:</td>
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<tr>
<td>MacConkey agar</td>
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<td>+</td>
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<tr>
<td>Picric acid</td>
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<td>5 mg EMB ml⁻¹</td>
<td>+</td>
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<td>API ZYM results</td>
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<td>Lipase (C14)</td>
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<td>aryiamidase</td>
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<td>MIC (µg ml⁻¹)</td>
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<td>8</td>
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</tr>
<tr>
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<td>4</td>
<td>8</td>
<td>2</td>
</tr>
<tr>
<td>Sul</td>
<td>&gt;128</td>
<td>&gt;128</td>
<td>&gt;128</td>
</tr>
<tr>
<td>Tob</td>
<td>0.5</td>
<td>&gt;32</td>
<td>&gt;32</td>
</tr>
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</table>

*R, rough; Y, yellow; W, white.
†N, non-photochromogenic.
‡Ami, amikacin; Cef, cefoxitin; Cip, ciprofloxacin; Cla, clarithromycin; Dox, doxycycline; Imi, imipenem; Mox, moxifloxacin; Rif, rifampicin; Sul, sulfamethoxazole; Tob, tobramycin.
accumulation, urease activity and tellurite reductase, but was positive for nitrate reductase, Tween 80 hydrolysis, heat-stable catalase and pyrazinamidase activity. Generally, strain 299T grew more slowly than M. koreense and M. triviale, and distinct differences were found in the activity of arylsulfatase (in 14 days) and growth patterns on the medium supplemented with PNB (500 mg ml\(^{-1}\)), picric acid (5 mg ml\(^{-1}\)) and EMB (5 mg ml\(^{-1}\)). Lipase and valine arylamidase tests were negative, those for alkaline phosphatase, esterase (C4) and esterase lipase (C8) were positive, and those for leucine arylamidase and cysteine arylamidase were weakly positive. Although strain 299T was generally similar to M. koreense DSM 45576\(^T\) and M. triviale ATCC 23292\(^T\), some unique phenotypic traits – including very slow growth in culture (more than 4 weeks), no arylsulfate activity (in 14 days), no growth in PNB media, MacConkey agar or picric acid media, and growth in EMB media – were observed. Cultural and biochemical characteristics that differentiated strain 299T from M. koreense and M. triviale are shown in Table 1.

Although antibiotic susceptibility tests generally did not provide decisive information regarding mycobacterial taxonomy, it should be noted that strain 299\(^T\) had extremely low MIC values for two types of aminoglycoside drugs, amikacin (4 \(\mu\)g ml\(^{-1}\)) and tobramycin (0.5 \(\mu\)g ml\(^{-1}\)), and for two types of fluoroquinolone drugs, ciprofloxacin (0.25 \(\mu\)g ml\(^{-1}\)) and moxifloxacin (0.125 \(\mu\)g ml\(^{-1}\)), compared with those of M. koreense and M. triviale (Table 1).

HPLC was used to analyse mycolic acid patterns of strain 299\(^T\), M. koreense DSM 45576\(^T\) and M. triviale ATCC 23292\(^T\) as described by Butler et al. (1992) or as described in ‘Division of Tuberculosis Elimination’ (http://www.cdc.gov/nchhstp/Default.htm). For HPLC analysis, M. koreense DSM 45576\(^T\) and M. triviale ATCC 23292\(^T\) were cultured on 7H10 agar plates supplemented by OADC for 2 weeks at 37 °C under aerobic conditions and strain 299\(^T\) was cultured for 6 weeks. Low- and high-molecular-mass standards (Ribi ImmunoChem) were added for peak identification. To identify and quantify mycolic acids and to assign these to Mycobacterium species based on mycolic acid patterns, the Microbial Identification system (MIDI Inc.) was used. Also using the HPLC mycobacterium library (available at http://www.MycobacToscana.it), the mycolic acid patterns obtained were compared with data for previously described species.

The mycolic acid patterns of strain 299\(^T\) showed two significantly separated clusters of peaks after HPLC elution, with the most prominent peaks in the first cluster. The first cluster in the HPLC patterns of strain 299\(^T\) was not observed in those of M. triviale ATCC 23292\(^T\) (Fig. 1). The HPLC patterns of M. koreense DSM 45576\(^T\) showed similar patterns to those of strain 299\(^T\), with two clusters of peaks, but with low intensity of peaks (Kim et al., 2012). Also, in the second cluster, unique peaks were detected in strain 299\(^T\) (retention times of 5.629 and 6.003 min), compared with M. koreense and M. triviale. These unique HPLC profiles distinguished strain 299\(^T\) from M. koreense and M. triviale.

Fatty acid methyl esters were obtained from the biomass of M. koreense DSM 45576\(^T\) and M. triviale ATCC 23292\(^T\) as described by Minninkin (1988). For fatty acid analysis, strain 299\(^T\) and M. triviale ATCC 23292\(^T\) were cultured in 7H9 broth supplemented with ADC for 6 and 2 weeks, respectively, at 37 °C under aerobic conditions. Extracted samples were separated by GC (model 5898A; Hewlett Packard) and analysed by using the Sherlock Microbial ID System (MIS).
Fatty acid analysis showed that the predominant fatty acid of strain 299\textsuperscript{T} was C\textsubscript{16:0} (37.68\%). The fatty acid profile comprised unbranched saturated and unsaturated fatty acid esters with chain lengths of 12, 14, 15, 16, 17, 18 and 20 carbon atoms, and tuberculostearic acid (C\textsubscript{18:0} 10-ME TBSA; 7.02\%) was also detected. Using the M17H10 3.80 database and the MYCO6 method, the strain was identified as belonging to the Mycobacterium avium–intracellulare–scrofulaceum complex (0.285 similarity index). The fatty acid profile of strain 299\textsuperscript{T} is detailed in Table S1 (available in IJSEM Online).

For MALDI-TOF MS, lipids were extracted with CHCl\textsubscript{3}/CH\textsubscript{3}OH (1:1 v/v, adding 0.5\,μl 2,5-dihydroxybenzoic acid) from 30 ml 7H9 broth cultures of strain 299\textsuperscript{T}, M. koreense DSM 45576\textsuperscript{T} and M. triviale ATCC 23292\textsuperscript{T}. MALDI-TOF MS was performed on the extracted samples with a Voyager DE-STR MALDI-TOF instrument (Perseptive Biosystems) equipped with a pulsed nitrogen laser emitting at 337 nm as described by Pérez et al. (2004).

The MALDI-TOF MS profiles of strain 299\textsuperscript{T}, M. koreense DSM 45576\textsuperscript{T} and M. triviale ATCC 23292\textsuperscript{T} were analysed and compared. The profiles of strain 299\textsuperscript{T} and M. koreense showed three distinct clusters of peaks ranging from m/z 1067 to m/z 1259 (1st cluster), m/z 1415 to m/z 1488 (2nd cluster) and m/z 1568 to m/z 1752 (3rd cluster). The profiles of strain 299\textsuperscript{T} showed that the 1st cluster had the highest intensity, whereas for M. koreense the 3rd cluster had the highest intensity. In the case of M. triviale, the profiles showed a distinct cluster of peaks ranging from m/z 1378 to m/z 1507 (2nd cluster) (Fig. S1). All the HPLC fatty acid and MALDI-TOF analyses were performed in duplicate.

For molecular taxonomic study, chromosomal DNA of strain 299\textsuperscript{T} was extracted by the bead beater-phenol extraction method as previously reported (Kim et al., 2005). The purified genomic DNA was used as a template for PCR amplifications of three independent gene targets, the 16S rRNA, hsp65 and rpoB genes. The almost-complete 16S rRNA gene, partial hsp65 gene and partial rpoB gene sequences were amplified as described previously (Kim et al., 1999, 2005; Roth et al., 1998; Springer et al., 1996). PCR products of all target genes were cloned into pCR 2.1-TOPO vector (Invitrogen). Three independent white colonies of strain 299\textsuperscript{T} were chosen and sequenced (Kim et al., 2005). The 16S rRNA gene sequence of strain 299\textsuperscript{T} obtained in this study was compared with the GenBank database using the BLAST program (http://www.ncbi.nlm.nih.gov/blast/).

The 16S rRNA (1404 bp), hsp65 (603 bp) and rpoB (306 bp) gene sequences of strain 299\textsuperscript{T} and other mycobacterial strains, which include a wide range of both slowly and rapidly growing mycobacteria, were aligned by using the multiple alignment algorithm in the MEGALIGN software package as previously described (Kim et al., 1999, 2005; Turenne et al., 2001). Evolutionary distance matrices were generated according to the Jukes–Cantor model (Jukes & Cantor, 1969). Phylogenetic trees were inferred from the three target gene sequences using the neighbour-joining (Saitou & Nei, 1987) and maximum-parsimony (Fitch, 1971) methods in the MEGA version 4.0 software (Kumar et al., 2008). The 16S rRNA gene sequence and hsp65 gene sequence of Tsukamurella paurometabola NCTC 10741\textsuperscript{T} and KCTC 9821\textsuperscript{T} or the rpoB gene sequence of Rhodococcus equi ATCC 10146\textsuperscript{T} were retrieved from GenBank and used as outgroups. In addition, concatenated trees using 16S rRNA, rpoB and hsp65 gene sequences were constructed. Constructed trees and their topologies were evaluated by bootstrap analysis based on 1000 resamplings (Felsenstein, 1985).

The 16S rRNA gene sequence of strain 299\textsuperscript{T} was used for a BLAST search using the GenBank database. The result showed the closest match (99.4\%) to Mycobacterium sp. NLA001000736 (GenBank accession no. HM627011), which was isolated from a sputum sample and recently sequenced from the Netherlands. The 16S rRNA gene sequence of strain 299\textsuperscript{T} differed from Mycobacterium sp. NLA001000736 by two gaps and 8 bp substitutions among 1464 bp. Compared with the 16S rRNA sequence of M. koreense DSM 45576\textsuperscript{T} (GenBank accession no. JF271826), strain 299\textsuperscript{T} differed by two gaps and 9 bp substitutions among 1464 bp. In the hypervariable regions of the 16S rRNA gene sequence, the strain 299\textsuperscript{T} sequence was identical to those of M. koreense DSM 45576\textsuperscript{T} and M. triviale ATCC 23291\textsuperscript{T} but differed from those of the type strains of four M. terrae-related species, namely Mycobacterium arupense, M. nonchromogenicum, M. terrae and the recently described Mycobacterium senense (Fig. 2).

The neighbour-joining tree based on the multiply aligned 16S rRNA gene sequences of strain 299\textsuperscript{T} and other Mycobacterium species indicated a close relationship between strain 299\textsuperscript{T} and two other M. triviale-related strains, M. triviale ATCC 23292\textsuperscript{T} and M. koreense DSM 45576\textsuperscript{T}, within the slowly growing Mycobacterium species. The grouping of strain 299\textsuperscript{T} together with M. triviale ATCC 23292\textsuperscript{T} and M. koreense DSM 45576\textsuperscript{T} was strongly supported by recapitulation in the maximum-parsimony tree and the high bootstrap value (99\%) (Fig. 3a). Compared with the type strains of M. koreense and M. triviale, strain 299\textsuperscript{T} showed 16S rRNA gene sequence similarity values ranging from 98.0\% (M. triviale) to 99.5\% (M. koreense) (Table 2).

Trees based on the 306 bp rpoB gene (Fig. 3b) and 603 bp hsp65 gene sequences (Fig. 3c) also showed that strain 299\textsuperscript{T} is closely related to M. triviale ATCC 23292\textsuperscript{T} and M. koreense DSM 45576\textsuperscript{T}, which is strongly supported by recapitulation in the maximum-parsimony tree (bootstrap values of 74\% in the rpoB tree and 98\% in the hsp65 tree) (Fig. 3b, c). Moreover, the phylogenetic clusters comprising strains 299\textsuperscript{T}, M. triviale ATCC 23292\textsuperscript{T} and M. koreense DSM 45576\textsuperscript{T} were also found in the tree based on the concatenated sequences (2292 or 2293 bp)
of the 16S rRNA, rpoB and hsp65 genes, showing 100 %
bootstrap support (Fig. 3d). Collectively, our phylo-
genetic analysis based on three independent genes indicated
strains 299\textsuperscript{T}, \textit{M. triviale} ATCC 23292\textsuperscript{T} and \textit{M. koreense}
DSM 45576\textsuperscript{T} belong to the same cluster within the \textit{M. triviale}
subclade.

Note that there was incongruence in the topology of the
cluster belonging to the \textit{M. triviale} subclade between the
trees based on the \textit{hsp65} and \textit{rpoB} gene sequences. The \textit{hsp65} tree (but not the \textit{rpoB} tree) showed that the
cluster of \textit{M. triviale} subclade strains was positioned at
the border between rapid- and slow-growing mycobac-
teria, as in the 16S rRNA gene sequence-based tree (Fig.
3a–c). Sequence comparisons between strain 299\textsuperscript{T} and
strains belonging to the \textit{M. terrae} complex showed
sequence similarity values ranging from 89.2 % (\textit{M. nonchromogenicum})
to 94.4 % (\textit{M. koreense}) in the \textit{rpoB}
gene and 87.1 % (\textit{M. arupense}) to 96.7 % (\textit{M. koreense})
in the \textit{hsp65} gene (Tables S2 and S3).

DNA–DNA hybridization was carried out to determine
the DNA relatedness between strain 299\textsuperscript{T} and
\textit{M. koreense} DSM 45576\textsuperscript{T}. Briefly, extracted genomic DNA of the two strains was
blotted onto Hybond-N+ nylon membranes (Amersham
Pharmacia Biotech) in three replicates after denaturation
using NaOH solution and heating at 80 °C. Each DNA
sample (4 μg) was digested using the \textit{Hae}III restriction
enzyme and fragmented DNA was used individually as a
labelled DNA probe for cross-hybridization. Random
primed DNA labelling with digoxigenin (DIG)-dUTP
and detection of hybrids by enzyme immunoassay on
nylon membrane were performed using a DIG High Prime
DNA Labelling kit (Roche Applied Science) according to
the manufacturer’s instructions and standard procedures
(Sambrook & Russell, 2001). The hybridization signals
were captured using a scanner (HP Scanjet 3770) and
analysed using Adobe Photoshop (Ver 7.0). The signal
produced by hybridization of the probe to the homolog-
ous target DNA was taken to be 100 % and signal
intensities by the self-hybridization of the series of
dilutions were used for calculation of levels of DNA
relatedness between strain 299\textsuperscript{T} and \textit{M. koreense}
DSM 45576\textsuperscript{T}. The DNA–DNA hybridization tests were confirmed
by cross-hybridization.

The mean (±SD) DNA–DNA relatedness between strain
299\textsuperscript{T} and the type strain of \textit{M. koreense} DSM 45576\textsuperscript{T} was

<table>
<thead>
<tr>
<th>Strain</th>
<th>Percentage similarity (%)</th>
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<tbody>
<tr>
<td>Strain 299\textsuperscript{T}</td>
<td>–</td>
</tr>
<tr>
<td>\textit{M. koreense} DSM 45576\textsuperscript{T}</td>
<td>–</td>
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<tr>
<td>\textit{M. triviale} ATCC 23292\textsuperscript{T}</td>
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Table 2. 16S rRNA gene sequence similarities among strain 299\textsuperscript{T}, \textit{M. koreense} DSM 45576\textsuperscript{T} and \textit{M. triviale} ATCC 23292\textsuperscript{T}
42.79 ± 6.83, below the 70 % DNA–DNA relatedness which is generally used for species delineation (Rossello-Mora & Amann, 2001).

Taken together, the distinct sequence characteristics of the three independent genes (16S rRNA, rpoB and hsp65) together with unique HPLC profiles of mycolic acids, MALDI-TOF profiles of lipids, and phenotypic traits including very slow growth strongly support the conclusion that strain 299T belongs to a distinct mycobacterial species within the M. triviale subclade, for which the name Mycobacterium parakoreense sp. nov. is proposed.
Description of *Mycobacterium parakoreense* sp. nov.

*Mycobacterium parakoreense* [pa.ra.ko.re.en’se. Gr. prep. *para* beside, alongside of, near, like; N.L. neut. adj. *koreense* of or belonging to Korea, and also a bacterial specific epithet; N.L. neut. adj. *parakoreense* near (*Mycobacterium* *koreense*).

Cells are acid–alcohol-fast bacilli. Morphology is generally rod-shaped, with frequent curved shapes. Spores and filaments are not present. The optimal growth temperature is 37 °C. There is no growth at either 25 or 45 °C. On Middlebrook 7H10 agar medium, mature colonies develop in about four or more weeks. Colonies grown on Middlebrook 7H10 agar medium are usually rough, white- or yellow-pigmented forms under both dark and photo-induced conditions. No growth is observed on MacConkey agar medium, with 500 mg PNB or 5 mg picric acid ml⁻¹. Tolerates 5% NaCl, EMB at 5 mg ml⁻¹ and TCH at...
10 mg ml$^{-1}$. Negative for arylsulfatase, niacin accumulation, urease activity and tellurite reductase. Positive for nitrate reductase, Tween 80 hydrolysis, heat-stable catalase and pyrazinamidase activity. Has extremely low MIC values for two types of aminoglycoside drugs, amikacin (4 μg ml$^{-1}$) and tobramycin (0.5 μg ml$^{-1}$), and for two types of fluoroquinolone drugs, ciprofloxacin (0.25 μg ml$^{-1}$) and moxifloxacin (0.125 μg ml$^{-1}$). HPLC analysis shows unique mycolic acid and MALDI-TOF profiles distinct from those of \textit{M. koreense} and \textit{M. triviale}. Genetically, the organism has unique 16S rRNA, hsp65 and rpoB gene sequences. Phylogenetic analyses show that it belongs to the slowly growing mycobacteria and is closely related to \textit{M. koreense}. However, the DNA–DNA relatedness between the type strain and that of \textit{M. koreense} DSM 45576$^T$ is below the 70 % which is generally accepted for species delineation.

The type strain, 299$^T$ (= DSM 45575$^T$ = KCTC 19818$^T$), was isolated from human sputum in Seoul, Korea.

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**References**


