Novel mycolic acid-containing bacteria in the family Segniliparaceae fam. nov., including the genus Segniliparus gen. nov., with descriptions of Segniliparus rotundus sp. nov. and Segniliparus rugosus sp. nov.

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Four strains of novel, rapidly growing, acid–alcohol-fast-staining bacteria were characterized with a polyphasic approach. Isolates were received by the Centers for Disease Control and Prevention from domestic health department laboratories for reference testing as unidentifiable, clinical mycobacteria. Bacteria were rod-shaped and produced non-pigmented (white to beige), non-photochromogenic, smooth or wrinkled-rough colonies on Middlebrook 7H10 and 7H11 media at 33 °C. The smooth and wrinkled colony forms were representative of two species with 68 ± 0 and 72 ± 0 mol% DNA G+C content. The cell wall contained meso-diaminopimelic acid and mycolic acids. Species were characterized by cellular fatty acids of C10:0, C14:0, C16:1ω9t, C16:0, C18:1ω9c and 10-methyl C18:0 (tuberculostearic acid). HPLC analysis of mycolic acids produced a novel late-emerging, genus-specific mycolate pattern. TLC analysis demonstrated a novel α+ mycolate. Species were 98-99% similar by comparison of 16S rRNA gene sequences; however, the DNA–DNA association was <28%. Phylogenetic analysis of 16S rRNA gene sequences demonstrated an association with Rhodococcus equi, although a DNA–DNA relatedness value of 2% did not support a close relationship. PCR analysis of a proposed, selected actinomycete-specific 439 bp fragment of the 65 kDa heat-shock protein was negative for three of the four isolates. The creation of Segniliparaceae fam. nov. is proposed to encompass the genus Segniliparus gen. nov., including two novel species, the type species Segniliparus rotundus sp. nov. and Segniliparus rugosus sp. nov., with the respective type strains CDC 1076T (ATCC BAA-972T = CIP 108378T) and CDC 945T (ATCC BAA-974T = CIP 108380T).

INTRODUCTION

Unique HPLC mycolic acid patterns were discovered during routine identification testing of isolates with the standard method for identification of mycobacteria (Butler et al., 1996). The HPLC patterns were developed from clinical isolates submitted from public health laboratories to the Diagnostic Mycobacteriology Section (DMS) at the US Centers for Disease Control and Prevention (CDC) as...
difficult-to-identify mycobacteria, suspected of involvement in respiratory mycobacteriosis. Comparison of the HPLC patterns with standard reference chromatograms of known mycolic-acid-containing bacteria maintained at the DMS showed they were of ‘no common pattern’ (NCP) and assigned the DMS series NCP 215 (Floyd et al., 2000). A retrospective search of HPLC mycolic acid patterns generated by the DMS from 1994 to the present day revealed that similar patterns had been detected on seven occasions. Four viable cultures, representative of the HPLC patterns, were recovered for comparative studies. 16S rRNA gene sequence patterns had been detected on seven occasions. Four numbers representing four of the isolates were available for additional comparative studies. Information of case histories was not expected, the isolates were recovered in Middlebrook 7H9 broth and incubated at 37°C according to the manufacturer’s instructions.

Susceptibility testing was performed using serial twofold broth microdilution for amikacin (1–128 μg ml⁻¹), cefoxitin (2–256 μg ml⁻¹), ciprofloxacin (0–12–16 μg ml⁻¹), clarithromycin (0–03–64 μg ml⁻¹), doxycycline (0–25–128 μg ml⁻¹), imipenem (1–64 μg ml⁻¹), sulfamethoxazole (1–64 μg ml⁻¹) and tobramycin (1–16 μg ml⁻¹) and interpreted with the criteria used for rapidly growing mycobacteria, nocardiae and other aerobic actinomycetes by standard procedures used by DMS (NCCLS, 2003).

**Chemotaxonomic analysis.** Diaminopimelic acid was analysed from acidic hydrolysates with TLC on aluminium high-performance cellulose sheets (Merck) in methanol/distilled water/6 M HCl/pyridine (80: 26: 4: 10, by vol.) as described previously for aerobic actinomycetes (Staneck & Roberts, 1974).

High-molecular-mass, alkyl-branched, 3-hydroxy mycolic acids as p-bromophenacyl esters (MAPBE) were examined with HPLC for genus and species characteristics as described previously (Butler et al., 1996; Butler & Guthertz, 2001). In addition, chemical functional groups of mycolic acid methyl esters (MAMES), derivatized with freshly prepared N-methyl-N-nitroso-p-toluenesulphonamide, were detected as described previously (Lévy-Frebault et al., 1986). Mycolic acids extracted by alkaline and acid methanolation were analysed with a two-dimensional TLC (2D-TLC) system involving a triple development with petroleum ether/acetone (95:5, v/v), followed with a single development, at 90° to the first direction, in toluene/acetone (97:3, v/v), as described previously (Minnikin et al., 1975, 1985b; Dobson et al., 1985). MAMES were also examined by one-dimensional TLC (1D-TLC) with the solvent systems petroleum ether/diethyl ether (85:15, v/v), petroleum ether/ethyl acetate (96:4, v/v) and with petroleum ether/diethyl ether (90:10, v/v) compared with dichloromethane (Minnikin et al., 1975). Components were sprayed with phosphomolybdic acid (Alltech Associates) and charred at 110°C for 15 min for visualization of mycolic acid spots. Chemical functional groups of mycolic acids were designated as: α-mycocenes (I), α’-mycolates (II), methoxymycolates (III) and ketomycolates (IV), as described previously (Lévy-Frebault et al., 1986). The designation, α’-mycolates (I) was used to indicate unsaturated mycolates with carbon chains longer than mycobacteria α-mycocenes.

**Heat-shock protein (HSP) and cholesterol oxidase (COX) gene analysis.** PCR amplification of a 439 bp fragment of the 65 kDa HSP gene with forward and reverse primers TB11 and TB12 and restriction analysis with *MspI* and *HinfI* for differentiation of selected actinomycetes was performed as described previously (Steingrube et al., 1997). The specificity of the reaction was challenged by reducing the annealing temperature to 55°C. In addition, PCR amplification of a 959 bp fragment of the *choE* gene encoding the suggested *R. equi* virulence factor, COX, was performed with *R. equi*-specific forward and reverse prime, COX-F and COX-R, as described by Ladrón et al. (2003). Nucleotide confirmation of amplicons was done by PCR and sequencing using the PCR amplification primers with HotStarTaq Master Mix kit according to the manufacturer’s instructions (Qiagen). Sequencing was performed

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**METHODS**

**Bacterial strains, media and morphology.** The DMS chemically analysed the mycobacteria from strains upon receipt from original Löwenstein–Jensen (LJ) slants for reference identification testing using HPLC (Butler & Guthertz, 2001). Colony morphology and pigmentation were recorded upon receipt. The cultures, sources and state of origin, if given, were documented as follows: CDC 1178, FL; CDC 544 (a duplicate culture of CDC 563), sputum, CA; CDC 434, LA; CDC 945, sputum, AL; CDC 1076, sputum, TN; CDC 413, nasal region, MO; CDC 606, bronchial wash, PA. Only the underlined mycolic-acid-containing bacteria using a polyphasic taxonomic approach.

**Biochemical and antimicrobial susceptibility tests.** Tests used for NTM were growth rate, pigmentation, acid-fastness, 3- and 14-day arylsulphatase activity, niacin production, 7- and 14-day sodium chloride tolerance on LJ and American Trudeau Society (ATS) media, semi-quantitative catalase detection, growth on MacConkey agar without crystal violet, iron uptake, nitrate reduction, tellurite reduction, Tween opacity, Tween hydrolysis and urea hydrolysis. Tests were performed and interpreted as described by Kent & Kubic (1985). General tests for actinomycetes included growth with lysozyme in 21 days, growth at 25, 35 and 45°C on HI agar and tryptone glucose yeast extract (TGY) agar. Hydrolysis of acetamide, adenosine, casein, citrate, ascellin, hypoxanthine, tyrosine and xanthine and utilization/acid production with adonitol, L-arabinose, cellobiose, dulcitol, i-erythritol, D-fructose, D-galactose, D-glucose, glycerol, i-myo-inositol, lactose, maltose, mannitol, mannosone, melibiose, raffinose, L-rhamnose, sodium citrate, salicin, sucrose, α-sorbitol and trehalose were determined by the methods of Berd (1973), Kent & Kubic (1985) and Yassin et al. (1995). In addition, an API CORYNE strip identification system (bioMérieux) was utilized for detection of enzyme activities and carbohydrate utilization at 33°C according to the manufacturer’s instructions.

**CHEMOTAXONOMIC ANALYSIS.** Diaminopimelic acid was analysed from acidic hydrolysates with TLC on aluminium high-performance cellulose sheets (Merck) in methanol/distilled water/6 M HCl/pyridine (80: 26: 4: 10, by vol.) as described previously for aerobic actinomycetes (Staneck & Roberts, 1974).

Cellular fatty acid methyl esters (FAMEs) were analysed by GLC and, when applicable, were confirmed by MS as described previously (Weyant et al., 1996). Initial identification of fatty acids was with commercial software (MIDI). The trans isomer of hexadecenoic acid (C16:1ω9t) acid (reported by MIDI software as summed feature 3, which comprised i-2-OH 15 : 0 and/or 16 : 1o9t) was confirmed by acetylation as described previously (Weyant et al., 1996).

High-molecular-mass, alkyl-branched, 3-hydroxy mycolic acids as p-bromophenacyl esters (MAPBE) were examined with HPLC for genus and species characteristics as described previously (Butler et al., 1996; Butler & Guthertz, 2001). In addition, chemical functional groups of mycolic acid methyl esters (MAMES), derivatized with freshly prepared N-methyl-N-nitroso-p-toluenesulphonamide, were detected as described previously (Lévy-Frebault et al., 1986). Mycolic acids extracted by alkaline and acid methanolation were analysed with a two-dimensional TLC (2D-TLC) system involving a triple development with petroleum ether/acetone (95:5, v/v), followed with a single development, at 90° to the first direction, in toluene/acetone (97:3, v/v), as described previously (Minnikin et al., 1975, 1985b; Dobson et al., 1985). MAMES were also examined by one-dimensional TLC (1D-TLC) with the solvent systems petroleum ether/diethyl ether (85:15, v/v), petroleum ether/ethyl acetate (96:4, v/v) and with petroleum ether/diethyl ether (90:10, v/v) compared with dichloromethane (Minnikin et al., 1975). Components were sprayed with phosphomolybdic acid (Alltech Associates) and charred at 110°C for 15 min for visualization of mycolic acid spots. Chemical functional groups of mycolic acids were designated as: α-mycocenes (I), α’-mycolates (II), methoxymycolates (III) and ketomycolates (IV), as described previously (Lévy-Frebault et al., 1986). The designation, α’-mycolates (I) was used to indicate unsaturated mycolates with carbon chains longer than mycobacteria α-mycocenes.

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with a CEQ 8000 (Beckman Coulter) Genetic Analysis System with the CEQ Dye Terminator Cycle Sequencing Quick Start kit according to the manufacturer’s instructions.

**DNA extraction.** Bacteria were inoculated from 7H11 agar slants into 200 ml 7H9 broth containing 1-0 ml Tween 80 and 3-0 g glycine and grown for 1–2 days at 35 °C, before being harvested by centrifugation at 3793 g for 30 min. DNA was purified from lysed protoplasts as described previously by Lasker et al. (1992). Repeat extractions were performed with a 20% (w/v) SDS solution to improve the DNA yield, a method adapted from Loeffelholz & Scholl (1989).

**DNA reassociation and G+C content.** DNA–DNA relatedness was determined with the hydroxyapatite method with an optimum reassociation temperature of 70 °C (Brenner et al., 1983; Wayne et al., 1987; Stackebrandt et al., 2002). G+C content was determined spectrophotometrically using the thermal denaturation method as described previously (Mandel et al., 1970). Samples were tested three times.

**16S rRNA gene sequencing and phylogenetic analysis.** Procedures for cellular disruption, extraction, PCR and sequencing reactions for determination of the consensus sequence of the 16S rRNA gene with a 373A DNA Sequencer (Applied Biosystems Division) were described previously (Floyd et al., 1996). Nucleotide comparisons were conducted at GenBank with the BLAST program for the study isolates with consensus strands of nearly complete 16S rRNA gene sequences for CDC 413 (1450 bp), CDC 606 (1433 bp), CDC 945T (1458 bp) and CDC 1076T (1450 bp). PHYLIP (Felsenstein, 1993) analysis tools at the Ribosomal Database Project II (RDP; http://rdp.cme.msu.edu) were used for automatic alignment of imported sequences to an established online database of aligned 16S rRNA gene sequences (Cole et al., 2003). Only sequences that contained a base in every position were used in the alignments at RDP. In addition, manual multiple-sequence alignments were analysed with the Wisconsin Package for Sequence Analysis, version 10.3 (Accelrys), with PILEUP as described previously (Floyd et al., 2000). Maximum-parsimony analysis was done with the PAUPSearch program using a heuristic tree search option. A phylogenetic tree was constructed from a Jukes and Cantor evolutionary distance matrix (Jukes & Cantor, 1969) by the Saitou and Nei neighbour-joining method (Saitou & Nei, 1987) with the programs in PHYLIP. The study isolates were compared with representative species of mycolic-acid-containing genera, selected to demonstrate reported branches of the 16S rRNA gene phylogenetic tree. The confidence of the tree branching was estimated by bootstrap analysis with 100 resamplings of the data.

**RESULTS AND DISCUSSION**

**Phenotypic characteristics**

Isolates originated from different geographical regions in the United States and were detected seven times in 10 years. The environmental niche was not determined, but isolates were recovered from non-sterile human sources: three from sputa, one from a nasal region, one from a bronchial wash and two unknown. Additional clinical information was not available. The four isolates available for this study grew aerobically and produced rod-shaped bacilli with occasional v-forms but no true branching and no mycelium or spores. Cell walls contained mycolic acids and bacilli were intensely stained acid–alcohol-fast. Smooth, domed (SmD) colonies were formed by isolates CDC 1076T and CDC 413, whereas isolates CDC 945T and CDC 606 formed wrinkled-rough (WrRg) colonies. The bacilli forming the SmD colonies demonstrated consistent rod lengths of 1–0–1.3 μm by 0.4 μm (Fig. 1a, b). The bacilli of the WrRg colonies were irregular-length rods, 0.76–1.93 x 0.4 μm (Fig. 1c, d). In addition, cells from strains CDC 1076T and CDC 413 were easily dispersed and formed ‘smooth’ culture suspensions in 7H9 broth, in contrast with CDC 945T and CDC 606, which aggregated and produced ‘rough’ cultures. Visible colonies developed in 3–4 days on 7H10 or 7H11 agar at 33 °C. Growth on chocolate agar was mucoid and embedded in the agar surface. SmD colonies were dense and could be pushed intact across the surface of the 7H10 plates, whereas WrRg colonies were creamy and smeared when pushed. Colonies were non-pigmented (white to beige), non-photochromogenic and did not produce any diagnostic odour. CDC 945T grown for >4 weeks at <33 °C produced a diffusible pinkish colour throughout 7H10 agar and at the

![Fig. 1. (a, c) Scanning electron micrographs of CDC 1076T (a; bar, 5 μm) and CDC 945T (c; bar, 2 μm) grown at 33 °C in 7H9 broth for 3 days. (b, d) Colony morphology for CDC 1076T (b) and CDC 945T (d) grown for 7 days on 7H11.](http://ijs.sgmjournals.org)
outer edge of mature growth on LJ medium. In addition, this isolate developed light buff-coloured colonies with age on LJ and after >5 weeks the colonies demonstrated a ‘greening effect’ from uptake of malachite green. Generally, the study isolates demonstrated weak or no arylsulfatase activity in 3 days but were positive in 14 days. They did not produce niacin and developed activity in 3 days but were positive in 14 days. They did not produce acid with D-glucose, maltose and trehalose but not hypoxanthine, tyrosine or xanthine. They utilized and produced acid with D-glucose, maltose and trehalose but not with adonitol, L-arabinose, cellobiose, citrate, dulcitol, i-erythritol, galactose, i-myoinositol, lactose, mannose, melibiose, raffinose, L-rhamnose, salicin or sodium citrate and were variable for use of D-fructose, glycerol, mannitol, D-sorbitol and sucrose. Growth was observed at 25, 35 and 45 °C on TGY but was retarded on HI. CDC 1076 and CDC 413 developed SmD colonies slowly on LJ or ATS with NaCl (>8 days), did not produce a full growth swirl on MacConkey agar, did not reduce tellurite and were negative or weakly positive for Tween opacity but utilized and produced acid from D-fructose compared with the isolates that produced WrRg colonies. CDC 945 reduced nitrate, took up iron, grew in lysozyme, did not hydrolyse Tween and utilized mannitol and D-sorbitol compared with the other isolates (Table 1).

The API CORYNE test kit numerical profile was 2040000 and did not correspond to any organism in the bioMérieux database. The study isolates were positive for β-glucosidase and pyrazinamidase activities and negative for alkaline phosphatase, β-galactosidase, β-glucuronidase, x-glucosidase, N-acetyl-β-glucosaminidase and pyrrolidonyl arylamidase activity at 33 °C. Detection of carbohydrate utilization for the study isolates was unreliable with this system.

**Drug susceptibilities**

Strains CDC 1076T and CDC 413 were susceptible to amikacin, cefoxitin, clarithromycin, ciprofloxacin, doxycycline, imipenem and sulfamethoxazole at or below the respective MIC breakpoints but intermediate to tobramycin. CDC 945T and CDC 606 were susceptible to amikacin and sulfamethoxazole at or below the respective MIC breakpoints, but intermediate to cefoxitin and resistant to clarithromycin, doxycycline and tobramycin. Specifically, CDC 945T was intermediate to imipenem and resistant to ciprofloxacin. CDC 606 was susceptible to ciprofloxacin and imipenem at or below the respective MIC breakpoint.

**Chemotaxonomic characteristics**

The diamino acid in the cell wall was meso-diaminopimelic acid.

The study strains shared a common fatty acid profile, characterized by major amounts of C10:0 (13–36%), C14:0 (6–25%), C16:1 (6–12%), C16:0 (9–28%), C18:1ω9c (6–15%) and 10-methyl C18:0 (tuberculostearic acid, 4–21%). The profile differed from *R. equi* in the

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**Table 1. Differential morphological and physiological characteristics of study isolates**

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<th>Property</th>
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<th>3</th>
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<td>WrRg</td>
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presence of C10:0 (0 vs 13–36% for the study isolates). The pyrolysis cleavage product of the β-hydroxy position of the mycolic acids yielded a C24:0 acid-methyl ester, similar to Mycobacterium species (Lechevalier et al., 1971). The identities of C22:0 and C24:0 compounds were confirmed by MS. The GLC detection of mycolic acid hydrolysis products and tuberculostearic acid confirmed that the isolates were within the suprageneric cluster of mycolic-acid-containing taxa.

HPLC analysis revealed three distinctive, genus-/species-specific chemotaxonomically relevant mycolic acid patterns, distinguished by the unique occurrence of high-molecular-mass, non-polar, late-emerging MAPBE peaks. Similar peak times were demonstrated in different patterns by comparison of retention times to the internal standard for comparative peaks; however, patterns differed visually in peak area, peak height and number of peaks (Fig. 2). The MAPBE peak groups eluted in a range starting from 6:03 or 7:24 min to the internal standard at 9:45 min and generally appeared specific for the colony types. Two groups of MAPBE peaks were invariant for all patterns and appeared in a time range 7:24–9:45 min or 8:18–9:45 min. Only those isolates that developed SmD colonies, CDC 413 and CDC 1076T, produced three MAPBE peak groups and displayed early elution times starting at 6:03 min (Fig. 2a). Strains CDC 544, CDC 606 and CDC 945T developed WrRg colonies and produced partially separated MAPBE peaks, i.e. doublet peaks in the first group and unresolved peaks in the second group (Fig. 2b). The remaining HPLC pattern was noted by DMS on initial culture analysis for CDC 434, an isolate that formed WrRg colonies, and CDC 1178, an isolate whose colony morphology was not recorded. These isolates developed two MAPBE peak groups with partially resolved peaks (Fig. 2c). This HPLC pattern was consistent with an additional novel species; unfortunately, isolates were not recovered for confirmation studies.

In this liquid chromatography system, the late elution of peaks was indicative of non-polar compounds associated with the presence of long-carbon-chain mycolic acids (Takayama et al., 1979; Minnikin et al., 1985a; Butler et al., 1986). As a result of this elution, the high-molecular-mass internal standard, a C110 non-mycolate compound (Corixa Corp.), co-eluted as a shoulder peak with the final MAPBE peak from the study samples (Fig. 2). This time of elution suggested that the number of carbon atoms in the final peak group was greater than the mycobacteria C90 mycolic acids but less than the C110 standard. In general, MAPBE peaks noted at 6:03 and 7:24 min for the study isolates overlapped the known peak times for some species of Rhodococcus, Tsukamurella and Mycobacterium, although HPLC chromatograms produced from species of Rhodococcus or Tsukamurella do not show late-emerging peaks. In fact, peaks detected later than 7:5 min represent species of mycobacteria; however, their patterns consistently terminate 0:5 to approx. 1 min prior to the elution of the standard, a result unlike the patterns of the study isolates (Butler et al., 1993; Butler & Guthertz, 2001). Neither GLC nor HPLC analysis assigned the study isolates to any established mycolic-acid-containing taxa, although the chromatographic results were similar to those expected from species of Mycobacterium.

1D-TLC separation of chemical functional groups of mycolic acids from the study isolates authenticated the high-molecular-mass, non-polar mycolic acids, shown in Supplementary Figs S1 and S2, available in IJSEM Online. The mycolic acids demonstrated an increased mobility compared with mycolates from representatives of...
Corynebacterium, Rhodococcus and Gordonia, and were comparable with extracts from Tsukamurella or Mycobacterium. Extracts from CDC 945T and CDC 1076T revealed two mycolic acid spots, whereas CDC 413 and CDC 606 revealed three mycolic acid spots on 1D-TLC (Supplementary Fig. S2). The migration of the mycolic acids was in the range of, but slightly greater than, C70–C90 mycolates of mycobacteria. 2D-TLC analysis of alkaline and acid methanolylates revealed identical migration spots, unaffected by the lysis conditions, and were comparable with mycolic acids lacking oxygen functions, other than the hydroxy group of the mycolic acid. Consequently, acidic methanolation did not alter the number of chemical functional groups observed with TLC and established the absence of oxygenated epoxymycolates (data not shown). Comparison of chromatographic migration rates of authentic compounds from representative mycobacteria demonstrated the absence of oxygenated ketomycolates (IV) (Supplementary Fig. S2). The absence of oxygenated methoxymycolates (III) and ketomycolates (IV) was demonstrated by comparison of their co-migration with a lack of group migration of the mycolates for the study isolates with either solvent development (Supplementary Fig. S3a, b; lanes 1 and 4). Strain CDC 606 developed a faint band with the solvent dichloromethane that migrated in a range similar to methoxymycolates, but this was not observed with other developments. Alignment of mycolic acid chemical functional groups of the study isolates validated the presence of non-oxygenated mycolates and they were designated α+ -mycolic acids (I1), α-mycolic acids (I) and α′-mycolic acids (II). The detection of non-oxygenated α+ -mycolates was unique to the study isolates. The confirmation of mycolic acids in the cell wall provided a definitive characteristic for association of the study isolates with related taxa, including Corynebacterium (with some exceptions), Gordonia, Rhodococcus, Williamsia, Skermania, Dietzia, Nocardia, Tsukamurella or Mycobacterium. The detection of multiple mycolic acid chemical functional groups with TLC has been shown to be a diagnostic feature for Mycobacterium, although exceptions producing only α-mycolic acids are Mycobacterium fallax and Mycobacterium triviale. In addition, species of Tsukamurella are known to produce two-spot patterns representative of α- and α′-mycolic acids (Yassin et al., 1997). Overall, the TLC mycolic acid patterns developed from the study isolates were similar to those found with species of Tsukamurella or Mycobacterium.

Genetic characteristics

PCR amplification of a 439 bp region of the 65 kDa HSP gene with the proposed, albeit selected, aerobic actinomydinate-specific primers TB11 and TB12 did not produce amplicons for three of the four study isolates; CDC 945T was the exception. The apparent nucleotide polymorphism in the priming regions was unaffected by reducing the stringency of the test by lowering the binding temperature to 55 °C. Restriction analysis with MspI and HinfI of the single amplicon produced from isolate CDC 945T produced novel fragments of 250/105/75 bp and 400/40 bp, respectively, and supported the uniqueness of the study isolates.

CDC 1076T and CDC 413 had identical 16S rRNA gene sequences, as did CDC 945T and CDC 606. The two proposed species demonstrated a difference of 15 bp within the first 500 bp of their consensus strands and an overall similarity of 98-9 %. This separation of the proposed species was supported by <28 % DNA–DNA relatedness. Comparisons of the nearly complete 16S rRNA gene sequences to sequences at GenBank revealed that CDC 1076T was 94-8 % (a 78 bp difference) and CDC 945T was 94-5 % (a 79 bp difference) similar to the nearest genetic neighbour, R. equi ATCC 6939T. This genetic alignment with R. equi and other Rhodococcus species was unexpected, as the physiological and chemical properties clearly delineated the study isolates from this group. Furthermore, overall sequence comparisons of the almost complete consensus strands for R. equi (GenBank accession no. X80614) compared with CDC 1076T (accession no. AY608918) or CDC 945T (accession no. AY608920) were highly dissimilar. Additional genetic dissimilarity was shown with PCR amplification of a 959 bp DNA fragment of the choE gene previously determined to be R. equi-specific. This amplification test was negative for the study isolates. Although a prominent band was found that migrated in close proximity to the reported location of the 959 bp amplicon, it produced a different migration rate when co-analysed with the specific choE fragment (data not shown). Furthermore, conclusive identification of both of the amplicons was done by sequencing with the same primers used for PCR and revealed that this band was a PCR artefact and the choE gene fragment was only present in the R. equi control, an indication that the study isolates were not strains of R. equi.

Comparison of 16S rRNA gene sequences with other mycolic-acid-containing genera demonstrated that CDC 1076T showed higher similarity than CDC 945T. Nevertheless, CDC 1076T was genetically less related to Dietzia maris (93-2 %), Tsukamurella paurometabola (92-5 %), Nocardia brasiensis (92-0 %), Skermania piniformis (91-9 %), Gordonia sputi (91-4 %) and Mycobacterium fortuitum (91-0 %) than to R. equi (94-8 %). Moreover, sequence comparisons using the RDP database of online sequences, pre-aligned to the secondary structure model of the ribosomal subunit, demonstrated low alignment scores (based upon occurrence of unique 8-base oligomers) of 0-79 and 0-78 with R. equi ATCC 6939T. These low similarity scores were consistent with divergent 16S rRNA gene alignments demonstrated at GenBank.

The phylogeny of the genus Rhodococcus currently remains unresolved, but the R. equi taxon segregates on a distinct, monophyletic 16S rRNA tree branch and has been suggested to occupy a novel ancestral evolutionary position (Rainey et al., 1995; Stackebrandt et al., 1988; McMinn et al., 2000). The unanticipated branching position of the study isolates on the same clade as R. equi suggested a similar association
Fig. 3. Neighbour-joining phylogenetic tree constructed from aligned 16S rRNA gene sequences at the online site for the RDP. See Methods for additional information. Rooted with Skermania piniformis. Bar, 0·01 substitutions per nucleotide position.

(Fig. 3). The application of different treeing methods did not affect this branching; even manual alignment of multiple sequences using less-restrictive alignment criteria resulted in similar branching (data not shown). A bootstrap value of 100 % was revealed for the node of the study isolates and R. equi, but a low statistical value of 59 % for their branching with the other Rhodococcus was unsupportive. Moreover, a lack of close genetic association was substantiated by DNA–DNA hybridization values of 2 % for R. equi and the study isolates. The G + C contents of strains CDC 1076T and CDC 945T were respectively 68·0 and 72·0 mol%. These values, the presence of mycolic acids and the occurrence of meso-diaminopimelic acid in the cell wall peptidoglycan were consistent with high G + C, mycolic-acid-containing aerobic actinomycetes in the suborder Corynebacterineae (Stackebrandt et al., 1997).

The divergent genetic relationships combined with phenotypic characteristics and chemotaxonomic data supported the creation of a new genus containing two novel species. The family name was formed according to Rules 9 and 21a of the International Code of Nomenclature of Bacteria (Lapage et al., 1992).

Description of Segniliparaceae fam. nov.

Segniliparaceae (Segni.li.par.a’ceae. N.L. masc. n. Segnilipus type genus of the family; -aceae ending to denote a family; N.L. fem. pl. n. Segniliparaceae the Segnilipus family).

Segniliparaceae (Segni.li.par.a’rus. L. adj. segnis slow; Gr. adj. liparos fat/fatty; N.L. masc. n. Segnilipus the slow fatty one, the one with slow fats, to indicate the possession of slowly reacting fatty acids, i.e. late-eluting mycolic acids detected with HPLC).

Aerobic, brightly staining, acid–alcohol-fast, rod-shaped cells. No spores or aerial mycelium, non-motile, occasional v-shaped rods but no true branching. Grows in 3–4 days on LJ, 7H10 and 7H11 at 33 °C. Colonies are non-pigmented, non-photochromogenic and do not produce a diagnostic odour. Cell wall contains mycolic acids, tuberculostearic acid and meso-diaminopimelic acid. Arylsulfatase activity and growth tolerance with sodium chloride are positive in 14 days. Niacin production is negative. Semi-quantitative catalase produces >45 mm bubbles. Cells grow on mycobacterial media and TGY but growth on HI is poor. Urea is hydrolysed but acetamide, adenine, casein, citrate, ascorcin, hypoxanthine, tyrosine and xanthine are not. D-Glucose, maltose and trehalose are used as carbon sources and produce acid, whereas adonitol, L-arabinose, cellobiose,
The type species of the genus is Segniliparus rotundus. The mental habitat is unknown. DNA hybridization is the proposed genus on an extended, distinct tree clade with R. equi. rRNA gene sequence phylogenetic analysis demonstrates Segniliparus rotundus (ro.tun.) and are uniform in size, 1 μm. Species has the genus characteristics. Acid-alcohol-fast rods are 0-76–1.93 × 0.4 μm. Colonies are wrinkled-rough with smooth, undulating edges. Young colonies are creamy and smear when pushed. Optimal grow on 7H11 agar occurs at 33 °C. Cells aggregate, forming clumps in 7H9 broth. Arylsulfatase activity is negative or weakly positive at 3 days and positive at 14 days. Grows on LJ and ATS with sodium chloride in 7 days. Grows in 3 days on MacConkey agar. May be weakly positive or positive for iron uptake. May or may not reduce nitrate, but reduces tellurite. Tween opacity is positive, Tween hydrolysis is variable. May or may not grow in lysozyme in 21 days. Utilizes and produces acid from carbon source glycerol and is variable for d-fructose and sucrose. Utilizes and produces acid from carbon source glycerol and is variable for d-fructose and sucrose. Partially susceptible to ciprofloxacin and imipenem. HPLC mycolic acid pattern is a double cluster of contiguous eluting peaks starting at approx. 7-2 min and ending with the last peak co-eluting with the internal standard. The DNA G+C content is 72 mol%. The type strain, CDC 945T (= ATCC BAA-974T =CIP 108380T), was isolated from human sputum. An additional strain is CDC 606 (= ATCC BAA-975 = CIP 108381).

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References


