Rhodococcus gordoniae sp. nov., an actinomycete isolated from clinical material and phenol-contaminated soil

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The taxonomic relationships of two actinomycetes provisionally assigned to the genus Rhodococcus were determined using a polyphasic taxonomic approach. The generic assignment was confirmed by 16S rRNA gene similarity data, as the organisms, strains MTCC 1534 and W 4937T, were shown to belong to the Rhodococcus rhodochrous subclade. These organisms had phenotypic properties typical of rhodococci; they were aerobic, Gram-positive, weakly acid-fast actinomycetes that showed an elementary branching-rod–coccus growth cycle and contained meso-diaminopimelic acid, arabinose and galactose in whole-organism hydrolysates, N-glycolated muramic acid residues, dehydrogenated menaquinones with eight isoprene units as the predominant isoprenologue and mycolic acids that co-migrated with those extracted from the type strain of R. rhodochrous. The strains had identical phenotypic profiles and belong to the same genomic species, albeit one distinguished from Rhodococcus pyridinivorans, with which they formed a distinct phyletic line. They were also distinguished from representatives of all of the species classified in the Rhodococcus 16S rRNA gene tree using a set of phenotypic features. The genotypic and phenotypic data show that the strains merit recognition as a novel species of Rhodococcus. The name proposed is Rhodococcus gordoniae sp. nov., with the type strain W 4937T (=DSM 44689T =NCTC 13296T).

The application of polyphasic procedures led to marked improvements in the classification of Rhodococcus and related genera (Goodfellow et al., 1998, 1999). The taxonomic status of rhodococcal species with validly published names is underpinned by a wealth of genotypic and phenotypic data (Goodfellow et al., 2002, 2004; Takeuchi et al., 2002; Zhang et al., 2002); representatives of these taxa can be assigned to four subclades in the Rhodococcus 16S rRNA gene tree (Rainey et al., 1995; McMinn et al., 2000; Goodfellow et al., 2004). The improved classification of the genus provides a sound framework for the recognition of additional Rhodococcus species, including ones of clinical and industrial significance. Rhodococci show remarkable metabolic diversity, as exemplified by their ability to transform nitriles (Bunch, 1998) and degrade xenobiotic compounds (Dabbs, 1998; Goodfellow et al., 2004). In contrast, Rhodococcus equi strains cause infections in humans, notably in immunocompromised hosts (Kedlaya et al., 2001; Weinstock & Brown, 2002), though there are a few reports of infections caused by members of other Rhodococcus species (Spark et al., 1993; Cuello et al., 2002).

The present polyphasic study was designed to determine the taxonomic position of strains MTCC 1534 and W 4937T. Isolate W 4937T had been distinguished from representatives of Rhodococcus species using phenotypic and ribotyping data (Spark et al., 1993). The two organisms were found to form a novel species of Rhodococcus, for which the name Rhodococcus gordoniae sp. nov. is proposed, with organism W 4937T as the type strain.

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Abbreviation: A2pm, diaminopimelic acid.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains MTCC 1534 and W 4937T are AY233202 and AY233201.
Strain MTCC 1534 (Microbial Type Culture Collection, Chandigarh, India) was isolated on M3 agar (Rowbotham & Cross, 1977) that had been inoculated with a suspension of phenol-contaminated soil from near Chandigarh, India, and incubated at 28 °C for 4 days, and strain W 4937T was isolated from a blood culture of an immunocompetent patient with fatal pneumonia associated with adult respiratory disease syndrome, as described previously (Spark et al., 1993). The organisms were maintained on glucose/yeast extract agar (GYEA; Gordon & Mihm, 1962) at room temperature and as glycerol suspensions (20 %, v/v) at −20 °C.

The organisms and appropriate marker strains were examined for a range of phenotypic properties (Table 1) using standard procedures (Goodfellow et al., 1990). Biomass for chemotaxonomic studies was prepared following growth of the isolates in shake flasks of GYE broth for 5 days at 28 °C; after checking for purity, the biomass was harvested by centrifugation, washed twice in distilled water and freeze-dried. Established HPLC and TLC procedures were used to determine the diagnostic isomers of diaminopimelic acid (A2pm) (Staneck & Roberts, 1974), major whole-organism sugars (Schaal, 1985), predominant isoprenoid quinones (Collins, 1994) and muramic acid type (Uchida et al., 1999). The alkaline methanolysis procedure was used to detect mycolic acids (Minnikin et al., 1980).

Genomic DNA was isolated, purified and sequenced after Kim et al. (1998). The resultant 16S rRNA gene sequences of strains MTCC 1534 and W 4937T were aligned manually with corresponding sequences of representatives of the genera classified in the family Corynebacterineae, retrieved from the DDBJ, EMBL and GenBank databases, by using the AL 16S program (Chun, 1995). Evolutionary trees were inferred using the least-squares (Fitch & Margoliash, 1967) and neighbour-joining (Saitou & Nei, 1987) treeing algorithms from the PHYLIP suite of programs (Felsenstein, 1993). Evolutionary distance matrices for the least-squares and neighbour-joining methods were prepared after Jukes & Cantor (1969). The topologies of the resultant trees were evaluated by bootstrap analyses (Felsenstein, 1985) of the neighbour-joining data based on 1000 resamplings using the SEQBOOT and CONSENSE programs from the PHYLIP package.

Table 1. Characteristics that distinguish strains MTCC 1534 and W 4937T from the type strains of species classified in the R. rhodochrous 16S rDNA subclade

<table>
<thead>
<tr>
<th>Characteristic</th>
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<th>6</th>
<th>7</th>
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<tbody>
<tr>
<td>Morphogenetic sequence*</td>
<td>EB-R–C</td>
<td>R–C</td>
<td>H–R–C</td>
<td>EB-R-C</td>
<td>EB-R–C</td>
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<tr>
<td>Aesculin hydrolysis</td>
<td>–</td>
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<td>–</td>
<td>+</td>
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<td>Urea hydrolysis</td>
<td>–</td>
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<td>Degradation of:</td>
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<td>DNA</td>
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<td>Starch</td>
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<td>Uric acid</td>
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<td>Growth on sole carbon sources</td>
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<tr>
<td>Arbutin</td>
<td>+</td>
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<td>Glycerol</td>
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<td>+</td>
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<td>meso-Inositol</td>
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<td>Methyl α-D-glucoside</td>
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<td>At 0.1% (w/v):</td>
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<td>m-Hydroxybenzoic acid</td>
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<td>+</td>
<td>–</td>
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<td>+</td>
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<td>p-Hydroxybenzoic acid</td>
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<td>+</td>
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<td>Monoethanolamine</td>
<td>+</td>
<td>+</td>
<td>–</td>
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<td>+</td>
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<tr>
<td>Sodium benzoate</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Sodium butyrate</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Sodium gluconate</td>
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<td>–</td>
<td>–</td>
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</table>

Chromosomal DNA for DNA–DNA hybridization studies was extracted from strains MTCC 1534 and W 4937T and the type strain of *Rhodococcus pyridinivorans* following growth in trypticase soy broth for 3 days at 35 °C and purification using universal bacterial DNA isolation procedures, modified for Gram-positive bacteria that produce copious amounts of exopolysaccharides or capsular material by the addition of hexadecyltrimethylammonium bromide (Graves & Swaminathan, 1993). Repeat extractions were performed with 20% (w/v) SDS to improve the DNA yield as adapted from Loeffelholz & Scholl (1989). DNA–DNA relatedness studies between the test strains and *R. pyridinivorans* KCTC 0647BP were carried out using a hydroxyapatite procedure (Brenner et al., 1982).

Almost complete 16S rRNA gene sequences were obtained for strains MTCC 1534 and W 4937T; comparison of these sequences with corresponding data from representatives of the suborder *Corynebacterineae* confirmed that they belong to the genus *Rhodococcus* (data not shown). Similarly, the results of the chemotaxonomic and morphological studies, as given in the species description, are consistent with the assignment of the strains to this genus (Goodfellow et al., 1998).

It is evident from Fig. 1 that the tested strains belong to the *Rhodococcus rhodochrous* 16S rDNA subclade (Rainey et al., 1995; McMinn et al., 2000; Goodfellow et al., 2004), a relationship that is supported by the results with all three treeing algorithms, by a high bootstrap value in the neighbour-joining analysis and by the high 16S rRNA gene similarities found between members of the *R. rhodochrous* subclade and strains MTCC 1534 and W 4937T (96.9–99.1%). Strains MTCC 1534 and W4937T shared a 16S rDNA nucleotide similarity of 99.9%, a value that corresponds to 2 nt differences at 1421 locations, and are most closely related to *R. pyridinivorans* PDB9F and *R. rhodochrous* DSM 43274T. The test strains showed respective 16S rDNA similarities to the *R. pyridinivorans* strain of 99.1 and 98.8%, values that correspond to 13 and 15 nt differences at 1403 sites; the corresponding values to the *R. rhodochrous* strain were respectively 99.0 and 99.1%, values equivalent to 12 and 14 nt. It is also evident from Fig 1 that *R. rhodochrous* DSM 43274T, formerly *Rhodococcus roseus* Tsukamura et al. 1991, is more closely related to *R. pyridinivorans* PDB9F (99.7% similarity, 5 differences) than to *R. rhodochrous* DSM 43241T (99.2% similarity, 12 differences). These data suggest that strain DSM 43274T should be reclassified as *R. pyridinivorans*, though comparative studies with the type strain of this species are needed to prove the point.

16S rDNA similarity values between 99-0 and 99-5% have been reported for representatives of several species of *Rhodococcus* (Yoon et al., 2000; Goodfellow et al., 2002, 2004) that share DNA–DNA relatedness values well below the 70% cut-off point recommended for the delineation of bacterial species (Wayne et al., 1987). Strains MTCC 1534 and W 4937T shared a DNA–DNA relatedness value of 100% and showed a corresponding value of 60% with the type strain of *R. pyridinivorans*; results that show that the test strains form a distinct genomic species. It is evident from Table 1 that strains MTCC 1534 and W 4937T gave identical phenotypic profiles that separates them from representatives of species classified in the *R. rhodochrous* 16S rDNA subclade.

The present study shows that strains MTCC 1534 and W 4937T have properties consistent with their assignment to the same species in the genus *Rhodococcus*. They are particularly closely related to *R. pyridinivorans* and *R. rhodochrous*, but can be distinguished from these species by using genotypic and phenotypic data. It is proposed that strains W 4937T (= DSM 44689T = NCTC 13296) and MTCC 1534 be assigned to the genus *Rhodococcus* as *Rhodococcus gordoniae* sp. nov., with strain W 4937T as the type strain.

**Description of Rhodococcus gordoniae sp. nov.**

*Rhodococcus gordoniae* (gor.don’i.ae. N.L. gen. n. gordoniae of Gordon, named after Ruth Gordon, a celebrated microbial systematist).

The description is based upon information taken from this study and from Spark et al. (1993). Aerobic, Gram-positive, catalase-positive actinomycete that forms branched filaments that fragment into coccobacillary elements. Acid-fast when grown on 7H10 agar and stained using the modified Kinyoun procedure. Neither aerial hyphae nor diffusible pigments are formed. Produces raised, shiny, pink- to coral-pigmented colonies with filamentous edges on blood and chocolate agar plates incubated at 36 °C. Grows on MacConkey agar supplemented with crystal violet; grows poorly on Lowenstein–Jensen medium with 5% (w/v)
sodium chloride. Degrades tyrosine but not casein, hypo-
xanthine or xanthine. Hydrolyses aesculin weakly and is
indole- and urease-negative. Hydrogen sulphide is not
produced. Reduces nitrate to nitrite and grows in the
presence of lysozyme. Does not exhibit equi-factors. Acid
is produced from L-arabinose, D-fructose, D-galactose,
D-glucose, glycerol, D-mannitol, D-mannose, salicin,
D-sorbitol, D-sucrose, D-trehalose and D-xyllose, but not
from D-cellobiose, inositol, D-maltose or L-rhamnose.
Resistant to clindamycin and norfloxacin but susceptible
to amikacin, amoxicillin/clavulinate, ampicillin, ampicillin/
sulbactam, cephalothin, cefotaxime, ceftriaxone, ciproflox-
acin, doxycycline, erythromycin, gentamicin, imipenem,
iminopen, minocycline, oxacillin, penicillin, rifampicin, sulmethylene-
xazole, tetracycline, trimethoprin/sulfamethoxazole and
vancomycin. Additional phenotypic properties are shown
in Table 1. Characterized by the presence of meso-A2pm,
arabinose and galactose in whole-organism hydrolysates,
contains N-glycolated muramic acid residues, predominant
proportions of dehydrogenated menaquinones with eight
isoprene units and mycolic acids that co-migrate with those
of the type strain of \textit{R. rhodochrous}.

The type strain, strain W 4937T (= DSM 44689T = NCTC
13296\textsuperscript{T}), was isolated from a blood culture of a previously
healthy patient who died of pneumonia-associated adult
respiratory distress syndrome. Strain MTCC 1534 (= DSM
44690), which is clearly very closely related to strain W
4937\textsuperscript{T}, was isolated from a markedly different habitat,
namely phenol-contaminated soil. This organism can degrade
phenol as a sole carbon source at very high concentrations
(> 25 mM) in a minimal medium (data not shown).

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