Rubrobacter xylanophilus sp. nov., a New Thermophilic Species Isolated from a Thermally Polluted Effluent

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One strain of a thermophilic, slightly halotolerant bacterium was isolated from a thermally polluted industrial runoff near Salisbury, United Kingdom. This organism, strain PRD-1^T (T = type strain), for which we propose the name *Rubrobacter xylanophilus* sp. nov., produces short gram-positive rods and coccoid cells and forms pink colonies. The optimum growth temperature is approximately 60°C. Unusual internal branched-chain fatty acids (namely, 12-methylhexadecanoic acid and 14-methyloctadecanoic acid) make up the major acyl chains of the lipids. The results of our 16S rRNA sequence comparisons showed that strain PRD-1^T is related to *Rubrobacter radiotolerans* and that these two organisms form a deep evolutionary line of descent within the gram-positive *Bacteria*.

Over the past 20 years the microbiology of thermophiles has been dominated by the isolation and characterization of thermophilic *Archaea* species, many of which grow at extreme temperatures. During this time, however, many new thermophilic *Bacteria* species, several belonging to the gram-positive phylum, have been isolated from a wide range of thermal environments. The majority of the thermophilic gram-positive *Bacteria* species that have been described belong to the genera *Bacillus*, *Alicyclobacillus*, and *Clostridium* and other recently proposed genera, some of which were formerly included in the genus *Clostridium* (3, 5, 18, 28, 33, 40).

The species *Rubrobacter radiotolerans*, which was initially named *Arthrobacter radiotolerans*, was described on the basis of one strain isolated from a hot spring in Japan after gamma irradiation of water samples (35, 44). *R. radiotolerans* is gram positive, has an optimum growth temperature of about 48°C, and forms short pleomorphic rod-shaped cells. It is also highly radiotolerant and possesses unique internally branched fatty acids.

A pink-pigmented strain was recently isolated from a thermally polluted runoff from a carpet factory in the United Kingdom. This organism is more thermophilic than *R. radiotolerans* and also possesses unusual branched-chain fatty acids. On the basis of morphological and biochemical characteristics, chemotaxonomic parameters, and 16S rRNA gene sequence data, we propose that this organism, strain PRD-1^T (T = type strain), belongs to a new species, *Rubrobacter xylanophilus*.

MATERIALS AND METHODS

Isolation of strain PRD-1^T. Strain PRD-1^T was isolated from thermally polluted runoff (temperature, 50°C) from a carpet factory in Wilton, Wiltshire, United Kingdom. This strain was isolated by spreading a biofilm sample with a glass rod on tryptone soya agar. After incubation at 50°C for 5 days, one pink-pigmented colony appeared on a culture plate and was purified on the same medium. *R. radiotolerans* DSM 46359^T was obtained from the Deutsche Sammlung von Mikroorganismen und Zellkulturen, Braunschweig, Germany. Strains PRD-1^T and DSM 46359^T were routinely grown in *Thermus* medium containing 1.0 g of yeast extract per liter and 1.0 g of tryptone medium (41) per liter and were stored at –80°C in the same medium containing 15% glycerol.

Corynebacterium xerosis ATCC 373^T, which was obtained from the American Type Culture Collection, Rockville, Md., *Corynebacterium* sp. strain DSM 20146, and *Mycobacterium smegmatis* ATCC 19420^T were used as reference strains during extraction and chromatographic separation of mycolic acids. These organisms were grown in the appropriate growth media as described in the *DSM Catalogue of Strains* (6).

Morphological and biochemical characteristics. Cell morphology and motility were examined by phase-contrast microscopy during the exponential growth phase in *Thermus* medium. Unless indicated otherwise, biochemical and tolerance tests were performed as described by Santos et al. (31) and Manaia and da Costa (19) at 45°C for *R. radiotolerans* and at 60°C for strain PRD-1^T for up to 5 days. The temperature ranges for growth of *R. radiotolerans* and strain PRD-1^T were determined by incubating the organisms at temperatures between 35 and 75°C in 300-ml metal-capped Erlenmeyer flasks containing 100 ml of *Thermus* medium in a reciprocal water bath shaker. The pH range for growth of strain PRD-1^T was determined in *Thermus* medium buffered at pH 6.0 and 6.5 with 20 mM MES [2-(*N*-morpholino)ethanesulfonic acid], at pH 7.0 to 8.5 with 20 mM HEPES [*N*-(2-hydroxyethyl)piperazine-*N'*-2-ethanesulfonic acid], and at pH 9.0, 9.5, and 10.0 with 20 mM CAPSO [3-(cyclohexylamino)-2-hydroxy-1-propanesulfonic acid].

Single-carbon-source assimilation experiments were performed by using a minimal medium composed of CMD (2) basal salts medium to which filter-sterilized carbon sources (2.0 g liter⁻¹), yeast extract (0.2 g liter⁻¹), and ammonium sulfate (0.5 g liter⁻¹) were added. Growth was examined turbidimetrically at 610 nm after growth for 7 days in metal-capped tubes (16 by 160 mm) containing 5.0 ml of medium incubated at 60°C in a water bath. The negative controls contained no carbon source; growth was also monitored in *Thermus* medium.

Determination of the G+C content of DNA. Cells from a 500-ml overnight culture were harvested by centrifugation and resuspended in 10 ml of 50mM Tris-HCl (pH 8.0). Then lysozyme (10 mg) was added, and the cell suspension was incubated for 45 min at 4°C. Proteinase K and sodium dodecyl sulfate were added to final concentrations of 1 mg ml⁻¹ and 0.2%, respectively, and the mixture was kept on ice for an additional 2 h. The proteins were denatured by adding an equal volume of 2 M sodium perchlorate and then an equal volume of chloroform and gently mixing the preparation until a stable emulsion was obtained. The suspension was centrifuged at 12,000 × g for 5 min at 4°C, and the aqueous layer was recovered. After 2 volumes of ice-cold ethanol was added, the DNA was spooled, dried in a stream of air, and dissolved in 5 ml of TE buffer (10 mM Tris-HCl, 1.0 mM EDTA; pH 8.0). DNase-free RNase (1.0 mg) was added, and the mixture was incubated overnight at 4°C. An equal volume of phenol was added, and the suspension was mixed gently. The resulting mixture was centrifuged at 5,000 × g for 5 min at 4°C, and the top layer was recovered. The remaining phenol was removed by washing the preparation three times with ether. The DNA was spooled as described above and resuspended in 0.1 × SSC (1 × SSC is 0.015 M trisodium citrate plus 0.15 M NaCl).

The DNA base composition was determined by the thermal denaturation method of Marmur and Doty (20) in 0.1 × SSC by using a Pye Unicam SP-100 spectrophotometer equipped with a temperature program controller. The G+C content was determined from the denaturation temperature by the method of Gillis et al. (9). The denaturation temperature of *Escherichia coli* B (= ATCC

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23226) in $0.1\times$ SSC was determined to be 73.2°C. Other G+C contents were derived from this value.

Genomic DNA isolation, PCR amplification and determination of the 16S ribosomal DNA sequence. Genomic DNA was isolated by the C TAB (hexadecyl trimethylammonium bromide) method of Wilson (42). Nearly complete 16S rRNA genes were amplified by PCR (22, 30) by using forward primer 16F27 (5'-AGAGTTTGATCMTGGCTCAG-3' [M = A or C]; hybridizing at positions 8 to 27 [*E. coli* 16S rRNA gene sequence numbering]) and reverse primer 16R1525 (5'-AAGGAGGTGWTCCAGCC-3' [W = A or T]; hybridizing at the complement of positions 1525 to 1541). PCR amplification was performed by using a GeneAmp 9600 apparatus (Perkin-Elmer Corp., Norwalk, Conn.) and the reaction conditions described previously (14).

PCR DNA products were purified by using Centricon-100 microconcentrators (Amicon GmbH, Witten, Germany). The purified PCR DNA was sequenced directly by using an Applied Biosystems model 373A DNA sequencer, the protocol recommended by the manufacturer (Perkin-Elmer Applied Biosystems GmbH, Weiterstadt, Germany) for *Taq* cycle sequencing, and fluorescent-dye-labeled dideoxynucleotides. The sequences of both strands of the PCR-amplified 16S ribosomal DNAs were determined by using the forward and reverse oligonucleotide primers described previously (17). The sequence data obtained were aligned with reference rRNA sequence data that were maintained in an in-house database by using the evolutionarily conserved primary sequence and secondary structure as references (10, 43). Evolutionary distances were calculated from masked sequence pair levels of similarity (17), and a correction factor for back mutations was included (13). We used a weighted, least-squares distance method (24) to generate dendrograms.

Polar lipid, lipoquinone, mycolic acid, and peptidoglycan analyses and fatty acid composition. The cultures used for polar lipid analysis were grown in 1-liter Erlenmeyer flasks containing 200 ml of *Thermus* medium at 45°C for *R. radiotolerans* and at 60°C for strain PRD-1^T in a reciprocal water bath shaker until the late exponential phase of growth. Harvesting of the cultures, extraction of the lipids, and two-dimensional thin-layer chromatography were performed as described previously (7, 27).

Lipoquinones were extracted from freeze-dried cells and were purified by thin-layer chromatography as described by Tindall (36). The lipoquinones were separated with a Gilson high-performance liquid chromatograph by using a reverse-phase (RP18) Spherisorb S5 ODS2 column and methanol-heptane (10:2, vol/vol) as the mobile phase and were detected at 269 nm.

Mycolic acids were extracted from cells obtained from growth in the appropriate growth medium by using the method of Minnikin et al. (21). Thin-layer chromatography was performed on Silica Gel 60 plates (10 by 20 cm; thickness, 0.25 mm; Merck) by using triple development with petroleum ether (bp, 60 to 80°C)-acetone (95:5, vol/vol). The components were revealed with molybdophosphoric acid.

Peptidoglycan was prepared and peptidoglycan structure was determined as described by Schleifer and Kandler (32). Freeze-dried cells (1 mg) were hydrolyzed in 0.2 ml of 4 N HCl at 100°C for 16 h (total hydrolysate) or for 45 min (partial hydrolysate). The diamino acid content was determined by one-dimensional thin-layer chromatography on cellulose plates by using methanol-pyridine-water-10 N HCl (32:4:7:1, vol/vol) solvent system. Amino acids and peptides were identified after two-dimensional thin-layer chromatography performed with the solvent systems of Schleifer and Kandler (32) by their mobilities and staining properties with ninhydrin.

For the fatty acid analysis *R. radiotolerans* was grown at 37 and 45°C and strain PRD-1^T was grown at 45 and 60°C until the mid-exponential phase of growth. Fatty acid methyl esters were obtained from fresh wet biomass by saponification, methylation, and extraction by using the method of Kuykendall et al. (16). Fatty acid methyl esters were separated with a Hewlett-Packard model 5890 gas chromatograph fitted with a 5% phenylmethyl silicone capillary column (Ultra 2; 0.2 mm by 25 m; Hewlett-Packard) by using the protocol of the Microbial Identification System (MIDI; Microbial ID, Inc., Newark, Del.). The fatty acid methyl esters were identified and quantified and a numerical analysis of the fatty acid profiles was performed by using the MIDI software package.

Mass spectrometry. Gas chromatography-mass spectrometry (GC-MS) was performed with a model MS80 RFA mass spectrometer (Kratos, Ltd., Manchester, United Kingdom) interfaced with a Carlo Erba model 5160 capillary gas chromatograph equipped with a type BPX-5 column (0.2 mm by 25 m; SGE Ltd., Milton Keynes, United Kingdom). Spectra were recorded by using electron ionization at an ionization energy of 70 eV, chemical ionization, and isobutane as the reagent gas. Negative ion fast atom bombardment mass spectra of isoprenoid quinones were obtained with the same instrument by using 3-nitrobenzyl alcohol as the liquid matrix. Samples were trimethylsilylated by treating them with 100 μ l of bis-(trimethylsilyl)-trifluoroacetamide (Fluka, Buchs, Switzerland) for 30 min at 60°C. Picolinyl esters were prepared by the method of Harvey (12) as modified by Wait and Hudson (39).

Nucleic acid sequence accession numbers. The 16S ribosomal DNA sequence data are available from EMBL under accession number X87134 for *R. radiotolerans* DSM 46359^T and accession number X87135 for strain PRD-1^T.

FIG. 1. Effect of temperature on the growth rates of *R. radiotolerans* (○) and strain PRD-1^T (●).

RESULTS AND DISCUSSION

Morphological and biochemical characteristics. Strain PRD-1^T produced very short, gram-positive, nonmotile, rod-shaped cells and a few spherical cells in older cultures on solid medium. The colonies of strain PRD-1^T were light pink after growth at 60°C, but bright pink after growth at 45°C. The colonies of *R. radiotolerans* were bright pink.

The following media were tested initially to determine whether they supported growth of strain PRD-1^T and *R. radiotolerans*: nutrient broth, tryptic soy broth, medium 162 (4), and *Thermus* medium. Both organisms grew on all of the media, but growth was excellent on *Thermus* medium, and this medium was used throughout this study. Several biochemical characteristics, such as the presence of cytochrome oxidase and catalase, reduction of nitrate to nitrite, the inability to ferment carbohydrates, and the ability to grow in *Thermus* basal salts medium containing only (NH₄)₂SO₄ (0.5 g liter⁻¹) and an appropriate carbon source, were identical in strain PRD-1^T and *R. radiotolerans*. The growth temperature range was the most outstanding differential characteristic for the two organisms (Fig. 1). Strain PRD-1^T had an optimum growth temperature of about 60°C and did not grow at temperatures above 70°C, while *R. radiotolerans* had an optimum growth temperature between 45 and 50°C and did not grow at temperatures above 55°C. Strain PRD-1^T hydrolyzed xylan, while *R. radiotolerans* did not. Several other phenotypic differences, including utilization of inositol by strain PRD-1^T, distinguished strain PRD-1^T from *R. radiotolerans* (Table 1).

GC-MS. The fatty acid composition of strain PRD-1^T was determined by gas chromatography, but several chromatographic peaks did not match the equivalent chain lengths of the fatty acid methyl esters in the MIDI database; we used GC-MS to identify these peaks. One peak eluted between 16:0 and 17:0 and had an electron ionization mass spectrum typical of a 17-carbon fatty acid (Table 2). The presence of a methyl branch at position 12 was indicated by three weak ions at *m/z* 227 (cleavage distal to the methyl group, forming a stabilized carbocation), *m/z* 195 (loss of CH₃OH from *m/z* 227), and *m/z* 177 (loss of H₂O from *m/z* 195), which identified the compound as 12-methylhexadecanoic acid (12-methyl-16:0). Similar fragment ions were present in the mass spectrum of a minor peak that had a molecular ion at *m/z* 298, which was identified as 12-methylheptadecanoic acid (12-methyl-17:0). The equivalent chain length of the other component (*M*⁺ at *m/z* 312) was less than 19:0, suggesting that the structure was branched. This compound was identified as 14-methyloctadecanoic acid (14-methyl-18:0) by fragment ions at *m/z* 255, 223, and 205 (29). A

TABLE 1. Characteristics that distinguish *R. radiotolerans* from strain PRD-1^{Ta}

Organism	Growth at 60°C	Hydrolysis of xylan	Utilization of the following carbon sources:											
			D-Galactose	D-Xylose	D-Melibiose	Glycerol	Galactitol	D-Mannitol	Ribitol	myo-Inositol	Acetate	Succinate	L-Glutamine	Acetamide
<i>R. radiotolerans</i>	— ^b	—	—	—	—	+	—	+	+	—	—	—	—	—
Strain PRD-1 ^T	+	+	+	+	+	—	w	—	—	+	w	+	w	+

^a Both organisms grew in medium containing 6% NaCl; degraded hide powder azure, gelatin, hippurate, arbutin, and esculin; were cytochrome oxidase, catalase, and β-galactosidase positive; produced nitrite; and utilized D-glucose, D-mannose, D-fructose, L-arabinose, L-rhamnose, sucrose, D-trehalose, D-raffinose, lactose, D-cellobiose, erythritol, pyruvate, malate, L-glutamate, L-asparagine, L-proline, and salicin. Neither organism hydrolyzed elastin, fibrin, casein, starch, cellulose, tyrosine, olive oil, or Tween 80; degraded *p*-nitrophenyl-α-glucopyranoside, *p*-nitrophenyl-β-glucopyranoside, *p*-nitrophenyl-laurate, or *p*-nitrophenyl-palmitate; had DNase or α-galactosidase; or utilized D-sorbitol, citrate, and L-serine.

^b —, negative; +, positive; w, weakly positive.

small peak that eluted immediately before 14-methyl-18:0 was identified by GC-MS as 12-methyloctadecanoic acid (12-methyl-18:0).

The identities of the components described above were confirmed by the results of GC-MS of their picolinyl esters. In the mass spectrum of the picolinyl ester of 14-methyl-18:0 the low abundance of *m/z* 318 and the interval of 28 between *m/z* 304 and 332 confirmed the presence of a methyl group in position 14 (Fig. 2). The structure of 12-methyl-16:0 was also deduced from the mass spectrum of its picolinyl ester. The major fatty acid methyl esters of *R. radiotolerans* were identified by their mass spectra as 12-methyl-16:0 and 14-methyl-18:0.

The retention times of several unidentified peaks in the gas chromatograms increased after trimethylsilylation, indicating that hydroxyl groups were present. The results of an analysis in which electron ionization and chemical ionization GC-MS was used suggested that these peaks corresponded to long-chain primary alcohols containing 16, 17 (two isomers), 18, and 19 carbon atoms. Since the retention times of these compounds were somewhat less than those of the available straight-chain standards, their alkyl chains were probably branched. Mass spectrometry of the trimethylsilyl derivatives did not, however, result in full characterization of the alkyl chains.

Several late-eluting peaks detected in the gas chromatograms had mass spectra dominated by a fragment ion at *m/z* 205, which is known to be characteristic of 1-*O*-alkyl-2,3-di-*O*-

trimethylsilyl glycerols (8). The results of chemical ionization GC-MS indicated that the two most abundant compounds were a pair of isomeric 1-heptadecyl-2,3-di-*O*-trimethylsilyl glycerols. In the absence of authentic standards, it was not possible to determine if the alkyl chains were branched from the mass spectra of the trimethylsilyl ether derivatives. A component with a mass spectrum very similar to the mass spectra of the C₁₇ alkyl glycerols was also present in the spectrum of the *R. radiotolerans* extract.

Respiratory quinone, polar lipid, cell wall, mycolic acid, and fatty acid compositions. The only respiratory lipoquinone detected in strain PRD-1^T and *R. radiotolerans* was menaquinone 8. The identity of the quinone was confirmed by negative ion fast ion bombardment mass spectrometry, which revealed a molecule that had a molecular anion at *m/z* 716.7, which is consistent with the presence of menaquinone 8. Mycolic acids were not detected in either of the organisms.

The thin-layer chromatography polar lipid patterns of the two organisms were also similar and consisted of five phospholipids, including phosphatidylglycerol and diphosphatidylglycerol, one phosphoglycerolipid, and one glycolipid (Fig. 3). These results are in agreement with the results of Suzuki et al. (35).

The peptidoglycan of strain PRD-1^T contained lysine, glutamic acid, and alanine at a molar ratio of 1:1:3. The results of an analysis of partial hydrolysates of the peptidoglycan were compatible with the identification of the peptidoglycan as type A3α (L-Lys-L-Ala) as defined by Schleifer and Kandler (32), and the strain PRD-1^T peptidoglycan was identical to the peptidoglycan of *R. radiotolerans* (35).

The fatty acid compositions of strain PRD-1^T and *R. radiotolerans* were also qualitatively very similar. At the optimum growth temperature 14-methyl-18:0 was the major fatty acid in strain PRD-1^T (53% of the total acyl groups), while 12-methyl-16:0 accounted for about 12% of the acyl groups; 12-methyl-17:0 and 12-methyl-18:0 were present in relatively minor amounts. In contrast to strain PRD-1^T, 12-methyl-16:0 accounted for 54% and 14-methyl-18:0 accounted for 11% of the acyl chains in *R. radiotolerans* grown at 45°C. The difference in the fatty acid compositions of the two organisms was not due to different growth temperatures since 14-methyl-18:0 was also the major fatty acid after growth of strain PRD-1^T at 45°C (Table 2). Several of the fatty acids identified in this study (namely, 12-methyl-17:0, 12-methyl-18:0, and 14-methyl-18:0) and the long-chain alcohols were not found previously in *R. radiotolerans* (35), and we did not detect anteiso-19:0 or 2-OH-17:0.

Internally branched fatty acids with a methyl group at position 10 are common in mycolic acid-containing gram-positive bacteria (1, 15, 34) and are present in some sulfate-reducing bacteria belonging to the *Proteobacteria* (37). In *Thermomicrobium roseum* 12-methyl-18:0 is the major fatty acid, and other internally branched fatty acids are also present (26), but this

TABLE 2. Fatty acid and long-chain fatty alcohol compositions of *R. radiotolerans* and strain PRD-1^T, at two growth temperatures

Fatty acid or fatty alcohol	Equivalent chain length	% of total in:			
		<i>R. radiotolerans</i>		Strain PRD-1 ^T	
		37°C	45°C	45°C	60°C
16:0 alcohol	15.549	14.5	4.5	1.3	ND ^a
16:0	16.000	4.5	6.6	5.3	2.3
17:0 alcohol ^b	16.080	8.7	4.7	4.8	ND
12-methyl-16:0	16.504	38.3	54.1	9.8	12.4
Unknown 1	16.559	ND	ND	0.9	ND
Unknown 2	16.862	ND	ND	1.6	1.2
17:0	17.000	ND	ND	0.9	1.2
Unknown 3	17.047	ND	ND	1.0	ND
12-methyl-17:0	17.460	ND	ND	1.9	1.7
18:0 alcohol	17.577	17.7	10.5	8.9	2.2
18:0	18.000	3.0	4.6	13.1	20.0
19:0 alcohol	18.099	5.1	3.9	8.2	2.3
12-methyl-18:0	18.430	ND	ND	3.0	3.6
14-methyl-18:0	18.512	8.3	11.2	37.1	53.0
19:0	19.000	ND	ND	0.9	ND

^a ND, not detected.

^b Two isomers were detected by GC-MS.

FIG. 2. Mass spectrum of the picolinyl ester derivative of 14-methyl-18:0 obtained from strain PRD-1^T.

bacterium belongs to the green nonsulfur bacteria and is not related to *R. radiotolerans* and strain PRD-1^T.

16S rRNA sequence analysis. PCR amplification of the 16S rRNA genes between nucleotides 28 and 1524 allowed us to determine 1,508 nucleotide positions or approximately 98% of the complete gene (estimated by comparison with the 16S rRNA gene of *E. coli*). Masked sequence comparisons of the 16S ribosomal DNA of strain PRD-1^T with reference sequence data (25, 38) demonstrated that PRD-1^T clusters with the gram-positive bacteria and is most similar to the species having high G+C contents, which is consistent with the results of gram staining and with the G+C content of the DNA (67.6 mol%). However, PRD-1^T was not particularly closely related to any of the previously described reference organisms for which sequence data were available. The levels of sequence similarity ranged from 75 to 80% with the most closely related high-G+C-content gram-positive species. On the basis of fatty acid results, Tindall suspected that strain PRD-1^T was closely related to *R. radiotolerans* (36a). Therefore, the 16S rRNA gene sequence of *R. radiotolerans* was determined to compare it with the strain PRD-1^T sequence. Our results showed that the level of sequence similarity between the two organisms was about 90%. Figure 4 shows that strain PRD-1^T and *R. radiotolerans* form a very deeply branching line of descent within the high-G+C-content gram-positive bacteria.

The level of 16S rRNA gene sequence similarity between strain PRD-1^T and *R. radiotolerans* (90%) is relatively low for species belonging to the same genus (3). However, we decided to place strain PRD-1^T in the genus *Rubrobacter* at this time because of the lack of differentiating phenotypic and chemotaxonomic characteristics that would justify creation of another genus, and we propose the name *Rubrobacter xylanophilus* for this organism.

Description of *Rubrobacter xylanophilus*. *Rubrobacter xylanophilus* (xy. la. no' phi. lus. Gr. n. xylon, wood; M. L. n. xylanum, xylan; Gr. adj. philos, liking, friendly to; M. L. adj. xylanophilus,

liking xylan). *R. xylanophilus* forms pleomorphic short rod-shaped cells that are 0.9 to 1.0 μm wide and 1.0 to 3.0 μm long; coccoid cells are also present. Gram positive. Colonies on *Thermus* medium incubated at 60°C for 7 days are 1.5 to 2.0 mm in diameter, circular, convex, smooth, opaque, and light pink. Motility and endospores are not observed. Strain PRD-1^T is aerobic, and no growth occurs under anaerobic conditions. Growth occurs in basal salts medium containing $(\text{NH}_4)_2\text{SO}_4$ and an appropriate carbon source. The optimum temperature for growth is about 60°C; no growth occurs at temperatures below 40°C or above 70°C. The optimum pH for growth is 7.5 to 8.0; growth does not occur at pH values below 6.0 or above 10.0. Cytochrome oxidase, catalase, and β -galactosidase are present. Xylan is hydrolyzed. Non-fermentative. Nitrate is reduced to nitrite. Growth occurs in *Thermus* medium containing 6.0% NaCl. Gelatin, hide powder azure, arbutin, esculin, and hippurate are hydrolyzed. Growth occurs on many common sugars, organic acids, and amino acids. The major respiratory

FIG. 3. Two-dimensional thin-layer chromatography of polar lipids of *R. radiotolerans* and strain PRD-1^T. Components were revealed by staining the preparations with 5% molybdophosphoric acid in ethanol and heating them at 160°C. Abbreviations: PL, phospholipid; PGL, phosphoglycolipid; GL, glycolipid; PG, phosphatidylglycerol; DPG, diphosphatidylglycerol.

FIG. 4. Rooted dendrogram (based on pairwise 16S rRNA gene sequence comparisons) showing the derived phylogenetic relationships of strain PRD-1^T, *R. radiotolerans*, and reference strains of the gram-positive phylum. Reference sequence data were obtained from the European Molecular Biology Laboratory (11, 23) and Ribosomal Database Project (25) databases. The scale bar indicates the average number of substitutions per nucleotide position. The root organism was *E. coli*.

quinone is menaquinone 8, and the major fatty acid is 14-methyl-18:0. The peptidoglycan type is type A3 α . The G+C content of the DNA is 67.6 mol%. The natural habitat of *R. xylanophilus* is not known.

Type strain PRD-1 has been deposited in the Deutsche Sammlung von Mikroorganismen und Zellkulturen as strain DSM 9941.

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REFERENCES

- Brennan, P. J. 1988. *Mycobacterium* and other actinomycetes, p. 203–298. In C. Ratledge and S. G. Wilkinson (ed.), *Microbial lipids*, vol. 1. Academic Press, London.
- Castenholz, R. W. 1969. Thermophilic blue-green algae and the thermal environment. *Bacteriol. Rev.* 33:476–504.
- Collins, M. D., P. A. Lawson, A. Willems, J. J. Cordoba, J. Fernandez-Garayzabal, P. Garcia, J. Cai, H. Hippe, and J. A. E. Farrow. 1994. The phylogeny of the genus *Clostridium*: proposal of five new genera and eleven new species combinations. *Int. J. Syst. Bacteriol.* 44:812–826.
- Degryse, E., N. Glansdorff, and A. Pirard. 1978. A comparative analysis of extreme thermophilic bacteria belonging to the genus *Thermus*. *Arch. Microbiol.* 177:189–196.
- Demharter, W., R. Hensel, J. Smida, and E. Stackebrandt. 1989. *Sphaerobacter thermophilus* gen. nov., sp. nov. A deeply rooting member of the actinomycetes subdivision isolated from thermophilically treated sewage sludge. *Syst. Appl. Microbiol.* 11:261–266.
- Deutsche Sammlung von Mikroorganismen und Zellkulturen. 1993. DSM catalogue of strains. Deutsche Sammlung von Mikroorganismen und Zellkulturen, Braunschweig, Germany.
- Donato, M. M., E. A. Seleiro, and M. S. da Costa. 1990. Polar lipid and fatty acid composition of strains of the genus *Thermus*. *Syst. Appl. Microbiol.* 13: 234–239.
- Egge, H. 1983. Mass spectrometry of ether lipids, p. 17–47. In H. K. Mangold and F. Paltauf (ed.), *Ether lipids: biochemical and biomedical aspects*. Academic Press, Inc., New York.
- Gillis, M., J. De Ley, and M. de Cleene. 1970. The determination of molecular weight of bacterial genome DNA from renaturation rates. *Eur. J. Biochem.* 12:143–153.
- Gutell, R. R., B. Weiser, C. R. Woese, and H. F. Noller. 1985. Comparative anatomy of 16S-like ribosomal RNA. *Prog. Nucleic Acid Res. Mol. Biol.* 32: 155–216.
- Hamm, G. H., and G. N. Cameron. 1986. The EMBL data library. *Nucleic Acids Res.* 14:5–9.
- Harvey, D. J. 1982. Picolinyl esters as derivatives for the structural determination of long chain branched and unsaturated fatty acids. *Biomed. Mass Spectrom.* 9:33–38.
- Jukes, T. H., and C. R. Cantor. 1969. Evolution of protein molecules, p. 21–132. In H. N. Munro (ed.), *Mammalian protein metabolism*. Academic Press, Inc., New York.
- Karlson, U., D. F. Dwyer, S. W. Hooper, E. R. B. Moore, K. N. Timmis, and L. D. Eltis. 1993. Two independently regulated cytochromes *p*-450 in a *Rhodococcus rhodochrous* strain that degrades 2-ethoxyphenol and 4-methoxybenzoate. *J. Bacteriol.* 175:1467–1474.
- Kroppenstedt, R. M. 1985. Fatty acid and menaquinone analysis of actinomycetes and related organisms, p. 173–200. In M. Goodfellow and D. E. Minnikin (ed.), *Chemical methods in bacterial systematics*. Academic Press, Inc., London.
- Kuykendall, L. D., M. A. Roy, J. J. O'Neill, and T. E. Devine. 1988. Fatty acids, antibiotic resistance, and deoxyribonucleic acid homology groups of *Bradyrhizobium japonicum*. *Int. J. Syst. Bacteriol.* 38:358–361.
- Lane, D. J. 1991. 16S/23S sequencing, p. 115–175. In E. Stackebrandt and M. Goodfellow (ed.), *Nucleic acid techniques in bacterial systematics*. John Wiley & Sons, Chichester, United Kingdom.
- Lee, Y.-E., M. K. Jain, C. Lee, S. E. Lowe, and J. G. Zeikus. 1993. Taxonomic distinction of saccharolytic thermophilic anaerobes: description of *Thermoanaerobacterium xylanolyticum* gen. nov., sp. nov., and *Thermoanaerobacterium saccharolyticum* gen. nov., sp. nov.; reclassification of *Thermoanaerobium brockii*, *Clostridium thermosulfurogenes*, and *Clostridium thermohydrosulfuricum* E100-69 as *Thermoanaerobacter brockii* comb. nov., *Thermoanaerobacterium thermosulfurigenes* comb. nov., and *Thermoanaerobacter thermohydrosulfuricus* comb. nov., respectively; and transfer of *Clostridium thermohydrosulfuricum* 39E to *Thermoanaerobacter ethanolicus*. *Int. J. Syst. Bacteriol.* 43:41–51.
- Manaia, C. M., and M. S. da Costa. 1991. Characterization of halotolerant *Thermus* isolates from shallow marine hot springs on S. Miguel, Azores. *J. Gen. Microbiol.* 137:2643–2648.
- Marmur, J., and P. Doty. 1962. Determination of the base composition of deoxyribonucleic acid from its thermal denaturation temperature. *J. Mol. Biol.* 5:109–118.
- Minnikin, D. E., I. G. Hutchinson, and A. B. Caldicott. 1980. Thin-layer chromatography of methanolysates of mycolic acid-containing bacteria. *J. Chromatogr.* 188:221–233.
- Mullis, K. B., and F. Faloona. 1987. Specific synthesis of DNA *in vitro* via a polymerase-catalysed chain reaction. *Methods Enzymol.* 155:335–350.

23. Neess, J. M., Y. Van de Peer, P. De Rijk, S. Chapelle, and R. De Wachter. 1993. Compilation of small ribosomal subunit RNA structures. *Nucleic Acids Res.* **21**:3025–3049.
24. Olsen, G. J. 1987. The earliest phylogenetic branchings: comparing rRNA-based evolutionary trees inferred with various techniques. *Cold Spring Harbor Symp. Quant. Biol.* **52**:825–838.
25. Olsen, G. J., N. Larsen, and C. R. Woese. 1991. The Ribosomal Database Project. *Nucleic Acids Res.* **19**:2017–2021.
26. Pond, J. L., T. A. Langworthy, and G. Holzer. 1986. Long-chain diols: a new class of membrane lipids from a thermophilic bacterium. *Science* **231**:1134–1136.
27. Prado, A., M. S. da Costa, and V. M. C. Madeira. 1988. Effect of growth temperature on the lipid composition of two strains of *Thermus* sp. *J. Gen. Microbiol.* **134**:1653–1660.
28. Rainey, F. A., and E. Stackebrandt. 1993. Transfer of the type species of the genus *Thermobacteroides* to the genus *Thermoanaerobacter* as *Thermoanaerobacter acetothylacus* (Ben-Bassat and Zeikus 1981) comb. nov., description of *Coprothermobacter* gen. nov., and reclassification of *Thermobacteroides proteolyticus* as *Coprothermobacter proteolyticus* (Ollivier et al. 1985) comb. nov. *Int. J. Syst. Bacteriol.* **43**:857–859.
29. Ryhage, R., and E. Stenhagen. 1960. Mass spectrometry in lipid research. *J. Lipid Res.* **1**:361–390.
30. Saiki, R. K., D. H. Gelfand, S. Stoffel, S. J. Scharf, R. Higuchi, G. T. Horn, K. B. Mullis, and H. A. Ehrlich. 1988. Primer-directed enzymatic amplification of DNA with a thermo-stable DNA polymerase. *Science* **239**:487–491.
31. Santos, M. A., R. A. D. Williams, and M. S. da Costa. 1989. Numerical taxonomy of *Thermus* isolates from hot springs in Portugal. *Syst. Appl. Microbiol.* **12**:310–315.
32. Schleifer, K.-H., and O. Kandler. 1972. Peptidoglycan types of bacterial cell walls and their taxonomic implications. *Bacteriol. Rev.* **36**:407–477.
33. Sharp, R. J., P. W. Riley, and D. White. 1992. Heterotrophic thermophilic bacilli, p. 19–50. *In* J. K. Kristjansson (ed.), *Thermophilic bacteria*. CRC Press, Inc., London.
34. Stackebrandt, E., R. M. Kroppenstedt, K.-D. Jahnke, C. Kemmerling, and H. Gürtler. 1994. Transfer of *Streptosporangium viridogriseum* (Okuda et al. 1966), *Streptosporangium viridogriseum* subsp. *kofuense* (Nonomura and Ohara 1969), and *Streptosporangium albidum* (Furamai et al. 1968) to *Kutzneria* gen. nov. as *Kutzneria viridogrisea* comb. nov., *Kutzneria kofuensis* comb. nov., and *Kutzneria albida* comb. nov., respectively, and emendation of the genus *Streptosporangium*. *Int. J. Syst. Bacteriol.* **44**:265–269.
35. Suzuki, K., M. D. Collins, E. Iijima, and K. Komagata. 1988. Chemotaxonomic characterization of a radiotolerant bacterium, *Arthrobacter radiotolerans*: description of *Rubrobacter radiotolerans* gen. nov., comb. nov. *FEMS Microbiol. Lett.* **52**:33–40.
36. Tindall, B. J. 1989. Fully saturated menaquinones in the archaeobacterium *Pyrobaculum islandicum*. *FEMS Microbiol. Lett.* **60**:251–254.
- 36a. Tindall, B. J. Personal communication.
37. Vainshtein, M., H. Hippe, and R. M. Kroppenstedt. 1992. Cellular fatty acid composition of *Desulfovibrio* species and its use in classification of sulfate-reducing bacteria. *Syst. Appl. Microbiol.* **15**:554–566.
38. Van de Peer, Y., I. Van den Broeck, P. de Rijk, and R. de Wachter. 1994. Database on the structure of small ribosomal subunit RNA. *Nucleic Acids Res.* **22**:3488–3494.
39. Wait, R., and M. J. Hudson. 1985. The use of picolinyl esters for the characterization of microbial lipids; application to *Campylobacter* species. *Lett. Appl. Microbiol.* **1**:95–99.
40. Wiegel, J. 1992. The anaerobic thermophilic bacteria, p. 105–184. *In* J. K. Kristjansson (ed.), *Thermophilic bacteria*. CRC Press, Inc., London.
41. Williams, R. A. D., and M. S. da Costa. 1992. The genus *Thermus* and related microorganisms, p. 3745–3753. *In* A. Balows, H. G. Trüper, M. Dworkin, W. Harder, and K. H. Schleifer (ed.), *The prokaryotes*, 2nd ed. Springer-Verlag, New York.
42. Wilson, K. 1987. Preparation of genomic DNA from bacteria, p. 241–242. *In* F. M. Ausubel, R. Brent, R. E. Kingston, D. D. Moore, J. G. Seidman, J. A. Smith, and K. Struhl (ed.), *Current protocols in molecular biology*. John Wiley & Sons, Inc., New York.
43. Woese, C. R., R. Gutell, R. Gupta, and H. F. Noller. 1983. Detailed analysis of the higher-order structure of 16S-like ribosomal ribonucleic acids. *Microbiol. Rev.* **47**:621–669.
44. Yoshinaka, T., K. Yano, and H. Yamaguchi. 1973. Isolation of a highly radioresistant bacterium, *Arthrobacter radiotolerans* nov. sp. *Agric. Biol. Chem.* **37**:2269–2275.