Geodermatophilus telluris sp. nov., an actinomycete isolated from Saharan desert sand

Maria del Carmen Montero-Calasanz, 1,2 Markus Göker, 1 Gabriele Pötter, 1 Manfred Rohde, 3 Cathrin Spröer, 1 Peter Schumann, 1 Hans-Peter Klenk 1 and Anna A. Gorbushina 4

1Leibniz Institute DSMZ – German Collection of Microorganisms and Cell Cultures, Inhoffenstraße 7B, 38124 Braunschweig, Germany
2IFAPA – Instituto de Investigación y Formación Agraria y Pesquera, Centro Las Torres-Tomejil, Ctra. Sevilla-Cazalla de la Sierra, Km 12.2, 41200 Alcalá del Río, Seville, Spain
3HZI – Helmholtz Centre for Infection Research, Inhoffenstraße 7, 38124 Braunschweig, Germany
4BAM – Federal Institute for Material Research and Testing, 12205 Berlin, Germany
5Free University of Berlin, Department of Biology, Chemistry and Pharmacy and Department of Earth Sciences, 12249 Berlin, Germany

A novel Gram-positive, multiloculated thalli-forming, aerobic, actinobacterial strain, CF9/1/1 T, was isolated in 2007 during environmental screening for xerophilic fungi in arid desert soil from the Sahara desert, Chad. The isolate grew best at a temperature range of 20–35 °C and at pH 6.0–8.5 and with 0–4% (w/v) NaCl, forming black-coloured and irregular colonies on GYM agar. Chemotaxonomic and molecular characteristics of the isolate matched those described for members of the genus Geodermatophilus. The DNA G + C content of the novel strain was 75.4 mol%. The peptidoglycan contained meso-diaminopimelic acid as a diagnostic diamino acid. The main phospholipids were diphosphatidylglycerol, phosphatidylcholine, phosphatidylethanolamine, phosphatidylglycerol, a not yet structurally identified aminophospholipid and a small amount of phosphatidylinositol; MK-9(H4) was identified as the dominant menaquinone and galactose was a diagnostic sugar. The major cellular fatty acids were branched-chain saturated acids: iso-C16 : 0 and iso-C15 : 0. The 16S rRNA gene sequence of the isolate showed 94.6–97.0% sequence similarities with those of five members of the genus: Geodermatophilus ruber DSM 45317 T (94.6%), Geodermatophilus obscurus DSM 43160 T (94.8%), Geodermatophilus siccatus DSM 45419 T (96.2%), Geodermatophilus nigrescens DSM 45408 T (96.7%) and Geodermatophilus arenarius DSM 45418 T (97.0%). Based on the evidence from this polyphasic taxonomic study, a novel species, Geodermatophilus telluris sp. nov., is proposed; the type strain is CF9/1/1 T (= DSM 45421 T = CCUG 62764 T).

Although Geodermatophilus obscurus, the type species of the genus Geodermatophilus (= ‘earth Dermatophilus’) was described 44 years ago by George M. Luedemann (Luedemann, 1968), the family name Geodermatophilaceae was first proposed (but at that time not validly published) by Normand et al. (1996). The name was validly published a decade later by Normand (2006). In addition to the type genus Geodermatophilus, the Geodermatophilaceae also contains the genera Blastococcus and Modestobacter. Sharing the harsh conditions of low availability of water and nutrients, these two genera are mainly known as inhabitants of rock surfaces, whereas Geodermatophilus prefers arid soils as natural habitats (Urzi et al., 2001). The family is rather poorly sampled and studied, with only one type-strain genome sequenced, that of G. obscurus (Ivanova et al., 2010), and a total, at the time of writing of only ten species with validly published names. In addition to the type species G. obscurus, the genus Geodermatophilus currently contains the following species with validly published names: Geodermatophilus ruber (Zhang et al., 2011), Geodermatophilus nigrescens (Nie et al., 2012; Euzéby, 2012), Geodermatophilus arenarius (Montero-Calasanz et al., 2012; Euzéby, 2013a) and Geodermatophilus siccatus (Montero-Calasanz et al., 2013; Euzéby, 2013b). An analysis of 16S rRNA reference sequences using the
Greengenes database (DeSantis et al., 2006) and latest metagenomic studies on dust originating from deserts (Giongo et al., 2013) revealed the existence of several more isolates and as yet uncultured phylotypes in soil and on rock surfaces (for an overview see Ivanova et al., 2010; Urzi et al., 2001). The novel organism described in this report represents a genomically distinct novel lineage from a screening of Saharan desert sand that falls into the genus *Geodermatophilus* in 16S rRNA-based phylogenies.

Representative sand samples were taken in the Saharan desert of the Republic of Chad near to Vers Ourba and axenic cultures of xerophiles were isolated from these samples with classical enrichment procedures (Giongo et al., 2013). Portions of sand were suspended in physiological saline, shaked for one hour at 26 °C and kept overnight at 4 °C and then again shaken for two hours before being streaked out on R2A and TSA plates and incubated at 25 °C for 3–10 days (for details see Giongo et al., 2013). Purified isolates were stored in Microbank Blue Colour Beads (Pro-Lab Diagnostics) before accessions into the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ) open collection. To determine their morphological characteristics, strain CF9/1/1T was cultivated on trypticase soy broth agar (DSMZ medium 535) and GYM *Streptomyces* medium (DSMZ medium 65). The colony features were observed under a binocular microscope according to the protocol of Pelczar (1957). Exponentially growing bacterial cultures were observed with an optical microscope (AxioScope A1, Zeiss) with ×100 magnification and phase-contrast illumination. Micrographs of bacterial cells were taken with a field-emission scanning electron microscope (FE-SEM Merlin, 2015). Catalase activity was tested by the observation of bubbles following the addition of drops of 3 % H₂O₂. Activity of oxidase was analysed using filter-paper disks (Sartorius grade 388) impregnated with 1 % solution of N,N,N',N'-tetramethyl-p-phenylenediamine (Sigma–Aldrich); a positive test was indicated by the development of a blue-purple colour after applying biomass on the filter paper. Whole-cell amino acids and sugars were extracted with methanol as described by Collins et al. (1977) and analysed by HPLC (Kroppenstedt, 1982). For the extraction of peptidoglycan hydrolysates (6 M HCl, 100 °C for 16 h) was examined by TLC according to the protocol of Schleifer & Kandler (1972). All physiological tests were performed at 28 °C using *G. obscurus* G-20° (DSM 43160T), *G. ruber* CPC2 201356T (DSM 45317T), *G. nigrescens* YIM 75980T (DSM 45408T), *G. arenarius* CF5/4T (DSM 45418T) and *G. siccatus* CF6/1T (DSM 45419T) in parallel assays. The exported measurement data were further analysed with the omop package for R (Vaas et al., 2012), using its functionality for merging subsequent measurements of the same plate, statistically estimating parameters from the respiration curves, such as the maximum height, and automatically discretizing these values into negative, weak and positive reactions, respectively. Each strain was studied in two independent repetitions (yielding a total of six recorded runs per strain), and reactions with a distinct behaviour between the two repetitions were regarded as ambiguous. Enzyme activities were tested using API ZYM galleries according to the instructions of the manufacturer (bioMérieux). Further biochemical tests were performed as described by Tindall et al. (2007), including cellulase activity (15.2.18) and methyl red and Voges–Proskauer reactions (15.2.52 and 15.2.82). Whole-cell amino acids and sugars were prepared according to the protocol of Lechevalier & Lechevalier (1970) and analysed by TLC (Staneck & Roberts, 1974). Polaris lipids were extracted, separated by two-dimensional TLC and identified according to the method of Minnikin et al. (1984) as modified by Kroppenstedt & Goodfellow (2006). For the extraction of menaquinones, freeze-dried cell material was extracted with methanol as described by Collins et al. (1977) and analysed by HPLC (Kroppenstedt, 1982). For the extraction and analysis of cellular fatty acids, the physiological age of the strains was standardized by always choosing the same sector (the last quadrant streak) on Gym agar plates held at 28 °C (four days). Analysis was conducted using the Microbial Identification System (MIDI) Sherlock Version 4.5 (method TSBA40, TSBA6 database) as described by Sasser (1990). The composition of peptidoglycan hydrolysates (6 M HCl, 100 °C for 16 h) was examined by TLC according to the protocol of Schleifer & Kandler (1972). All physiological tests were performed at 28 °C using *G. obscurus* G-20° (=DSM 43160T), *G. ruber* CPC2 201356T (=DSM 45317T), *G. nigrescens* YIM 75980T (=DSM 45408T), *G. arenarius* CF5/4T (=DSM 45418T) and *G. siccatus* CF6/1T (=DSM 45419T) in parallel assays. The G+C content of the chromosomal DNA was determined by...
HPLC according to the protocol of Mesbah et al. (1989). Genomic DNA extraction, PCR-mediated amplification of the 16S rRNA gene and purification of the PCR product was carried out as described by Rainey et al. (1996). Phylogenetic analysis was based on an alignment inferred with POA version 2.0 (Lee et al., 2002) and filtered with GBLOCKS (Castresana, 2000). Phylogenetic trees were inferred under maximum-likelihood and maximum-parisimony as optimality criteria using RAxML version 7.2.8 (Stamatakis et al., 2008) and PAUP* 4b10 (Swofford, 2002), respectively. Bootstrap support values were calculated using the bootstrapping criterion (Pattengale et al., 2009) as implemented in RAxML and 1000 replicates in the case of PAUP*. Rooting was done using the midpoint method (Hess & De Moraes Russo, 2007) and then checked for its agreement with the classification. Pairwise similarities were calculated from exact pairwise sequence alignments using the Smith–Waterman algorithm as implemented in the EMBOSS suite (Rice et al., 2000). To assess the occurrence of the novel strain in environmental samples, the 16S rRNA sequence was compared using NCBI BLAST (Altschul et al., 1990) under default settings (e.g. considering only the high-scoring segment pairs from the best 250 hits) with the most recent release of the Greengenes database (DeSantis et al., 2006) and the relative frequencies of taxa and environmental samples were determined, weighted by BLAST scores (Ivanova et al., 2010). DNA–DNA hybridization tests were performed as described by De Ley et al. (1970) with the modifications suggested by Huss et al. (1983) using a Cary 100 Bio UV/VIS instrument.

Cells of strain CF9/1/1T were cuboidal and Gram-positive. They were observed forming dimers, tetrads and higher aggregates (Fig. 1). The motile zoospores were elliptical; septated filaments from zoospore germination were observed. The colonies were black-coloured, irregular, multicellular and opaque with a dry surface and an irregular margin. Strain CF9/1/1T grew best at 20–35 °C; no growth was observed below 15 °C and above 40 °C. Growth was observed in the presence of 0–4% NaCl but not 8% NaCl and pH 6.0–8.5. More details about the strain’s phenotypic features are presented in Table 1 in comparison with the other species of the genus Geodermatophilus; see also Fig. S1 (available in IJSEM Online) for the heatmap obtained from the OmniLog phenotyping results. Analysis of cell-wall components revealed the presence of meso-diaminopimelic acid (meso-DAP), which is in line with the other species of the genus Geodermatophilus (Montero–Calasanz et al., 2013), whose cell-wall type is type III (Lechevalier & Lechevalier, 1970). Strain CF9/1/1T displayed MK-9(H4) (84.7%) as the dominant menaquinone, complemented by small amounts of MK-9 (4.1%), MK-8(H4) (3.6%) and an unknown MK (3.6%). The major fatty acids were the saturated branched-chain acids iso-C16:0 (44.8%), iso-C15:0 (21.4%). The phospholipid pattern consisted of diphosphatidylglycerol (DPG), phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylinositol (PI), an unknown aminophospholipid (PN) and a small amount of phosphatidylglycerol (PG) (Fig. 2). Whole-cell sugar analysis revealed galactose as a diagnostic sugar, complemented by glucose and traces of ribose and mannose (Lechevalier & Lechevalier, 1970). The DNA G+C content was 75.4 mol%.

The almost complete (1528 bp) 16S rRNA gene sequence of strain CF9/1/1T was determined. The 16S rRNA sequence of the G. arenarius DSM 45418T was the most similar sequence (97.0%) within the members of the genus Geodermatophilus; G. ruber DSM 45317T (94.6%), G. obscurus DSM 43160T (94.8%), G. siccatus DSM 45419T (96.2%) and G. nigrescens DSM 45408T (96.7%). Both maximum-likelihood and maximum-parsimony phylogenies placed CF9/1/1T within a group also containing the type strains of G. arenarius and G. nigrescens with high support (99%/100%) (Fig. 2). The 16S rRNA analysis thus leaves little doubt that the novel strain belongs to the genus Geodermatophilus and forms a species of its own. However, the degree of 16S rRNA gene sequence difference of strain CF9/1/1T from the type strain of G. arenarius (which is also the sister group of the novel strain in Fig. 2) indicated the need to prove the genomic distinctness of the type strain representing the novel species by DNA–DNA hybridizations. Strain CF9/1/1T displayed a DNA–DNA relatedness of 34.3 ± 0.7% with G. arenarius, this value being far below the threshold value of 70% recommended by Wayne et al. (1987) for a decision on the species status of novel strains.

The Greengenes analysis indicated that sequences of this taxon have not been detected as yet in environmental samples.

Several phenotypic characteristics apart from the phylogenetic analysis based on 16S rRNA gene sequences also support the distinctiveness of strain CF9/1/1T from all other species of the genus Geodermatophilus (see Table 1). Based on the phenotypic and genotypic data presented above, we propose that strain CF9/1/1T represents a novel species within the genus Geodermatophilus, with the name Geodermatophilus telluris sp. nov.
Table 1. Differential phenotypic characteristics of strain CF9/1/1T and the type strains of the other species of the genus Geodermatophilus

Strains: 1. *G. telluris* sp. nov. CF9/1/1T (=DSM 45421T); 2. *G. obscurus* G-20T (=DSM 43160T); 3. *G. ruber* CPCC 201356T (=DSM 45317T); 4. *G. nigrescens* YIM 75980T (=DSM 45408T); 5. *G. arenarius* CF5/4T (=DSM 45418T); 6. *G. siccatus* CF6/1T (=DSM 45419T). All data are from this study. +, Positive reaction; −, negative reaction; +/−, ambiguous; MK, menaquinones. i-, iso-branched, ai-, anteiso-branched; DPG, diphosphatidylglycerol; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PC, phosphatidylcholine; PI, phosphatidylinositol; PL, unknown phospholipid; PN, unknown aminophospholipid; PGL, unknown phosphoglycolipid.

<table>
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<th>Characteristic</th>
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<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
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<tr>
<td>Colony colour on GYM</td>
<td>Black</td>
<td>Black</td>
<td>Light-red, red</td>
<td>Light-red, black</td>
<td>Light-red, brown</td>
<td>Light-red, black</td>
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<tr>
<td>Colony surface on GYM</td>
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<td>Dry</td>
<td>Moist</td>
<td>Moist</td>
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<td>Moist</td>
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<td>Nitrate reduction</td>
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<td>−</td>
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<td>Degradation of:</td>
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<td>Starch</td>
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<td>+</td>
<td>−</td>
<td>+</td>
<td>−</td>
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<td>Growth at pH 5</td>
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<td>+</td>
<td>+</td>
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<td>D-Salicy</td>
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<td>Oxidase activity</td>
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<td>−</td>
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<td>Catalase activity</td>
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<td>−</td>
<td>−</td>
<td>+</td>
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<td>Predominant menaquinone(s)*</td>
<td>MK-9(H4), MK-9(H4), MK-9(H2), MK-9(H2), MK-9(H4), MK-9(H4), MK-8(H4), MK-9(H4), MK-8(H4)</td>
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<tr>
<td>Major fatty acids†</td>
<td>i-C15:0, i-C16:0, C17:1ω8c</td>
<td>i-C15:0, i-C16:0, C17:1ω8c, ai-C15:0</td>
<td>i-C15:0, i-C16:0, C17:1ω8c, ai-C15:0</td>
<td>i-C15:0, i-C16:0, ai-C15:0</td>
<td>i-C15:0, i-C16:0, ai-C15:0</td>
<td>i-C15:0, i-C16:0, C17:1ω8c</td>
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</table>

*Only components with ≥5 % peak area ratio are shown.
† Only components making up ≥10 % peak area ratio are shown.
Description of *Geodermatophilus telluris* sp. nov.

*Geodermatophilus telluris* (tel.lu ris. L. gen. n. *telluris* of the soil or earth, referring to the source of organism).

Colonies are black-coloured, multilocalized, of irregular shape, with a dry surface and an irregular margin. Cells are Gram-positive, catalase and oxidase-negative. No diffusible pigments are produced on any medium tested. Utilizes dextrin, maltose, trehalose, cellobiose, sucrose, turanose, D-glucose, D-galactoside, D-galacturonic acid, L-galactonic acid-1, D-gluconic acid, myo-inositol, L-arginine, L-lysine, L-histidine, L-pyroglutamic acid, L-serine, D-serine, inosine, L-aspartic acid, L-glutamic acid, lactic acid, acetic acid, propionic acid, L-glutamic acid, L-proline, L-alanine, L-histidine, L-pyroglutamic acid, p-hydroxyphenylactic acid, bromosuccinic acid, L-α-hydroxybutyric acid, and DL-malic acid as sole carbon source for energy and growth, but not glycyl-L-proline, L-alanine, L-histidine, L-pyroglutamic acid, D-serine, L-serine, D-serine, and threonine. The predominant menaquinone is MK-9(H4). The main phospholipids are diphasphatidylglycerol, phosphatidylethanolamine, phosphatidylinositol and an unknown amino-phospholipid as well as a trace of phosphatidylglycerol. Cellular fatty acids consist mainly of branched-chain saturated acids: iso-C16:0 and iso-C15:0.

The type strain, CF9/1/1<sup>T</sup> (=DSM 45421<sup>T</sup> = CCUG 62764<sup>T</sup>), was isolated in 2007 from sand of the Saharan desert collected near Vers Ourba, Republic of Chad. The type strain has a genomic DNA G+C content of 75.4 mol%.

**Acknowledgements**

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