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

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A novel Gram-positive, multiloculated thalli-forming, aerobic, actinobacterial strain, CF9/1/1T, 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, phosphatidylinositol, a not yet structurally identified aminophospholipid and a small amount of phosphatidylglycerol; 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 45317T (94.6 %), *Geodermatophilus obscurus* DSM 43160T (94.8 %), *Geodermatophilus siccatus* DSM 45419T (96.2 %), *Geodermatophilus nigrescens* DSM 45408T (96.7 %) and *Geodermatophilus arenarius* DSM 45418T (97.0 %). Based on the evidence from this polyphasic taxonomic study, a novel species, *Geodermatophilus telluris* sp. nov., is proposed; the type strain is CF9/1/1T (=DSM 45421T=CCUG 62764T).

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 (Urzı", 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; Ůrţi 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 Ourbâ 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, shaken 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 accession into the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ) open collection. To determine their morphological characteristics, strain CF9/1/1 T 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, Zeiss). Gram reaction was performed using the KOH test (Beams & Ward, 1991).

Microplates were inoculated with a cell suspension made in physiological saline, shaken 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). 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. Catalase activity was tested by the observation of bubbles following the addition of drops of 3 % H2O2.

Growth rates were determined on plates of GYM medium for temperatures from 10 to 50 °C in steps of 5 °C and for pH values 5.0 to 9.0 (in steps of 0.5 pH units) on modified ISP2 medium (Shirling & Gottlieb, 1966) by adding NaOH or HCl, respectively, because the use of a buffer system inhibited growth of the cultures. Degradation of specific substrates was examined using agar plates with various basal media, considering the appearance of clear zones around the colonies as a positive result: casein degradation was tested on plates containing milk powder (5 % w/v), NaCl (0.5 %) and agarose (1 %); tyrosine degradation was investigated as previously described (Gordon & Smith, 1955) on plates containing peptone (0.5 %), beef extract (0.3 %), L-tyrosine (0.5 %) and agarose (1.5 %); the decomposition of xanthine and hypoxanthine was tested by the same test, replacing L-tyrosine with hypoxanthine or xanthine (0.4 %); starch degradation was tested on plates containing nutrient broth (0.8 %), starch (1 %) and agarose (1.5 %), these plates were developed by flooding with iodine solution (1 %). The utilization of carbon compounds and acid production were determined using API 20 NE strips (bioMérieux) and GEN III Microplates in an OmniLog device (Biolog). The GEN III Microplates were inoculated with a cell suspension made in a ‘gelling’ inoculating fluid (IF) at a cell density of 80–83 % T, except for G. arenarius plates that were filled to a cell density of 90 % T. As the cultures were respiring (and growing) comparatively slowly, each plate was measured in three subsequent runs by restarting the OmniLog device twice, yielding a total running time of 10 days in phenotype microarray mode at 28 °C. The exported measurement data were further analysed with the omp 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). Polar 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 T (=DSM 43160 T), G. ruber CPCC 201356 T (=DSM 45317 T), G. nigrescens YIM 75980 T (=DSM 45408 T), G. arenarius CF5/4 T (=DSM 45418 T) and G. siccatus CF6/1 T (=DSM 45419 T) 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-parsimony as optimality criteria using RAxML version 7.2.8 (Stamatakis, 2006) and PAUP* 4b10 (Swofford, 2002), respectively. Bootstrap support values were calculated using the bootstrapping criterion (Pattengale et al., 2008) and PAUP* 4b10 (Swofford, 2002), respectively. 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, multiocular 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 (Montoro–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. S2). 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 CF9/1/1<sup>T</sup> and the type strains of the other species of the genus *Geodermatophilus*

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<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>
<td>Dry</td>
<td>Dry</td>
<td>Moist</td>
<td>Moist</td>
<td>Moist</td>
<td>Moist</td>
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<td>Nitrate reduction</td>
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<td>−</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>+</td>
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<td>Degradation of:</td>
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<tr>
<td>Starch</td>
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<td>+</td>
<td>−</td>
<td>+</td>
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<td>Growth at pH 5</td>
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<td>+</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>−</td>
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<td>Utilization of:</td>
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<td>Gentiobiose</td>
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<td>+/−</td>
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<td>D-Salolin</td>
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<td>+/−</td>
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<td>L-Fucose</td>
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<td>Inosine</td>
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<td>−</td>
<td>−</td>
<td>+</td>
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<tr>
<td>D-Arabitol</td>
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<td>+</td>
<td>−</td>
<td>+/−</td>
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<td>myo-Inositol</td>
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<td>+</td>
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<td>Quinic acid</td>
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<td>+</td>
<td>−</td>
<td>−</td>
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<td>Oxidase activity</td>
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<td>+</td>
<td>+</td>
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<td>−</td>
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<td>Catalase activity</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>+</td>
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<td>+</td>
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<tr>
<td>Predominant menaquinone(s)*</td>
<td>MK-9(H&lt;sub&gt;4&lt;/sub&gt;)</td>
<td>MK-9(H&lt;sub&gt;4&lt;/sub&gt;), MK-9(H&lt;sub&gt;2&lt;/sub&gt;)</td>
<td>MK-9(H&lt;sub&gt;4&lt;/sub&gt;)</td>
<td>MK-9(H&lt;sub&gt;4&lt;/sub&gt;)</td>
<td>MK-9(H&lt;sub&gt;4&lt;/sub&gt;), MK-9(H&lt;sub&gt;0&lt;/sub&gt;)</td>
<td>MK-9(H&lt;sub&gt;4&lt;/sub&gt;), MK-9(H&lt;sub&gt;0&lt;/sub&gt;)</td>
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<tr>
<td>Polar lipids</td>
<td>DPG, PE, PG, PC, PI, PN</td>
<td>DPG, PE, PG, PC, PI, PN</td>
<td>DPG, PE, PG, PC, PI, PN</td>
<td>DPG, PE, PG, PC, PI, PN</td>
<td>DPG, PE, PG, PC, PI, PN</td>
<td>DPG, PE, PG, PC, PI, PN</td>
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<tr>
<td>Major fatty acids†</td>
<td>i-C&lt;sub&gt;15:0&lt;/sub&gt;, i-C&lt;sub&gt;16:0&lt;/sub&gt;</td>
<td>i-C&lt;sub&gt;15:0&lt;/sub&gt;, i-C&lt;sub&gt;16:0&lt;/sub&gt;, C&lt;sub&gt;17:1偶校键&lt;/sub&gt;</td>
<td>i-C&lt;sub&gt;15:0&lt;/sub&gt;, i-C&lt;sub&gt;16:0&lt;/sub&gt;, C&lt;sub&gt;17:1偶校键&lt;/sub&gt;</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.1u ris. L. gen. n. telluris of the soil or earth, referring to the source of organism).

Colonies are black-coloured, multilobated, of irregular shape, with a dry surface and an irregular margin. Cells are Gram-positive, catalase and oxidase-negative. No diffusible compound. The predominant menaquinone is MK-9(H4). As a diamino acid, with galactose as a diagnostic sugar. The main phospholipids are diphosphatidylglycerol, phosphatidylglycerol, phosphatidylylcholine, 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, CF9/1/1T (=DSM 45421T=CCUG 62764T), 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%.

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References


Geodermatophilus telluris sp. nov.

Greengenes, a chimera-checked 16S rRNA gene database and workbench compatible with ARB. *Appl Environ Microbiol* 72, 5069–5072.


