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 (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 phyotypes 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, shaken for one hour at 26 °C and then 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 CF9/1/1T 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, Zeiss). Gram reaction was performed using the KOH test described by Gregersen (1978). The motility of the cells was observed on modified ISP2 (Shirling & Gottlieb, 1966) swarming agar (0.3 %, w/v) at pH 7.2 that contained (l-lysine (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 opm 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-20T (=DSM 43160T), G. ruber CPCC 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
Cells of strain CF9/1/1^T 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 CF9/1/1^T 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-Calahasan et al., 2013), whose cell-wall type is type III (Lechevalier & Lechevalier, 1970). Strain CF9/1/1^T 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 diphasphatidylglycerol (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 CF9/1/1^T was determined. The 16S rRNA sequence of the *G. arenarius* DSM 45418^T was the most similar sequence (97.0 %) within the members of the genus *Geodermatophilus*; *G. ruber* DSM 45317^T (94.6 %), *G. obscurus* DSM 4316^T (94.8 %), *G. sicatius* DSM 45419^T (96.2 %) and *G. nigrescens* DSM 45408^T (96.7 %). Both maximum-likelihood and maximum-parsimony phylogenies placed CF9/1/1^T 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 CF9/1/1^T 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 CF9/1/1^T 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 CF9/1/1^T from all other species of the genus *Geodermatophilus*; see Table 1. Based on the phenotypic and genotypic data presented above, we propose that strain CF9/1/1^T represents a novel species within the genus *Geodermatophilus*, with the name *G. 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*

<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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<tbody>
<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>
<td>Moist</td>
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<tr>
<td>Nitrate reduction</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>+</td>
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<tr>
<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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<tr>
<td>Growth at pH 5</td>
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<td>+</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>−</td>
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<tr>
<td>Utilization of:</td>
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<tr>
<td>Gentiobiose</td>
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<td>+/−</td>
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<td>−</td>
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<td>+/−</td>
<td>−</td>
<td>+</td>
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<td>−</td>
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<tr>
<td>l-Fucose</td>
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<td>+/−</td>
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<td>l-Rhamnose</td>
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<td>−</td>
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<td>Inosine</td>
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<td>d-Sorbitol</td>
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<td>Quinic acid</td>
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<td>Oxidase activity</td>
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<td>−</td>
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<tr>
<td>Catalase activity</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Predominant menaquinone(s)*</td>
<td>MK-9(H4)</td>
<td>MK-9(H4), MK-9(H2), two MK</td>
<td>MK-9(H4)</td>
<td>MK-9(H4)</td>
<td>MK-9(H4), MK-9(H0), MK-8(H4)</td>
<td>MK-9(H4), MK-9(H0), MK-8(H4)</td>
</tr>
<tr>
<td>Major fatty acids†</td>
<td>i-C_{15:0}, i-C_{16:0}</td>
<td>i-C_{15:0}, i-C_{16:0}, C_{17:1}ω8c</td>
<td>i-C_{15:0}, i-C_{16:0}, C_{17:1}ω8c, ai-C_{15:0}</td>
<td>i-C_{15:0}, i-C_{16:0}, C_{17:1}ω8c, ai-C_{15:0}</td>
<td>i-C_{15:0}, i-C_{16:0}, C_{17:1}ω8c, ai-C_{15:0}</td>
<td>i-C_{15:0}, i-C_{16:0}, C_{17:1}ω8c</td>
</tr>
</tbody>
</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, multiloculated, 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 L-histidine, L-pyroglutamic acid, D-serine, inosine, D-galactoside, inosine, glycerol, L-arginine, pectin, D-gluconic acid, -D-glucuronic acid, D-mannose, D-fructose, D-galactose, L-serine, D-arabitol, D-sorbitol, D-mannitol, glycerol, L-arginine, pectin, D-glucosic acid, -triazine, D-glucuronic acid, D-galactoside, D-glucosamidase, D-glucosidase, D-mannosidase and D-fucosidase. NaCl tolerance ranges from 0 to 4% (w/v). Cell growth temperature and pH ranges are from 15 to 40 °C and pH 6.0 to 8.5, respectively. The peptidoglycan in the cell wall contains meso-diaminopimelic acid as a diamino acid, with galactose as a diagnostic sugar compound. The predominant menaquinone is MK-9(H4). The main phospholipids are diphosphatidylglycerol, phosphatidylcholine, phosphatidyethanolamine, 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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