Geodermatophilus normandii sp. nov., isolated from Saharan desert sand

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A novel Gram-reaction-positive actinobacterial strain, designated CF5/3T, was isolated from a sand sample obtained in the Sahara Desert, Chad. The greenish-black-pigmented isolate was aerobic and exhibited optimal growth from 25–40 °C at pH 6.0–10.0 with 0–1 % (w/v) NaCl. Chemotaxonomic and molecular characteristics of the isolate matched those described for members of the genus Geodermatophilus. The DNA G+C content of the genome of the novel strain was 75.5 mol%. The peptidoglycan contained meso-diaminopimelic acid as diagnostic diamino acid. The main phospholipids were diphosphatidylglycerol, phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol and a minor fraction of phosphatidylglycerol. MK-9(H4) was the dominant menaquinone, and galactose was detected as a diagnostic sugar. The major cellular fatty acids were branched-chain saturated acids: iso-C15:0 and iso-C16:0. Analysis of 16S rRNA gene sequences showed 95.6–98.8 % pairwise sequence identity with the type strains of Geodermatophilus species. The isolate represents a novel species, Geodermatophilus normandii, with the type strain CF5/3T (=DSM 45417T=CCUG 62814T=MTCC 11412T).

The family Geodermatophilaceae was originally proposed by Normand et al. (1996), but a formal description of the family name was only published a decade later (Normand, 2006). At the time of writing, the family comprises the genera Blastococcus, Modestobacter and Geodermatophilus (type genus). The genus Geodermatophilus was first proposed by Luedemann (1968) and the name was accepted in the Approved Lists of Bacterial Names by Skerman et al. (1980). Members of this genus are frequently isolated from arid soils (Urzi et al., 2001), although some have also been isolated from rhizosphere soil (Zhang et al., 2011; Jin et al., 2012). Nevertheless, this genus was for a long time poorly studied and sampled due to challenges in culturing (Urzi et al., 2004).

Ten named species have been classified in the genus Geodermatophilus: G. obscurus (Luedemann, 1968), G. ruber (Zhang et al., 2011), G. nigrescens (Nie et al., 2012), G. arenarius (Montero-Calasanz et al., 2012, 2013b), G. siccatus (Montero-Calasanz et al., 2013f, g), G. saharensis (Montero-Calasanz et al., 2013c, d), G. telluris (Montero-Calasanz et al., 2013e), ‘G. tzadiensis’ (Montero-Calasanz et al., 2013a), G. soli and G. terrae (Jin et al., 2013). The genome of only one type strain, that of G. obscurus, has been sequenced (Ivanova et al., 2010). Moreover, three named subspecies have been identified: ‘G. obscurus subsp. amargosae’, ‘G. obscurus subsp. utahensis’ and ‘G. obscurus subsp. dictysporus’ (Luedemann, 1968). This study describes the taxonomic position of a novel species in the genus Geodermatophilus based on a polyphasic approach.

During an environmental screening of arid soils in the Sahara Desert (Republic of Chad) in 2007, representative sand samples were collected near Oure Cassoni. Portions of sand were suspended in physiological saline, shaken for 1 h
at 26 °C and kept overnight at 4 °C, then shaken for an additional 2 h before being streaked out on R2A (DSMZ medium 830) and trypticase soy broth (TSB; DSMZ medium 535) plates and incubated at 25 °C for 3–10 days (for details see Giongo et al., 2013). Purified strain CF5/3T was stored in Microbank Blue Colour Beads (Pro-Lab Diagnostics) before accession into the DSMZ open collection. Cultural characteristics were tested on the following media (http://www.dsmz.de/?id=441) for 15 days: GYM Streptomyces (DSMZ medium 65), TSB agar, GPHF (DSMZ medium 553), R2A, GEO (DSMZ medium 714), PYGV (DSMZ medium 621) and Luedemann (DSMZ medium 877). To determine morphological characteristics, strain CF5/3T was cultivated on TSB agar and GYM Streptomyces medium. Colony features were observed at 4 and 15 days under a binocular microscope according to Pelczar (1957). Exponentially growing bacterial cultures were observed with an optical microscope (AxioScope A1, Zeiss) with a 100-fold magnification and phase-contrast illumination. Micrographs of bacterial cells grown on GYM Streptomyces broth for 4 days were taken with a field-emission scanning electron microscope (Merlin, Zeiss). Gram reaction was performed using the KOH test as described by Gregersen (1978). Cell motility was observed on modified ISP2 (Shirling & Gottlieb, 1966) swarming agar (0.3 %, w/v) at pH 7.2 that contained (1−1) 4.0 g dextrin, 4.0 g yeast extract and 10.0 g malt extract. Oxidase activity was analysed using filter paper discs (Sartorius grade 388) soaked in a 1 % solution of N,N,N,N′-tetratethyl-p-phenylenediamine (Sigma-Aldrich); a positive test was defined by the development of a blue–purple colour after applying biomass on the filter paper. Catalase activity was determined based on formation of bubbles following the addition of 3 % H2O2 (1 drop). Growth rates were determined on plates of GYM medium at temperatures from 10–50 °C in 5 °C increments and at pH 4.0–12.5 (in increments of 0.5 pH units) on modified ISP2 medium (Shirling & Gottlieb, 1966) by adding NaOH or HCl, since the use of a buffer system inhibited growth of the cultures. Degradation of specific substrates was examined using agar plates with various basal media: casein degradation was tested on plates containing milk powder (5 %, w/v), NaCl (0.5 %) and agarose (1 %); tyrosine degradation was investigated as described by Gordon & Smith (1955) on plates containing peptone (0.5 %), beef extract (0.3 %), L-tyrosine (0.5 %) and agarose (1.5 %); xanthine and hypoxanthine decomposition 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 %) then developed by flooding with 1 % iodine solution. For all tests, a positive result was defined by the appearance of clear zones around the colonies. Utilization of carbon compounds and production of acid were tested using API 20 NE strips (bioMérieux) and GEN III Microplates in an OmniLog device (Biolog). The GEN III Microplates were inoculated with cells suspended in the viscous inoculating fluid (IF C) provided by the manufacturer at a cell density of 80–83 % T, with the exception of G. arenarius, which was inoculated at a cell density of 90 % T. As growth rates were relatively slow, 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. Data were exported and analysed using the opm package for R (Vaas et al., 2012). Each strain was studied in two independent experiments, yielding a total of six recorded runs per strain. Reactions with a distinct behaviour between the two experiments were regarded as ambiguous. Enzymic activity was screened using API ZYM galleries according to manufacturer’s instructions (bioMérieux). Whole-cell amino acids and sugars were prepared according to Lechevalier & Lechevalier (1970), followed by TLC analysis (Staneck & Roberts, 1974). Polar lipids were extracted, separated by two-dimensional TLC and identified according to procedures outlined by Minnikin et al. (1984) with modifications proposed by Kroppenstedt & Goodfellow (2006). Additionally, choline-containing lipids were detected by spraying with Dragendorff reagent (Merck). Menaquinones (MK) were extracted from freeze-dried cell material using methanol as described by Collins et al. (1977) and analysed by HPLC (Kroppenstedt, 1982). For extraction and analysis of cellular fatty acids, physiological age of each strain was standardized by consistently choosing the last quadrant streaked on GYM agar plates incubated at 28 °C for 4 days. Analysis was conducted using the Microbial Identification System (MIDI) Sherlock software 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 as described by Schleifer & Kandler (1972). All physiological tests were performed at 28 °C using G. obscurus DSM 43160T (G-20T), G. ruber DSM 45317T (CPCC 201356T), G. nigrescens DSM 45408T (YIM 75980T), G. arenarius DSM 45418T (CF5/4T), G. siccatus DSM 45419T (CF6/1T) G. saharensis DSM 45423T (CF5/5T) G. telluris DSM 45421T (CF9/1T) and G. tzadiensis DSM 45416 (CF5/2) in parallel assays. All chemotaxonomic tests were conducted with the same reference strains under standardized conditions. G+C content of chromosomal DNA was determined by HPLC according to 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 by POA software version 2.0 (Lee et al., 2002) and filtered with GBLOCKS (Castresana, 2000). Phylogenetic trees were inferred with maximum-likelihood and maximum-parsimony approaches 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 checked for agreement with the phylogenetic classification. Pairwise similarities were calculated from exact pairwise sequence alignments using the Smith-Waterman algorithm within the European Molecular
CF5/3T cells were pleiotrophic and Gram-reaction-positive with cell dimensions ranging from 0.2–0.6 × 1.1–1.8 μm in 4-day-old cultures (Fig. 1). Individual cells and large aggregates were both observed, confirming reports by Ishiguro & Wolfe (1970) of synchronous morphogenesis on unspecific media. Motile zoospores were circular or elliptical; septated filaments from zoospore germination were observed. Young colonies were light red in colour and turned greenish-black at maturity. Colonies were convex, nearly circular and opaque with a moist surface and an entire margin. Optimal growth was observed on GYM Streptomyces medium. Moderate growth was observed on both R2A medium and TSB agar, but not on GPHT, GEO, PYGV or Luedemann media. CF5/3T grew best at 25–40 °C; no growth was observed below 15 °C or above 40 °C. Growth occurred in the presence of 0–1 % NaCl, but not 4–8 % NaCl, and between pH 6.0–10.0. Results from phenotype microarray analysis are shown as a heatmap in Fig. S1 (available in IJSEM Online) in comparison to other named type strains of the genus Geodermatophilus. A summary of select differential phenotypic characteristics is presented in Table 1. Analysis of cell wall components revealed the presence of DL-diaminopimelic acid (cell wall type III), which is consistent with other species of the genus Geodermatophilus (Lechevalier & Lechevalier, 1970; Montero-Calasanz et al., 2013a). Strain CF5/3T displayed primarily MK-9(H4) (75.2 %) but also MK-9 (11.3 %), an unknown MK (5.4 %) and MK-8(H4) (4.3 %). The major fatty acids were saturated branched-chain acids such as iso-C15:0 (26.8 %) and iso-C16:0 (23.3 %), as well as the monounsaturated straight-chain C17:1ω8c (7.9 %) and, in decreasing order, iso-C17:0, anteiso-C17:0, C18:1ω9c, iso-H-C16:1ω7c, C17:0 and iso-C17:1ω9c (all <6 %). The phospholipid pattern consisted of diphosphatidylglycerol, phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol and a small amount of phosphatidylglycerol (Fig. S2). Whole-cell sugar analysis revealed galactose as diagnostic sugar (Lechevalier & Lechevalier, 1970) but also glucose, ribose and a yet unidentified sugar. Genomic DNA G+C content was 75.5 mol%.

The almost complete (1469 bp) 16S rRNA gene sequence of strain CF5/3T was determined. The 16S rRNA gene sequence showed the highest similarity with the homologous genes of G. saharenensis (98.8 %), ‘G. tzadiensis’ (98.7 %), G. nigrescens (98.7 %), G. siccatum (97.9 %), G. arenarius (97.8 %) and G. telluris (97.4 %). CF5/3T and all listed closely related type strains were placed within the same phylogenetic group by both maximum-likelihood and maximum-parsimony estimations (Fig. 2). The 16S rRNA gene sequence analysis thus strongly supports that the novel strain belongs to the genus Geodermatophilus. However, similarities in 16S rRNA gene sequence between CF5/3T and closely related strains indicated the need to prove the genomic distinctness of the type strain representing the novel species by DNA–DNA hybridization. CF5/3T displayed a percentage of DNA–DNA relatedness of 15.8 ± 3.8 % with G. saharenensis, 28.9 ± 1.4 % with ‘G. tzadiensis’, 25.6 ± 3.4 % with G. nigrescens, 30.1 ± 0.9 % with G. siccatum, 45.7 ± 3.2 % with G. arenarius and 20.8 ± 0.9 % with G. telluris. All values are far below the 70 % threshold value recommended by Wayne et al. (1987) to confirm the species status of a novel strain.

Apart from the phylogenetic analysis based on 16S rRNA gene sequences, several phenotypic characteristics support the distinctiveness of strain CF5/3T from all other named species of the genus Geodermatophilus (Table 1). Based on phenotypic and genotypic data presented, we propose that strain CF5/3T represents a novel species within the genus Geodermatophilus, with the name Geodermatophilus normandii sp. nov.

**Description of Geodermatophilus normandii sp. nov.**

Geodermatophilus normandii (nor.mand’i.i. N.L. gen. masc. n. normandii of Normand, named for Philippe Normand, a microbiologist at University Claude Bernard Lyon I, in recognition of his contributions to the microbiology field including his characterization of suborder Frankineae and, in particular, the family Geodermatophilaceae).

Colonies are greenish-black in colour, nearly circular with a moist surface and an entire margin. Cells are Gram-reaction-positive, catalase-positive and oxidase-negative. No diffusible pigments are produced on any medium tested. Utilizes dextrin, maltose, trehalose, cellobiose, sucrose, turanose, methyl β-D-galactoside, D-glucose, D-mannose, D-fructose, D-galactose, L-rhamnose, inosine, glycerol, L-aspartic acid, L-glutamic acid, L-pyroglutamic acid, L-serine, pectin, D-gluconic acid, glucuronamide,
Table 1. Differential phenotypic characteristics of strain CF5/3<sup>T</sup> and representative strains of other species of the genus Geodermatophilus

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<td>Colony colour on GYM</td>
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<td>Colony surface on GYM</td>
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<td>Predominant menaquinone(s)*†</td>
<td>MK-9(H&lt;sub&gt;4&lt;/sub&gt;)</td>
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<td>Major fatty acids‡</td>
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*Components are listed in decreasing order of quantity.
†Only components making up ≥5% peak area ratio are shown.
‡Only components making up ≥10% peak area ratio are shown.
methyl pyruvate, L-malic acid, Tween 40, γ-amino-N-butyric acid, αβ-hydroxybutyric acid, α-ketobutyric acid, acetoacetic acid, propionic acid, acetic acid and sodium formate as sole carbon source for energy and growth, but not stachyose, raffinose, α-lactose, N-acetyl-D-galactosamine, N-acetylneuraminic acid, L-fucose, sodium lactate, D-mannitol, D-arabitol, myo-inositol, D-aspartic acid, D-serine, gelatin, glyceryl-L-proline, L-alanine, L-arginine, L-histidine, guanidine hydrochloride, D-galactouronic acid, L-galacturonic acid, γ-lactone, D-glucuronic acid, mucic acid, quinic acid, D-saccaric acid, p-hydroxyphenylacetic acid, L-lactic acid, citric acid, α-ketoglutaric acid, D-malic acid and bromosuccinic acid. Acid is produced from inosine, L-aspartic acid, L-glutamic acid, L-pyroglutamic acid, L-arginine, glucuronamide and γ-amino-N-butyric acid, which can be used as sole nitrogen sources, but not from N-acetyl-D-galactosamine, D-aspartic acid, D-serine, glyceryl-L-proline, L-alanine, L-arginine, L-histidine or guanidine hydrochloride. Positive for ascinulin and starch degradation. Negative for the reduction of nitrate and denitrification, gelatin hydrolysis, indole production and degradation of casein, tyrosine, xanthine and hypoxanthine. Tests for alkaline phosphatase, esterase lipase (C8), valine arylamidase, leucine arylamidase and αβ-glucosidase are positive. Tests are negative for acid phosphatase, naphthol-AS-Bl-phosphohydrolase, esterase (C4), lipase (C14), urease, cystine arylamidase, trypsin, z-chymotrypsin, αβ-galactosidase, β-glucuronidase, N-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase. NaCl tolerance ranges from 0–1 % (w/v). Cell growth ranges from 15–40 °C and pH 6.0–10.0. The peptidoglycan in the cell wall contains meso-diaminopimelic acid as diamino acid, with galactose as a diagnostic sugar compound. The predominant menaquinone is MK-9(H4). The main polar lipids are diphasphatidyglycerol, phosphatidylcholine, phosphatidylethanolamine and phosphatidylglycerol with a minor fraction of phosphatidyglycerol. Cellular fatty acids consist mainly of branched-chain saturated acids: iso-C15:0 and iso-C16:0.

The type strain, CF5/3T (=DSM 45417T=CCUG 62814T =MTCC 11412T), was isolated in 2007 from sand of the Sahara Desert collected in Ouré Cassoni (15° 42’ 44” N 23° 3’ 1” E), Republic of Chad. The type strain has a genomic DNA G+C content of 75.5 mol%.

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References


