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ORIGINAL PAPER

Geodermatophilus arenarius sp. nov., a xerophilic actinomycete isolated from Saharan desert sand in Chad

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Abstract A novel Gram-positive, aerobic, actinobacterial strain, CF5/4^T, was isolated in 2007 during an environmental screening of arid desert soil in Ouré Cassoni, Chad. The isolate grew best in a temperature range of 28–40 °C and at pH 6.0–8.5, with 0–1 % (w/v) NaCl, forming brown-coloured and nearly circular 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 75.9 mol %. The peptidoglycan contained *meso*-diaminopimelic acid as diagnostic diaminoacid. The main phospholipids were phosphatidylethanolamine, phosphatidylcholine, phosphatidylinositol, diphosphatidylglycerol and a small amount of phosphatidylglycerol; MK-9(H_4) was identified as the dominant menaquinone and galactose as diagnostic sugar. The major cellular fatty acids were branched-chain saturated acids: *iso*-$C_{15:0}$ and *iso*-$C_{16:0}$. The 16S rRNA gene showed 96.2–98.3 % sequence identity with the three members of the genus *Geodermatophilus: G. obscurus* (96.2 %), *G. ruber* (96.5 %), and *G. nigrescens* (98.3 %). Based on the chemotaxonomic results, 16S rRNA gene sequence analysis and DNA–DNA hybridization with the type strain of *G. nigrescens,* the isolate is proposed to represent a novel species, *Geodermatophilus arenarius* (type strain CF5/4^T = DSM 45418^T = MTCC 11413^T = CCUG 62763^T).

Keywords Xerophiles · Actinomycetes · *Geodermatophilaceae* · Taxonomy · Sahara desert

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The INSDC accession number for the 16S rRNA gene sequence of strain CF5/4^T is HE654547.

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Introduction

Although *Geodermatophilus obscurus*, the type species of the genus *Geodermatophilus* (="earth *Dermatophilus*") was described already 44 years ago by Luedemann (1968),

the family *Geodermatophilaceae* was first proposed (but invalidly named) only by Normand et al. (1996), and formally described a decade later by Normand (2006). Besides the type genus *Geodermatophilus,* the *Geodermatophilaceae* contain only the genera *Blastococcus* and *Modestobacter*. Since they share the harsh (=extreme) conditions of low availability of water and nutrients, these two genera are known as inhabitants of rock surfaces, whereas *Geodermatophilus* prefers arid soils as natural habitats (Urzì et al. 2001). The family is until now rather poorly sampled and studied, with a total of only nine named species and only one type strain genome sequenced, that of *G. obscurus* (Ivanova et al. 2010). In addition to the type species *G. obscurus,* the genus *Geodermatophilus* currently contains only two more species: *G. ruber* (Zhang et al. 2011) and *G. nigrescens* (Nie et al. 2012; List Editor 2012). But an analysis of 16S rRNA reference sequences using the Greengenes database (DeSantis et al. 2006) and latest metagenomic studies on dust originated from deserts (Giongo et al. 2012) revealed the existence of several more isolates and yet uncultured phylotypes in soil and on rock surfaces (for an overview, see Ivanova et al. 2010; Urzì et al. 2001). The novel organism described in this report represents a genomically distinct novel lineage from a screening of Sahara desert sand that falls into the genus *Geodermatophilus* in 16S rRNA-based phylogenies. Our own (yet unpublished) data indicate that there are even more cultures in the DSMZ open collection that represent distinct *Geodermatophilus* lineages.

Methods

Sample collection and culture conditions

Representative sand samples were taken from the Saharan desert of the Republic of Chad near Ouré Cassoni and axenic cultures of xerophiles were isolated from these samples with classical enrichment procedures (Giongo et al. 2012). Portions of sand were suspended in physiological saline, shaken for 1 h at 26 °C and kept overnight at 4 °C and then again shaken for 2 h before streaked out on R2A and TSA plates and incubated at 25 °C for 3–10 days (for details, see Giongo et al. 2012). Purified isolates were stored in Microbank™ Blue Colour Beads (Pro-Lab Diagnostics, Richmond, Canada) before accession into the DSMZ open collection.

Morphological procedures

To determine its morphological characteristics, strain CF5/4^{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 Pelczar (1957). Exponentially growing bacterial cultures were observed with an optical microscope (Zeiss AxioScope A1) with a 100-fold magnification and phase-contrast illumination. Micrographs of bacterial cells were taken with a field-emission scanning electron microscope (FE-SEM Merlin, Zeiss, Germany). Gram reaction was performed using the KOH test described by Gregersen (1978). The motility of the cells was observed on modified ISP2 (Shirling and Gottlieb 1966) swarming agar (0.3 %, w/v) at pH 7.2 that contained (l^{-1}) 4 g dextrin, 4 g yeast extract and 10 g malt extract.

Physiological analysis

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 result 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 % H_2O_2. 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 5.0–9.0 (in steps of 0.5 pH units) on modified ISP2 medium (Shirling and Gottlieb 1966) by adding NaOH or HCl, respectively, since the use of a buffer system inhibited the bacterial growth. Considering the appearance of clear zones around the colonies as a positive result, degradation of specific substrates was examined as follows: 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 and 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 by hypoxanthine or xanthine (0.4 %); starch degradation was tested on plates containing nutrient broth (0.8 %), starch (1 %) and agarose (1.5 %), developing these plates by flooding in 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 Inc., Hayward, CA, USA). 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 strain CF5/4^{T} plates that were filled with a cell density of 90 % T because of overexpression when a higher concentration was used. 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 theses values into negative, weak, and positive reactions. 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), methyl red and Voges–Proskauer reactions (15.2.52 and 15.2.82). All physiological tests were performed at 28 °C using *G. obscurus* G-20^T (DSM 43160), *G. ruber* CPCC 201356^T (DSM 45317) and *G. nigrescens* YIM 75980^T (DSM 45408) in parallel assays.

Chemotaxonomy

Whole-cell amino acids and sugars were prepared according to Lechevalier and Lechevalier (1970) and analysed by thin-layer chromatography (Staneck and Roberts 1974). Phospholipids were extracted, separated by two-dimensional thin-layer chromatography and identified according to Minnikin et al. (1984) as modified by Kroppenstedt and 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, cells were grown on GYM medium at 28 °C for 4 days 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 N HCl, 100 °C for 16 h) was examined by thin-layer chromatography according to Schleifer and Kandler (1972).

Genetic and phylogenetic analysis

The G + C content of the 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 were 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 (ML) and maximum-parsimony (MP) 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 bootstopping 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 and 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). 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. To assess the occurrence of the novel strain in environmental samples, the 16S rRNA sequences were compared using NCBI BLAST (Altschul et al. 1990) under default settings (e.g., considering only the high-scoring segment pairs (HSPs) 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).

Results and discussion

Morphological and biochemical characteristics

Cells of strain CF5/4^T were pleomorphic and Gram-positive. They were observed individually or forming high aggregates (Fig. 1). The motile zoospores were circular or elliptical; septated filaments from zoospore germination were observed. The young colonies were light red-coloured turning into brown-coloured colonies at maturity. All colonies were convex, circular and opaque with a moist surface and an entire margin. Strain CF5/4^T grew best at

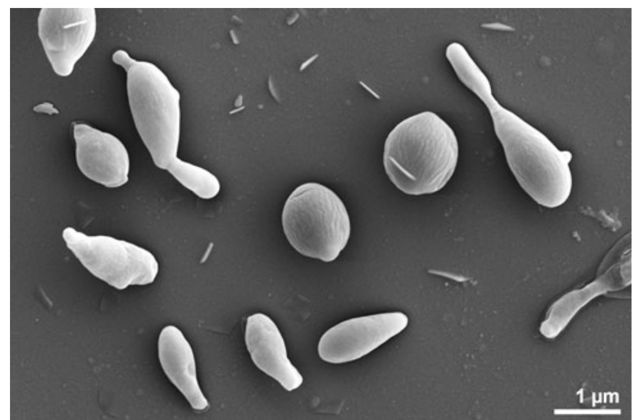

Fig. 1 Scanning electron micrograph showing strain CF5/4^T grown on Glucose–Yeast–Malt medium (DSMZ medium 65, GYM) for 4 days at 28 °C

Table 1 Differential phenotypic characteristics of strain CF5/4^T and the type strains of other *Geodermatophilus* species

Characteristics	1	2	3	4
Colony colour on GYM	Light red, brown	Black	Light red, red	Light red, black
Colony surface on GYM	Moist	Dry	Moist	Moist
Nitrate reduction	–	–	–	+
Degradation of:				
Starch	+	+	–	+
Gelatin	+	–	–	–
NaCl range (w/v)				
4 %	–	+/–	+	+
Growth at pH 5	+	+	+	–
Utilization of:				
D-Raffinose	–	–	+/–	–
α-D-Lactose	+	–	–	+/–
D-Mannose	+	+	–	+
L-Rhamnose	+	–	–	+
D-Sorbitol	–	+	–	–
D-Arabitol	–	+	–	+/–
myo-Inositol	+	+	–	–
Glycyl-L-proline	+	–	–	+/–
L-Alanine	+	–	+/–	–
L-Histidine	–	–	+	–
D-Glucuronic acid	+	–	–	–
Mucic acid	–	–	+	–
L-Lactic acid	+	+	+	–
Oxidase activity	–	–	+	–
Predominant menaquinone (s)[a]	MK-9(H_4), MK-8(H_4), MK-9 (H_0)	MK-9(H_4), MK-9(H_2), 2 MK	MK-9(H_4)	MK-9(H_4)
Polar lipids	DPG, PE, PG PC, PI	DPG, PE, PG, PC, PI	DPG, PE, PG, PC, PI, PL, PGL	DPG, PE, PG, PC, PI
Major fatty acids[b]	*i*-$C_{15:0}$, *i*-$C_{16:0}$	*i*-$C_{15:0}$, *i*-$C_{16:0}$, $C_{17:1\omega8c}$	*i*-$C_{15:0}$, *i*-$C_{16:0}$, $C_{17:1\omega8c}$, *ai*-$C_{15:0}$	*i*-$C_{15:0}$, *i*-$C_{16:0}$

Strains: 1 *G. arenarius* sp. nov. CF5/4^T (DSM 45418^T), 2 *G. obscurus* G-20^T (DSM 43160^T), 3 *G. ruber* CPCC 21356^T (DSM 45317^T), 4 *G. nigrescens* YIM 75980^T (DSM 45408^T). All physiological 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 phospholipids, *PGL* unknown phosphoglycolipid

[a] Only components making up ≥5 % peak area ratio are shown

[b] Only components making up >10 % peak area ratio are shown

28–40 °C; no growth was observed below 15 °C and above 40 °C. Growth was observed in the presence of 0–1 % NaCl but not in 4–8 % NaCl and pH 5.0–8.5. More details about the strain's phenotypic features are presented in Table 1 in comparison to the type strains of the other species in the genus; see also supplementary Fig. S1 for a heatmap of the phenotyping results.

Chemotaxonomic characteristics

Analysis of cell-wall components revealed the presence of *DL*-diaminopimelic acid (*meso*-DAP), which is in line with the other species of the genus *Geodermatophilus* (Nie et al. 2012), whose cell-wall type is III (Lechevalier and Lechevalier 1970). Strain CF5/4^T displayed primarily menaquinone MK-9(H_4) (82.5 %) but also MK-8(H_4) (7.1 %), MK-9(H_0) (6.8 %), an unknown menaquinone (2.3 %) and MK-9(H_2) (1.4 %). The major fatty acids were the saturated branched-chain acids *iso*-$C_{15:0}$ (30.5 %), *iso*-$C_{16:0}$ (21.0 %), *anteiso*-$C_{17:0}$ (8.3 %), and *iso*-$C_{17:0}$ (6.8 %), as well as *iso*-H-$C_{16:1}$ (6.0 %), $C_{17:1\ \omega8c}$ (5.7 %) and $C_{17:1\ \omega9c}$ (5.5 %) (see supplementary Table S1). The phospholipid pattern consisted of phosphatidylethanolamine (PE), phosphatidylcholine (PC), phosphatidylinositol (PI), diphosphatidylglycerol (DPG) and

small amounts of phosphatidylglycerol (PG) (see Supplementary Fig. S2). Whole-cell sugar analysis revealed the presence of galactose as a diagnostic sugar (Lechevalier and Lechevalier 1970), as well as glucose and traces of ribose. The DNA G + C content was 75.9 %.

Molecular analysis

The almost complete (1511 bp) 16S rRNA gene sequence of the strain CF5/4^T was determined, with *G. nigrescens* 16S rRNA as the most similar sequence: 98.3 % identity. Both maximum-likelihood and maximum-parsimony phylogenies placed strain CF5/4^T as the sister group of *G. nigrescens* with maximum support (Fig. 2), leaving no doubt that the novel strain belongs to the genus *Geodermatophilus* and indicating that it could form a species of its own. Strains CF5/4^T and *G. nigrescens* YIM 75980^T shared 44.2 % DNA–DNA relatedness in reciprocal measurements of 40.7 % and 47.6 %, respectively. This value of genomic relatedness is far below the threshold value of 70 % recommended by Wayne et al. (1987) as indicating species status. Therefore, strain CF5/4^T was considered to represent a separate species in the genus *Geodermatophilus*.

In the Greengenes analysis, the most frequently occurring genera were *Micromonospora* (20.3 %), *Geodermatophilus* (18.1 %), *Jiangella* (13.2 %), *Dactylosporangium* (11.6 %) and *Actinomadura* (7.0 %) (108 hits in total). Regarding the six hits to sequences from other members of the genus *Geodermatophilus*, the average identity within HSPs was 97.0 %, whereas the average coverage by HSPs was 96.6 %. Among all other species, the one yielding the highest score was *Geodermatophilus obscurus* (X92359), which corresponded to an identity of 97.5 % and an HSP coverage of 98.8 %. (Note that the Greengenes database uses the INSDC (=EMBL/NCBI/DDBJ) annotation, which is not an authoritative source for nomenclature or classification.) The highest scoring environmental sequence was HM366507 (Greengenes short title 'Effects Asian dust and air masses on airborne urban aerosols clone ADB-47′), which showed an identity of 96.5 % and an HSP coverage of 98.0 %. Thus, environmental samples which yielded hits of a higher score than the highest scoring species were not found, indicating that the novel strain was not yet detected in environmental samples.

Several phenotypic characteristics apart from the phylogenetic analysis based on 16S rRNA gene sequences also support the distinctiveness of strain CF5/4^T from all other *Geodermatophilus* species (see Table 1). Based on the phenotypic and genotypic data presented above, we propose that strain CF5/4^T represents a novel species within the genus *Geodermatophilus*, with the name *Geodermatophilus arenarius* sp. nov.

Description of *Geodermatophilus arenarius* sp. nov

(*a.re.na'ri.us*. L. masc. adj. *arenarius* pertaining to sand, referring to the source of organism). Colonies are brown-coloured, convex, of circular shape with a moist surface and an entire margin. Cells are Gram-positive, catalase positive and oxidase negative. No diffusible pigments are produced on any medium tested. Utilizes dextrin, D-maltose, D-trehalose, D-cellobiose, sucrose, D-turanose, D-salicin, α-D-glucose, D-mannose, D-fructose, D-galactose, L-rhamnose, gentiobiose, D-fucose, glycerol, α-D-lactose, *N*-acetyl-D-glucosamine, α-keto-butyric acid, myo-inositol, L-lactic acid, acetic acid, propionic acid, L-glutamic acid, tween 40, L-serine, methyl pyruvate, sodium lactate, glycyl-proline, D-glucuronic acid, L-alanine, 3-*O*-methyl-glucose, potassium tellurite, α,β-hydroxy-butyric acid and D,L-malic acid as sole carbon source for energy and growth, but not D-arabitol, D-sorbitol, γ-amino-butyric acid, D-mannitol, *N*-acetyl-β-D-mannosamine, quinic acid, *N*-acetyl-D-galactosamine, p-hydroxy-phenylacetic acid, bromo succinic acid, D-saccharic acid, L-arginine, pectin, D-gluconic acid, *N*-acetyl neuraminic acid, acetoacetic acid, α-keto-glutaric acid, glucuronamide, inosine, L-fucose, fusidic acid, stachyose,

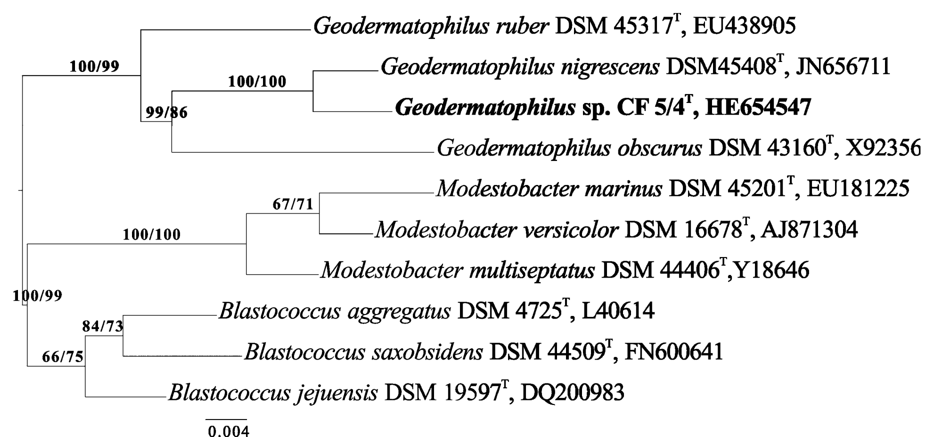

Fig. 2 Maximum likelihood phylogenetic tree inferred from 16S rRNA gene sequences, showing the phylogenetic position of strain CF5/4^T relative to the type strains within the family *Geodermatophilaceae*. The branches are scaled in terms of the expected number of substitutions per site. Support values from maximum-likelihood (*left*) and maximum-parsimony (*right*) bootstrapping are shown above the branches if equal to or larger than 60 %

D-raffinose, D-melibiose, D-serine, D-glucose-6-phosphate, D-fructose-6-phosphate, D,L-aspartic acid, L-histidine, L-pyroglutamic acid, formic acid, D-galacturonic acid, L-galactonic, acid-γ-lactone, *N*-acetyl neuraminic acid, D-lactic acid methyl ester, mucic acid and citric acid. Acid is produced from L-glutamic acid, L-serine, glycyl-L-proline, L-alanine and *N*-acetyl-D-glucosamine and can be used as sole nitrogen sources, but not L-arginine, L-histidine, γ-amino-butyric acid, L-pyroglutamic acid, D-serine, inosine, *N*-acetyl-β-D-mannosamine, *N*-acetyl-D-galactosamine and D,L-aspartic acid. Positive for gelatine hydrolysis, aesculin and starch degradation but negative for the reduction of nitrate and denitrification, methyl red and Voges–Proskauer tests, indole production and casein, tyrosine, cellulose, xanthine and hypoxanthine degradation. Tests for alkaline phosphatase, esterase lipase (C_8), esterase (C_4), leucine arylamidase and α-glucosidase are positive. Tests for acid phosphatase, naphthol-AS-BI-phosphohydrolase, β-glucosidase, valine arylamidase, lipase (C_{14}), urease, cystine arylamidase, trypsin, α-chymotrypsin, α-galactosidase, β-galactosidase, β-glucuronidase, *N*-acetyl-β-glucosamidase, α-mannosidase and α-fucosidase are negative. NaCl tolerance ranges from 0 to 1 % (w/v). Cell growth ranges from 15 to 40 °C and pH 5.0 to 8.5. The peptidoglycan in the cell-wall contains *meso*-diaminopimelic acid as diamino acid, with galactose as diagnostic sugar compounds. The predominant menaquinone is MK-9(H_4). The main polar lipids are diphosphatidylethanolamine, phosphatidylcholine, phosphatidylinositol and diphosphatidylglycerol with a minor fraction of phosphatidylglycerol. Cellular fatty acids consist mainly of branched-chain saturated acids: *iso*-$C_{15:0}$ and *iso*-$C_{16:0}$. The type strain, CF5/4^T (=DSM 45418^T = MTCC 11413^T = CCUC 62763^T), has a genomic DNA G + C content of 75.9 mol %, and was isolated in 2007 from sand of the Saharan desert collected near Ouré Cassoni, Chad.

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