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

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Abstract A novel Gram-type-positive, aerobic, actinobacterial strain, designated CF11/1^T, was isolated from a sand sample obtained in the Sahara Desert, Chad. The black-pigmented isolate was aerobic and exhibited optimal growth from 25–35°C at pH 6.0–8.0 and with 0–8% (w/v) NaCl, indicating that it was a halotolerant mesophile. Chemotaxonomic and molecular characteristics of the isolate matched those described for members of the genus *Geodermatophilus*. The G+C content in the genome was 74.4 mol%. The peptidoglycan contained *meso*-diaminopimelic acid as diagnostic diaminoacid. The main phospholipids were diphosphatidylglycerol, phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol and a minor fraction of phosphatidylglycerol; MK-9(H_4) was the dominant menaquinone, and galactose was detected as a diagnostic sugar. The major cellular fatty acid was branched-chain saturated acid iso-$C_{16:0}$. Analysis of 16S rRNA gene sequences showed 95.3-98.6% pairwise sequence identity with the members of the genus *Geodermatophilus.* Based on phenotypic and chemotaxonomic properties, as well as phylogenetic distinctiveness, the isolate represents a novel species, *Geodermatophilus africanus,* with the type strain CF11/1^T (DSM 45422 = CCUG 62969 = MTCC 11556)

Keywords Actinomycetes*,* *Geodermatophilaceae*, taxonomy, Osmotolerant*,* Phenotype Microarray

Introduction

The family *Geodermatophilaceae* was originally proposed in 1996 by Normand et al.*,* but a formal description of the family name was only published after a decade (Normand 2006). Today, the family comprises the genera *Blastococcus, Modestobacter* and *Geodermatophilus* (as type genus). *Geodermatophilus* was first proposed by Luedemann (1968) and accepted in the Approved Lists of Bacterial Names by Skerman et al. (1980). The members of this genus are frequently isolated from arid soils (Urzì 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 (Urzì et al. 2004). ~~Ten~~ Eleven named species have been classified in the genus *Geodermatophilus*: *Geodermatophilus~~.~~ obscurus* (Luedemann 1968), *Geodermatophilus ~~G.~~ ruber* (Zhang ***et al***. 2011), *Geodermatophilus ~~G.~~ nigrescens* (Nie ***et al***. 2012), *Geodermatophilus~~G.~~ arenarius* (Montero-Calasanz et al. 2012; Validation list no. 150 March 2013), *Geodermatophilus~~G.~~ siccatus* (Montero-Calasanz et al. 2013a; Validation list no. 151 May 2013), *Geodermatophilus~~G.~~ saharensis* (Montero-Calasanz et al. 2013b; Validation list no. 151 May 2013), *Geodermatophilus tzadiensis* (Montero-Calasanz et al. 2013c), ~~'~~*Geodermatophilus~~G.~~ telluris*~~'~~ (Montero-**Calasanz et al.** ~~2013e~~2013d~~),~~ ~~'*Geodermatophilus~~G. tzadiensis'* (Montero-Calasanz *et al*. 2013d~~),~~ '*Geodermatophilus~~G.~~ soli'*~~,~~ ~~and~~ '*Geodermatophilus~~G.~~ terrae*' (Jin et al. 2013) and `*Geodermatophilus normandii´* (Montero-Calasanz et al. 2013e). The genome of only type-strain, *G. obscurus,* has been sequenced so far (Ivanova et al. 2010)~~:~~. Moreover, ~~three~~ four named subspecies have been identified but with their names not yet validly published: `~~-~~*Geodermatophilus~~G.~~ obscurus* subsp. *amargosae´*, `*Geodermatophilus~~G.~~ obscurus* subsp.~~—~~ *utahensis´*~~,~~ ~~and~~ `*Geodermatophilus~~G.~~ obscurus* subsp. *dictyosporus'* (Luedemann 1968) and `*Geodermatophilus obscurus* subsp. *everesti*' (Ishiguro and Fletcher, 1975; Normand and Benson 2012). This study describes the taxonomic position of a novel halotolerant species in the genus *Geodermatophilus* based on a polyphasic approach.

Material and methods

Sample collection and culture conditions

During an environmental screening of arid surface soils in the Sahara Desert (Republic of Chad) in 2007, representative red sand samples (sand grain diameter 1-2 mm) were collected near Ourba (for details see Favet et al. 2013). 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. 2012). Purified strain CF 11/1^{T} was stored in Microbank™ Blue Colour Beads (Pro-Lab Diagnostics, Richmond, Canada) before accession into the DSMZ open collection.

Phenotypic procedures

Cultural characteristics were tested on GYM *Streptomyces* medium (DSMZ medium 65), TSB agar, GPHF medium (DSMZ medium 553), R2A medium, GEO medium (DSMZ medium 714), PYGV medium (DSMZ medium 621) and Luedemann medium (DSMZ medium 877) for 15 days. To determine its morphological characteristics, strain CF11/1^{T} was cultivated on 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 (Zeiss AxioScope A1) with a 100-fold magnification and phase-contrast illumination. Micrographs of bacterial cells grown on GYM *Streptomyces* broth after 7 days 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). Cell motility was observed on modified ISP2 (Shirling and Gottlieb 1966) swarming agar (0.3%, w/v) at pH 7.2 that contained (l^{-1}) 4.0 g dextrin, 4.0 g yeast extract and 10.0 g malt extract. Oxidase activity was analysed using filter-paper disks (Sartorius grade 388) soaked in a 1% solution of N,N,N',N'-tetramethyl-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 drops of 3% H_2O_2 (1 drop). Growth rates were determined on plates of GYM medium for temperatures from 10 to 50°C at 5°C increments and for pH values 4.0-12.5 (in increments of 0.5 pH units) on modified ISP2 medium ~~(Shirling and 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 previously described (Gordon and 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 by 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 in 1% iodine solution. For all tests, a positive result was defined by the appearance of clear zones around the colonies. The 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 Inc., Hayward, CA, USA). 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 75-79 % T for strain CF11/1^{T}, at 90 % T for *G. arenarius* CF5/4^{T} and at 80-83 % T for the rest of reference strains. 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 was 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. Enzymatic activity was screened using API ZYM galleries according to manufacturer instructions (bioMérieux). All physiological tests were performed at 28°C using *G. obscurus* DSM 43160~~G-20~~^{T} ~~(DSM 43160)~~, *G. ruber* DSM 45317~~CPCC 201356~~^{T} ~~(DSM 45317)~~, *G. nigrescens* DSM 45408~~YIM 75980~~^{T} ~~(DSM 45408)~~, *G. arenarius* DSM 45418~~CF5/4~~^{T}, ~~(DSM 45418),~~ *G. siccatus* DSM 45419~~CF6/1~~^{T} ~~(DSM 45419)~~, *G. saharensis* DSM 45423~~CF5/5~~^{T} ~~(DSM 45423)~~, *G. tzadiensis* DSM 45416^{T}, ~~'~~*G. telluris* DSM 45421~~' CF9/1/1~~^{T} ~~(DSM 45421)~~, *G. soli* DSM 45843^{T}, *G. terrae* DSM 45844^{T} and *G. normandii* DSM 45417^{T} ~~'*G. tzadiensis*' CF5/2~~^{T} ~~(DSM 45416)~~ in parallel assays.

Chemotaxonomic analysis

Whole-cell amino acids and sugars were prepared according to Lechevalier and Lechevalier (1970), followed by thin layer chromatography (TLC) analysis (Staneck and 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 and Goodfellow (2006). Additionally, choline-containing lipids were detected by spraying with Dragendorff reagent (Merck) (Tindall 1990). Menaquinones (MK) were extracted from freeze-dried cell material using methanol as described by Collins et al. (1977) and analysed by high-performance liquid chromatography (HPLC) (Kroppenstedt 1982). For extraction and analysis of cellular fatty acids, the physiological age of each strain was standardised 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 Version 4.5 (method TSBA40, TSBA6 database) as described by Sasser (1990). The composition of peptidoglycan hydrolysates (6 N HCl, 100 ºC for 16 h) was examined by TLC as described by Schleifer and Kandler (1972). All chemotaxonomic tests were conducted with the same reference strains under standardised conditions.

Genetic and phylogenetic analysis

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 version 2.0 (Lee et al. 2002) and filtered with GBLOCKS (Castresana 2000). Phylogenetic trees were inferred with maximum-likelihood (ML) and maximum-parsimony 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 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 Biology Open Software (EMBOSS) suite (Rice et al. 2000). DNA-DNA hybridization tests were performed by double reciprocal analysis as described by De Ley et al. (1970) with the modifications suggested by Huss et al. (1983) using a Cary 100 Bio UV/VIS (Biotech).

Results and discussion

CF11/1^T cells were Gram-type-positive, pleiotrophic and ~~and~~with dried aspect ~~Gram-type-positive~~. Individual cells, dimers and large aggregates were both observed, confirming reports by Ishiguro and Wolfe (1970) of synchronous morphogenesis on unspecific media ~~(Fig. 1)~~. Motile zoospores were circular or elliptical; septated filaments from zoospore germination were observed (Fig. 1). Colonies were black-coloured, irregular, multiocular and opaque with a dry surface and an irregular margin. Similar appearances were observed in colonies of *G. osbcurus* and *G. telluris* under the same growth conditions (Table 1). Moderate growth was observed on GYM *Streptomyces* medium and R2A medium, but not on TSB agar, GPHF, GEO, PYGV and Luedemann media. CF11/1^T grew best at 25-35°C; no growth was observed below 20°C or above 37°C. Growth was observed in the presence of 0-8% NaCl, regarding as a halotolerant bacteria, and between pH 6.0-8.0. Results from phenotype microarray analysis are shown as a heatmap in the supplementary material (Fig. S1) in comparison to other 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 and Lechevalier 1970; Montero-Calasanz et al. ~~2013d~~2013e). Strain CF11/1^T displayed primarily MK-9(H_4) (87.2%), in agreement with it reported for the family *Geodermatophilaceae* (Normand, 2006), but also MK-8(H_4) (4.5%), MK-9(H_0) (3.0%), MK-9(H_2) (2.1%) and an unknown MK (3.2%). Similar patterns were already observed for species *G. arenarius* (Montero-Calasanz et al. 2012) and *G. tzadiensis* (Montero-Calasanz et al. 2013e). Most major fatty acids were saturated branched-chain acids: iso-$C_{16:0}$ (36.2%), anteiso-$C_{17:0}$ (8.3%), anteiso-$C_{15:0}$ (7.7%) and iso-$C_{15:0}$ (7.1%), complemented by the monounsaturated iso-H-$C_{16:1}$ (7.9%) and $C_{16:1\omega7c}$ (7.2%). The phospholipid pattern consisted of diphosphatidylglycerol (DPG), phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylinositol (PI) and a small amount of phosphatidylglycerol (PG) (see Supplementary Fig. S2) and is accordance with profiles obtained for the other *Geodermatophilus* species investigated in this study (Table 1). Whole-cell sugar analysis revealed galactose as diagnostic sugar (Lechevalier and Lechevalier 1970), but also glucose and traces of ribose. Genomic G + C content was 74.4 mol %.

The almost complete (1511 bp) 16S rRNA gene sequence of strain CF11/1^T was determined. The 16S rRNA gene sequences showed the highest similarity with the homologous genes of *G. siccatus* (98.6%), *G. tzadiensis* (98.3%), *G. normandii* (98.2%), *G. arenarius* (97.8%), *G. nigrescens* (97.5%), *G. saharensis* (97.4%), *G. obscurus* (97.2%), *G. ruber* (97.0%) and *G. telluris* (97.0%) 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 sequences analysis thus strongly supports that strain CF11/1^T belongs to the genus *Geodermatophilus*. However, similarities in 16S rRNA gene sequence between CF11/1^T and closely related type strains indicated the need to prove the genomic distinctness of the type strain representing the novel species by DNA-DNA hybridizations. CF11/1^T displayed a percentage of DNA-DNA relatedness of 32.3 ± 0.7 with *G. siccatus*, 23.5 ± 3.4 with *G. tzadiensis*, 19.3 ± 5.1 with *G. normandii*, 20.9 ± 0.5 with *G. nigrescens*, and 28.2 ± 1.0 with *G. obscurus*. DNA-DNA hybridizations of CF11/1^T with the type strains of *G. arenarius*, *G. saharensis*, *G. ruber* and *G. telluris* were not conducted, because our hands-on experience from over thirty DDHs between pairs of strains related by 97-99 % 16S rRNA gene sequence identity in the genus *Geodermatophilus* clearly

confirmed the observation reported by Stackenbrandt and Ebers (2006) and Meier-Kolthoff et al. (2013) that such strains generally result in DNA-DNA hybridization values below the 70% threshold 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 CF11/1^T from all other named *Geodermatophilus* species (Table 1). Based on the phenotypic and genotypic data presented, we propose that strain CF11/1^T represents a novel species within the genus *Geodermatophilus*, with the name *Geodermatophilus africanus* sp. nov.

Description of *Geodermatophilus africanus* sp. nov.

Geodermatophilus africanus (af.ri.ca'nus. L. masc. adj. *africanus*, of Africa).

Colonies are black-coloured, irregular, multiocular with a dry surface. Cells are Gram-type-positive, catalase positive and oxidase negative. No diffusible pigments are produced on any medium tested. Utilizes sodium lactate, D-serine, guanidine hydrochloride, D-galacturonic acid, D-glucuronic acid, glucuronamide, L-malic acid, bromo-succinic acid, potassium tellurite, β-hydroxy-butyric acid, acetoacetic acid and butyric acid as sole carbon source for energy and growth, but not D-maltose, β-gentiobiose, sucrose, D-turanose, stachyose, D-raffinose, α-D-lactose, D-melibiose, β-methyl-D-galactoside, D-salicin, N-acetyl-D-glucosamine, N-acetyl-β-D-mannosamine, N-acetyl-D-galactosamine, N-acetyl-neuraminic acid, D-mannose, D-fructose, D-galactose, 3-O-methyl-D-glucose, D-fucose, L-fucose, L-rhamnose, inosine, D-sorbitol, D-mannitol, D-arabitol, myo-inositol, glycerol, D-glucose-6-phosphate, D,L-aspartic acid, D-serine, gelatin, glycyl-L-proline, L-alanine, L-arginine, ~~L-aspartic acid,~~ L-glutamic acid, L-histidine, L-pyroglutamic acid, L-serine, pectin, L-galactonic acid-ϒ-lactone, D-gluconic acid, mucic acid, quinic acid, D-saccharic acid, D-lactic acid methyl ester, L-lactic acid, citric acid, α-keto-glutaric acid, D-malic acid, α-keto-butyric acid, propionic acid, acetic acid and sodium formate, considering as ambiguous the utilization of dextrin, D-trehalose, D-cellobiose, D-glucose, D-fructose-6-phosphate, p-hydroxy-phenylacetic acid, methyl pyruvate, tween 40, ϒ-amino-N-butyric acid and α-hydroxy-butyric acid. Acid is produced from D-serine and guanidine hydrochloride and can be used as sole nitrogen sources, but not L-arginine, L-glutamic acid, L-serine, glycil-L-proline, L-alanine, L-histidine, L-pyroglutamic acid, inosine, N-acetyl-D-glucosamine, N-acetyl-β-mannosamide, N-acetyl-D-galactosamide and ~~L,~~D,L-aspartic acid and ambiguous the utilization of ϒ-amino-N-butyric. Negative for the reduction of nitrate and denitrification, gelatine hydrolysis, indole production and degradation of casein, tyrosine, aesculin, starch, xanthine and hypoxanthine. Tests for alkaline phosphatase, esterase lipase (C8) and leucine arylamidase are positive. Tests are negative for ~~A~~acid phosphatase, Naphthol-AS-BI-phosphohydrolase, esterase (C4), lipase (C14), valine arylamidase, urease, cystine arylamidase, trypsin, α-chymotrypsin, α,ß-galactosidase, ß- glucuronidase, α,ß-glucosidase, N-acetyl-ß-glucosamidase, α-mannosidase and α-fucosidase. NaCl tolerance ranges from 0-8% (w/v). Cell growth ranges from 20-37°C and pH 6.0-8.0. 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 diphosphatidylglycerol, phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol with minor fraction of phosphatidyglycerol. Cellular fatty acids

consist mainly of the branched-chain saturated acid iso-$C_{16:0}$. The type strain has a genomic DNA G+C content of 74.4 mol%.

The type strain, CF11/1^T = DSM 45422 = CCUG 62969 = MTCC 11556 was isolated in 2007 from sand of the Sahara Desert collected in Ourba (N15.23.905, E22.42.297), Republic of Chad (N15.23.905, E22.42.297 836). The INSDC accession number for the 16S rRNA gene sequence of strain CF11/1^T is HE654550.

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Fig. 1. Scanning electron micrograph of strain CF11/1^T grown on GYM medium for 7 days at 28 °C.

Fig. 2. Maximum likelihood phylogenetic tree inferred from 16S rRNA gene sequences, showing the phylogenetic position of strain CF11/1^T relative to the type strains within the family *Geodermatophilaceae*. The branches are scaled in terms of the expected number of substitutions per site (see size bar). Support values from maximum-likelihood (left) and maximum-parsimony (right) bootstrapping are shown above the branches if equal to or larger than 60%.

Table 1. Differential phenotypic characteristics of strain CF11/1^T and the type strains of other *Geodermatophilus* species.

**Strains: 1, *G. africanus* sp. nov. CF11/1^T ~~(DSM 45422)~~; 2, *G. obscurus* DSM 43160~~G-20^T (DSM 43160)~~; 3, *G. ruber* DSM 45317~~CPCC 21356^T (DSM 45317)~~; 4, *G. nigrescens* DSM 45408~~YIM 75980^T (DSM 45408)~~; 5, *G. arenarius* DSM 45418~~CF5/4^T (DSM 45418)~~:; 6, *G. siccatus* DSM 45419~~CF6/1^T (DSM 45419)~~; 7, *G. saharensis* DSM 45423~~CF5/5^T (DSM 45423)~~; 8, ~~'~~*G. telluris*~~'~~ DSM 45421~~CF9/1/1^T (DSM 45421)~~; 9, ~~'~~*G. tzadiensis* DSM 45416~~' CF5/2^T (DSM 45416)~~; 10,~~ `~~*G. soli*´ DSM 45843$^T$; 11, ~~and~~ `*G. terrae*´ DSM 45844^T; 12, `*G. Normandii*´ DSM 45417$^T$ ~~were not included in the comparison because they are further distant species (Fig. 2) and were not yet available in the collection~~. All physiological data are from this study, except chemotaxonomic data of `*G. soli*´ and `*G. terrae*´.~~

Characteristics	1	2	3	4	5	6	7	8	9	10	11	12
Colony colour on GYM	Black	Black	Light-red, red	Light-red, black	Light-red, brown	Light-red, black	Light-red, black	Black	Green-black	Light-red	Light-red	Green-Black
Colony surface on GYM	Dry	Dry	Moist	Moist	Moist	Moist	Moist	Dry	Moist	Moist	Moist	Moist
~~Nitrate reduction~~	~~-~~	~~-~~	~~-~~	~~+~~	~~-~~	~~+~~	~~-~~	~~-~~			~~-~~	
~~Degradation of:~~												
~~Starch~~	~~-~~	~~+~~	~~-~~	~~+~~	~~+~~	~~+~~	~~+~~	~~+~~			~~+~~	
~~Gelatin~~	~~-~~	~~-~~	~~-~~	~~-~~	~~+~~	~~-~~		~~+~~			~~+~~	
NaCl range (w/v)												
1.00%	+	+	+	+	+	-	+	+	+	+/-	+	+
4.00%	+	+/-	+	+	-	-	+	+	+/-	-	-	-
8.00%	+	+/-	-	-	-	-	-	-	-	-	-	-
Sucrose	-	+	+	+	+	+	+	+	+	+	+	+
D-Mannose	-	+	-	+	+	+	+	+	+	+	+	+
D-Fructose	-	+	+	+	+	+	+	+	+	+	+	+
D-Galactose	-	+	+	+/-	+	+	+	+	+	+/-	+	+
Inosine	-	-	+	+/-	-	-	+	-	+	+	-	+
Sodium Lactate	+	+/-	+	+	+	+	+	+	-	+/-	+	-
D-Serine	+	+	-	-	-	-	-	-	-	-	-	-
D-Mannitol	-	+	-	+/-	-	+	+	+	+	+	+	-

Guanidine Hydrochloride	+	-	-	-	-	-	-	-	-	-	-	+/-
Pectin	-	+	-	+	-	+	+	+	+	±	±	±
D-Galacturonic acid	+	-	-	+/-	-	+	-	-	+/-	-	-	-
D-Gluconic acid	-	+	-	+	-	+	+	+	+	+/-	-	±
Glucuron-amide	+	+	+	-	-	+	-	-	+	-	-	±
Quinic acid	-	+	+	-	-	+	-	+	-	±	±	-
L-Malic acid	+	+	+	+	+	+	+	+	+	±	±	±
Bromo-succinic acid	+	-	+	+	-	+	+	+	+	±	±	-
Potassium tellurite	+	+/-	+	+	+	-	+	+	+	±	±	+/-
β-Hydroxy-Butyric Acid	+	+	-	+	+	+	+	+	+	±	±	±
α-Keto-Butyric acid	-	-	-	+	+	+	+	+	+/-	±	±	±
Acetoacetic acid	+	+	+	+	-	+	+/-	+/-	+	±	+/-	±
Propionic acid	-	+	+	+	+	+	+	+	+/-	±	+/-	±
Butyric acid	+	+	+	+/-	-	+/-	+	+	+	+/-	±	+/-
~~Oxidase activity~~	~~-~~	~~-~~	~~+~~	~~-~~	~~-~~	~~-~~	~~-~~	~~-~~			~~-~~	
~~Catalase activity~~	~~+~~	~~+~~	~~+~~	~~+~~	~~+~~	~~+~~	~~-~~	~~-~~			~~+~~	
Predominant menaquinone (s)[a]	MK-9(H_4)	MK-9(H_4), MK-9(H_2), 2 MK	MK-9(H_4)	MK-9(H_4)	MK-9(H_4), MK-8(H_4), MK-9 (H_0)	MK-9(H_4), MK-8(H_4), MK-9	MK-9(H_4), MK-8(H_4)	MK-9(H_4)	MK-9(H_4), MK-9(H_0), MK	MK-9(H_4), MK-9(H_0)[†]	MK-9(H_4), MK-9(H_0)[†]	MK-9(H_4)
Phospho~~Polar~~ lipids*	DPG, PC, PE, PI, PG	DPG, PE, PG, PC, PI	DPG, PE, PG, PC, PI, 2PL	DPG, PE, PG, PC, PI	DPG, PE, PG PC, PI	DPG, PE, PG, PC, PI	DPG, PC, PI, PE, PG	DPG, PE, PG, PC, PI, PN	DPG, PE, PG PC, PI	DPG, PME, PE, 3PL, PI[†]	DPG, PME, PE, PI, 5PL[†]	DPG, PC, PE, PI, PG
Major fatty acids[b]	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-$C_{16:0}$	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-$C_{16:0}$, i-H-$C_{16:1}$	i-$C_{15:0}$, i-$C_{16:0}$	i-$C_{15:0}$, i-$C_{16:0}$	i-$C_{15:0}$, i-$C_{16:0}$, $C_{16:1\omega7c}$, i-$C_{17:0}$[†]	i-$C_{14:0}$, i-$C_{15:0}$, i-$C_{16:0}$, $C_{16:0}$[†]	i-$C_{15:0}$, i-$C_{16:0}$

+, 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 amino-phospholipid; ~~PGL, unknown phosphoglycolipid.~~ PME, phosphatidyl-N-methylethanolamine.

[a], only components making up ≥ 5% peak area ratio are shown;

[b], only components making up ≥ 10% peak area ratio are shown

*, the components are listed in decreasing order of quantity.

[†], Data taken from Jin et al. (2012)

Supplementary Fig. S1. The parameter "Maximum Height" estimated from the respiration curves as measured by an OmniLog phenotyping device and discretized and visualized as a heatmap using the `opm` package. Plates and substrates are rearranged according to their overall similarity (as depicted using the row and column dendrograms). Orange colour indicates positive reaction; purple colour indicate negative reaction; white colour indicate ambiguous reaction. Letters (A/B) indicate each replicate of experiment.

Supplementary Fig. S2. Polar lipids profile of *Geodermatophilus africanus* sp. nov. CF11/1^T, after separation by two-dimensional TLC. Plate was sprayed with molydatophosphoric acid for detection of total polar lipid. DPG, diphosphadidylglycerol; PG, phosphadidylglycerol; PE, phosphatidethanolamine; PC, phosphatidylcholine; PI, phosphatidylinositol; GL, unknown glycolipid; L, unknown lipid.