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ORIGINAL PAPER

# Description of *Geodermatophilus bullaregiensis* sp. nov.

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**Abstract** The taxonomic position of an aerobic actinobacterial strain, BMG841$^T$, isolated from the Bulla Regia monument (Tunisia) and exhibiting a high resistance to gamma-radiation (D10 ~9 kGy) was determined using polyphasic approach. The optimal growth range was found to be 25–35 °C at pH of 7.0–8.5. The strain was observed to form black dry colonies. Chemotaxonomic characteristics of the isolate showed a cell wall type III, with galactose and glucose as diagnostic sugars; phosphatidylcholine, phosphatidylinositol, diphosphatidylglycerol, phosphatidylethanolamine and an unidentified glycolipid as main polar lipids; and MK-9($H_4$) as the predominant menaquinone. The major cellular fatty acids were identified as iso-$C_{16:0}$ and iso-$C_{15:0}$. Phylogenetic analysis indicated that strain BMG841$^T$ represents a novel member of the genus *Geodermatophilus* with high 16S rRNA gene sequence identity with *Geodermatophilus saharensis* (98.28 %). Based on phylogenetic and phenotypic analysis, strain BMG841$^T$ is proposed as the type strain (=DSM 46841$^T$ = CECT 8821$^T$) of a novel species, *Geodermatophilus bullaregiensis*.

**Keywords** Geodermatophilus · Gamma radiation-resistant · Monument

## Introduction

The genus *Geodermatophilus* was proposed by Luedemann (1968) to accommodate aerobic, Gram-positive actinomycetes with DL-2,6-diaminopimelic acid (DL-

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DAP) in the cell wall peptidoglycan and MK-9(H4) as the predominant menaquinone. Currently the genus comprises eighteen species, which have been isolated from Desert sands (Montero-Calasanz et al. 2012), soil (Luedemann 1968; Nie et al. 2012; Jin et al. 2013; Montero-Calasanz et al. 2014a; Bertazzo et al. 2014), rhizosphere (Zhang et al. 2011), sediment (Qu et al. 2013) and altered stones (Montero-Calasanz et al. 2014b; Hezbri et al. 2015). Members of the genus *Geodermatophilus* are well known as gamma-radiation resistant actinobacteria with LD10s around 8–9 kGy (Gtari et al. 2012; Montero-Calasanz et al. 2014b; Hezbri et al. 2015). In this paper, we describe the polyphasic characterisation of the type strain of a gamma radiation-resistant new species, *Geodermatophilus bullaregiensis*, isolated from the marble monument of Bulla Regia located in North Western Tunisia.

## Materials and methods

### Isolation and culture of strain

Dust sampled from crevices of a marble rock surface located in the ruin of Bulla Regia, a Roman City situated in North Western Tunisia, was suspended in physiological saline solution and shaken overnight at 28 °C before being streaked out on Luedemann medium (Luedemann 1968) and on R2A (DSMZ medium 830) supplemented with cycloheximide at 0.01 %. Strain BMG841$^T$ was isolated and maintained on Luedemann medium after 10 days incubation. Colonies and general cultural characteristics were observed from cultures growing at 28 °C for 7 days on different media: GYM *Streptomyces* medium (DSMZ medium 65), R2A medium (DSMZ medium 830) and Luedemann medium (DSMZ medium 877).

### Phenotypic tests

Morphological characteristics were observed by using light microscopy (Zeiss AxioScope A1) and a field-emission scanning electron microscope (FE-SEM Merlin, Zeiss, Germany) after 7 days growth at 28 °C on GYM *Streptomyces* medium. Gram reaction was carried out by the standard Gram staining procedure (Gram 1884). Oxidase activity 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 defined by the development of a blue-purple colour after applying biomass to the filter paper. Catalase activity was determined based on formation of bubbles following the addition of 1 drop of 3 % $H_2O_2$. Enzymatic activities were tested using *API ZYM* galleries according to the instructions of the manufacturer (bioMérieux). Oxidation of carbon and nitrogen compounds was tested using GEN III Microplates in an Omnilog device (BIOLOG Inc., Hayward, CA, USA) in comparison with the reference strains in parallel assays: *Geodermatophilus africanus* DSM 45422$^T$ (Montero-Calasanz et al. 2013d), *G. amargosae* DSM 46136$^T$ (Montero-Calasanz et al. 2014a), *G. arenarius* DSM 45418$^T$ (Montero-Calasanz et al. 2012), *G. dictyosporus* DSM 43161$^T$ (Montero-Calasanz et al. 2015), *G. nigrescens* DSM 45408$^T$ (Nie et al. 2012), *G. normandii* DSM 45417$^T$ (Montero-Calasanz et al. 2013b), *G. saharensis* DSM 45423$^T$ (Montero-Calasanz et al. 2013c), *G. telluris* DSM 45421$^T$ (Montero-Calasanz et al. 2013e) and *G. tzadiensis* DSM 45416$^T$ (Montero-Calasanz et al. 2013a). The GEN III microplates were inoculated with cells suspended in a viscous inoculating fluid (IF C) provided by the manufacturer at a cell density of at 83–84 % Transmittance (T) for strain BMG841$^T$, at 70 % T for *G. amargosae* DSM 46136$^T$, at 75–79 % T for *G. africanus* DSM 45422$^T$, at 90 % T for *G. arenarius* DSM 45418$^T$ and at 80–83 % T for *G. dictyosporus* DSM 43161$^T$ and the remaining reference strains. The strains were studied in two independent technical replicates. Data were exported and analysed using the opm package for R (Vaas et al. 2012; Vaas et al. 2013) v.1.0.6.

The temperature range and optimum for growth were tested at 5–45 °C on plates of GYM *Streptomyces* medium and at 50–60 °C on modified *Streptomyces* medium by adding $MgCl_2$ and substituting agar with GELRITE (Sigma-Aldrich) (Shungu et al. 1983). The pH range was investigated between pH 4.0 and 12.5 at intervals of 0.5 pH units. 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 determined as previously described by Gordon and Smith (1955) on plates containing peptone (0.5 %), beef extract (0.3 %), L-tyrosine (0.5 %) and

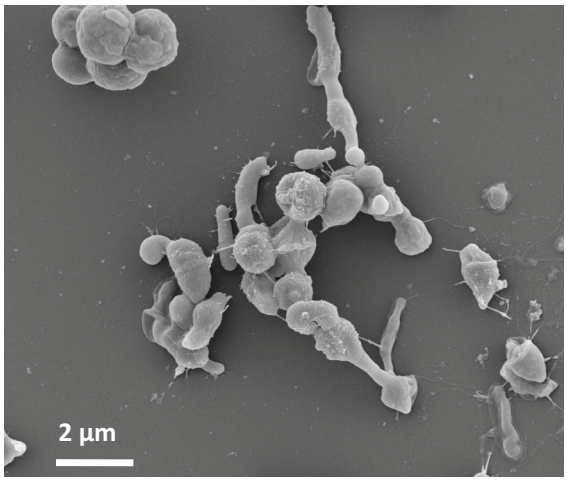


**Fig. 1** Scanning electron micrograph of strain BMG841$^T$ grown for 7 days at 28 °C on GYM *Streptomyces* medium and showing masses of cuboid and elliptical cells together with short septated filaments. Hezbri et al. (2015)

agarose (1.5 %); xanthine and hypoxanthine decomposition (0.4 %) were examined using the same basal medium; 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.

## Chemotaxonomic analyses

Biomass for chemotaxonomic and genotypic studies was obtained by cultivation in shaken flasks (~150 rpm) containing GYM *Streptomyces* broth at 28 °C for 7 days. The diagnostic isomer of diaminopimelic acid in whole-cell hydrolysates (6 N HCl, 100 °C for 16 h) of strain BMG841$^T$ was identified by TLC on cellulose plates using the solvent system of Schleifer and Kandler (1972). Sugar analysis of whole-cell hydrolysates (1 N $H_2SO_4$, 95 °C for 2 h) was carried out as described by Staneck and Roberts (1974). About 100 mg of freeze-dried cells were used for the extraction of polar lipids followed by two-dimensional TLC separation and identification according to Minnikin et al. (1984) with modifications of Kroppenstedt and Goodfellow (2006). Choline-containing lipids were detected by spraying with Dragendorff's reagent (Merck) (Tindall 1990). Menaquinones (MK) were extracted from about 300 mg of freeze-dried cell material using the method of Collins et al. (1977) and analysed by high-performance liquid chromatography (HPLC) (Groth et al. 1997). Cellular fatty acids were prepared by harvesting 40 mg of bacterial cells from the third quadrant of the streaked plate followed by saponification and methylation of the cells. The extraction and analysis of cellular fatty acids was conducted using the Microbial Identification System (MIDI) Sherlock Version 6.1 (method TSBA40, ACTIN6 database) as described by Sasser (1990). All chemotaxonomic analyses were conducted under standardized conditions with strain BMG841$^T$ and cultures of the same set of reference strains as listed in Table 1.

## Phylogenetic analyses

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). The identification of strain BMG841$^T$ based on the 16S rRNA gene sequence was performed using Ez-Taxon (Kim et al. 2012) and RDP-II (Maidak et al. 2001) servers. Pairwise similarities were calculated as recommended by Meier-Kolthoff et al. (2013). Sequence analyses and phylogenetic reconstruction were performed using MEGA 6.0 (Tamura et al. 2013). The stability of relationships was assessed by 1000 replicates through bootstrap analysis (Felsenstein 2005).

## Radiation resistance assay

Ionizing-radiation resistance analysis was performed on strains BMG841$^T$ and *Geodermatophilus obscurus* DSM 43160$^T$, used as a control, following the protocol outlined by Gtari et al. (2012). Non-sporulating cultures were obtained by growth in Luedemann medium containing tryptose (Difco, Detroit, USA) for 5 days at 28 °C (Ishiguro and Wolfe 1970). Collected cells were then washed twice with 0.9 % NaCl, homogenized and subsequently re-suspended in saline solution. One milliliter of cell suspension of each strain was introduced into separate sterile 1.5-mL Eppendorf tubes and subsequently exposed to 1–10 kGy, at a dose rate of 63,319 Gy min$^{-1}$, in a $^{60}$Co irradiator. Subsequently, the cell suspensions were cooled directly on ice, tenfold dilution series were prepared and plated in triplicate on solid Luedemann medium, and incubated at 28 °C. After a

**Table 1** Phenotypic characteristics of strain BMG841$^T$ and related *Geodermatophilus* species

| Characteristics | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|---|
| Colony colour on GYM | Black | Light-orange, black | Black | Black | Light-red, red | Light-red, black | Light-red, brown | Light-red, black | Light-red, black | Light-red, greenish-black |
| Colony surface on GYM | Dry | Moist | Dry | Dry | Moist | Moist | Moist | Moist | Moist | Moist |
| pH optimum | 7.0–8.5 | 6.0–9.5 | 6.5–8.0 | – | 7.0–7.5 | 7.0 | 6.0–8.5 | 6.0–8.5 | 6.0–8.0 | 6.0–8.5 |
| Temperature optimum (°C) | 25–35 | 25–35 | 25–35 | 24–28 | 20–32 | 37 | 28–40 | 20–37 | 20–35 | 25–37 |
| Utilizaton of | | | | | | | | | | |
| D-Maltose | + | +/– | + | + | + | + | + | + | + | + |
| D-Cellobiose | – | +/– | + | + | + | + | + | – | + | + |
| Turanose | +/– | + | + | + | + | + | + | – | + | + |
| α-D-Lactose | +/– | +/– | + | – | – | +/– | + | – | + | – |
| D-Mannose | + | – | + | + | – | + | + | + | + | + |
| D-Galactose | + | + | + | + | + | +/– | + | + | + | + |
| L-Rhamnose | + | + | + | – | – | + | + | – | + | + |
| Sodium lactate | – | + | – | +/– | + | + | + | + | + | – |
| D-Arabitol | + | +/– | – | + | – | +/– | – | + | + | – |
| D-Glucose-6-phosphate | +/– | +/– | + | – | – | + | – | – | – | – |
| L-Arginine | + | – | + | – | – | – | – | + | + | + |
| L-Glutamic acid | + | +/– | – | + | + | + | + | + | + | – |
| L-Histidine | – | – | – | – | + | – | – | – | + | – |
| Pectin | + | + | – | + | – | + | – | + | + | + |
| Methyl pyruvate | + | – | – | – | + | +/– | + | + | + | – |
| L-Lactic acid | + | – | – | + | + | – | + | + | +/– | + |
| D-Malic acid | + | – | + | + | + | +/– | – | + | + | +/– |
| Predominant menaquinone(s)[a] | MK-9(H4), MK-9(H0), MK-9(H2) | MK-9($H_4$) | MK-9($H_4$), MK-9($H_0$) | MK-9($H_4$), MK-9($H_2$), MK-8($H_4$) | 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($H_0$) | MK-9($H_4$), MK-8($H_4$) | MK-9($H_4$), MK-9($H_0$) |
| Phospholipids[b] | PC, PI, DPG, PE, GL | PC, DPG, PI, PE, APL, GL | PC, DPG, PI, PE | DPG, PC, PE, PI, PG | DPG, PE, PC, PI, 2PL,PG | DPG, PE, PC, PI, PG | PE, PC, DPG, PI, PG, | PE, PC, PI, DPG, PG | DPG, PC, PI, PE, PG | DPG, PC, PE, PI, PG |
| Major fatty acids[c] | i-$C_{15:0}$, i-$C_{16:0}$ | i-$C_{16:0}$, i-$C_{15:0}$, i-H-$C_{16:1}$ | i-$C_{15:0}$, i-$C_{16:0}$ | i-$C_{15:0}$, i-$C_{16:0}$, $C_{17:1w8c}$ | i-$C_{15:0}$, ai-$C_{15:0}$, i-$C_{16:0}$, ai-$C_{17:0}$, $C_{17:1w8c}$ | i-$C_{15:0}$, i-$C_{16:0}$ | i-$C_{15:0}$, i-$C_{16:0}$ | i-$C_{15:0}$,i-$C_{16:0}$, $C_{17:1w8c}$ | i-$C_{15:0}$, i-$C_{16:0}$, i-H-$C_{16:1}$ | i-$C_{15:0}$, i-$C_{16:0}$ |

**Table 1** continued

| Characteristics | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 |
|---|---|---|---|---|---|---|---|---|---|
| Colony colour on GYM | Black | Light red | Light red | Black | Light-red, greenish-black | Coral Pink | Black | Coral Pink | Light-red, greenish-black |
| Colony surface on GYM | Dry | Moist | Moist | Dry | Moist | Moist | Dry | Moist | Moist |
| pH optimum | 6.0–8.5 | 7.0 | 7.0 | 6.0–8.0 | 6.0–10.0 | 7.0 | 6.0–12.0 | 6.5–8.5 | 7.0–9.5 |
| Temperature optimum (°C)[f] | 20–35 | 30 | 30 | 25–35 | 25–40 | 28 | 25–35 | 20–30 | 25–30 |
| Utilizaton of | | | | | | | | | |
| D-Maltose | + | + | + | − | + | + | − | + | + |
| D-Cellobiose | + | + | + | +/− | + | + | − | + | + |
| Turanose | + | + | + | − | + | + | + | + | − |
| α-D-Lactose | − | + | + | − | − | +/− | +/− | + | +/− |
| D-Mannose | + | + | + | − | + | − | +/− | + | + |
| D-Galactose | + | +/− | + | − | + | − | +/− | − | + |
| L-Rhamnose | + | + | + | − | + | − | − | + | + |
| Sodium lactate | + | +/− | + | + | − | + | +/− | + | − |
| D-Arabitol | + | − | − | − | − | + | − | +/− | − |
| D-Glucose-6-phosphate | − | − | +/− | − | +/− | − | − | +/− | − |
| L-Arginine | + | − | − | − | − | − | − | − | + |
| L-Glutamic acid | + | + | + | − | + | + | +/− | + | − |
| L-Histidine | − | − | − | − | − | − | +/− | − | − |
| Pectin | + | + | + | − | + | + | − | + | + |
| Methyl pyruvate | + | + | + | +/− | + | + | +/− | + | + |
| L-Lactic acid | + | + | + | − | − | − | +/− | − | +/− |
| D-Malic acid | + | + | + | − | − | − | − | − | + |
| Predominant menaquinone(s)[a] | MK-9($H_4$) | MK-9($H_4$), MK-9($H_0$), MK-9($H_2$) | MK-9($H_4$), MK-9($H_0$) | MK-9($H_4$) | MK-9($H_4$) | MK-9 ($H_4$), MK-9($H_0$) | MK-9($H_4$) | MK-9($H_4$), MK-9($H_0$), MK-10($H_4$), MK-9($H_2$) | MK-9($H_4$) |

**Table 1** continued

| Characteristics | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 |
|---|---|---|---|---|---|---|---|---|---|
| Phospholipids[b] | DPG, PC, PE, PI, APL, PG | DPG, PME, PE, PI, 3PL | DPG, PME, PE, PI, 5PL | DPG, PC, PE, PI, PG | DPG, PC, PE, PI, PG | DPG, PE, PI, PIM[e] | DPG, PC, PE, PI, PG | DPG, PE, PE-OH, PI, GPL, PL | PE, PC, PI, DPG |
| Major fatty acids[c] | i-$C_{15:0}$, i-$C_{16:0}$ | i-$C_{15:0}$, i-$C_{16:0}$, i-$C^{d}_{17:0}$ | i-$C_{15:0}$, i-$C_{16:0}$, $C^{d}_{18:1w9c}$ | i-$C_{16:0}$ | i-$C_{15:0}$, i-$C_{16:0}$ | i-$C_{15:0}$, i-$C_{16:0}$, $C^{e}_{17:1w8c}$ | i-$C_{15:0}$, i-$C_{16:0}$ | i-$C_{16:0}$, i-$C_{15:0}$, $C_{17:1\omega 8c}$ | i-$C_{15:0}$, i-$C_{16:0}$, $C_{17:1w8c}$ |

All physiological data are from this study

*Strain 1 G. bullaregiensis* sp. nov. BMG841$^{T}$, *Strain 2 G. aquæductus* DSM 46834$^{T}$, *Strain 3 G. dictyosporus* DSM 43161$^{T}$, *Strain 4 G. obscurus* DSM 43160$^{T}$, *Strain 5 G. ruber* DSM 45317$^{T}$, *Strain 6 G. nigrescens* DSM 45408$^{T}$, *Strain 7 G. arenarius* DSM 45418$^{T}$, *Strain 8 G. siccatus* DSM 45419$^{T}$, *Strain 9 G. saharensis* DSM 45423$^{T}$, *Strain 10 G. tzadiensis* DSM 45416$^{T}$, *Strain 11 G. telluris* DSM 45421$^{T}$, *Strain 12 G. soli* DSM 45843$^{T}$, *Strain 13 G. terrae* DSM 45844$^{T}$, *Strain 14 G. africanus* DSM 45422$^{T}$, *Strain 15 G. normandii* DSM 45417$^{T}$, *Strain 16 G. taihuensis* DSM 45962$^{T}$, *Strain 17 G. amargosae* DSM 46136$^{T}$, *Strain 18 G. brasiliensis* DSM 44526$^{T}$, *Strain 19 G poikilotrophi* DSM 44209$^{T}$

*MK* menaquinones, *DPG* diphosphatidylglycerol, *PE* phosphatidylethanolamine, *PME* phosphatidyl-*N*-methylethanolamine, *PE-OH* hydroxy-phosphatidylethanolamine, *PG* phosphatidylglycerol, *PC* phosphatidylcholine, *PI* phosphatidylinositol, *PIM* phosphatidylinositol mannoside, *PL* unidentified phospholipid, *APL* unidentified amino-phospholipid, *GL* unknown glucolipid, *i-* iso-branched, *ai-* anteiso branched

+ positive reaction, − negative reaction, +/− ambiguous

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

[b] The components are listed in decreasing order of quantity

[c] Only components making up ≥10 % peak area ratio are shown

[d] Data taken from Jin et al. (2013)

[e] Data taken from Qu et al.(2013)

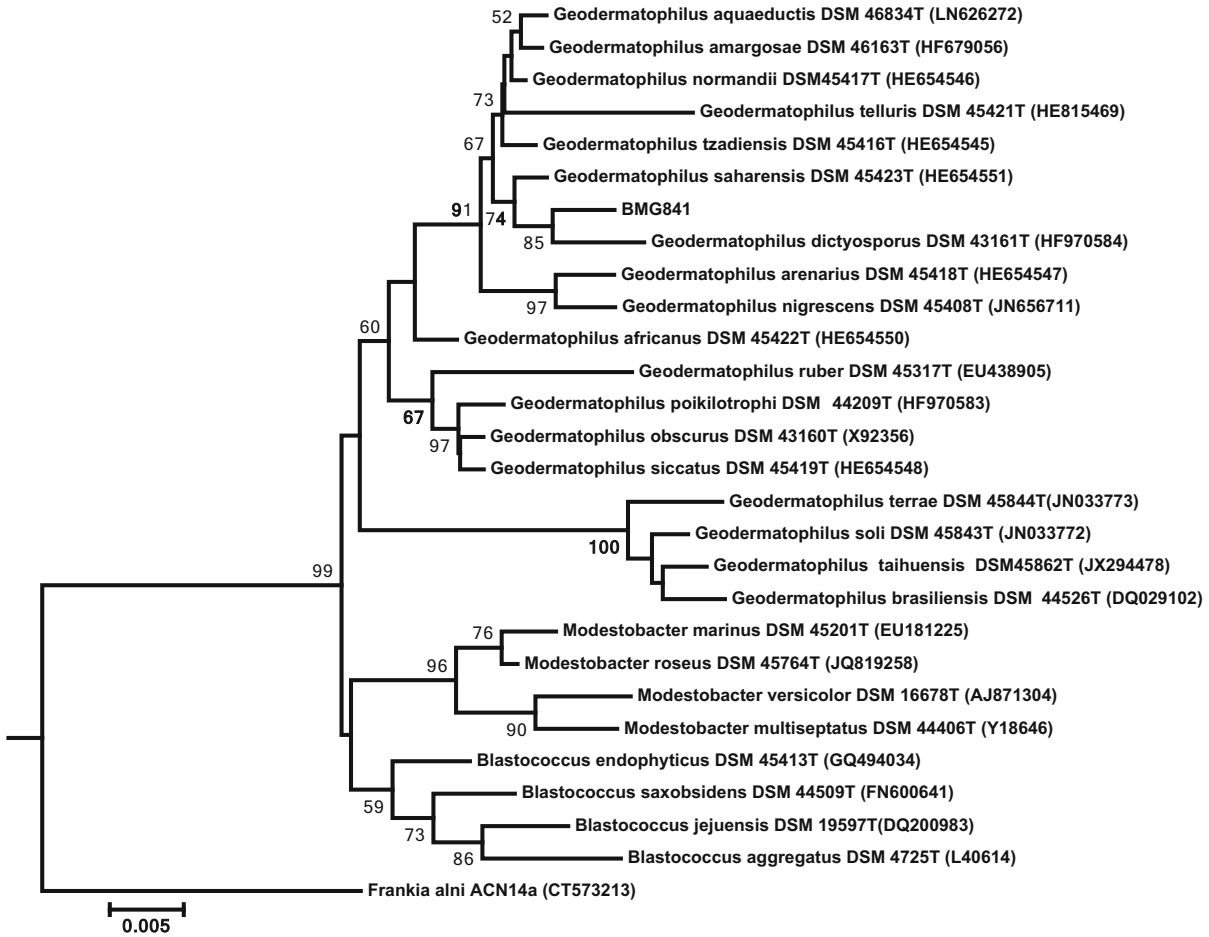


**Fig. 2** Phylogenetic position of strain BMG841$^T$ within the family *Geodermatophilaceae* based on 16S rRNA gene sequences. Only bootstrap values higher than 50 % are shown above the branches. Hezbri et al. (2015)

2-week period, CFUs were counted and the survival fractions were calculated based on a non-irradiated control by using the R software packages mgcv (Wood 2014) and lethal (Hofner 2014) as described by Montero-Calasanz et al. (2014b).

## Results and discussion

Strain BMG841$^T$ was observed to exhibit good growth on Luedemann and GYM *Streptomyces* media and moderate growth on R2A medium. No diffusible pigments were observed. Multilocular colonies were observed with a maximum diameter of 2.3 mm and intense black pigmentation with very dry surfaces and irregular margins. Cells of strain BMG841$^T$ were observed to be pleiomorphic and Gram stain-positive. Individual and cells aggregated in cauliflower-like clumps, together with groups of cuboid cells (Fig. 1), were observed as described by Ishiguro and Wolfe (1970). Strain BMG841$^T$ was found to grow in the presence of up to 4 % NaCl but not with 8 % NaCl. The temperature range for growth was found to range from 10 to 40 °C (with 25 to 35 °C as optimum) and the pH range from 6.5 to 10.5 (with 7.0–8.5 as optimum). Results from phenotype microarray analysis are shown as a heatmap in the supplementary material (Fig. S1) in comparison to the reference type strains of the genus *Geodermatophilus*. A summary of selected differential phenotypic characteristics is presented in Table 1.

Chemotaxonomic data of strain BMG841$^T$ are in agreement with those previously described for the members of the genus *Geodermatophilus* (Hezbri et al.

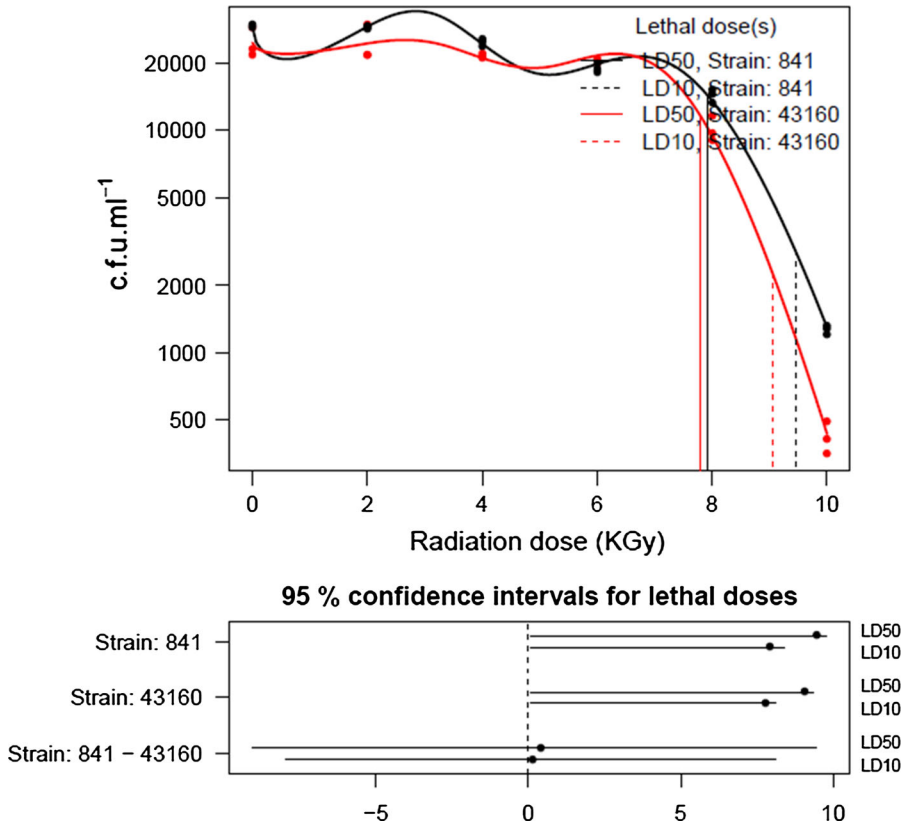


**Fig. 3** Survival of strains BMG841$^T$ and *G. obscurus* DSM 43160$^T$ following gamma-radiation exposure and estimation from mean c.f.u. ml$^{-1}$. LD50 and LD10 values are indicated in the upper panel of each figure. Y-axis is on a logarithmic scale. Hezbri et al. (2015)

2015). The cell wall peptidoglycan was found to be type III, containing DL-DAP as the diagnostic diamino acid (Lechevalier and Lechevalier 1970; Montero-Calasanz et al. 2015). The whole-cell sugar analysis revealed the presence of glucose and galactose as diagnostic sugars (Lechevalier and Lechevalier 1970), along with traces of ribose and mannose. The predominant menaquinone was identified as MK-9($H_4$) (71.5 %) as reported by Normand (2006) for all the members of the family *Geodermatophilaceae*. However, MK-9($H_0$) and MK-9($H_2$) were also present as minor components (17.2 and 8.6 %, respectively). These results are in accordance with those found for the strain *Geodermatophilus soli* DSM 45843$^T$ (Jin et al. 2013). The major fatty acids were identified as iso-$C_{16:0}$ (38.6 %) and iso-$C_{15:0}$ (20.4 %), consistent with the profiles of strains *G. dictyosporus* DSM 43161$^T$ (Montero-Calasanz et al. 2015), *G. nigrescens* DSM 45408$^T$ (Nie et al. 2012), *G. arenarius* DSM 45418$^T$ (Montero-Calasanz et al. 2012), *G. tzadiensis* DSM 45416$^T$ (Montero-Calasanz et al. 2013a), *G. telluris* DSM 45421$^T$ (Montero-Calasanz et al. 2013e), *G. normandii* DSM 45417$^T$ (Montero-Calasanz et al. 2013b) and *G. amargosae* DSM 46136$^T$ (Montero-Calasanz et al. 2014a) (Table 1). The polar lipid profile was found to contained phosphatidylcholine, phosphatidylinositol, diphosphatidylglycerol, phosphatidylethanolamine and an unidentified glycolipid (Supplementary Fig. S2), similar to the pattern obtained by Montero-Calasanz et al. (2015) for *G. dictyosporus* DSM 43161$^T$.

Analysis of the 16S rRNA gene sequence of strain BMG841$^T$ (1458 bp; GenBank/EMBL/DDBJ accession number LN626271) supports the affiliation of strain BMG841$^T$ to the genus *Geodermatophilus* (Fig. 2), with the strain being closely related to the type strain of *G. saharensis* (98.3 % sequence similarity). Meier-Kolthoff et al. (2013) reported that an Actinobacterial-specific 16S rRNA threshold of 99.0 % with a maximum probability of error of 1.0 %

can be assumed to correspond to DNA–DNA hybridization values below the recommended 70 % threshold recommended to assign a given strain to a new prokaryotic species (Wayne et al. 1987). Therefore, DNA–DNA hybridizations with the two closely related neighbours, *G. saharensis* and *G. dictyosporus* appear to be dispensable based on the 16S rRNA sequence similarity, and strain BMG841$^T$ can be proposed as the type strain of a novel species of the genus *Geodermatophilus.*

With an LD10 around 9 KGy (Fig. 3), strain BMG841$^T$ showed only a minor difference in inactivation kinetics compared to the reference strain *G. obscurus* DSM 43160$^T$ used as a positive control (Gtari et al. 2012). Compared to the model radioresistant bacteria *Deinococcus radiodurans* (LD10 $\sim$10 kGy) and the actinobacterial species *Rubrobacter xylanophilus* (LD10 $\sim$5.5 kGy), *Kineococcus radiotolerans* (LD10 $\sim$2 kGy) (Sghaier et al. 2008), *Geodermatophilus poikilotrophi* (Montero-Calasanz et al. 2014b), *G. dictyosporus* (Montero-Calasanz et al. 2015), *G. aquaeductis* (Hezbri et al. 2015) and *G. obscurus* DSM 43160$^T$ (Gtari et al. 2012), strain BMG841$^T$ can be considered as a new highly radioresistant representative of the actinobacteria.

On the basis of the genotypic and phenotypic data, strain BMG841$^T$ can be clearly distinguished from its phylogenetically close relatives. Therefore, strain BMG841$^T$ represents a novel species of the genus *Geodermatophilus*, for which we propose the name *Geodermatophilus bullaregiensis* sp. nov.

## Description of *Geodermatophilus bullaregiensis* sp. nov.

*Geodermatophilus bullaregiensis* (bul.la.re.gi.en'sis. L. n. Bulla Regia, a Roman town in Northern Africa, today North-Western Tunisia; N.L. masc. adj. *bullaregiensis*, derived from Bulla Regia referring to the origin of isolation).

Colonies are black-coloured, irregular, multilocular with a dry surface. Cells are Gram-stain positive, catalase positive and oxidase negative. No diffusible pigments are produced on any of the tested media. Temperature range for growth ranges from 10 to 40 °C and the pH range from 6.5 to 10.5. Can oxidize several carbon and nitrogen sources (Table 1), degrades aesculin but is negative for nitrate reduction and denitrification, indole production and degradation of casein, tyrosine, starch, xanthine, gelatine and hypoxanthine. Tests for alkaline phosphatase and leucine arylamidase are positive but those for esterase (C4), esterase lipase (C8), valine arylamidase, ß-galactosidase and α- and ß-glucosidase acid phosphatase, Naphthol-AS-BI-phosphohydrolase, lipase (C14), cystine arylamidase, trypsin, α-chymotrypsin, α-galactosidase, ß-glucuronidase, *N*-acetyl-ß-glucosamidase, α-mannosidase, urease, and α-fucosidase are negative. The peptidoglycan in the cell wall contains *meso*-diaminopimelic acid as the diamino acid, with glucose and galactose as diagnostic sugars. The predominant menaquinone is MK-9($H_4$). The main polar lipids are phosphatidylcholine, phosphatidylinositol, diphosphatidylglycerol, phosphatidylethanolamine and an unidentified glycolipid. Cellular fatty acids consist mainly of the branched-chain saturated acids iso-$C_{16:0}$ and iso-$C_{15:0}$.

The type strain is BMG841$^T$ (=DSM 46841$^T$ - = CECT 8821$^T$). The INSDC accession number of the 16S rRNA gene sequence of the type strain is LN626271.

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