Longimicrobium terrae gen. nov., sp. nov., an oligotrophic bacterium of the under-represented phylum Gemmatimonadetes isolated through a system of miniaturized diffusion chambers

Javier Pascual,† Marina García-López, Gerald F. Bills‡ and Olga Genilloud

Correspondence
Olga Genilloud
olga.genilloud@medinaandalucia.es

A novel chemo-organoheterotroph bacterium, strain CB-286315T, was isolated from a Mediterranean forest soil sampled at the Sierra de Tejeda, Almijara and Alhamia Natural Park, Spain, by using the diffusion sandwich system, a device with 384 miniature diffusion chambers. 16S rRNA gene sequence analyses identified the isolate as a member of the under-represented phylum Gemmatimonadetes, where 'Gemmatirosa kalamazoonensis' KBS708, Gemmatimonas aurantiaca T-27T and Gemmatimonas phototrophica AP64T were the closest relatives, with respective similarities of 84.4, 83.6 and 83.3 %. Strain CB-286315T was characterized as a Gram-negative, non-motile, short to long rod-shaped bacterium. Occasionally, some cells attained an unusual length, up to 35–40 μm. The strain showed positive responses for catalase and cytochrome-c oxidase and division by binary fission, and exhibited an aerobic metabolism, showing optimal growth under normal atmospheric conditions. Strain CB-286315T was also able to grow under micro-oxic atmospheres, but not under anoxic conditions. The strain is a slowly growing bacterium able to grow under low nutrient concentrations. Major fatty acids included iso-C17 : 1v9c, summed feature 3 (C16 : 1v7c and/or iso-C15 : 02-OH), C16 : 0 and iso-C17 : 0. The major polar lipids were phosphatidylglycerol, phosphatidylethanolamine, phosphatidylcholine, two unidentified glycolipids and three phospholipids. The major isoprenoid quinone was MK-8 and the diagnostic diamino acid was meso-diaminopimelic acid. The DNA G+C content was 67.0 mol%. Based on a polyphasic taxonomic characterization, strain CB-286315T represents a novel genus and species, Longimicrobium terrae gen. nov., sp. nov., within the phylum Gemmatimonadetes. The type strain of Longimicrobium terrae is strain CB-286315T (= DSM 29007T = CECT 8660T). In order to classify the novel taxon within the existing taxonomic framework, the family Longimicrobiaceae fam. nov., order Longimicrobiales ord. nov. and class Longimicrobia classis nov. are also proposed.

The phylum Gemmatimonadetes is a cosmopolitan bacterial taxon, members of which inhabit a wide variety of ecological niches (DeBruyn et al., 2011; Hanada & Sekiguchi, 2014; Zhang et al., 2003) including terrestrial and marine environments (Kamagata, 2010). Although they are found worldwide, members of the phylum are usually found at low frequency in soil microbial communities, with relative abundances ranging from 0.2 to 6.5 % (DeBruyn et al., 2011). Presently, >13 000 environmental 16S rRNA gene sequences available in the SILVA SSU Ref 123 database are likely to be associated with the phylum Gemmatimonadetes (Quast et al., 2013). According to the environmental 16S rRNA gene sequences, the phylum Gemmatimonadetes can be divided into five major lineages at the class level (groups 1–5) (Hanada & Sekiguchi, 2014). Group 1 (class Gemmatimonadetes) is the numerically dominant lineage, and the majority of sequences are associated with soils, while the remaining sequences have been retrieved from...
activated sludge in wastewater treatment systems (Hanada & Sekiguchi, 2014). The class Gemmatimonadetes is the only one represented by cultured strains to date. Within this class, two genera, Gemmatimonas and ‘Gemmatirosa’, have been proposed. The first genus harbours two species, Gemmatimonas aurantiaca (Zhang et al., 2003) and Gemmatimonas phototrophica (Zeng et al., 2015), both only represented by the type strains. Gemmatimonas aurantiaca T-27T was isolated from a laboratory-scale anaerobic–aerobic sequential batch reactor operated under excess biological phosphorus removal (Zhang et al., 2003), whereas Gemmatimonas phototrophica AP64T, a microaerophilic, bacteriochlorophyll a-containing bacterium, was isolated from a freshwater lake (Zeng et al., 2015). The genus ‘Gemmatirosa’ comprises a single species, ‘Gemmatirosa kalamazoonensis’, of which the proposed type strain KBS708 was isolated from an organically managed agricultural soil (DeBruyn et al., 2013). However, the names of the genus and species have not yet been validly published (http://www.bacterio.net/index.html). Almost all of the environmental sequences from groups 2, 3 and 5 have a terrestrial origin, whereas group 4 includes sequences recovered from marine sediments and sponges (Hanada & Sekiguchi, 2014).

The taxonomic and, probably, the metabolic diversity of the members of this phylum may be as extensive as that of other well-studied phyla such as Proteobacteria and Actinobacteria (Hanada & Sekiguchi, 2014). Despite the progress made so far to understand the ecology of the Gemmatimonadetes through high-throughput sequencing technologies, full exploration of their metabolic and physiological capacities still requires in vitro culture. Because of the difficulty in culturing members of the Gemmatimonadetes, improved and/or alternative culture strategies are needed (Alain & Querelle, 2009; Overmann, 2013). In this context, our group developed the diffusion sandwich system (DSS), an array of 384 miniature diffusion chambers, to isolate recalcitrant bacteria. DSS is based on the concept of the Ichip (Ling et al., 2015; Nichols et al., 2010), but it is engineered in a standard Society for Biomolecular Sciences (SBS) format to facilitate high-throughput microbial isolation and subsequent transfers. The device consists of a sandwich of three perforated stainless-steel layers and two polycarbonate semipermeable membranes that retain bacterial cells embedded in a gel matrix in each miniature chamber, preventing microbial cells from migrating in and out, but allowing diffusion of nutrients, signal molecules and waste products between the chambers and the environment. After incubation buried in a simulated soil environment, the DSS is disassembled and individual gel plugs containing grown bacterial colonies are removed from the miniaturized chambers by pushing them out using a stainless steel replicator and dispersing them in 384-well culture microplates filled with R2A broth medium.

In the course of the DSS proof-of-concept validation, strain CB-286315T was isolated from a soil sample collected from the Sierra de Tejeda, Almijara and Alhama Natural Park, a typical Mediterranean forest ecosystem located in Granada, Spain (36° 54’ 34.4’’ N 3° 51’ 52.9’’ W, 972 m above sea-level), in February 2013. At the time of collection, the temperature was 10 °C and the soil temperature (~10 cm depth) was 7 °C. The soil pH was 8.5 in deionized water, and its water content was 11.5 %. For bacterial isolation, cells were embedded in 2.5 % (w/v) gellan gum (Gelzan CM Gelrite; Sigma-Aldrich) at an average of one cell per diffusion chamber. The DSS was buried 10 cm deep in a simulated soil environment that consisted of a 20-L plastic box filled with soil from the same location, and incubated under an alternating 12-h cycle of 10 and 18 °C for 2 months. Strain CB-286315T was isolated from one gellan-gum plug by transferring the material from the diffusion chamber to synthetic R2A broth medium (DSMZ medium 830). The 384-well culture microplate was initially incubated in the dark for 12 weeks at 18 °C and 60 % relative humidity with 150 r.p.m. orbital shaking and then transferred to fresh medium and incubated for another 4 weeks under the same conditions. Finally, strain CB-286315T was streaked onto R2A medium solidified with 1.5 % (w/v) noble agar (Sigma-Aldrich) and its purity was checked throughout the study by Gram staining and colonial micromorphology. The diffusion of low-molecular-mass compounds, e.g. nutrients available in the natural environment or signal molecules produced by other microorganisms, that is favoured by the DSS suggests that the isolation of CB-286315T might not be the result of a dilution-to-extinction factor favoured by the DSS, where numerically dominant taxa are isolated at higher frequency, but instead is a true enrichment facilitated by biotic and abiotic conditions reproduced from its natural environment.

The reference strains used in this taxonomic study were Gemmatimonas aurantiaca DSM 14586T and ‘Gemmatirosa kalamazoonensis’ ATCC BAA-2150. Unless otherwise noted, CB-286315T and the reference strains were grown under normal atmospheric conditions at 25 °C using R2A medium solidified with 1.5 % (w/v) noble agar or in R2A broth for 15 days at 28 °C in the dark for inoculum or sample preparation. Since Gemmatimonas phototrophica AP64T only grew well on agar plates (Zeng et al., 2015), its inclusion in parallel analyses based on broth cultures was not possible. However, Gemmatimonas phototrophica AP64T was characterized by Zeng et al. (2015) under comparable growth conditions.

Strain CB-286315T was found to be a Gram-negative bacterium according to a 3 % KOH assay (Powers, 1995) and Gram staining and oxidase-positive and catalase-positive based on tests with 1 % (w/v) N,N,N’,N’-tetramethyl p-phenylenediamine dihydrochloride and 3 % (v/v) hydrogen peroxide solutions, respectively.

Cell morphology was examined using phase-contrast, transmission electron and scanning electron microscopy (see supplementary methods available in the online Supplementary Material). Motility was checked by observing the bacteria in a wet mount with phase-contrast
microscopy and using semi-solid agar stabs (2 g agar \( \text{L}^{-1} \)) (Tittsler & Sandholzer, 1936). Cells were found to be non-motile, unlike *Gemmatimonas aurantiaca* DSM 14586\(^T\) and *Gemmatimonas phototrophica* AP64\(^T\) (Table 1). Cells were irregular-rod-shaped (1.3–15 \( \mu \text{m} \) long, 0.4–0.8 \( \mu \text{m} \) wide) (Table 1). Occasionally, some cells reached an unusual length, up to 35–40 \( \mu \text{m} \), during the exponential phase (Fig. 1). Septa could not be observed in elongated cells with transmission electron microscopy, suggesting these cells to be single cell units (Fig. 1). Further molecular studies based on targeting membrane proteins present in cell division septa will be carried out in order to confirm the unusual cell size. Cells divided by binary fission and, unlike members of the genera *Gemmatimonas* and ‘*Gemmatirosa*’, membrane vesicles with lipid bilayers budding off the cells were not evident with scanning or transmission electron microscopy (Fig. 1). In addition, strain CB-286315\(^T\) synthesized an unknown granular material intra-cellularly that was excreted externally. This material adhered to the cell surface as an extracellular matrix and, together with pilus-like structures, led to the formation of cellular aggregates (Fig. 1). The composition of the granular material, how it is excreted, the ultrastructure of the pilus-like structures and the biological role of this coating remain to be investigated. Capsules and endospores were not observed after staining the cells with Indian ink and malachite green, respectively. Intracellular polyphosphate accumulation was suggested by DAPI (4,6-diamidino-2-phenylindole) staining (DeBruyn et al., 2013; Zhang et al., 2003) and by the presence of electron-dense inclusion bodies in transmission electron micrographs (Fig. 1). These polyphosphate granules may represent a reservoir of inorganic phosphorus that can be used by bacteria under low-phosphate conditions. *Gemmatimonas aurantiaca* DSM 14586\(^T\) and ‘*Gemmatirosa kalamazoonensis*’ ATCC BAA-2150 are also characterized by their capacity to accumulate polyphosphate.

After 15 days on solid R2A noble agar, growth appeared as small, circular, convex, entire, translucent, pale salmon-pigmented colonies, 0.9–1.3 mm in diameter. In broth medium, cells did not flocculate and showed a whitish-pink colour. ‘*Gemmatirosa kalamazoonensis*’ ATCC BAA-2150 flocculated in broth medium, whereas *Gemmatimonas phototrophica* AP64\(^T\) was reported to be unable to grow in microaerobic and anaerobic atmospheres, bacterial cultures were incubated on solid R2A noble agar at 25 °C for 30 days. *Gemmatimonas aurantiaca* and ‘*Gemmatirosa kalamazoonensis*’ are also chemo-organoheterotrophic bacteria, but the former grew optimally under aerobic conditions (Zhang et al., 2003), like strain CB-286315\(^T\), whereas the latter preferred micro-oxic conditions (DeBruyn et al., 2013). *Gemmatimonas phototrophica* is a micro-aerophilic, bacteriochlorophyll \( \alpha \)-containing bacterium with a facultatively photoheterotrophic metabolism. *Gemmatimonas phototrophica* AP64\(^T\) is the only known representative of the phylum *Gemmatimonadetes* able to undergo photosynthesis. The organization and phylogeny of its photosynthesis genes suggest an ancient acquisition of the photosynthesis gene cluster via horizontal transfer from purple phototrophic bacteria (Zeng et al., 2014). The inability to grow under anoxic conditions and the lack of a fermentative metabolism are traits shared by all known members of the phylum.

Growth ranges and optima of temperature, salinity and pH were determined under normal atmospheric conditions in R2A broth medium at 25 °C. For each pH range, the appropriate buffer was used: MES for pH 5, 6 and 6.5, HEPES for pH 7, 7.5 and 8, HEPPS for pH 8.5 and CHES for pH 9 and 10 (from Sigma-Aldrich; 10 mM each). To test the salinity range, R2A broth medium was supplemented with increasing concentrations of NaCl, ranging from 0 to 1.5 % in increments of 0.1 % (w/v). Growth was determined by measuring the OD\(_{660}\). Optimal growth was defined as \( \geq 75 \) % of the highest growth rate achieved. Strain CB-286315\(^T\) was able to grow between 10 °C (weak growth) and 33 °C with a temperature optimum of 25–28 °C. Comparable range and optimum values were reported for other species of the *Gemmatimonadetes* (Table 1). Growth of strain CB-286315\(^T\) was observed between pH 6.0 and 9.0 and was optimal at pH 7.0–7.5, these results also being similar to those reported for other species of the *Gemmatimonadetes* (Table 1). Strain CB-286315\(^T\) was able to grow when R2A medium was supplemented with up to 0.4 % (w/v) NaCl (final concentration). Optimum growth was reached with 0.1 % (w/v) NaCl. None of the other strains tolerated NaCl concentrations higher than 1 % (w/v), with *Gemmatimonas aurantiaca* DSM 14586\(^T\) being the most halotolerant [up to 0.7 % (w/v) NaCl]. All strains showed optimal growth at the lowest NaCl concentrations tested (Fig. 1). Doubling time of growth under optimal conditions was 13.5 h, and strain CB-286315\(^T\) could therefore be considered a slowly growing bacterium, like *Gemmatimonas aurantiaca* T-27\(^T\) (12 h; Zhang et al., 2003).

For physiological tests, commercial miniaturized API 20NE, API 20E and API ZYM galleries (bioMérieux) were used in triplicate, following the instructions of the manufacturer. API 20NE and API 20E tests were examined after 48 h, except for assimilation/oxidation of substrates, which was examined after 2 weeks, and exoenzymic activities of the API ZYM gallery were revealed after 5 h of
Table 1. Differential characteristics of strain CB-286315T and type strains of other species of the phylum Gemmatimonadetes

Strains: 1, CB-286315T; 2, Gemmatimonas aurantiaca T-27T; 3, Gemmatimonas phototrophica AP64T (data from Zeng et al., 2015); 4, 'Gemmatirosa kalamazoonensis' KBS708. Data for growth, oxygen requirement, phenotypic properties and enzyme activities in columns 2 and 4 were obtained in this study from Gemmatimonas aurantiaca DSM 14586T and 'Gemmatirosa kalamazoonensis' ATCC BAA-2150 under comparable growth conditions; other data were taken from the original species descriptions (Zhang et al., 2003; DeBruyn et al., 2013). +, Positive; −, negative; w, weakly positive; v, variable response; NA, no data available; DAP, diaminopimelic acid. All strains form circular colonies.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isolation</td>
<td>Forest soil</td>
<td>Wastewater reactor</td>
<td>Freshwater lake</td>
<td>Agricultural soil</td>
</tr>
<tr>
<td>Source</td>
<td>DSS</td>
<td>NM-1 agar plate</td>
<td>Half-strength standard R2A agar plate</td>
<td>VL55 noble agar plate</td>
</tr>
<tr>
<td>Incubation time (days)</td>
<td>60</td>
<td>14</td>
<td>21–42</td>
<td>21–42</td>
</tr>
<tr>
<td>Colonies on solid media</td>
<td>Convex, entire, translucent, pale salmon, 0.9–1.3 mm diameter</td>
<td>Smooth, faintly orange to pink, 1–2 mm diameter</td>
<td>Tiny (~0.3 mm), smooth, red, 2 mm diameter</td>
<td>Smooth and convex, opaque, pink, &lt;2 mm diameter</td>
</tr>
<tr>
<td>Growth in broth media</td>
<td>Does not flocculate, whitish pink</td>
<td>NA</td>
<td>Does not grow well</td>
<td>Flocculates, light to dark pink</td>
</tr>
<tr>
<td>Cell shape</td>
<td>Short to long rods</td>
<td>Short rods</td>
<td>Short to long rods</td>
<td>Short to long rods</td>
</tr>
<tr>
<td>Cell size (μm)</td>
<td>0.4–0.6 × 1.3–15*</td>
<td>0.7 × 2.5–3.5</td>
<td>0.3–0.5 × 1–12</td>
<td>0.5–0.7 × 1–16</td>
</tr>
<tr>
<td>Budding morphology</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Motility</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Catalase production</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>Temperature range (optimum) (°C)</td>
<td>10–33 (25–28)</td>
<td>22–33 (30)†</td>
<td>16–30 (25–30)</td>
<td>18–37 (33)‡</td>
</tr>
<tr>
<td>pH range (optimum)</td>
<td>6.0–9.0 (7.0–7.5)</td>
<td>6.5–9.5 (7.0)§</td>
<td>6.0–9.0 (7.0–7.5)</td>
<td>5.0–7.0 (6.0)</td>
</tr>
<tr>
<td>NaCl concentration range (% w/v)</td>
<td>0–0.4</td>
<td>0–0.71</td>
<td>0–0.2</td>
<td>0–0.4</td>
</tr>
<tr>
<td>Metabolism</td>
<td>Chemoheterotroph</td>
<td>Chemoheterotroph</td>
<td>Facultative photoheterotroph</td>
<td>Chemoheterotroph</td>
</tr>
<tr>
<td>Doubling time (h)</td>
<td>14</td>
<td>12</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Oxygen requirement</td>
<td>Aerobic</td>
<td>Aerobic</td>
<td>Microaerophilic</td>
<td>Microaerophilic</td>
</tr>
<tr>
<td>Preferred mode</td>
<td>Microaerophilic</td>
<td>Microaerophilic</td>
<td>None</td>
<td>Microaerophilic</td>
</tr>
<tr>
<td>Other tolerated mode</td>
<td>NA</td>
<td>NA</td>
<td>−</td>
<td>NA</td>
</tr>
<tr>
<td>Hydrolysis of gelatin</td>
<td>+</td>
<td>V</td>
<td>NA</td>
<td>+</td>
</tr>
<tr>
<td>Enzyme activities</td>
<td>Esterase (C4)</td>
<td>Esterase lipase (C8)</td>
<td>Valine arylamidase</td>
<td>N-Acetyl-β-glucosaminidase</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td></td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>w</td>
<td>−</td>
<td>w</td>
</tr>
<tr>
<td>Major quinone</td>
<td>MK-8</td>
<td>MK-9</td>
<td>MK-8</td>
<td>MK-9</td>
</tr>
<tr>
<td>Diagnostic diamin acid</td>
<td>meso-DAP</td>
<td>No DAP isomers detected</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>DNA G+C content (mol%)</td>
<td>67.0</td>
<td>64.3§</td>
<td>64.4§</td>
<td>72.0§</td>
</tr>
</tbody>
</table>

*Cells can occasionally elongate up to 35–40 μm.
†Reported as 22–33 °C (optimum 30 °C) by Zhang et al. (2003).
‡Reported as 20–40 °C (optimum 37 °C) by DeBruyn et al. (2013).
§Reported as pH 6.0–10.0 (optimum pH 7.0–7.5) by Zeng et al. (2015).
||Reported as 0–0.8% (w/v) NaCl by Zeng et al. (2015).
*Based on published genome sequences.
incubation. Strain CB-286315<sup>T</sup> showed a negative response for reduction of nitrates, indole production, fermentation of glucose, arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, citrate utilization, H₂S production, tryptophan deaminase, acetoin production and urease. However, it showed a positive result for gelatinase, like ‘Gemmatirosa kalamazoonensis’ ATCC BAA-2150 and Gemmatimonas aurantiaca DSM 14586<sup>T</sup> (Table 1). In API 20NE, strain CB-286315<sup>T</sup> showed variable responses for the assimilation of D-mannitol and maltose and, in API 20E, it showed a variable response for oxidation of L-arabinose. The small number of carbon sources assimilated or oxidized in API 20NE or API 20E, respectively, might be caused by either the high concentration of nutrients provided in these commercial kits or its inability to use the provided substrates. Among the exoenzymic activities, strain CB-286315<sup>T</sup> showed a preference towards phosphate esters (alkaline and acid phosphatases and naphthol-AS-BI-

**Fig. 1.** Cell morphology of strain CB-286315<sup>T</sup> grown on R2A agar or in R2A broth at 28 °C for 15 days. (a, b) Phase-contrast photomicrographs of cells immobilized on agarose-covered slides (1 %, w/v). The images were taken with a Zeiss Axioskop microscope equipped with an Olympus DP-12 camera (×1000 magnification). (c, d) Transmission electron micrographs. (e–h) Scanning electron micrographs. PI, Polyphosphate inclusions, which appear as electron-dense inclusion bodies; PLI, pilus-like structures; UP, unidentified polymer. The unidentified polymer with a granular structure is synthesized intracellularly and excreted outside and adheres to the cell surface. The polymer, along with pilus-like structures, forms an extracellular matrix that leads to the formation of cellular aggregates. Bars, 10 μm (a, b), 1 μm (c, h), 2 μm (d, f), 500 nm (e) and 5 μm (g).
phosphohydrolase), esterases [esterase (C4) and esterase lipase (C8)] and leucyl aryiamidase, showing variable activity for α-glucosidase. *Gemmatimonas aurantiaca* DSM 14586<sup>T</sup> and *Gemmatiersona kalamazoonensis* ATCC BAA-2150 showed a positive response for alkaline phosphatase, naphthol-AS-BI-phosphohydrolase and leucyl aryiamidase and a weak response for acid phosphatase (Table 1). In addition, *Gemmatimonas aurantiaca* DSM 14586<sup>T</sup> showed positive responses for esterase (C4), esterase lipase (C8), α-chymotrypsin, valine aryiamidase and N-acetyl-β-glucosaminidase and weak activity for cystine aryiamidase and trypsin. *Gemmatiersona kalamazoonensis* ATCC BAA-2150 showed weak responses for valine aryiamidase and N-acetyl-β-glucosaminidase. Other exoenzymic activities were reported as negative.

Individual carbon substrates used for growth were analysed in triplicate using the basal medium VL70 (DSMZ medium 1266 without added glucose and buffered at pH 7.0 with 10 mM HEPES). The final concentration of each substrate is detailed in Table S1, available in the online Supplementary Material. Substrates were scored as supporting growth when the ratio between the final OD<sub>660</sub> (mean of parallel incubations) and the initial OD<sub>660</sub> control value (without the addition of substrate) was higher than 1.5 after 30 days of incubation. Strain CB-286315<sup>T</sup> grew on 56 of 73 carbon sources tested, showing weak growth on 11 of them. The strain was able to utilize a wide range of substrates, mainly complex protein substrates, simple sugars, sugar alcohols, organic acids and complex carbohydrate polymers such as cellulose, laminarin, starch and xylan. The full list of sole substrates used by strain CB-286315<sup>T</sup> is detailed in the species description. *Gemmatimonas aurantiaca* DSM 14586<sup>T</sup> and *Gemmatiersona kalamazoonensis* ATCC BAA-2150 showed narrower ranges of preferred sole carbon sources (Table S2). *Gemmatimonas phototrophica* AP64<sup>T</sup> was characterized as using just a few substrates for growth, mainly protein-containing complex substrates such as yeast extract and peptone (Zeng et al., 2015).

The ability of strain CB-286315<sup>T</sup> to grow on serially diluted complex culture media was tested in order to quantify its oligotrophic metabolism. It was able to grow in the following media: 1 × R2A broth, 1/5 R2A broth, 1/10 R2A broth, 1/50 R2A broth, 1 × nutrient broth (NB; BD Difco), 1/10 NB, 1/10 trypsinase soy broth (TSB; BD Difco) and 1/10 brain heart infusion broth (BD Difco), showing a weak response on 1/100 R2A broth. No growth was observed in 1 × TSB or 1 × brain heart infusion broth. These results confirm the oligotrophic metabolism of strain CB-286315<sup>T</sup>, which grows only at low nutrient concentrations and not in rich media.

The molar G+C content of genomic DNA was analysed by HPLC by the Identification Service of the DSMZ. The polar lipid composition of strain CB-286315<sup>T</sup> was determined. For fatty acid methyl ester (FAME) analysis, fatty acids were extracted, saponified and methylated according to standard protocols (MIDI Microbial Identification System) (Sasser, 1990), and individual FAMEs were identified using the Microbial Identification System using the TSBA50 library (MIDI). The major fatty acids of strain CB-286315<sup>T</sup> were iso-C<sub>17</sub>:<sub>0</sub>3OH (47.5 %), summed feature 3 (C<sub>16</sub>:<sub>0</sub>3OH and/or iso-C<sub>15</sub>:<sub>0</sub> 2-OH: 13.6 %), C<sub>16</sub>:<sub>0</sub> (7.7 %), iso-C<sub>17</sub>:<sub>0</sub> (7.4 %) and iso-C<sub>15</sub>:<sub>0</sub> (6.1 %) (Table S3). The FAME profile of *Gemmatimonas aurantiaca* DSM 14586<sup>T</sup> reported in this study was comparable to that originally reported by Zhang et al. (2003), and was characterized by major amounts of iso-C<sub>15</sub>:<sub>0</sub> 3OH, summed feature 3 (C<sub>16</sub>:<sub>0</sub>3OH and/or iso-C<sub>15</sub>:<sub>0</sub> 2-OH) and iso-C<sub>13</sub>:<sub>0</sub>. However, Zeng et al. (2015) reported a FAME profile of *Gemmatimonas aurantiaca* T-27<sup>T</sup> different from that reported by Zhang et al. (2003). Zeng et al. (2015) speculated that the reason for these differences could be the use of different growth conditions (e.g. bacteria grown on solid medium vs liquid medium). However, since our results are in agreement with those reported by Zhang et al. (2003), we hypothesize that the differences in this specific case were more related to how the fatty acids were processed and further analysed than to the growth conditions. *Gemmatimonas phototrophica* AP64<sup>T</sup> was characterized by major amounts of C<sub>16</sub>:<sub>1</sub>c, C<sub>14</sub>:<sub>0</sub>c, C<sub>18</sub>:<sub>1</sub>ω9c and C<sub>16</sub>:<sub>0</sub>. In the case of *Gemmatiersona kalamazoonensis* KBS708, the dominant FAMEs were iso-C<sub>17</sub>:<sub>0</sub>3OH, iso-C<sub>15</sub>:<sub>0</sub> and C<sub>16</sub>:<sub>1</sub>c. Based on these results, we confirmed that members of the phylum *Gemmatimonadetes* are characterized by large amounts of C<sub>16</sub>:<sub>1</sub>c. 3OH. The methyl-branched saturated fatty acid iso-C<sub>17</sub>:<sub>0</sub> 2-OH was also a major FAME in *Gemmatimonas aurantiaca* DSM 14586<sup>T</sup> and *Gemmatiersona kalamazoonensis* KBS708. Polar lipid composition, isoprenoid quinones and the diagnostic dimano acid of the peptidoglycan were analysed by the Identification Service of the DSMZ. The polar lipid composition of strain CB-286315<sup>T</sup> was analysed by two-dimensional TLC (Bligh & Dyer, 1959; Tindall et al., 2007). The major polar lipids of the strain were phosphatidylglycerol, phosphatidylethanolamine, phosphatidylycholine, two unidentified glycolipids and three phospholipids. In addition, four unidentified lipids and one atypical glycolipid were reported at lower concentrations (Fig. S1). The large number of unidentified polar lipids synthesized by strain CB-286315<sup>T</sup>, including an atypical lipid, confirmed its taxonomic novelty. Further characterization of these unidentified polar lipids will be carried out in a future study. The polar lipid profiles of
other members of the Gemmatimonadetes have not yet been analysed, and the profile of strain CB-286315T therefore represents the only data available for the phylum. Iso- 
prenequinones were extracted from dried biomass with chloroform/methanol (2:1, v/v) (Collins & Jones, 1981) and analysed by HPLC (Tindall, 1990). The predominant respiratory quinone of CB-286315T was MK-8 (100 %), which is in agreement with Gemmatimonas phototrophi 
a gene sequence similarity, respectively. 
from public databases (Munoz et al., 2011; Quast et al., 2013) and their accession numbers are provided with the phylogenetic trees calculated with the neighbour-joining, maximum-parsimony and maximum-likelihood algorithms (Figs 2 and S2). According to the EzTaxon-e database (Kim et al., 2012), the closest type strains to strain CB-286315T were ‘Gemmatirosa kalamazo 
se’ KBS708 (GenBank accession no. HM154525), Gemmati 
onas aurantiaca T-27T (AB072735) and Gemmatimonas phototrophi 
a gene sequence identity of 92.7 %. 
ial genome of strain CB-286315T was clone FCPS453 (GenBank accession no. EF516348), which was recovered from a grassland soil, sharing 16S rRNA gene sequence identity of 92.7 %. Group 3 appeared as an external lineage to groups 2, 4 and 5. Whereas groups 2, 3 and 5 were considered terrestrial groups, group 4 is a marine benthic group (Hanada & Sekiguchi, 2014). The class Gemmatimonadetes (group 1), including the genera Gemmatimonas and ‘Gemmatirosa’, appeared as an external clade. To the best of our knowledge, strain CB-286315T is the first cultured representative of group 3, and therefore represents an important discovery taking into account the great phylo 
genetic distance from its closest cultured relatives (group 1). 
This discovery demonstrates the usefulness of the DSS as a tool for the isolation and further in vitro ‘domestication’ of recalcitrant bacterial taxa. 
According to the thresholds delineated by Yarza et al. (2014) and Rosselló-Mora & Amann (2013) to delimit higher taxonomic levels based on 16S rRNA gene sequence similarities, strain CB-286315T belongs to an independent class within the phylum Gemmatimonadetes(<86 % sequence identity). In addition to this phylogenetic conclusion, this phylogenetic distance was correlated with a large number of significant chemotaxonomic and phenoty 
pic differences from Gemmatimonas aurantiaca T-27T and Gemmatimonas phototrophi 
a gene, for which we propose the name of Longimicrobium terrae gen. nov., sp. nov. In addition, we propose the formal description of the novel family Longimicrobiaceae fam. nov., order Longimicrobiales ord. nov. and class Longimicrobia classis nov. to accommodate the genus Longimicrobium. 

**Description of Longimicrobium gen. nov.**

Longimicrobium (Lon.gi.mi.cro’bi.um. L. adj. longus long; N.L. neut. n. micro 
bum; N.L. neut. n. Longimicro 
bium long microbe, referring to the capacity of the type species to form very long rods). 
Gram-negative, non-spore-forming, non-motile, short to 
long rod-shaped bacteria. Cells divide by binary fission. Aerobic chem 
organoheterotrophs with optimal growth under normal atmospheric conditions. Also able to grow under micro-oxic atmospheres, but not under anoxic con 
ditions. Store inorganic phosphorus as intracellular polyphosphate granules. Mesophilic, neutrophilic or slightly alkaliphilic, and non-halophilic bacteria. Positive for cata 
lase and cytochrome-c oxidase. Slow-growing bacteria able to grow at low nutrient concentrations. Major fatty acids include iso-C17:1ω9c, summed feature 3 (C16:1ω7c and/or iso-C15:0 2-OH), C16:0 and iso-C17:0. Major polar lipids are phosphatidylglycerol, phosphatidylethano 
lamine, phosphatidylcholine, two unidentified glycolipids and three phospholipids. The major isoprenoid quinone is MK-8. The diagnostic diamino acid is meso-diaminopimel 
ic acid. The type species is Longimicrobium terrae.
Description of *Longimicrobium terrae* sp. nov.

*Longimicrobium terrae* (ter'rae. L. gen. n. terrae of the soil, the source of the type strain).

Has the following characteristics in addition to the characteristics that define the genus. Cells are short to long rods (1.3–15 μm long and 0.4–0.6 μm wide) that occur as cellular aggregates. Occasionally, cells can elongate up to 35–40 μm. On solid medium, colonies are small, circular, convex, entire, translucent and pale salmon-pigmented (0.9–1.3 mm in diameter). In liquid medium, cells do not flocculate and are a whitish pink. Growth is observed at
10–33 °C (optimum 25–28 °C), at pH 6.0–9.0 (optimum pH 7.0–7.5) and in media containing up to 0.4 % (w/v) NaCl [optimum 0.1 % (w/v) NaCl]. Doubling time under optimal conditions is 13.5 h. Displays activities of alkaline phosphatase, naphthol-AS-Bl-phosphohydrolase, esterase (C4), esterase lipase (C8), leucyl arylamidase and gelatinase. Variable response for 2-glucosidase. Shows no activity of lipase (C14), valine arylamidase, cystine arylamidase, trypsin, 2-chymotrypsin, 2-galactosidase, 2-gallactosidase, 2-glucuronidase, 2-glucosidase, N-acetyl-2-glucosaminidase, 2-mannosidase or 2-fucosidase. Shows negative responses for reduction of nitrates, indole production, fermentation of glucose, arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, L-lysine decarboxylase, ornithine decarboxylase, citrate utilization, H2S production, tryptophan deaminase, acetoin production and urease. Substrates used for growth include cornmeal, gelatin, peptone, peptonized milk, soybean flour (type not roasted), yeast extract, adonitol, cellulose, L-arabinose, D-arabinose, D-mannitol, D-sorbitol, D-galactose, D-glucose, lactose, maltose, D-mannose, melezitose, raffinose, trehalose, dextrin, D-sorbitol, galactitol, inulin, L-rhamnose, N-acetyl-D-glucosamine, laminarin, starch, sucrose, trehalose, xylan, L-arginine, L-glutamic acid, L-methionine, L-proline, alginic, CM-cellulose, polyethylene glycol, lactate, propionate and pyruvate. Weak responses are observed on glycerol, D-ribose, cellulbiose, ascuclin, melibiose, 2-cyclodextrin, DL-tryptophan, L-asparagine, L-leucine, L-phenylalanine and L-serine. No growth occurs on keratin, D-fructose, D-xylitol, D-glucose 6-phosphate, L-sorbitose, D-glucosamine, glycin, L-alanine, L-histidine, L-lysine, L-tyrosine, N-lipoic acid, 2,2-dimethyl succinate, citrate, succinate, D-glucuronate, 4-aminobenzoate, abietic acid, deoxycholic acid and diethanolamine. Grows in 1 × R2A broth, 1/5 R2A broth, 1/10 R2A broth, 1/50 R2A brain heart infusion broth. A weak response is observed in 1 × R2A broth, 1/5 R2A broth, 1/10 R2A broth, 1/10 brain heart infusion broth. A weak response is observed in 1/50 R2A broth. No growth is observed in 1 × trypticase soy broth and 1 × brain heart infusion broth. The type strain is CB-286315T (= DSM 29007T = CECT 8660T), isolated from a Mediterranean forest soil sampled in the Sierra de Tejeda, Almijara and Alhama Natural Park, Spain (36° 54’ 34.4” N 3° 51’ 52.9’ W, 972 m above sea-level). A miniature diffusion chamber system named the diffusion sandwich system (DSS) was used to isolate the strain. The DNA G+C content of the type strain is 67.0 mol%.

Description of Longimicrobiaceae fam. nov.

Longimicrobiaceae (Lon.gi.mi.cro.bi.a’ceae. N.L. neut. n. Longimicrobium type genus of the family; suff. -aceae ending to denote a family; N.L. neut. pl. n. Longimicrobiaceae the Longimicrobium family).

The description is the same as for the genus Longimicrobium. The type genus is Longimicrobium. The DNA G+C content of the type strain of the type species of the type genus is 67.0 mol%.

Description of Longimicrobiales ord. nov.

Longimicrobiales (Lon.gi.mi.cro.bi.a’les. N.L. neut. n. Longimicrobium type genus of the order; suff. -ales ending to denote an order; N.L. neut. pl. n. Longimicrobiales the order of the genus Longimicrobium).

The description is the same as for the genus Longimicrobium. The type genus is Longimicrobium gen. nov. The DNA G+C content of the type strain of the type species of the type genus is 67.0 mol%.

Description of Longimicrobia classis nov.

Longimicrobia (Lon.gi.mi.cro.bi’a. N.L. neut. n. Longimicrobium type genus of the type order of the class; suff. -ia ending to denote a class; N.L. neut. pl. n. Longimicrobia the class of the order Longimicrobiales).

The delineation of the class is primarily determined on the basis of phylogenetic analysis of 16S rRNA gene sequences and is equivalent to group 3 of the phylum Gemmatimonadetes proposed by Hanada & Sekiguchi (2014). The class currently comprises the sole order Longimicrobiales, which is also the type order.

Acknowledgements

This research was supported by the Non-Oriented Basic Research Program of the Spanish Ministry of Science and Innovation (project SAF2010-15010). The authors thank Professor Dr Aharon Oren and Professor Dr Bernhard Schink for their advice on the nomenclature. Mrs Cristina Carmona and Mr Bernabé Martos are appreciated. We are grateful to the administration of the Sierra de Tejeda, Almijara and Alhama Natural Park for permission to collect soil.

References

A new antibiotic kills pathogens without detectable
bacteria and yeasts.

Kim, O. S., Cho, Y. J., Lee, K., Yoon, S. H., Kim, M., Na, H., Park, S. C.,


