Gemmatimonas aurantiaca gen. nov., sp. nov., a Gram-negative, aerobic, polyphosphate-accumulating micro-organism, the first cultured representative of the new bacterial phylum *Gemmatimonadetes* phyl. nov.

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A phylogenetically novel aerobic bacterium was isolated from an anaerobic–aerobic sequential batch reactor operated under enhanced biological phosphorus removal conditions for wastewater treatment. The isolation strategy used targeted slowly growing polyphosphate-accumulating bacteria by combining low-speed centrifugations and prolonged incubation on a low-nutrient medium. The isolate, designated strain T-27^T, was a Gram-negative, rod-shaped aerobe. Cells often appeared to divide by budding replication. Strain T-27^T grew at 25–35 °C with an optimum growth temperature of 30 °C, whilst no growth was observed below 20 °C or above 37 °C within 20 days incubation. The pH range for growth was 6·5–9·5, with an optimum at pH 7·0. Strain T-27^T was able to utilize a limited range of substrates, such as yeast extract, polypepton, succinate, acetate, gelatin and benzoate. Neisser staining was positive and 4,6-diamidino-2-phenylindole-stained cells displayed a yellow fluorescence, indicative of polyphosphate inclusions. Menaquinone 9 was the major respiratory quinone. The cellular fatty acids of the strain were mainly composed of iso-C15 : 0, C16 : 1 and C14 : 0. The G+C content of the genomic DNA was 66 mol%. Comparative analyses of 16S rRNA gene sequences indicated that strain T-27^T belongs to candidate division BD (also called KS-B), a phylum-level lineage in the bacterial domain, to date comprised exclusively of environmental 16S rDNA clone sequences. Here, a new genus and species are proposed, *Gemmatimonas aurantiaca* (type strain T-27^T = JCM 11422^T = DSM 14586^T) gen. nov., sp. nov., the first cultivated representative of the *Gemmatimonadetes* phyl. nov. Environmental sequence data indicate that this phylum is widespread in nature and has a phylogenetic breadth (19 % 16S rDNA sequence divergence) that is greater than well-known phyla such as the *Actinobacteria* (18 % divergence).

INTRODUCTION

It is now well recognized that most micro-organisms in nature have not yet been cultivated (Amann *et al.*, 1995). In the past decade, culture-independent rRNA-based molecular methods have provided means of identifying the types of micro-organisms that occur in microbial communities without the need for cultivation (Amann *et al.*, 1995; Pace *et al.*, 1986; Hugenholtz & Pace, 1996). Consequently, the number of bacterial phyla (divisions) identified has more than tripled to over 30 due, in significant part, to culture-independent phylogenetic surveys of environmental microbial communities (Hugenholtz *et al.*, 1998; Hugenholtz, 2002). Approximately a third to one half of these phyla are yet to be represented by cultured organisms and are characterized only by environmental 16S rDNA clone sequences (Hugenholtz, 2002).

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Abbreviations: DAP, diaminopimelic acid; DAPI, 4,6-diamidino-2-phenylindole; EBPR, enhanced biological phosphate removal.

The GenBank/EMBJ/DDBJ accession number for the 16S rRNA gene sequence of strain T-27^T is AB072735.

Recently, a candidate division named the BD group was proposed by Hugenholtz *et al.* (2001) based on environmental sequence data. In their report, the BD group consisted of five 16S rDNA sequences from different environmental samples: two sequences from deep-sea sediments after which the group was named (Li *et al.*, 1999), two unpublished sequences from soil samples and one sequence from an enhanced biological phosphorus removal (EBPR) sludge (Hugenholtz *et al.*, 2001). The phylogenetic novelty of this group was recognized independently by other researchers at almost the same time and they named the lineage candidate division KS-B (Madrid *et al.*, 2001). Additional environmental sequences, particularly from soil and marine habitats, are expanding the BD group (see below), but no cultivated representative of the group has been identified up to now.

We have isolated a bacterium, designated strain T-27^T, from an anaerobic–aerobic sequential batch reactor operated under EBPR conditions as part of an ongoing survey of the diversity of this habitat. Phylogenetic analyses based on 16S rRNA gene sequences indicate that strain T-27^T belongs to the BD group. In this paper, we characterize strain T-27^T using a polyphasic approach and propose the name *Gemmatimonas aurantiaca* gen. nov., sp. nov. for the isolate. We also name the phylum that it represents *Gemmatimonadetes* phyl. nov.

METHODS

Sequencing batch reactor operation. The isolate was obtained from a laboratory-scale anaerobic–aerobic sequential batch wastewater treatment reactor operated under EBPR conditions (Liu *et al.*, 1997). A 2·5 l polycarbonate cylinder (inner diameter, 10 cm; depth, 36 cm) with a working volume of 1·8 l was used as a reactor for the enrichment of EBPR sludge. The reactor was operated in a cycle with three distinct periods, an anaerobic period (60 min), an aerobic period (90 min) and a settling period (30 min). A volume of 0·9 l of treated water was replaced with fresh synthetic wastewater to give a biochemical oxygen demand (BOD) loading rate of 0·5 kg m^{-3}. The synthetic wastewater contained 177 mg acetate, 55 mg peptone, 14·4 mg $CaCl_2$, 95·0 mg $MgSO_4.7H_2O$, 12·7 mg $(NH_4)_2SO_4$ and 21·1 mg KH_2PO_4 in 1 l tap water. The sludge retention time was controlled to 20 days and the sludge concentration remained at 4–5 g dry weight l^{-1}. After 2 months of operation, the mean phosphorus content in the sludge at the end of aerobic period was about 60 mg P (g dry weight)$^{-1}$.

Enrichment strategy and isolation. A sludge sample was taken from the reactor and diluted to a concentration of 1000 mg l^{-1} with a dilution buffer containing 20 mM sodium pyrophosphate and 20 mM EDTA (pH 7·0). After a short ultrasonic treatment (Tomy Seiko; model UR-200P) (equipped with a micro-tip; output 10 W, 15 s, constant pulsing) to disperse aggregates, the sludge suspension was centrifuged at 100 ***g*** for 5 min to remove remaining aggregates. The supernatant was then subjected to a further centrifugation at 1400 ***g*** for 5 min to pellet heavier individual cells. After washing three times with distilled water, the pelleted bacterial cells were diluted and spread onto plates of NM-1 medium [Nakamura *et al.*, 1995; containing (l^{-1}) 0·5 g glucose, 0·5 g polypepton, 0·5 g sodium glutamate, 0·5 g yeast extract, 0·44 g KH_2PO_4, 0·1 g $(NH_4)_2SO_4$, 0·1 g $MgSO_4.7H_2O$, 1 ml vitamin solution and 20 g agar]. The pH was adjusted to 7·0 with the addition of NaOH. The vitamin solution contained (l^{-1}): 1·0 g nicotinic acid, 1·0 g thiamin hydrochloride, 50 mg biotin, 0·5 g *p*-aminobenzoic acid, 10 mg vitamin B_{12}, 0·5 g calcium pantothenate, 0·5 g pyridoxine hydrochloride and 0·5 g folic acid. Strain T-27^T was obtained by picking single, non-transparent colonies from the agar plates after incubation at 25 °C for 2 weeks. Strain T-27^T was cultivated aerobically in liquid NM-1 medium at 30 °C for physiological and morphological analyses.

Microscopy. Cell morphology was examined under a phase-contrast microscope (Olympus AX80T). For electron microscopy of ultrathin sections, cells were embedded in Spurr medium (Kushida, 1980) after fixing with 2·5 % (v/v) glutaraldehyde and 1 % (w/v) osmium tetroxide. Photomicrographs were obtained with a Hitachi H-7000 transmission electron microscope operated at 75 kV. Scanning electron micrographs were obtained as described previously (Sekiguchi *et al.*, 2000).

Physiological and biochemical characterization. Gram staining was performed as described by Magee *et al.* (1975). Oxidase activity was determined by monitoring the oxidation of tetramethyl-*p*-phenylenediamine on filter paper and catalase activity was determined using a 3 % hydrogen peroxide solution (Smibert & Krieg, 1994). For determination of nitrate reduction, strain T-27^T was grown in NM-1 medium supplemented with 2·0 g $NaNO_3$ l^{-1} under anaerobic conditions. Fermentative growth was tested in a screw-capped tube filled with NM-1 medium in the presence of 0·2 % glucose and/or 0·2 % yeast extract. Neisser staining was performed as described by Jenkins *et al.* (1993). DAPI (4,6-diamidino-2-phenylindole) staining was performed by addition of 10 µl of a solution of 1 mg DAPI l^{-1} to 1 ml cell suspension and subsequent incubation for 5 min. DAPI-stained cells were observed under a fluorescence microscope (Olympus AX80T). Carbon source utilization was determined using a basal medium supplemented with test substrates (0·5 g l^{-1}). The basal medium contained (l^{-1}) 0·44 g KH_2PO_4, 0·1 g $(NH_4)_2SO_4$, 0·1 g $MgSO_4.7H_2O$ and 1 ml vitamin solution, the pH being adjusted to 7·0.

Quinone, fatty acid and cell wall analyses. Quinones were extracted with chloroform/methanol (2 : 1, v/v). The extract was purified by a Sep-Pak Plus column (Waters) and analysed by reverse-phase HPLC (Beckman System Gold with a Hewlett Packard Zorbox ODS column) for identification (Tamaoka *et al.*, 1983). Cellular fatty acid methyl esters were analysed by a Hitachi M7200A GC/3DQMS system equipped with a DB-5ms capillary column (30 m by 0·25 mm) coated with (5 %-phenyl)-methylpolysiloxane (J & W Scientific). The analytical procedure was described by Hanada *et al.* (2002a). The presence of diaminopimelic acid (DAP) isomers in the cell-wall peptidoglycan was determined by TLC with a silica gel plate (Merck; no. 5716) after hydrolysis with 6 M HCl at 100 °C for 18 h (Komagata & Suzuki, 1987).

DNA base composition. Total DNA of strain T-27^T was extracted according to the procedure of Saito & Miura (1963). Total DNA was digested with P1 nuclease using a Yamasa GC kit (Yamasa Shoyu). The G+C content was measured by HPLC (Kamagata & Mikami, 1991).

16S rRNA gene sequence and phylogenetic analysis. Cells of strain T-27^T were lysed by the method of Hiraishi (1992). A 16S rDNA fragment was amplified by PCR (Hiraishi *et al.*, 1994) using the universal primers 27F (5′-AGAGTTTGATCATGGCTCGA-3′; positions 8–27 in the *Escherichia coli* numbering system) and 1492R (5′-GGCTACCTTGTTACGACTT-3′; positions 1510–1492) (Weisburg *et al.*, 1991). The PCR product was sequenced directly on an ABI 377 DNA sequencer using a dRhodamine Dye Terminator cycle sequencing kit (Applied Biosystems). The compiled 16S rDNA sequence was aligned against an ARB dataset using the ARB program

package (http://www.arb-home.de/) and refined manually based on primary and secondary structural considerations. 16S rDNA sequences related to T-27^T were obtained from the public databases by similarity searches and added to the ARB database. Putative chimeric sequences were identified by partial treeing analysis as described previously (Hugenholtz & Huber, 2003). Twenty-seven *Gemmatimonadetes* reference sequences and a bacterial domain reference sequence dataset (Hugenholtz, 2002) representing most of the major recognized bacterial phyla were selected to generate a *de novo* evolutionary distance tree using the Olsen correction and neighbour joining (Fig. 3). The Lane mask (Lane 1991), available as a filter in ARB, or a *Gemmatimonas*-specific mask created in ARB based on 50 % minimal similarity was applied to the dataset to remove regions of ambiguous positional homology, leaving 1287 or 1358 bp, respectively, for comparative analyses. Sequence identities between 16S rRNA gene sequences were calculated from the 1287 bp dataset. Phylogenetic trees based on the 16S rDNA dataset and smaller datasets containing only the *Gemmatimonadetes* sequences and various outgroups were constructed by three different algorithms according to a previous report (Kim *et al.*, 2000); the neighbour-joining method (Saito & Nei, 1987) with the MEGA2 package (Kumar *et al.*, 2001), the maximum-parsimony method with the PAUP* 4.0 package (Swofford, 2002) and the maximum-likelihood method with the MOLPHY package (Adachi & Hasegawa, 1996). Bootstrap resampling analysis (Felsenstein, 1985) for 1000 replicates was performed with neighbour-joining and maximum-parsimony analyses to estimate the confidence of tree topologies. For maximum-likelihood analyses, local bootstrap probabilities were estimated by the RELL (resampling of estimated log-likelihood) method (Kishino *et al.*, 1990; Hasegawa & Kishino, 1994). In addition, posterior probabilities of branching points were estimated by Bayesian inference using MRBAYES version 2.01 (Huelsenbeck & Ronquist, 2001) with the default settings and general time-reversible model (16 substitution types, *nst*=6) and a gamma rate correction (rates=gamma) suggested for RNA data. A total of 100 000 generations were calculated for two truncated *Gemmatimonadetes* datasets, from which a tree was saved every 100 generations. The likelihoods of the trees (with WS3 or marine group A sequences used as outgroups) had converged on a stable value after 20 000 generations, and consensus trees were constructed from the final 800 trees in PAUP*.

RESULTS AND DISCUSSION

Isolation and morphology

Strain T-27^T was isolated from a laboratory-scale sequential batch wastewater treatment process operated under EBPR conditions. A strategy was taken to isolate slowly growing, polyphosphate-accumulating bacteria that may be important but uncharacterized members of EBPR communities. Low-speed centrifugations were applied initially to sonicated sludge samples to enrich for heavy and/or large cells that may contain various accumulated materials, such as polyphosphate, poly-β-hydroxyalkanoate and glycogen. Following enrichment, cells were plated onto a heterotrophic agar medium, NM-1, and incubated for up to 12 weeks. Pinpoint and slowly appearing colonies were preferentially selected from the isolation plates and purified through repeated isolation of single colonies on NM-1 media. Twenty isolates were obtained in this manner and checked for polyphosphate inclusions by Neisser staining. Among the Neisser-positive isolates (13/20), one strain, designated T-27^T, warranted further investigation based upon its novel 16S rDNA sequence.

After 2 weeks incubation, colonies of strain T-27^T were circular, smooth, faintly orange to pink and only 1–2 mm in diameter. This slow growth, resulting in pinpoint colonies, may explain why no closely related isolates have been obtained in pure culture previously. No diffusible pigment production was observed. Cells were rod-shaped, Gram-negative, motile, 0·7 μm in width and 2·5–3·2 μm in length (mean size 2·8 μm) (Fig. 1). Spore formation was not observed. Electron microscopy confirmed that cells of strain T-27^T possess a Gram-negative cell envelope, with the cytoplasmic and outer membranes readily visible (Fig. 2). Electron-dense and transparent inclusion bodies are present in TEM images (Fig. 2a). Cells reproduce by binary fission and often show budding morphology, suggesting asymmetrical cell division (Figs 1 and 2b).

Physiological and biochemical characteristics

Strain T-27^T grows at 25–35 °C with an optimum temperature of 30 °C; no growth was observed below 20 or above 37 °C during 20 days incubation. The pH range for growth is 6·5–9·5, with the optimum at pH 7·0. The minimum doubling time achieved was 12 h in liquid NM-1 medium (30 °C; pH 7·0). No growth was observed under anaerobic conditions: the isolate showed neither nitrate respiration (in the presence of 0·2 % nitrate) nor fermentative growth (with glucose or yeast extract). Catalase and oxidase were produced. Neisser staining was positive and cells fluoresced yellow with DAPI, indicative of polyphosphate accumulation.

Strain T-27^T was able to utilize a limited range of substrates as sole carbon sources: yeast extract, polypepton, succinate, acetate, gelatin and benzoate. The strain was also able to utilize the following substrates weakly: glucose, sucrose, galactose, melibiose, maltose, formate and β-hydroxybutyrate. The following were not utilized: fructose, mannose, lactose,

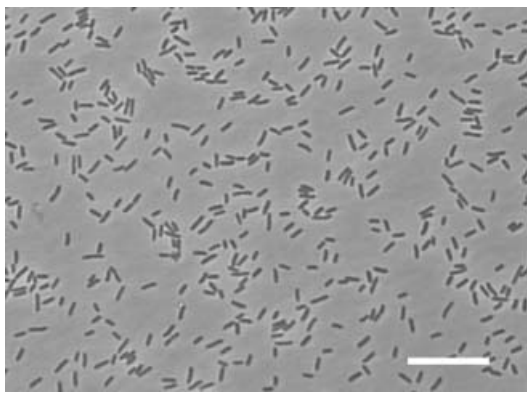

Fig. 1. Phase-contrast photomicrograph of cells of strain T-27^T grown aerobically in NM-1 liquid medium at 30 °C, often showing asymmetrical cell division. Bar, 10 μm.

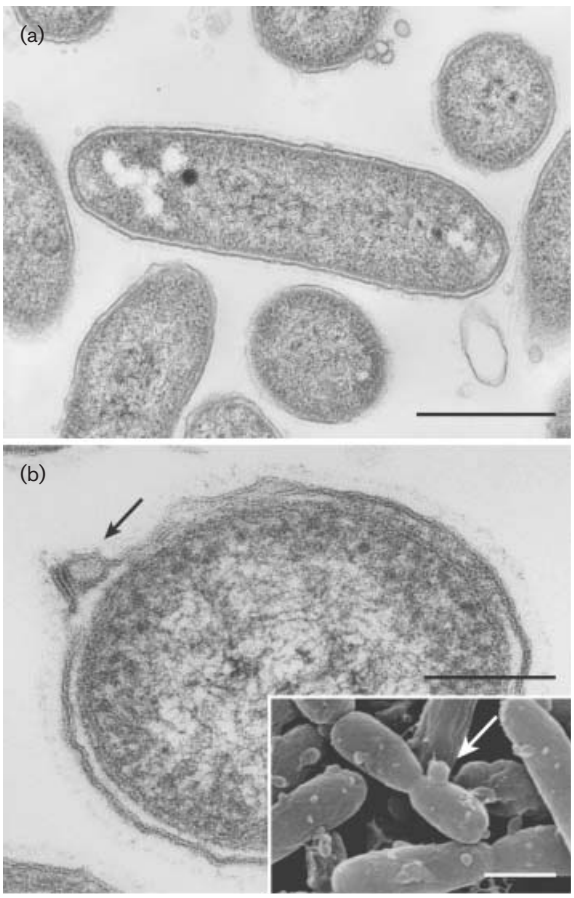

Fig. 2. Electron micrographs of cells of strain T-27^T. (a) Transmission electron micrograph showing a Gram-negative cell envelope structure. Bar, 0·5 μm. (b) Transmission and scanning (inset) electron micrographs of cells of strain T-27^T, suggesting asymmetric cell division. Budding structures are shown by arrows, Bar, 0·25 μm (inset, 0·5 μm).

trehalose, raffinose, arabinose, xylose, rhamnose, glycerol, ethanol, 1-propanol, erythritol, mannitol, sorbitol, lactate, citrate, pyruvate, propionate, malate, butyrate, glutamate, aspartate, alanine, starch, glycogen, gentiobiose, turanose, methyl pyruvate, 2-oxoglutarate, α-ketovalerate, serine, histidine, glycine, leucine and Casamino acids.

Chemotaxonomic characteristics

The major respiratory quinone of strain T-27^T is menaquinone (MK)-9. The dominant fatty acids of strain T-27^T are iso-C15 : 0 (45 % of total fatty acid methyl esters), C16 : 1 (27 %), C14 : 0 (12 %), C13 : 0 (9 %) and C16 : 0 (7 %). No DAP isomers were detected from the cell-wall peptidoglycan of strain T-27^T by DAP analysis. The G+C content of genomic DNA of strain T-27^T was 66·0 mol%.

Phylogenetic analysis

A nearly complete 16S rDNA sequence (1446 nucleotides) was obtained for strain T-27^T. Comparative sequence analysis indicated that strain T-27^T is not closely related to any known cultured micro-organisms in recognized bacterial phyla (⩽85 % sequence identity). Instead, it is a member of candidate division BD, a recently recognized phylum-level lineage in the domain *Bacteria*, to date containing only environmental clones obtained from a number of diverse habitats (Hugenholtz *et al.*, 2001; see below for a discussion of this phylum). The environmental clones most closely related to strain T-27^T (Ebpr21 and SBRH63, respectively 99 and 97 % sequence identity) were obtained from two geographically remote activated sludges operated under EBPR conditions (Hugenholtz *et al.*, 2001; Liu *et al.*, 2001). This suggests that strain T-27^T and related organisms may be ubiquitous polyphosphate-accumulating bacteria in EBPR processes. Polyphosphate accumulation of the strain was suggested by Neisser and DAPI staining and the presence of electron-dense inclusion bodies (Fig. 2a), although *in situ* polyphosphate accumulation remains to be verified.

How novel is strain T-27^T?

Based on the standard set of phenotypic tests conducted in this study, strain T-27^T is physiologically unremarkable. Many phylogenetically diverse bacteria are Gram-negative aerobic heterotrophs and a number of bacteria can accumulate polyphosphate, including '*Accumulibacter phosphatis*' (a member of the *Proteobacteria*; Hesselmann *et al.*, 1999), *Microlunatus phosphovorus* and *Tetrasphaera* species (members of the *Actinobacteria*) (Nakamura *et al.*, 1995; Maszenan *et al.*, 2000; Hanada *et al.*, 2002b). Strain T-27^T also has a pedestrian, if somewhat limited, carbon substrate utilization profile. Therefore, if isolates are screened using only phenotypic tests, phylogenetically novel micro-organisms may be overlooked. The chemotaxonomic and ultrastructural data obtained for strain T-27^T, however, hint at a more exotic ancestry. The strain has an unusual combination of dominant fatty acids: 45 % iso-C15 : 0 (usually characteristic of Gram-positive bacteria) and 27 % C16 : 1 (more often found in Gram-negative heterotrophs). Similarly, it is unusual for Gram-negative bacteria to lack DAP in their cell-wall peptidoglycan and to grow aerobically using MK-9 as the major respiratory quinone (D. C. White, personal communication). Also, the cell envelope is unusual in that the space between the membranes (periplasmic space) is atypically wide for Gram-negative organisms (R. Webb, personal communication). More detailed molecular analyses, including genome sequencing, will confirm (or deny) the evolutionary novelty of strain T-27^T indicated by its 16S rDNA sequence.

Phylogenetic delineation and environmental distribution of the phylum *Gemmatimonadetes*

The phylogenetic placement of the T-27^T 16S rDNA was determined by construction of a neighbour-joining tree with related environmental clones and representative sequences of recognized bacterial phyla (Fig. 3; based on the dataset of Hugenholtz, 2002). Bootstrap resampling of

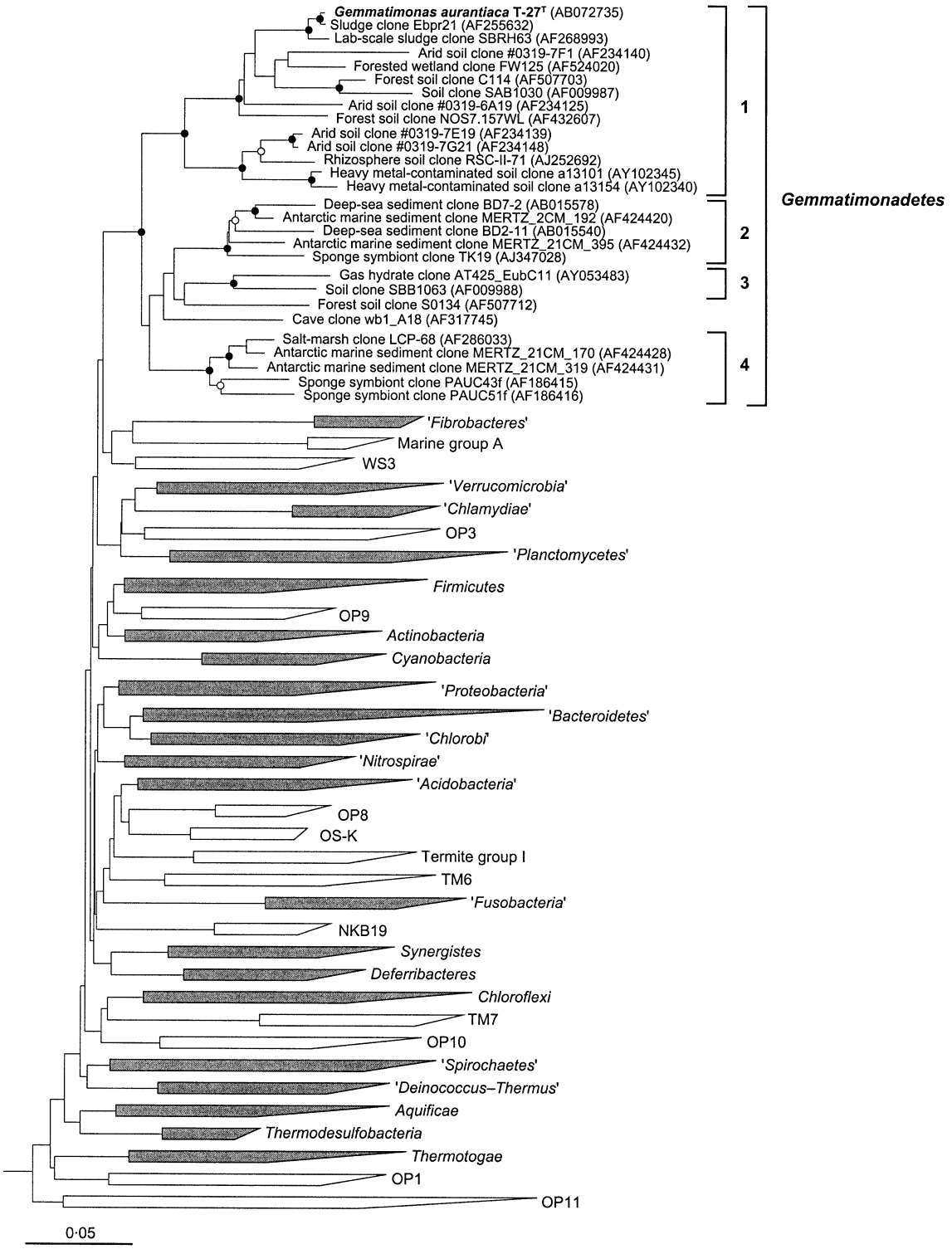

Fig. 3. Evolutionary-distance dendrogram of strain T-27^T and associated representatives of the phylum *Gemmatimonadetes* and other bacterial phyla (shown as wedges). Shaded wedges indicate phyla with cultivated representatives and unshaded wedges indicate phyla currently represented only by environmental sequences. The phylum *Gemmatimonadetes* and its subdivisions are indicated by brackets to the right. Branch points supported (bootstrap or posterior probability values $>90\%$) by all inference methods are indicated by solid circles, while open circles at branch points indicate $>75\%$ bootstrap or posterior probability support in most or all analyses. Branch points without circles were not resolved ($<75\%$) as specific groups in all analyses. Bar, 0·05 changes per sequence position.

the dataset using evolutionary distance, maximum parsimony and maximum likelihood and calculation of posterior probabilities using Bayesian inference confirmed that T-27^T reproducibly affiliates with sequences belonging to the BD group and that the group represents an independent phylum-level lineage in the bacterial domain (Fig. 3). This is consistent with previous analyses (Hugenholtz *et al.*, 2001; Madrid *et al.*, 2001; Hugenholtz, 2002). For this reason, we have renamed group BD the phylum *Gemmatimonadetes* phyl. nov., in accordance with its first cultured representative (see conclusions below).

Over 100 environmental clone sequences belonging to the *Gemmatimonadetes* were identified in the public databases by similarity searches and comparative analysis. Of these, the majority are partial length clones (<1000 bp; not shown), 27 are near full-length (mostly >1250 bp, shown in Fig. 3) and five are putatively chimeric as determined by partial treeing analysis (Table 1; Hugenholtz & Huber, 2003). The maximum 16S rDNA sequence divergence for the phylum is 19 %, which is greater than the divergence found in the *Actinobacteria* (18 %) but less than the phylum '*Proteobacteria*' (23 %; Dojka *et al.*, 2000). Four subdivisions of the *Gemmatimonadetes* could be defined in the present analysis (Fig. 3), i.e. lineages of two or more full-length 16S rDNA sequences within the phylum that are reproducibly monophyletic and unaffiliated with all other representatives of the phylum (Hugenholtz *et al.*, 1998). Interestingly, the subdivisions appear to be distinct in terms of where their representatives are found. Subdivision 1 clones were all obtained from terrestrial habitats and clones belonging to subdivisions 2 and 4 were all obtained from marine habitats (Fig. 3). This distinction may be due to sampling artefacts or may represent a real evolutionary split according to habitat type. However, subdivision 3, currently represented by only two full-length clones, is a mixture of one terrestrial (soil) and one marine (gas hydrate) clone (Fig. 3). Terrestrial habitats include soils (Axelrood *et al.*, 2002; Dunbar *et al.*, 2002; Holmes *et al.*, 2000; Valinsky *et al.*, 2002), activated sludges (Hugenholtz *et al.*, 2001; Liu *et al.*, 2001) and saline cave waters (Holmes *et al.*, 2001). Marine habitats include deep-sea sediments (Li *et al.*, 1999), gas hydrates (Lanoil *et al.*, 2001), arctic bacterioplankton (Bano & Hollibaugh, 2002), coastal mobile mud (Madrid *et al.*, 2001) and marine sponge symbionts (Hentschel *et al.*, 2002; Webster *et al.*, 2001). An rRNA clone, SB2 (accession no. AJ404563), obtained by RT-PCR from arthritis synovial tissue (Kempsell *et al.*, 2000), was identified as belonging to subdivision 1 of the *Gemmatimonadetes*, suggesting that members of this phylum may also be present in clinical niches. Note that the class *Gemmatimonadetes* classis nov. defined below is phylogenetically equivalent to subdivision 1 in Fig. 3.

It is foolhardy to extrapolate phenotypic traits determined from isolate T-27^T to the phylum that it represents, with the likely exception of cell envelope structure. Cell envelope type is largely consistent with phylum-level 16S rDNA

Table 1. Putative chimeric 16S rDNA sequences from the *Gemmatimonadetes* detected in the public databases

Putative chimeric sequence (accession no.)	Length (nt)	5′ Parent sequence		3′ Parent sequence		Approx. breakpoint‡	Reference
		Phylum (class)*	Closest BLAST match (%)†	Phylum (class)	Closest BLAST match (%)†		
Marine clone Arctic96AD-3 (AF354607)	1502	'*Proteobacteria*' ('*Gammaproteobacteria*')	Arctic96AD-9 (AF354608) (98 %)	*Gemmatimonadetes* (2)	BD7-2 (AB015578) (92 %)	935 (911)	Bano & Hollibaugh (2002)
Soil clone 384-2 (AF423257)	1472	*Gemmatimonadetes* (1)	#0319-7F1 (AF234140) (95 %)	'*Acidobacteria*' (6)	mb2431 (Z95736) (95 %)	435 (415)	Valinsky *et al.* (2002)
Marine sediment clone B2M68 (AF223301)	1098	*Gemmatimonadetes* (2)	BD2-11 (AB015540) (92 %)	'*Proteobacteria*' ('*Alphaproteobacteria*')	*Sinorhizobium* sp. JL84 (AY056832) (97 %)	1090 (677)	Cifuentes *et al.* (2000)
Mobile mud clone KS70 (AF328207)	991	Novel	BS003 (AY005448) (87 %)	*Gemmatimonadetes*	wb1_A18 (AF317745) (96 %)	1040 (520)	Madrid *et al.* (2001)
Sponge symbiont clone R33 (AF333535)	858	'*Proteobacteria*' ('*Gammaproteobacteria*')	*Balneatrix alpica* (Y17112) (87 %)	*Gemmatimonadetes* (2)	R171 (AF333522) (99 %)	1195 (660)	Webster *et al.* (2001)

*Where possible, the nomenclature of the taxonomic outline for *Bergey's Manual of Systematic Bacteriology* (Garrity *et al.*, 2001) has been used. Candidate phyla are named as described previously (Hugenholtz *et al.*, 1998; Hugenholtz, 2002).
†Closest match to a sequence of length comparable to that of the chimeric sequence; accession numbers and percentage similarities given in parentheses.
‡*E. coli* numbering; absolute nucleotide position in chimera is shown in parentheses.

phylogeny (Woese, 1987), so we predict that members of the *Gemmatimonadetes* will have a Gram-negative cell envelope that lacks DAP.

Taxonomic conclusions

The genotypic and phenotypic findings detailed above indicate that strain T-27^T is a novel and distinctive bacterium. Thus, we propose a new genus and species, *Gemmatimonas aurantiaca* gen. nov., sp. nov., to accommodate this isolate and create a new phylum, *Gemmatimonadetes* phyl. nov., with T-27^T as the sole cultured representative. The taxonomic ranks between phylum and genus are also proposed according to the precedent set for *Chrysiogenetes* (Garrity & Holt, 2001).

Description of *Gemmatimonadetes* phyl. nov.

Gemmatimonadetes (Gem.ma′ti.mo.na.det′es. N.L. fem. pl. n. *Gemmatimonas* type genus of the type order of the phylum; N.L. fem. pl. n. *Gemmatimonadetes* phylum of the genus *Gemmatimonas*).

The phylum *Gemmatimonadetes* is defined on a phylogenetic basis by comparative 16S rDNA sequence analysis of one isolated strain and uncultured representatives from multiple terrestrial and aquatic habitats. Gram-negative bacteria lacking DAP in their cell envelopes.

Type order: *Gemmatimonadales* ord. nov.

Description of *Gemmatimonadetes* classis nov.

The description is the same as for the phylum *Gemmatimonadetes*.

Type order: *Gemmatimonadales* ord. nov.

Description of *Gemmatimonadales* ord. nov.

Gemmatimonadales (Gem.ma′ti.mo.na.dal′es. N.L. fem. pl. n. *Gemmatimonas* type genus of the order; *-ales* ending to donate an order; N.L. fem. pl. n. *Gemmatimonadales* the order of the genus *Gemmatimonas*).

The description is the same as for the genus *Gemmatimonas*.

Type genus: *Gemmatimonas* gen. nov.

Description of *Gemmatimonadaceae* fam. nov.

Gemmatimonadaceae (Gem.ma′ti.mo.na.da′ce.ae. N.L. fem. n. *Gemmatimonas* type genus of the family; *-aceae* ending to donate a family; N.L. fem. n. *Gemmatimonadaceae* family of the genus *Gemmatimonas*).

The description is the same as for the genus *Gemmatimonas*.

Type genus: *Gemmatimonas* gen. nov.

Description of *Gemmatimonas* gen. nov.

Gemmatimonas (Gem.ma′ti.mo′nas. L. adj. *gemmatus* provided with buds; L. n. *monas* a unit; N.L. fem. n. *Gemmatimonas* a budding unit).

Gram-negative. Cells are motile, rod-shaped. Spores are not formed. Cells divide by binary fission but sometimes show budding forms. Mesophilic. Cells grow under aerobic conditions. The major respiratory quinone is MK-9. The main fatty acids are iso-C15 : 0, C16 : 1 and C14 : 0. The cell wall contains no DAP isomers. The G+C content of genomic DNA of the type species is 66·0 mol%. The type species is *Gemmatimonas aurantiaca*.

Description of *Gemmatimonas aurantiaca* sp. nov.

Gemmatimonas aurantiaca (au.ran.ti.a′ca. M.L. fem. adj. *aurantiaca* orange-coloured).

Cells are rod-shaped (0·7 μm in width and 2·5–3·2 μm in length). Growth occurs between 25 and 35 °C with the optimum at 30 °C. The pH range is 6·5–9·5. Optimum growth occurs at pH 7·0. The optimum doubling time of growth is 12 h. Catalase and oxidase are produced. Aerobe. No fermentative growth is observed. Nitrate reduction is negative. Neisser staining is positive. Cells are stained yellow with DAPI. The following can be utilized as sole carbon sources: yeast extract, polypepton, succinate, acetate, gelatin and benzoate. The following can be utilized weakly as sole carbon sources: glucose, sucrose, galactose, melibiose, maltose, formate and hydroxybutyrate. The following carbon sources are not utilized: fructose, mannose, lactose, trehalose, raffinose, arabinose, xylose, rhamnose, glycerol, ethanol, propanol, erythritol, mannitol, sorbitol, lactate, citrate, pyruvate, propionate, malate, butyrate, glutamate, aspartate, alanine, starch, glycogen, gentiobiose, turanose, methyl pyruvate, 2-oxoglutarate, α-ketovalerate, serine, histidine, glycine, leucine and Casamino acids.

The type strain, T-27^T (=JCM 11422^T=DSM 14586^T), was isolated from a laboratory-scale anaerobic–aerobic sequential batch reactor operated under EBPR conditions.

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