Olivibacter sitiensis gen. nov., sp. nov., isolated from alkaline olive-oil mill wastes in the region of Sitia, Crete

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A novel, Gram-negative, non-motile, non-sporulating, rod-shaped bacterium isolated from a viscous two-phase olive-oil mill waste ('alpeorujo') is described. The strain, designated AW-6^T, is an obligate aerobe, forming irregular, pigmented creamy white colonies. The pH and temperature ranges for growth were pH 5–8 and 5–45 °C, with optimal pH and temperature for growth of pH 6–7 and 28–32 °C, respectively. Strain AW-6^T was chemo-organotrophic and utilized mostly D(+)-glucose, protocatechuate and D(+)-xylose, followed by L-cysteine, D(–)-fructose, D(+)-galactose, L-histidine, lactose, sorbitol and sucrose. Menaquinone-7 was detected in the respiratory chain of strain AW-6^T. The major fatty acids of strain AW-6^T were C_{16:1}ω7c and/or iso-C_{15:0} 2-OH, iso-C_{15:0}, iso-C_{17:0} 3-OH and C_{16:0}. The closest phylogenetic relative of strain AW-6^T was clone BIti35 (89.7% 16S rRNA gene sequence similarity), while *Sphingobacterium thalpophilum* DSM 11723^T was the closest recognized relative within the *Sphingobacteriaceae* (88.2% similarity). Strain AW-6^T showed a low level of DNA–DNA relatedness to *S. thalpophilum* DSM 11723^T (33.8–37.0%). The DNA G+C content of strain AW-6^T was 45.6 mol%. Physiological and chemotaxonomic data further confirmed the distinctiveness of strain AW-6^T from members of the genera *Sphingobacterium* and *Pedobacter*. Thus, strain AW-6^T is considered to represent a novel species of a new genus within the family *Sphingobacteriaceae*, for which the name *Olivibacter sitiensis* gen. nov., sp. nov. is proposed. The type strain is AW-6^T (=DSM 17696^T=CECT 7133^T).

At the time of writing, the family *Sphingobacteriaceae* consists of the genera *Sphingobacterium* and *Pedobacter* (Steyn *et al.*, 1998). The genus *Sphingobacterium* includes the following recognized species: *Sphingobacterium antarcticum* (the corrected name of *S. antarcticus*), *S. faecium*, *S. multivorum*, *S. spiritivorum* and *S. thalpophilum* (Yabuuchi *et al.*, 1983; Shivaji *et al.*, 1992; Takeuchi & Yokota, 1992). Steyn *et al.* (1998) erected the genus *Pedobacter* initially to accommodate the species *Pedobacter africanus*, *P. heparinus*, *P. piscium* and *P. saltans*; seven further species, *Pedobacter caeni*, *P. cryoconitis*, *P. himalayensis*, *P. sandarakinus*, *P. aquatilis*, *P. ginsengisoli* and *P. roseus* have since

been added (Margesin *et al.*, 2003; Shivaji *et al.*, 2005; Vanparys *et al.*, 2005; Gallego *et al.*, 2006; Hwang *et al.*, 2006; Ten *et al.*, 2006; Yoon *et al.*, 2006).

Only a few bacteria isolated from olive-processing products and residues have been recognized (Chamkha *et al.*, 2001; Koussemon *et al.*, 2001). *Alkalibacterium* (Ntougias & Russell, 2001) and *Sporobacterium* (Mechichi *et al.*, 1999) are the only currently recognized genera; their type species, *Alkalibacterium olivapovliticus* and *Sporobacterium olearium*, were isolated from edible-olive wash-water and from an olive mill wastewater treatment digester, respectively. Alpeorujo is a liquid–solid waste produced by two-phase olive-oil decanters which is usually amended (before disposal) by Ca(OH)₂ to generate a moderately alkaline secondary waste (alkaline alpeorujo). To date, the only bacterial isolates recovered from alkaline alpeorujo have been either halotolerant alkaliphilic or halotolerant/alkali-tolerant micro-organisms (Ntougias *et al.*, 2006).

Here, we describe the morphological, physiological, chemotaxonomic and phylogenetic characteristics of a novel,

Abbreviation: ECL, equivalent chain-length.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain AW-6^T is DQ421387.

An electron micrograph showing the cell morphology of strain AW-6^T and a dendrogram based on data for 40 physiological and chemotaxonomic characteristics of strain AW-6^T, members of the genera *Sphingobacterium* and *Pedobacter* and *Flavobacterium mizutaii* are available as supplementary material in IJSEM Online.

non-halotolerant, non-alkalitolerant bacterium isolated from alkaline alpeorujó. Based on its unique taxonomic properties, we conclude that this isolate, designated strain AW-6^T, represents a novel species of a new bacterial genus.

Bacterial strain AW-6^T was isolated from alkaline alpeorujó, an olive by-product produced by an olive-oil mill equipped with a two-phase centrifugal system located at the premises of the Toplou Monastery, Sitia (35° 13' 12" N 26° 6' 6" E), north-east Crete, Greece. Alpeorujó, amended with 0.7 % w/w Ca(OH)₂, was obtained from an open-air settling pond near the olive-oil mill. The pH and the electrical conductivity of the alkaline alpeorujó (diluted in water at a volume ratio of 1 : 1) were 8.7 and 16.2 mS cm⁻¹, respectively.

For isolation, a 10 g sample of alkaline alpeorujó was mixed with 100 ml NaCl solution (8.5 g l⁻¹) followed by a tenfold dilution series (Ntougias *et al.*, 2006). Extract from alkaline alpeorujó (100 g per 1 litre of distilled water, stirred for 20 min, filtered and adjusted to pH 7) solidified with agar was used as the isolation medium (Ntougias *et al.*, 2006). After incubation for 1 week at 25 °C, bacterial isolate AW-6^T was obtained and subcultured on solid medium (by the addition of 17 g agar l⁻¹) containing 10 g glucose l⁻¹, 5 g yeast extract l⁻¹, 5 g peptone l⁻¹, 0.1 mM MgSO₄ and phosphate buffer at pH 7 (Ntougias *et al.*, 2006). Bacterial growth for maintenance (glycerol stocks or slants) was carried out at 25 °C (temperature of isolation), while the experimental temperature for growth was 28 °C (optimal temperature). Strain AW-6^T was grown on the medium described above unless otherwise specified.

The pH range for growth (single unit steps from pH 4 to 9) was examined by using the appropriate buffer solutions (Ntougias & Russell, 2000). Salt tolerance was investigated in the presence of 0, 30 and 50 g NaCl l⁻¹. Bacterial substrate utilization was tested in media (pH 7, with pH adjusted when necessary) containing a specific sugar, amino acid or other substrate (results are given in the species description below), 0.1 mM MgSO₄ and 0.05 g yeast extract l⁻¹ (no growth was observed in the medium containing the above concentration of yeast extract as the sole carbon source). The concentration of each compound tested was 0.05 M, unless the medium was saturated at a lower concentration, in which case a concentration up to the saturation point was used. The concentration of ethanol and glycerol was 0.5 % (v/v) and 0.5 g phenol l⁻¹ was also tested. Salts, metabolic compounds, yeast extract and the buffer were sterilized separately and mixed aseptically. Sensitive compounds were filter-sterilized. Incubations were performed at 3, 5, 10, 15, 20, 25, 28, 32, 37, 40, 45 and 50 °C to investigate the temperature range for growth. Anaerobic growth was examined by using the Anaerocult A system (Merck). Antibiotic susceptibility was investigated on medium containing 50 µg ml⁻¹ of the antibiotic tested (Ntougias *et al.*, 2006). Tween hydrolysis, catalase- and oxidase-activity, spore formation and Gram staining were also examined (Smibert & Krieg, 1994).

For transmission electron microscopy, suspensions of bacterial cells were placed on pyroxyline-coated copper grids (AEI) and negatively stained with 1 % (w/v) phosphotungstic acid (pH 7.0) in water for 10 s. Specimens were examined and photographed with a transmission electron microscope (9-S; Zeiss).

Genomic DNA was extracted according to Wilson (1992). The almost full-length 16S rRNA gene was amplified using universal primers A_{for}, 5'-GGAGAGTTAGATCTTGGCT-CAG-3' (sense; positions 6–27 according to the *Escherichia coli* numbering system) and C_{rev}, 5'-AGAAAGGAGGT-GATCCAGCC-3' (antisense; positions 1542–1525). A reaction mixture (50 µl) containing 1 µl (50 ng µl⁻¹) genomic DNA, 10 × PCR buffer (Finnzymes OY), 2 mM MgCl₂, 200 µM each dATP, dTTP, dCTP and dGTP, primers A_{for} and C_{rev} (0.5 µM each) and 1 U DNA polymerase (Dynazyme EXT-Finnzymes OY) was prepared (Ntougias & Russell, 2001). 16S rRNA gene amplification was performed by using a PTC-200 thermocycler (MJ Research Inc.) using a denaturation step of 2 min at 94 °C, followed by 35 cycles of 30 s denaturation at 94 °C, 30 s primer annealing at 55 °C and 1 min DNA chain extension at 72 °C. The PCR was terminated by 5 min DNA chain extension at 72 °C.

Three independent clones were obtained by cloning the respective amplicons in the pGEM-T Easy Vector (Promega) before insertion into DH5a competent cells. Plasmid DNA was purified by using the NucleoSpin plasmid Quick Pure kit (Macherey-Nagel). PCR sequencing using the fluorescence-labelled primers SP6 and T7 (Promega) and additional primers (5'-AGGCGGAATGAGGCAAGT-AGC-3' and 5'-TGCCAGAGTCCCCACCATAAC-3') was performed at the Institute of Molecular Biology and Biotechnology (IMBB), Heraklion, Greece, via a LI-COR Long ReadIR2 4200 automated sequencer.

The 16S rRNA gene sequences were assembled using the DNASTAR suite of programmes (DNASTAR Inc.). Similarity searches were carried out by BLAST analysis (<http://www.ncbi.nlm.nih.gov/blast/>) and the closest relatives to strain AW-6^T were included in further phylogenetic analyses. Alignment of the sequences and phylogenetic tree construction were performed by using the CLUSTAL W Submission Form (<http://www.ebi.ac.uk/clustalw/>) and TREECON for Windows (version 1.3b) (Van de Peer & de Wachter, 1993), respectively. Evolutionary distances were calculated according to Jukes & Cantor (1969) and the topology was inferred using the neighbour-joining method (Saitou & Nei, 1987) based on bootstrap analysis of 1000 trees. Phylogenetic trees were also generated by using the maximum-likelihood (DNAML; Cavalli-Sforza & Edwards, 1967) and maximum-parsimony (DNAPARS; Kluge & Farris, 1969) algorithms within the PHYLIP version 3.6 phylogenetic package (Felsenstein, 2004), including the SEQBOOT program for bootstrap analysis. Only almost full-length 16S rRNA gene sequences were used for tree construction. The

16S rRNA gene sequence (1477 bp) of strain AW-6^T was deposited in the GenBank database.

Numerical analysis was performed using the NTSYSpc package (version 2.2 for Windows; Exeter Software) (Rohlf, 2000). A binary 0/1 matrix was created based on the absence or presence, respectively, of physiological and chemotaxonomic characteristics. Similarities were calculated with the 'SimQual' option within NTSYSpc by employing the 'Simple Matching-SM' and the 'Jaccard-J' coefficients. For cluster analysis, the 'SAHN' option from the package was employed using the unweighted pair group method with arithmetic mean (UPGMA) for clustering.

Chemotaxonomic analyses (quinone and fatty acid methyl ester analyses), DNA–DNA hybridization experiments and DNA G+C content determination were carried out by the Identification Service of the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ), Braunschweig, Germany. DNA for spectroscopic DNA–DNA hybridization experiments and G+C content determination was isolated as described by Cashion *et al.* (1977). DNA–DNA hybridization experiments were performed as described by De Ley *et al.* (1970) and modified as suggested by Huß *et al.* (1983). DNA G+C content determination was carried out according to Tamaoka & Komagata (1984) and Mesbah *et al.* (1989). Fatty acid methyl esters were obtained as described by Kroppenstedt (1985) and Kämpfer & Kroppenstedt (1996) and by using minor modifications of the methods given by Miller (1982) and Kuykendall *et al.* (1988). Respiratory lipoquinone analyses were carried out by Dr B. J. Tindall and the Identification Service of

the DSMZ according to a standard procedure (Tindall, 1990a, b).

Morphological, cultural, physiological and biochemical characteristics of strain AW-6^T are given in the species description below. A transmission electron micrograph showing cell morphology is available as Supplementary Fig. S1 in IJSEM Online.

Only menaquinone-7 was detected in the respiratory chain of strain AW-6^T. The detailed fatty acid profile of strain AW-6^T was as follows: C_{16:1}ω7c and/or iso-C_{15:0} 2-OH (43.2 %), iso-C_{15:0} (29.3 %), iso-C_{17:0} 3-OH (11.2 %), C_{16:0} (5.5 %), iso-C_{17:1}ω9c (2.0 %), iso-C_{15:0} 3-OH (1.9 %), C_{16:1}ω5c (1.3 %), 1.1 % of an unknown fatty acid [equivalent chain length (ECL) of 16.582 as calculated by the Sherlock Microbial Identification System version 4.5], C_{16:0} 2-OH (0.9 %), 0.9 % of an unknown fatty acid (ECL of 13.565), C_{16:0} 3-OH (0.7 %), C_{16:1} 2-OH (0.6 %), C_{14:0} (0.5 %), anteiso B and/or iso I C_{17:1} (0.5 %), iso-C_{17:0} (0.4 %), and iso F C_{15:1} (0.3 %).

The phylogenetic placement of strain AW-6^T among members of the family *Sphingobacteriaceae* is illustrated in Fig. 1. Based on 16S rRNA gene sequence analysis, strain AW-6^T was associated, but not closely, with members of the genera *Sphingobacterium* and *Pedobacter* within this family. Its closest relatives, but still distinct from strain AW-6^T, were clone Bti35 (89.7 % 16S rRNA gene sequence similarity) and *Sphingobacterium daejeonense* (89.7 % similarity), while all recognized members of the genera *Sphingobacterium* and *Pedobacter* were placed in separate phylogenetic clusters. S.

Fig. 1. Phylogenetic position of strain AW-6^T and members of the family *Sphingobacteriaceae* based on 16S rRNA gene sequence analysis. Evolutionary distances were calculated using the method of Jukes & Cantor (1969) and the topology was inferred using the neighbour-joining method (Saitou & Nei, 1987) based on bootstrap analysis of 1000 trees. The 16S rRNA gene sequence of *Bacteroides caccae* ATCC 43185^T was arbitrarily chosen as the outgroup. Bar, 0.02 substitutions per site.

thalpophilum DSM 11723^T was the closest recognized relative within the *Sphingobacteriaceae* (88.2 % similarity).

The DNA G + C content of strain AW-6^T was 45.6 mol%. Levels of DNA–DNA relatedness between strain AW-6^T and *S. thalpophilum* DSM 11723^T were 33.8–37.0 %.

In contrast to *S. thalpophilum* (its closest relative within the genus *Sphingobacterium*), strain AW-6^T utilized L-cysteine, L-histidine and sorbitol, but not D(+)–cellobiose, glycerol, maltose or starch. Moreover, strain AW-6^T contained iso F C_{15:1}, C_{16:1} 2-OH, C_{16:1}ω5c, iso-C_{17:0}, iso-C_{17:1}ω9c, anteiso B and/or iso I C_{17:1} and an unknown fatty acid (ECL of 16.580). A slightly greater DNA G + C content for strain AW-6^T was also detected compared with *S. thalpophilum* (Takeuchi & Yokota, 1992; Steyn *et al.*, 1998). In contrast to *Pedobacter saltans* (its closest relative in the genus *Pedobacter*), strain AW-6^T was able to grow at 42 °C and utilized L-cysteine, L-histidine and sorbitol, but not glycerol or maltose. In addition, strain AW-6^T possessed iso F C_{15:1}, C_{16:1} 2-OH, iso-C_{17:0} and anteiso B and/or iso I C_{17:1}, but lacked anteiso-C_{15:0}. A higher DNA G + C content (>8.5 mol% greater) was also determined for strain AW-6^T compared with *P. saltans* (Steyn *et al.*, 1998).

Differential physiological and chemotaxonomic characteristics between strain AW-6^T and members of the genera *Sphingobacterium* and *Pedobacter* are presented in Table 1.

In contrast to members of the genus *Sphingobacterium* (excluding *S. antarcticum* isolates), strain AW-6^T utilized L-cysteine and sorbitol, but not starch, D(+)–cellobiose or maltose. Strain AW-6^T was distinct from all members of the family *Sphingobacteriaceae* in terms of its inability to utilize maltose and its ability to grow at 42 °C (apart from *S. thalpophilum*, which also grows at 42 °C). The major fatty acids of all members of the family *Sphingobacteriaceae* were iso-C_{15:0}, iso-C_{15:0} 2-OH, C_{16:1}ω7c and iso-C_{17:0} 3-OH (Steyn *et al.*, 1998; Margesin *et al.*, 2003; Shivaji *et al.*, 2005; Vanparys *et al.*, 2005; Yoon *et al.*, 2006). However, differences in the presence or absence of minor fatty acid components were identified between strain AW-6^T and members of the genera *Pedobacter* and *Sphingobacterium* (Table 1). Strain AW-6^T can be differentiated from *Sphingobacterium* and *Pedobacter* by the possession of C_{16:1} 2-OH, iso-C_{17:0}, and anteiso B and/or iso I C_{17:1} (Table 1). Moreover, iso F C_{15:1} was detected in cells of strain AW-6^T, but was absent from members of the genus *Sphingobacterium*. Combined physiological and chemotaxonomic data for strain AW-6^T distinguish it from members of the genera *Pedobacter* and *Sphingobacterium*, as is evidenced by its placement within a distinct cluster. A dendrogram based on combined physiological and chemotaxonomic data is available as Supplementary Fig. S2 in IJSEM Online. Levels of similarity between strain AW-6^T and recognized members of the family *Sphingobacteriaceae*

Table 1. Physiological and chemotaxonomic characteristics that differentiate strain AW-6^T from members of the genera *Sphingobacterium* and *Pedobacter*

Data for the genus *Sphingobacterium* are taken from Steyn *et al.* (1998). Data for the genus *Pedobacter* are taken from Steyn *et al.* (1998), Margesin *et al.* (2003), Shivaji *et al.* (2005), Vanparys *et al.* (2005) and Yoon *et al.* (2006). In all studies, cells for fatty acid methyl ester analysis were grown in peptone–yeast extract media at 28–30 °C. *S. antarcticum* was able to utilize sorbitol, but not starch although it was related to the genus *Pedobacter* rather than to the genus *Sphingobacterium* (see text, Fig. 1 and Supplementary Fig. S2 for details). +, Growth/presence; –, no growth/absence; v, variable.

Characteristic	Strain AW-6 ^T	<i>Sphingobacterium</i>	<i>Pedobacter</i>
Growth at 42 °C	+	v	–
Utilization of:			
D(+)–Cellobiose	–	+	v
L-Cysteine	+	–	v
L-Histidine	+	–*	–†
Maltose	–	+	+
Sorbitol	+	–‡	v
Starch	–	–‡	v
Detection of fatty acids:			
iso F C _{15:1}	+	–	v (0–1.4 %)
C _{16:1} 2-OH	+	–	–
iso-C _{17:0}	+	–	–
anteiso B/iso I C _{17:1}	+	–	–
DNA G + C content (mol%)	45.6	37.3–44.2	36.9–44.2

*Variable for members of the species *S. multivorum*.

†Variable for members of the species *P. africanus*.

‡Variable for members of the species *S. antarcticum*.

were even lower when the 'Jaccard' rather than 'Simple Matching' coefficient was employed. Moreover, the separate generic position of strain AW-6^T in the tree constructed on the basis of physiological and chemotaxonomic data is in agreement with the phylogenetic tree (Fig. 1 and Supplementary Fig. S2).

Numerical analysis placed *P. saltans* in the cluster consisting of *Sphingobacterium* species (Supplementary Fig. S2); *P. saltans* was also grouped outside the cluster that included the remaining *Pedobacter* species in the phylogenetic tree (Fig. 1). Furthermore, based on physiological, chemotaxonomic and phylogenetic analysis, *S. antarcticum* was shown to be related to the genus *Pedobacter* rather than to the genus *Sphingobacterium* (Fig. 1 and Supplementary Fig. S2). The only full-length sequence of *S. antarcticum* was recently submitted to GenBank (strain 6B1Y; GenBank accession number AJ576248) and it is almost identical to that of *Pedobacter piscium* (differing only by one gap and one unidentified nucleotide), indicating that the taxonomic status of this species should be re-examined. Steyn *et al.* (1998) reported that the misclassified species *Flexibacter canadensis* was located at the border of the genera *Sphingobacterium* and *Pedobacter*; however in our study, *F. canadensis* was placed in a separate branch of the phylogenetic tree with maximum bootstrap support (Fig. 1), thus questioning its taxonomic position within the family *Sphingobacteriaceae*. 'Candidatus comitans', which was not grown in pure culture, was proposed to be a member of the genus *Sphingobacterium* rather than *Pedobacter* (Jacobi *et al.*, 1996; Steyn *et al.*, 1998); however, the phylogenetic position of 'Candidatus comitans', based on the 16S rRNA gene sequence analysis performed in this study, was within the cluster formed by *Pedobacter* species (Fig. 1).

Yabuuchi *et al.* (1983) proposed the genus *Sphingobacterium*, the type genus of the family *Sphingobacteriaceae*, to accommodate some previously described members of the genus *Flavobacterium* with DNA G+C contents of 39–42 mol%. Strain AW-6^T has a DNA G+C content (45.6 mol%) slightly higher than that of recognized members of the family *Sphingobacteriaceae* (36.9–44.2 mol%; Steyn *et al.*, 1998). The closest match to strain AW-6^T based on DNA G+C content was *S. thalpophilum* (44.0–44.2 mol%). Steyn *et al.* (1998) reported that the genus *Sphingobacterium* is genotypically heterogeneous, with *S. thalpophilum* (the closest recognized species to strain AW-6^T within the family *Sphingobacteriaceae*) being its most remote species. In addition, a low (<70%) level of DNA–DNA relatedness (33.8–37.0%) was observed between strain AW-6^T and *S. thalpophilum* DSM 11723^T. Menaquinone-7 was detected in the respiratory chain of strain AW-6^T, a common characteristic of members of the family *Sphingobacteriaceae* (Yabuuchi *et al.*, 1983; Steyn *et al.*, 1998; Shivaji *et al.*, 2005).

Based on 16S rRNA gene sequencing, the phylogenetic position of strain AW-6^T among members of the family *Sphingobacteriaceae* was unique and distinct (Fig. 1).

Construction of phylogenetic trees using distance-matrix, character-based (parsimony) and maximum-likelihood methods all placed strain AW-6^T together with clone BIti35 (89.7% 16S rRNA gene sequence similarity) into a separate cluster, distinct from any recognized member of the family *Sphingobacteriaceae*. Clone BIti35 was obtained from a waste-gas-degrading community in an industrial biofilter (Friedrich *et al.*, 2002), while isolates PF-H (GenBank accession number DQ207363) and BPC8, other phylogenetic relatives (but still distinct) of strain AW-6^T, were isolated from Kartchner Caverns, Arizona, and a contaminated soil (Kanaly *et al.*, 2002), respectively. Strain AW-6^T was isolated from a sludge-like by-product of the olive-oil extraction industry, showing similarities in this respect to *Pedobacter* species, which have been isolated from soil/activated sludge and other environmental samples (Steyn *et al.*, 1998; Margesin *et al.*, 2003; Shivaji *et al.*, 2005; Vanparys *et al.*, 2005; Yoon *et al.*, 2006). In contrast, all *Sphingobacterium* species [apart from *S. antarcticum* (the taxonomic position of which needs to be reconsidered)] have been recovered from clinical specimens (Yabuuchi *et al.*, 1983; Steyn *et al.*, 1998).

This is the first report of a non-halotolerant, non-alkalitolerant bacterium isolated from alkaline alpeorajo. Despite the fact that all the previously isolated bacteria reported from alkaline alpeorajo could be effectively adapted to the moderate alkalinity and salinity of this environment (Ntougias *et al.*, 2006), strain AW-6^T failed to tolerate high pH and salinity. Furthermore, strain AW-6^T was able to grow only in protocatechuate when media containing phenolic compounds as the sole carbon and energy source were tested.

On the basis of the phenotypic, chemotaxonomic and phylogenetic data, we conclude that strain AW-6^T represents a novel species of a new genus within the family *Sphingobacteriaceae*, for which the name *Olivibacter sitiensis* gen. nov., sp. nov. is proposed.

Description of *Olivibacter* gen. nov.

Olivibacter [O.li.vi.bac'ter. L. n. *oliva* olive; N.L. masc. n. *bacter* a rod; N.L. masc. n. *Olivibacter* a rod-shaped bacterium from olives/olive processing by-product].

Cells are Gram-negative, non-motile and rod-shaped, developing in irregular, pigmented creamy white colonies. Endospores are not formed. Neutrophilic and non-halotolerant. Mesophilic and strictly aerobic. Oxidase- and catalase-positive. Chemo-organotrophic. Menaquinone-7 is present in the respiratory chain. The major fatty acids are C_{16:1}ω7c and/or iso-C_{15:0} 2-OH, iso-C_{15:0}, iso-C_{17:0} 3-OH, and C_{16:0}. The DNA G+C content is 45.6 mol%. The type species is *Olivibacter sitiensis*.

Description of *Olivibacter sitiensis* sp. nov.

Olivibacter sitiensis [si.ti.en'sis. N.L. masc. adj. *sitiensis* pertaining to Sitia (north-east Crete, Greece) the vicinity in which the olive-oil mill by-product was obtained].

A strict aerobe that does not grow at 3% (w/v) NaCl. The pH and temperature ranges for growth are 5–8 and 5–45 °C, respectively. The optimal pH and temperature for growth are 6–7 and 28–32 °C, respectively. Tween 20 is not hydrolysed. Utilizes mostly D(+)–glucose, protocatechuate and D(+)–xylose (OD₆₀₀ in the range 0.13–0.35), followed by L-cysteine, D(–)-fructose, D(+)–galactose, L-histidine, lactose, sorbitol and sucrose (OD₆₀₀ in the range 0.05–0.12). Acetate, D(+)–cellobiose, cinnamate, citrate, ethanol, ferulate, gallate (no growth was observed on solid medium), L-glutamine, glycerol, glycine, inositol, L-isoleucine, L-lysine, maltose, D-mannitol, L-methionine, phenol, L-proline, L-serine, starch, succinate, syringate, L-tryptophan, vanillate and *o*-vanillin are not utilized. Susceptible to ampicillin, bacitracin, chloramphenicol, penicillin, rifampicin, tetracycline and trimethoprim, but resistant to kanamycin, polymixin B and streptomycin (each at 50 µg ml^{–1}).

The type strain, AW-6^T (=DSM 17696^T=CECT 7133^T), was isolated from alkaline alpeorujo in the region of Sitia, Crete, Greece.

Acknowledgements

This work was partially funded by the EPAN-FP66 project (Greek General Secretariat of Research and Technology). We would like to thank Filotheos Spanoudakis and Manolis Aivaliotis (Toplou Monastery, Viokalliergites Sitias SA) for their kind collaboration throughout this project.

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