Arcobacter anaerophilus sp. nov., isolated from an estuarine sediment and emended description of the genus *Arcobacter*

T. S. Sasi Jyothsna,[1] K. Rahul,[2] E. V. V. Ramaprasad,[2] Ch. Sasikala[2] and Ch. V. Ramana[1]

Correspondence
Ch. V. Ramana
r449@sify.com;
sasi449@yahoo.ie

[1]Department of Plant Sciences, School of Life Science, University of Hyderabad, Hyderabad-500046, India

[2]Bacterial Discovery laboratory, Centre for Environment, Institute of Science & Technology, Jawaharlal Nehru Technological University Hyderabad, Hyderabad-500085, India

Two strains (JC83, JC84^T) of obligately anaerobic, H_2S-producing bacteria were isolated from estuarine sediment samples collected from Gangasagar, West Bengal, India. Cells were Gram-stain-negative, non-motile rods. Both strains were positive for oxidase, negative for catalase, hydrolysed casein, reduced nitrate and utilized citrate. Both strains grew chemoorganoheterotrophically with optimal pH of 7–8 (range 7–10) and at 30 °C (range 25–37 °C). $C_{16:1}\omega7c$, $C_{18:1}\omega7c$, $C_{16:0}$ and $C_{12:0}$ were the major fatty acids of both strains with minor amounts of $C_{14:0}$, $C_{12:0}$ 3-OH and $C_{18:0}$. Polar lipids of both strains included diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylglycerol, phosphatidylserine, phosphatidylcholine, phosphatidylinositol, an unidentified aminolipid (AL2), an unidentified phospholipid (PL2) and an unidentified lipid (L3). MK-6 was the major respiratory quinone. The DNA G+C content of strains JC83 and JC84^T was 25.0 and 24.6 mol%, respectively. The strains showed DNA reassociation >85 % (86.0 ± 0.5 %) (based on DNA–DNA hybridization). Based on 16S rRNA gene sequence analysis, both strains were identified as belonging to the family *Campylobacteraceae* of the class *Epsilonproteobacteria* with *Arcobacter marinus* CL-S1^T (95.4 % sequence similarity) as their closest phylogenetic neighbour. On the basis of morphological, physiological and chemotaxonomic characteristics as well as phylogenetic analysis, strains JC83 and JC84^T are considered to represent a novel species, for which the name *Arcobacter anaerophilus* sp. nov. is proposed. The type strain is JC84^T (=KCTC 15071^T=MTCC 10956^T=DSM 24636^T). An emended description of the genus *Arcobacter* is provided.

Tidal flat sediments are the most productive coastal marine ecosystems (Alongi, 1998) and are characterized by intense heterotrophic and phototrophic activity (Poremba *et al.*, 1999). As a consequence, high microbiological activity and rich diversity is expected in these sediments (Webster *et al.*, 2010). The prevalence of anoxic conditions and anaerobic electron acceptors such as nitrate, iron and manganese encourage dissimilatory sulfate reduction as the predominant anaerobic degradation process in such sediments (Jørgensen, 1982). Hence, an attempt was made to isolate sulfate-reducing bacteria from the tidal sediment samples of Gangasagar, India. Gangasagar, which is located in the Sundarbans (a mangrove ecosystem) of West Bengal, is an estuary where the river Ganges meets the Bay of Bengal. The two new isolates belonging to *Campylobacteraceae* reported in this communication were isolated from these samples. The family *Campylobacteraceae* comprises three genera, namely *Arcobacter*, *Campylobacter* and *Sulfurospirillum*.

At the time of writing, the genus *Arcobacter* (Vandamme *et al.*, 2005) comprised 17 recognized species, eight of which have been described recently: *Arcobacter defluvii* (Collado *et al.*, 2011), *Arcobacter trophiarum* (De Smet *et al.*, 2011), *Arcobacter molluscorum* (Figueras *et al.*, 2011a), *Arcobacter ellisii* (Figueras *et al.*, 2011b), *Arcobacter bivalviorum* and *Arcobacter venerupis* (Levican *et al.*, 2012), '*Arcobacter cloacae*' and '*Arcobacter suis*' (Levican *et al.*, 2013). Members of the genus *Arcobacter* were isolated from various environments,

Abbreviations: DPG diphosphatidylglycerol; ME, minimum-evolution; MP, maximum-parsimony; NJ, neighbour-joining; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; PS, phosphatidylserine.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA, *gyrB*, *rpoB* and *hsp60* gene sequences of strain JC84^T are FR686494, HE609033, HF968432 and HF968431, respectively.

One supplementary table and four supplementary figures are available with the online version of this paper.

many from plant and animal associations (Vandamme *et al.*, 1991; Figueras *et al.*, 2011a, b; Levican *et al.*, 2012). All reported members of the genus *Arcobacter* are motile by means of a single polar flagellum and are capable of growing under microaerobic conditions. *Arcobacter butzleri*, Arcobacter skirrowii, *Arcobacter cryaerophilus*, *Arcobacter nitrofigilis* (Vandamme *et al.*, 1992) and *Arcobacter halophilus* (Donachie *et al.*, 2005) show anaerobic growth, *Arcobacter cibarius* (Houf *et al.*, 2005), *Arcobacter mytili* (Collado *et al.*, 2009) and *Arcobacter defluvii* (Collado *et al.*, 2011) show weak or poor growth, while *Arcobacter marinus* (Kim *et al.*, 2010) cannot grow under anaerobic conditions. In this communication, we report two novel members of the genus *Arcobacter*, strains JC83 and JC84^T, isolated from two estuarine sediment samples collected from Gangasagar, Kolkata, West Bengal, India (GPS position of the sample collection site is 21° 882′ N 088° 164′ E.).

The initial enrichment for strains JC83 and JC84^T was carried out in Postgate's C broth (Postgate, 1984) supplemented with lactate (0.35 %, v/v) as the carbon source, NH_4Cl (0.1 %, w/v) as the nitrogen source and $FeSO_4 . 7H_2O$ (0.05 %, w/v) as the electron acceptor in fully filled screw cap test tubes (10 × 100 mm) and incubated at 28 ± 2 °C. Black coloured enrichments were obtained after 10 days of incubation, indicating the presence of sulfate-reducing bacteria. Purification of the bacteria was achieved by repeated streaking of the culture on agar slants in the above medium sealed with suba-seal and flushed with argon gas to maintain anaerobic conditions (Lakshmi *et al.*, 2011a, b). After 5 days of incubation, small, round transparent colonies were observed. The colonies initially formed a black zone around them and the whole medium turned black within the next 2 days of growth. Both strains grew well in nitrate medium containing (per litre) beef extract (3 g), peptone (5 g) and KNO_3 (1 g) under anaerobic conditions. The colonies on nitrate medium were tiny, round and pale yellow. As the strains could not withstand either refrigeration at 4 °C or lyophilization, pure cultures were maintained by frequent subculturing in broth or in agar slants under anaerobic conditions with subculturing done every 15 days in nitrate medium.

Genomic DNA was extracted and purified from strains JC83 and JC84^T according to the method of Marmur (1961) and the G+C content of the DNA was 25.0 and 24.6 mol%, respectively, as determined by HPLC (Mesbah *et al.*, 1989). Cell material for 16S rRNA gene sequencing was taken from a colony. DNA was extracted and purified by using a Qiagen genomic DNA extraction kit. Recombinant *Taq* polymerase (Genei) was used for PCR, which was carried out using primers F27 (5′-GTTTGATCCTGGCTCAG-3′) and R1489 (5′-TACCTTGTTACGACTTCA-3′) [positions 11–27 and 1489–1506, respectively, according to the *Escherichia coli* 16S rRNA gene numbering system of the International Union of Biochemistry (Brosius *et al.*, 1978; Lane *et al.*, 1985)]. PCR amplification was performed as described by Imhoff & Pfennig (2001) and Imhoff *et al.* (1998). 16S rRNA gene sequencing was performed using the BigDye Terminator v1.1 Sequencing kit (Applied Biosystems) in a 3730-DNA-Analyser (Applied Biosystems) as specified by the manufacturer. For sequencing, the primers F27, F790 (5′-GATACCCTGGTAGTCC-3′) and R1489 were used. Identification of phylogenetic neighbours and calculation of pairwise 16S rRNA gene sequence similarity were achieved using the EzTaxon-e server (Kim *et al.*, 2012). The CLUSTAL W algorithm of MEGA 5.2 was used for sequence alignments and MEGA 5.2 (Tamura *et al.*, 2011) software was used for phylogenetic analysis of the individual sequences. Distances were calculated by using the Kimura correction in a pairwise deletion manner (Kimura, 1980). Neighbour-joining (NJ), maximum-parsimony (MP) and minimum-evolution (ME) methods in the MEGA 5.2 software were used to reconstruct phylogenetic trees. Percentage support values were obtained using a bootstrap procedure.

The results of phylogenetic analysis of the 16S rRNA gene sequences (~1390 bp) suggested that strains JC83 and JC84^T were monophyletic with members of the genus *Arcobacter* including the type species, but they took up the most diverse phylogenetic position within the family *Campylobacteraceae* [the NJ tree is shown as Fig. 1; ME and MP trees had similar topologies (data not shown); the NJ tree including the type strains of additional species is shown in Fig. S1, available in IJSEM Online], and the sequence similarities with the nearest phylogenetic members are in agreement with the EzTaxon-e server result. The 16S rRNA gene sequence similarity between strains JC83 and JC84^T was 99.6 %. EzTaxon-e server search analysis revealed that both strains (JC83 and JC84^T) were related most closely to members of the genus *Arcobacter*; highest sequence similarity was observed with *A. marinus* CL-S1^T (95.5 %).

To further confirm the genus affiliation of strains JC83 and JC84^T, the housekeeping genes *rpoB*, *gyrB* and *hsp60* were also amplified and sequenced. The *rpoB* gene was amplified using the primers CamrpoB-L (5′-CCAATTTATGGATCAAAC-3′) and RpoB-R (5′- GTTGCATGTTNGNACCCAT-3′), the *gyrB* gene was amplified with the primers gyrB-Arc-7F (5′-GTTTAYCAYTTTGAAGGTGG-3′) and gyrB-Arc-14R (5′-CTAGATTTTTCAACATTTAAAAT-3′), and the *hsp60* gene was amplified with the primers hsp60F (5′-TTGAACT-TAAAAAAGCTTCGAG-3′) and hsp60R (5′-TCCATCAAC-ATCTTCAGCTAC-3′), and these were sequenced according to the methods described by Collado *et al.* (2009, 2011) and Figueras *et al.* (2011a).

The concatenated sequences of housekeeping genes *rpoB*, *gyrB* and *hsp60* were first aligned by CLUSTAL X and the alignments were improved by removing poorly aligned regions within each gene sequence by using the online program Gblocks (Castresana, 2000). A phylogenetic tree of the concatenated sequences was constructed using CLUSTAL W within MEGA 5.2 (Tamura *et al.*, 2011) and distances were calculated with default parameters. The robustness of the tree topologies was evaluated by 100 bootstrap replications. Bootstrap values were calculated

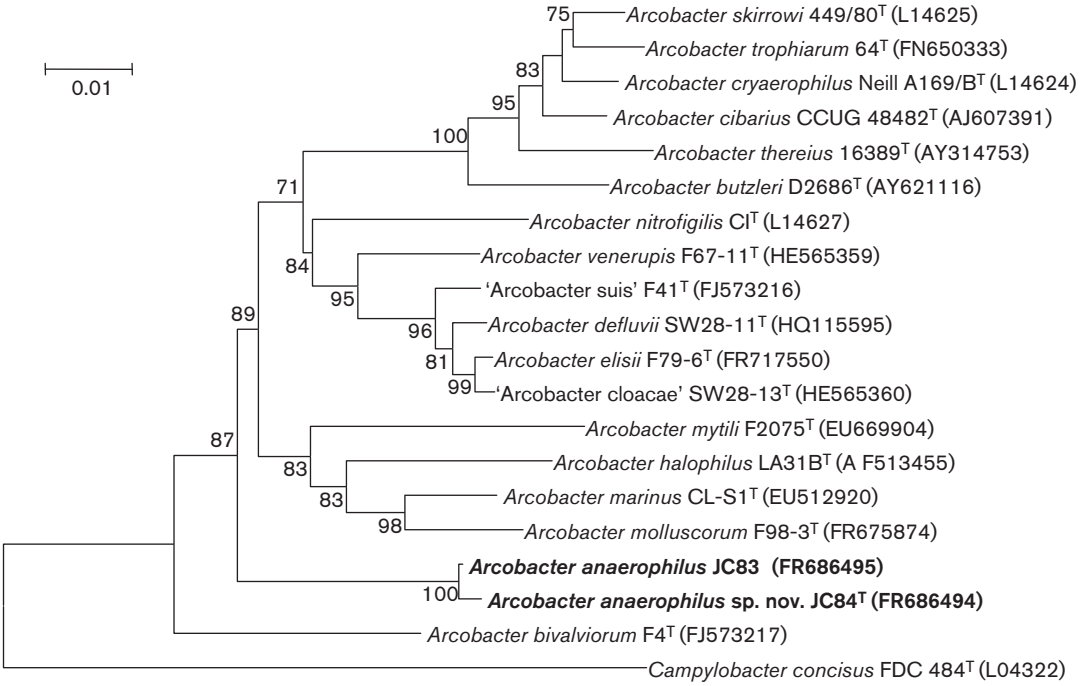

Fig. 1. Phylogenetic tree based on 16S rRNA gene sequences showing the relationship of strains JC83 and JC84^T with the type strains of all recognized species of the genus *Arcobacter*. The tree was constructed (1350 gap-free sites were compared) by the neighbour-joining method using the MEGA5.2 software. Bootstrap values (>70 %) based on 1000 replications are shown at the nodes of the tree. Bar, 1 substitution per 100 nt. *Campylobacter concisus* FDC 484^T was used as the outgroup.

using the NJ and MP methods in MEGA 5.2 to compare and validate the tree topologies reconstructed by the different algorithms. The concatenated gene sequences of *gyrB* (586 bp), *hsp60* (513 bp) and *rpoB* (573 bp) of strains JC83 and JC84^T were compared with those of the type strains along with additional strains in the genus *Arcobacter*, and the improved Gblocks concatenated sequences consisted of 1444 homologous positions. The constructed phylogenetic NJ tree (Fig. S2) clearly confirmed the genus affiliation of strains JC83 and JC84^T but distinct from other members of the genus *Arcobacter*.

The taxonomic relationship between strains JC83 and JC84^T was examined further using genome DNA–DNA hybridization. Genomic relatedness was determined by the membrane filter technique (Seldin & Dubnau, 1985; Tourova & Antonov, 1987) using a DIG High Prime DNA labelling and Detection Starter kit II (Roche). Hybridization was performed with three replications for each sample (control: reversal of the strains used for binding and labelling) and a mean DNA–DNA relatedness of >85 % (86.0±0.5 %) between strains JC83 and JC84^T was observed.

All morphological, physiological and biochemical analyses, unless otherwise mentioned, were performed for both strains JC83 and JC84^T separately in nitrate medium containing (per litre) beef extract (3 g), peptone (5 g) and KNO_3 (1 g) under anaerobic incubation conditions. *A. nitrofigilis* DSM 7299^T (representing the type species of the genus *Arcobacter*), *A. marinus* DSM 24769^T (=CL-S1^T), *A. molluscorum* LMG 25693^T (=F98-3^T) and *A. halophilus* DSM 18005^T (=LA31B^T) were used for comparative taxonomic analysis owing to their phylogenetic closeness to strains JC83 and JC84^T. On nitrate agar, anaerobically grown colonies of strains JC83 and JC84^T were pale yellow, circular, convex and with entire margin. On Postgate's C medium, colonies were tiny, circular and cream coloured which become milk white on exposure to air. Morphological properties (cell shape, cell size, motility) as observed under phase-contrast light microscopy (Olympus BH-2) indicated that individual cells of both strains were rods (1.0–2.0 μm long, 0.1–0.3 μm wide; Fig. S3) and non-motile. The absence of motility in both strains was confirmed through the hanging-drop method, and negatively stained cells observed by transmission electron microscopy (H-7500; Hitachi) indicated the absence of flagella.

Growth and tolerance of the strains at different temperatures, pH and salinity were determined in nitrate broth. Growth was measured turbidometrically at 540 nm from cultures which were centrifuged (15 000 r.p.m. for 15 min) and resuspended in distilled water. Anaerobically grown broth cultures were pale yellow, slightly gelatinous and sedimented at the bottom of the tube after 2 days of growth. NaCl was not required for growth of the two strains, although optimum growth was at 1–3 % and they

were able to tolerate up to 6 % (w/v). Both strains showed optimum growth at pH 7.0–8.0 (range pH 7–10) and 30 °C (range 25–37 °C). Chemoorganoheterotrophy was the only growth mode and both strains utilized a limited range of organic molecules (Table S1).

Phenotypic characterization was performed following the proposed minimal standards for describing new species of the family *Campylobacteraceae* (Ursing *et al.*, 1994). Growth was not possible on blood agar and no growth was observed in the presence of triphenyltetrazolium chloride (0.04 %). Oxidase was positive while catalase was negative for both strains. Oxidases are uncommon among strict anaerobic bacteria, although the presence of membrane-bound functional cytochrome *bd*-type oxidases in a few strict anaerobes helps in catalysing the reduction of low levels of dioxygen (Das *et al.*, 2005; Baughn & Malamy, 2004; Lemos *et al.*, 2001). Both strains hydrolysed casein and reduced nitrate. Both strains produced H_2S in Postgate's C medium, iron-bisulfite-pyruvate medium and sulfide-indole-motility agar. The *dsrAB* gene, which codes for sulfate reductase, could not be amplified using the primer set *dsr*1F (5′-AC[C/G]CACTGGAAGCACG-3′) and *dsr*4R (5′-GTGTAGCAGTTACCGCA-3′; Sasi Jyothsna *et al.*, 2008), while this gene was positive for *Desulfovibrio psychrotolerans* JS1^T, which was used as a positive control. Gelatin was not liquefied and indole was not produced from L-tryptophan by both strains. Lipase, amylase and urease activities were negative for both strains. Acid from glucose and acid phosphatase were not produced. Indoxyl acetate was hydrolysed by both strains.

For cellular fatty acids, anaerobically grown cells were harvested when growth of the cultures attained 70 % of maximal optical density (at their late exponential growth phase). Forty milligrams of bacterial cells was subjected to saponification and the methyl esters were analysed by GC (Agilent 6850) with Sherlock MIS software (Microbial ID; MIDI 6.0 version; peak identification was done based on the RTSBA6 database) [Sasser (1990); http://www.midi-inc.com/]. Analysis was outsourced to the Royal Research Laboratories, Secunderabad, India. $C_{16:1}\omega7c$ (25 %), $C_{18:1}\omega7c$ (34.4 %), $C_{16:0}$ (20.5 %) and $C_{12:0}$ (7.3 %) were the major (>5 %) cellular fatty acids of strain JC84^T. Minor (<5 %, >1 %) amounts of $C_{12:0}$ 3-OH (3.4 %), $C_{14:0}$ (3.5 %) and $C_{18:0}$ (2.3 %) were also present. The fatty acid profile of strain JC83 was qualitatively identical to that of strain JC84^T. The fatty acid profile of strain JC84^T was similar to those of *A. nitrofigilis* DSM 7299^T (=CI^T), *A. marinus* DSM 24769^T (=CL-S1^T), *A. molluscorum* LMG 25693^T (F98-3^T) and *A. halophilus* DSM 18005^T (=LA31B^T), all of which were analysed in this study (data not shown).

Polar lipids were extracted from 1 g freeze-dried cells with methanol/chloroform/saline (2 : 1 : 0.8, by vol.) as described by Kates (1986). The lipids were separated using silica gel TLC (Kieselgel 60 F_{254}; Merck) by two-dimensional chromatography using chloroform/methanol/water (65 : 25 : 4 by vol.) in the first dimension and chloroform/methanol/acetic acid/water (80 : 12 : 15 : 4 by vol.) in the second dimension (Tindall, 1990; Tindall *et al.*, 1987; Oren *et al.*, 1996). Total polar lipids were detected by spraying with 5 % ethanolic molybdophosphoric acid and further characterized by spraying with ninhydrin (specific for amino groups), molybdenum blue (specific for phosphates), Dragendorff (quaternary nitrogen) or α-naphthol (specific for sugars) (Kates, 1972; Oren *et al.*, 1996). Polar lipids of strains JC83 and JC84^T included diphosphatidylglycerol (DPG), phosphatidylethanolamine (PE), phosphatidylglycerol (PG), phosphatidylserine (PS), phosphatidylcholine (PC), phosphatidylinositol (PI), an unidentified amino lipid (AL2), an unidentified phospholipid (PL2) and an unidentified lipid (L3) (Fig. S4). The polar lipid profile of strain JC84^T was not in line with that of *A. nitrofigilis* DSM 7299^T (=CI^T), which contained PE, PG, PI, PS, an unidentified phospholipid (PL1), an unidentified aminolipid (AL1) and unidentified lipids (L1 and L2). The presence of DPG, PC, PL2, AL2 and L3 and absence of PL1, AL1, L1 and L2 in strains JC83 and JC84^T clearly distinguished them from *A. nitrofigilis* DSM 7299^T. In addition, the limited polar lipid profiles of *A. marinus* DSM 24769^T, *A. molluscorum* LMG 25693^T and *A. halophilus* DSM 18005^T, which were analysed in parallel, further differentiated strains JC83 and JC84^T from their nearest phylogenetic neighbours (Fig. S4).

Quinones from strains JC83 and JC84^T were extracted with a chloroform/methanol (2 : 1, v/v) mixture, purified by TLC and analysed by HPLC as described (Tamaoka *et al.*, 1983; Hiraishi & Hoshino, 1984; Hiraishi *et al.*, 1984) and were compared with those extracted from *A. nitrofigilis* DSM 7299^T, *A. marinus* DSM 24769^T, *A. molluscorum* LMG 25693^T and *A. halophilus* DSM 18005^T. Both novel strains had MK-6 (97 %) and MMK-6 (3 %) as respiratory quinones. The quinone compositions of strains JC83 and JC84^T were not in line with that of *A. nitrofigilis* DSM 7299^T, which had MK-6 (50 %), MK-7 (46 %) and MK-8 (4 %). However, the quinone compositions of *A. marinus* DSM 24769^T, *A. molluscorum* LMG 25693^T and *A. halophilus* DSM 18005^T were similar to those of strains JC83 and JC84^T.

The distinct polar lipid profile (Fig. S4), quinone composition and other phenotypic traits (Table 1), including lack of motility, obligate anaerobic growth, negativity for catalase, positivity for casein hydrolysis, ability to produce H_2S and citrate utilization, allows a clear separation of strains JC83 and JC84^T from the type strains of recognized species in the genus *Arcobacter*. Based on their molecular and phenotypic distinctiveness, strains JC83 and JC84^T are considered to represent a novel species of the genus *Arcobacter*, for which the name *Arcobacter anaerophilus* sp. nov. is proposed.

Description of *Arcobacter anaerophilus* sp. nov.

Arcobacter anaerophilus [a.na.e.ro′phi.lus. Gr. pref. *an* not; Gr. masc. n. *aer* air; N.L. masc. adj. *philus* (from Gr. masc. adj. *philos*) friend, loving; N.L. masc. adj. *anaerophilus* not air-loving].

Table 1. Characteristics that differentiate strain JC84^T from all other species of the genus *Arcobacter*

Strains: 1, JC84^T (strain JC83 has similar characters; data from this study); 2, *A. nitrofigilis* DSM 7299^T (this study); 3, *A. marinus* DSM 24769^T (this study); 4, *A. halophilus* DSM 18005^T (this study); 5, *A. molluscorum* LMG 25693^T (this study); 6, *A. bivalviorum* F4^T (Levican *et al.*, 2012); 7, *A. venerupis* F67-11^T (Levican *et al.*, 2012); 8, *A. cryaerophilus* Neill A 169/B^T (Vandamme *et al.*, 1991; Levican *et al.*, 2013); 9, *A. butzleri* D2686^T (Vandamme *et al.*, 1992; Levican *et al.*, 2013); 10, A. skirrowii Skirrow 449/80^T (Vandamme *et al.*, 1992; Levican *et al.*, 2013); 11, *A. cibarius* CCUG 48482^T (Houf *et al.*, 2005); 12, *A. mytili* F2075^T (Collado *et al.*, 2009); 13, *A. thereius* 16389^T (Houf *et al.*, 2009); 14, *A. trophiarum* 64^T (De Smet *et al.*, 2011); 15, *A. defluvii* SW28-11^T (Collado *et al.*, 2011); 16, *A. ellisii* F79-6^T (Figueras *et al.*, 2011b); 17, '*A. cloacae*' SW28-13^T (Levican *et al.*, 2013); 18, '*A. suis*' F41^T (Levican *et al.*, 2013). +, Positive; (+), weakly positive; −, negative; V, variable; V(+), variable where a majority of strains were positive; V(−), variable where a majority of strains were negative; ND, not determined. All strains were Gram-stain-negative and oxidase-positive.

Characteristic	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Motility	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Growth in/on:																		
Air at 37 °C	−	−	+	+	+	+	−	+	+	+	−	+	−	−	+	+	+	−
CO_2 at 37 °C*	−	−	+	+	+	+	+	V(+)	+	+	+	+	−	−	+	+	+	−
CO_2 at 42 °C*	−	−	−	−	−	−	−	−	V(+)	−	−	(+)	−	−	ND	ND	−	−
100 % Argon†	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Blood agar	−	+	+	+	+	+	+	+	+	+	+	+	V	+	+	+	+	+
NaCl (0.5 %)	+	+	−	−	−	+	+	+	+	+	+	+	+	+	ND	ND	+	+
NaCl (4 %)	+	+	+	+	+	+	−	−	−	+	−	+	−	V(−)	−	−	−	−
Glycine (1 %)	+	−	+	+‡	−	−	−	−	V(+)	−	−	+	+	ND	−	−	−	−
Resistance to cefoperazone (64 mg l^{-1})	−	−	−	−	+	−	−	+	+	+	+	−	+	+	V(+)	−	−	−
Enzyme activities																		
Catalase	−	+	−	−	+	+	+	+	(+)	+	V(+)	+	+	+	+	+	+	+
Urease	−	+	−	−	−	−	+	−	−	−	−	−	−	−	+	V (−)	−	−
Nitrate reduction	+	+	+	+	+	−	+	−	+	+	−	−	+	−	+	+	+	+
Indoxyl acetate hydrolysis	+	+	+	+	−	+	+	+	+	+	+	−	+	+	+	+	+	+
H_2S production	+	−	−	−	−	−	−	−	V(−)	−	−	−	−	−	−	−	−	−
Casein hydrolysis	+	−	−	−	−	−	−	ND	ND	ND	ND	ND	ND	ND	−	−	−	−
DNA G+C content (mol%)	24.6	28–31	28	35	29	ND	ND	28–31	28–29	29	26.8–27.3	26.9	28.5	28.5	ND	ND	ND	ND

*Microaerobic conditions.

†Obligate anaerobic growth under 100 % argon.

‡Growth on glycine, which was previously not observed by Donachie *et al.* (2005).

Gram-stain-negative, non-spore-forming, non-motile, non-flagellated rods. Cells are 1.0–2.0 μm long and 0.1–0.3 μm wide. On nitrate agar, anaerobically well grown colonies are pale yellow, circular, smooth with entire margin. Anaerobic broth culture is pale yellow, gelatinous and sediments at the bottom. Catalase-negative, oxidase-positive; utilizes citrate, hydrolyses casein, reduces nitrate and produces H_2S. Amylase, urease and gelatinase are not produced. Growth occurs on limited carbon sources and good growth occurs on complex media. NaCl is not essential for growth, but good growth occurs with 1–3 % (w/v) NaCl; tolerates up to 6 % NaCl. The following compounds are oxidized in the Biolog GN2 test system: L-arabinose, D-arabitol, cellobiose, lactose, D-mannose, melibiose, acetate, inosine and phenylethylamine. Major polar lipids include DPG, PE, PG and PS; PC, an unidentified aminolipid (AL2), an unidentified phospholipid (PL2), PI and an unidentified lipid (L3) are also present. Major fatty acids include $C_{16:1}\omega7c$, $C_{18:1}\omega7c$, $C_{16:0}$ and $C_{12:0}$. Minor amounts of $C_{12:0}$ 3-OH, $C_{14:0}$ and $C_{18:0}$ are also present. MK-6 is the major quinone and MMK-6 is present in minor amounts.

The type strain is JC84^T (=KCTC 15071^T=MTCC 10956^T=DSM 24636^T), which was isolated from a sediment sample of Gangasagar estuary, West Bengal, India. The DNA G+C content of the type strain is 24.6 mol%. JC83, isolated from the same region, is a second strain of the species. The DNA G+C of strain JC83 is 25 mol%.

Emended description of the genus *Arcobacter* Vandamme *et al.* 1991, 1992

The description is as given previously (Vandamme *et al.*, 1991, 1992) with the following modifications. Some species are obligate anaerobes and lack motility. Cells of some species are filamentous and are up to 7 μm long. Catalase is absent in some species. Hydrolysis of casein is variable and H_2S is produced by some species. The type species of the genus has MK-6 and MK-7 as major quinones with minor amounts of MK-8 while some species have MK-6 as the major respiratory quinone with minor amounts of MMK-6. $C_{16:1}\omega7c$ and $C_{18:1}\omega7c$ are the major fatty acids with minor amounts of $C_{12:0}$ 3-OH, $C_{14:0}$, $C_{18:0}$, $C_{14:1}\omega6c$ and $C_{18:1}\omega9c$ also present in some species. All species tested have PG and PE as major polar lipids. In addition, some species have DPG, PS, PC, PI, unidentified aminolipids (AL1, AL2), unidentified phospholipids (PL1, PL2) and unidentified lipids (L1, L2 and L3). The DNA base composition ranges from 24.6 to 31 mol% G+C.

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