Aminobacterium thunnarium sp. nov., a mesophilic, amino acid-degrading bacterium isolated from an anaerobic sludge digester, pertaining to the phylum *Synergistetes*

Olfa Hamdi,[1,2] Wajdi Ben Hania,[1] Anne Postec,[1] Hassib Bouallagui,[2] Moktar Hamdi,[2] Patricia Bonin,[1] Bernard Ollivier[1] and Marie-Laure Fardeau[1]

Correspondence
Marie-Laure Fardeau
marie-laure.fardeau@univ-amu.fr

[1]Aix Marseille Université, CNRS, Université de Toulon, IRD, Mediterranean Institute of Oceanography (MIO), UM 110, 13288 Marseille, France

[2]Laboratoire d'Ecologie et de Technologie Microbienne, Institut National des Sciences Appliquées et de Technologie, Centre Urbain Nord, BP 676, 1080 Tunis, Université de Carthage, Tunisia

A new Gram-staining-positive, non-sporulating, mesophilic, amino acid-degrading anaerobic bacterium, designated strain OTA 102^T, was isolated from an anaerobic sequencing batch reactor treating wastewater from cooking tuna. The cells were curved rods (0.6–2.5×0.5 μm) and occurred singly or in pairs. The strain was motile by means of one lateral flagellum. Strain OTA 102^T grew at temperatures between 30 and 45 °C (optimum 40 °C), between pH 6.0 and 8.4 (optimum pH 7.2) and NaCl concentrations between 1 and 5 % (optimum 2 %, w/v). Strain OTA 102^T required yeast extract for growth. Serine, threonine, glycine, cysteine, citrate, fumarate, α-ketoglutarate and pyruvate were fermented. When co-cultured with *Methanobacterium formicicum* as the hydrogen scavenger, strain OTA 102^T oxidized alanine, valine, leucine, isoleucine, aspartate, tyrosine, methionine, histidine and asparagine. The genomic DNA G+C content of strain OTA 102^T was 41.7 mol%. The main fatty acid was iso-$C_{15:0}$. Phylogenetic analysis of the 16S rRNA gene sequence indicated that strain OTA 102^T was related to *Aminobacterium colombiense* and *Aminobacterium mobile* (95.5 and 95.2 % similarity, respectively), of the phylum *Synergistetes*. On the basis of phylogenetic, genetic and physiological characteristics, strain OTA 102^T is proposed to represent a novel species of the genus *Aminobacterium*, *Aminobacterium thunnarium* sp. nov. The type strain is OTA 102^T (=DSM 27500^T=JCM 19320^T).

Together with carbohydrates and lipids, proteins comprise a major part of the organic load in urban and industrial wastewaters. In the case of wastewater from cooking tuna, 80 % of organic compounds consist mainly of proteins, the rest being fatty acids, fats and aromatic compounds (Chia & Wen, 2002).

Anaerobic protein degradation has been studied in particular in the animal gut, e.g. the rumen (Debroas & Blanchart, 1993), and in some industrial wastewaters (Baena *et al.*, 1998, 2000) with amino acid-fermenting or -oxidizing prokaryotes known to be involved in this process. Among them, members of the phylum *Synergistetes* comprising 13 genera and 22 species all share the ability to use these organic compounds. They have been isolated from various anaerobic environments, i.e. anaerobic digesters, human oral cavity and goat rumen (Honda *et al.*, 2013). Interestingly, an analysis of environmental 16S rRNA gene sequences revealed that these bacteria are present in 90 % of 93 anaerobic ecosystems tested (guts, soils, digestors, etc.), in spite of their low abundances (less than 1 % of total bacteria in each environment investigated) (Godon *et al.*, 2005) thus suggesting that they are widespread in the environment.

In this study, a novel mesophilic fermentative bacterium isolated from sludge samples of a Tunisian anaerobic bioreactor which pertains to the genus *Aminobacterium*, phylum *Synergistetes*, family *Synergistaceae* is proposed to represent a novel species.

The samples were collected under anaerobic conditions from the sludge of a 2 l anaerobic sequencing batch reactor

The GenBank /EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain OTA 102^T is KJ159211.

A supplementary figure and two supplementary tables are available with the online Supplementary Material.

(ASBR) (temperature: 37 °C, pH: 7.6, flow rate: 100 ml day^{-1}) fed continuously with wastewater from cooking tuna (NaCl 30 g l^{-1}) and transported to the laboratory at ambient temperature. Micro-organisms were isolated and cultivated under strict anaerobiosis, according to the Hungate technique (Hungate, 1969). The basal medium (BM) for isolation contained (g l^{-1}): NH_4Cl (1.0), K_2HPO_4 (0.3), KH_2PO_4 (0.3), KCl (0.1), $CaCl_2 . 2H_2O$ (0.1), NaCl (10), serine (1.0), yeast extract (Difco) (2.0), 10 mM, cysteine hydrochloride (0.5), 1 ml trace mineral element solution (Widdel & Pfennig, 1982) and 1 ml 0.1 % resazurin. The pH was adjusted to 7.6 with 10 M KOH solution. The basal medium was boiled under a stream of O_2-free N_2 gas, cooled to room temperature. Aliquots of 5 ml were dispensed into Hungate tubes, degassed under N_2/CO_2 (80 : 20, v/v) and subsequently sterilized by autoclaving at 120 °C for 20 min. Prior to inoculation, 0.1 ml 10 % (w/v) $NaHCO_3$, 0.1 ml 2 % (w/v) $Na_2S . 9H_2O$ and 0.1 ml 15 % (w/v) $MgCl_2 . 6H_2O$ were injected from sterile stock solutions into the tubes.

Enrichments were performed in Hungate tubes containing the basal medium inoculated with 10 % of sample and incubated at 37 °C. The culture was purified by repeated use of the Hungate Roll tubes method, using 1.6 % agar solid medium, and then transferred into liquid medium (Hungate, 1969). The pH, temperature and NaCl concentration ranges for growth were determined using the basal medium supplemented with 10 mM serine. For studies of NaCl requirements, NaCl was weighed directly in the tubes to produce concentrations ranging from 0 to 8 % NaCl before addition of basal medium without NaCl. To determine the optimum pH for growth, the pH of BM in Hungate tubes (5 ml) was adjusted by injecting appropriate aliquots of 0.1 M HCl (acidic pH), 10 % $NaHCO_3$ or 8 % Na_2CO_3 (basic pH) from sterile anaerobic stock solutions to produce a range between pH 5.2 and 9.2. Bacterial cultures were incubated at temperatures from 15 to 55 °C (at 5 °C intervals).

Cultures were subcultured into fresh medium at least twice under the same experimental conditions before determination of growth rates and substrates used.

The Gram reaction was determined with heat-fixed liquid cultures stained with Difco kit reagents.

For electron microscopy, exponentially grown cells were negatively stained with 1 % sodium phosphotungstic acid (pH 7.0) as described by Fardeau *et al.* (1997). Whole cells were observed with an EM 912 electron microscope (Zeiss) at an accelerating voltage of 75 kV.

For substrate-utilization studies, 0.2 % yeast extract was added to the basal medium. L-Amino acids (serine, threonine, glycine, cysteine, alanine, glutamate, valine, isoleucine, proline, methionine, aspartate, leucine, phenylalanine, histidine, asparagine, glutamine, arginine, lysine, histidine and tyrosine), organic acids (pyruvate, succinate, malate, fumarate, citrate, α-ketoglutarate lactate, acetate, propionate and butyrate) and sugars (glucose, sucrose, ribose, xylose, cellobiose, melibiose, maltose, galactose, mannose, arabinose, rhamnose, lactose, sorbose and mannitol) were each tested at a final concentration of 10 mM. Glycerol and ethanol were added to a final concentration of 5 mM, whereas biotrypcase, peptone, Casamino acids, gelatin and casein were each tested at a final concentration of 0.5 %. The electron acceptors tested were thiosulfate (10 mM), sulfate (10 mM), elemental sulfur (2 %), sulfite (2 mM), nitrite (2 mM) and nitrate (10 mM). Amino acid degradation via the Stickland reaction system was examined in medium containing specific amino acid combinations (electron donor, 10 mM; electron acceptor, 20 mM) supplemented with 0.2 % yeast extract, for which the following amino acid combinations were tested: alanine/glycine, alanine/serine, alanine/proline and alanine/arginine. End products of metabolism were measured by HPLC after 10 days of incubation at 37 °C (Fardeau *et al.*, 2000). *Methanobacterium formicicum* DSM 1535^T, which was used as the hydrogen scavenger in the co-culture, was purchased from the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ; Braunschweig, Germany). *M. formicicum* was precultured on H_2/CO_2 whereas our isolate (strain OTA 102^T) was precultured on 10 mM serine. Each culture (500 µl) was added to 5 ml of basal medium containing 10 mM substrate supplemented with 0.2 % yeast extract. Substrate utilization and final products were determined after 3 weeks of incubation as indicated above.

Cultures of strain OTA 102^T were stopped at the end of exponential phase and sent to DSMZ for fatty acid analysis. Fatty acids were extracted using the method of Miller (1982), with the modifications of Kuykendall *et al.* (1988), and the profile of cellular fatty acids was analysed by gas chromatography using the Microbial Identification System (MIDI, Sherlock Version 6.1; database, TSBA40; gas chromatograph, model 6890N, Agilent Technologies). Analysis of the polar lipids was carried out by the Identification Service of the DSMZ.

The determination of the G+C content of the DNA was made at the DSMZ. Genomic DNA for analysis of the base composition was isolated after disruption of bacterial cells by using a French press (Thermo Spectronic) and purified by chromatography on hydroxyapatite using the procedure of Cashion *et al.* (1977). The DNA G+C content was determined by using HPLC as described by Mesbah *et al.* (1989).

Methods for purification of the DNA, PCR amplification and sequencing of the 16S rRNA gene have been described previously (Thabet *et al.*, 2004). The most closely related sequences were retrieved from the Ribosomal Database Project (release 11) and GenBank using BLAST (Altschul *et al.*, 1997), then aligned using the SINA program (Pruesse *et al.*, 2012). Phylogenetic analyses were conducted in MEGA5 using the neighbour-joining method (Saitou & Nei, 1987; Tamura *et al.*, 2011). There were a total of 1128 positions in the final dataset. Tree topology was tested by the bootstrap method (1000 resamplings) (Felsenstein, 1985).

Several colonies developed after incubation at 37 °C and were picked separately. Colonies were round, smooth, lens-shaped and white with diameters ranging from 0.5 to 1.0 mm after 2–3 weeks of incubation at 37 °C. The process of serial dilution was repeated several times until the isolates were deemed to be axenic. Two strains were isolated and designated OAC 101 and OTA 102^T. Their morphology and metabolic profiles were similar and they also yielded the same phylogenetic inference. Only one strain (OTA 102^T) was selected and used for further metabolic and physiological characterization.

Cells of strain OTA 102^T were curved rods (0.6–2.5 × 0.5 μm) and Gram-staining-positive when grown on a medium containing serine as an energy source (Fig. S1a, available in the online Supplementary Material). Ultrathin sections showed a typical Gram-staining-positive cell wall with an electron-dense layer and the absence of an outer membrane (Fig. S1b). Cells were motile by means of a single lateral flagellum (data not shown).

Strain OTA 102^T was anaerobic but tolerated up to 1 % O_2 in the gas phase. The physiological optimal growth conditions were determined in duplicate experiments conducted in the basal medium containing serine (20 mM) and yeast extract (0.2 %) as previously described (Baena *et al.*, 1998; Fardeau *et al.*, 2000). The optimal temperature for growth was 40 °C (range 30–45 °C). Optimum pH was 7.2 (range pH 6.0–8.4). Strain OTA 102^T required NaCl for growth, (range 1–5 %) with an optimum at 2 %. Few of the tested compounds were used for growth. They included serine, cysteine, threonine, glycine, citrate and pyruvate, which were fermented into acetate, H_2 and CO_2; succinate and propionate were produced from α-ketoglutarate degradation, whereas malate and succinate resulted from fumarate fermentation (Table S1). In contrast to amino acids, poor growth was obtained on Casamino acids and peptone. No growth was observed on alanine, glutamate, valine, isoleucine, proline, methionine, aspartate, leucine, phenylalanine, histidine, asparagine, glutamine, arginine, lysine, histidine, tyrosine, succinate, malate, lactate, acetate, propionate, butyrate, glucose, sucrose, ribose, xylose, cellobiose, melibiose, maltose, galactose, mannose, arabinose, rhamnose, lactose, sorbose, mannitol, glycerol, ethanol, gelatin or casein. Although growth occurred in a minimal medium with serine as the only energy and carbon source, yeast extract or biotrypcase improved growth.

Thiosulfate, sulfate and sulfite, elemental sulfur, nitrate and nitrite did not serve as terminal electron acceptors. Under optimal conditions with serine as the electron donor, the maximum growth rate of strain OTA 102^T was 0.5 h^{-1}.

Phylogenetic analysis of the 16S rRNA gene sequence (1539 nt) indicated that strain OTA 102^T represented a member of the phylum *Synergistetes*, class *Synergistia*, order *Synergistales*, family *Synergistaceae* (Jumas-Bilak *et al.*, 2009) and genus *Aminobacterium* (Baena *et al.*, 1998) (Fig. 1). Strain OTA 102^T shared 95.5 % similarity with *Aminobacterium colombiense* (Baena *et al.*, 1998) and 95.2 % similarity with *Aminobacterium mobile* (Baena *et al.*, 2000).

Although chemotaxonomic differences were observed between the novel isolate, *Aminobacterium colombiense* and *Aminobacterium mobile* (Table 1), the cellular fatty acids profile (Table 2) of strain OTA 102^T consisting mainly of iso-$C_{15:0}$ was also in agreement with its classification as a member of the genus *Aminobacterium*. The DNA G+C content of strain OTA 102^T was 41.7 mol%. Both species of the genus *Aminobacterium* with validly published names (*Aminobacterium colombiense* and *Aminobacterium mobile*) were isolated from an anaerobic lagoon in a dairy wastewater treatment plant (Baena *et al.*, 1998, 2000) and similarly to strain OTA 102^T, they are recognized as amino acid-degrading bacteria. Some metabolic features are shared by other members of the phylum *Synergistetes*, including *Aminomonas paucivorans* (Baena *et al.*, 1999a), *Dethiosulfovibrio peptidovorans*, *Jonquetella anthropi* (Jumas-Bilak *et al.*, 2007), *Synergistes jonesii* (Vartoukian *et al.*, 2007) and *Aminivibrio pyruvatiphilus* (Honda *et al.*, 2013). In contrast to *Aminobacterium colombiense* and *Aminobacterium mobile*, strain OTA 102^T used citrate as an energy source with acetate being an end product of metabolism (Table S1). This is identical to what was observed for *Aminivibrio pyruvatiphilus* (Honda *et al.*, 2013), *Dethiosulfovibrio peptidovorans* and *Dethiosulfovibrio marinus* (Magot *et al.*, 1997). Strain OTA 102^T also fermented (i) fumarate to malate and succinate and (ii) α-ketoglutarate to succinate and propionate. Carbohydrates were not used. To our knowledge carbohydrate utilization in the phylum *Synergistetes* has only been observed for *Anaerobaculum thermoterrenum* (Rees *et al.*, 1997), *Anaerobaculum mobile* (Menes & Muxí, 2002), *Anaerobaculum hydrogeniformans* (Maune & Tanner, 2012), *Thermanaerovibrio velox* (Zavarzina *et al.*, 2000) and *Thermanaerovibrio acidaminovorans* (Baena *et al.*, 1999b).

Further experiments that we conducted with strain OTA 102^T demonstrated that it was able to oxidize the following amino acids: alanine, valine, leucine, isoleucine, aspartate, tyrosine, histidine, methionine, glutamate and asparagine in the presence of the hydrogen-consuming methanogenic archaeon, *M. formicicum* (Table S2). Acetate was produced from alanine, tyrosine, aspartate, histidine, asparagine and glutamate oxidation, while branched chain fatty acids were produced from their corresponding branched chain amino acids (e.g. isobutyrate from valine, isovalerate from leucine and 2-methylbutyrate from isoleucine). This metabolic profile with respect to amino acid utilization in the presence of an archaeal hydrogen scavenger was somewhat similar to that observed for *Aminivibrio pyruvatiphilus* (Honda *et al.*, 2013), *Aminobacterium colombiense* (Baena *et al.*, 1998) and *Aminobacterium mobile* (Baena *et al.*, 2000). However in contrast to strain OTA 102^T, these bacteria have not been reported to oxidize tyrosine. It is noteworthy that strain OTA 102^T performed the Stickland reaction using alanine as an electron donor and glycine or serine as terminal electron acceptors in a similar manner

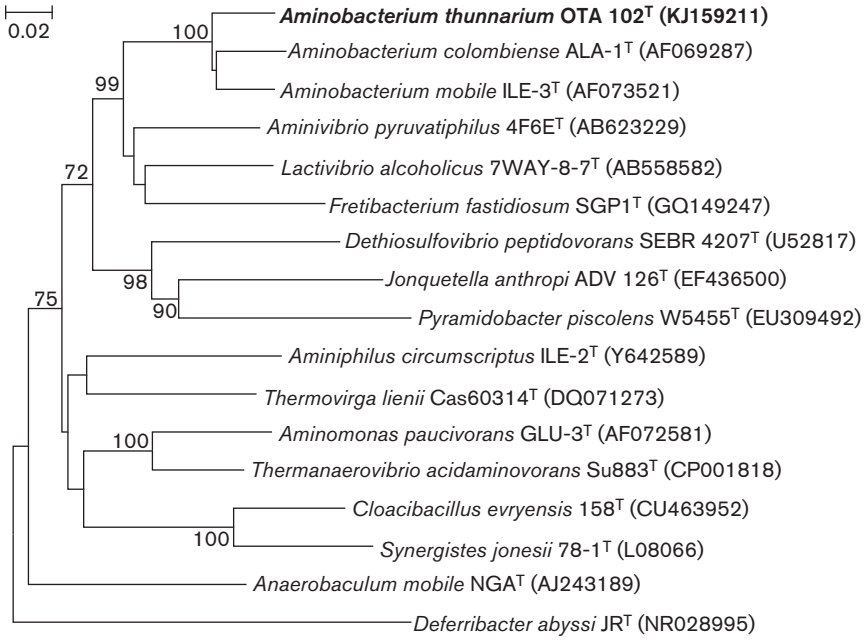

Fig. 1. Phylogenetic position of strain OTA 102^T within the family *Synergistaceae* based on 16S rRNA gene sequence analysis using the neighbour-joining method. The sequence of *Deferribacter abyssi* JR^T was used as outgroup. There were a total of 1128 positions in the final dataset. Numbers at nodes indicate bootstrap values above 70 % (1000 replicates). Bar, 0.02 substitutions per site.

as already described for members pertaining to amino acidolytic members of the class *Clostridia* such as *Clostridium sticklandii* and *Clostridium bifermentans* (Elsden & Hilton 1979; Mead, 1971).

A comparison of the main distinguishing characteristics of strain OTA 102^T from *Aminobacterium colombiense* or *Aminobacterium mobile* is given in Table 1. Based on phenotypic and genetic characteristics of strain OTA 102^T,

Table 1. Comparison of the main characteristics of strain OTA 102^T, *Aminobacterium colombiense* and *Aminobacterium mobile*

Strains: 1, OTA 102^T; 2, *Aminobacterium colombiense* DSM 12261^T; 3, *Aminobacterium mobile* DSM 12262^T. Results were examined after 3 weeks at 37 °C. The basal medium contained 0.2 % yeast extract and different substrates as indicated. Tubes containing basal medium with 0.2 % yeast extract and inocula without any substrate were used as controls. All values were corrected for the small amounts of products formed in the control tubes. Values are the means from duplicate experiments. +, Positive; −, negative; w, weakly positive.

Characteristic	1	2	3
Morphology	Curved rods	Curved	Curved
Motility	+	−	−
Gram staining	+	−	−
Size (μm)	0.6–2.5 × 0.5	3–4 × 0.2–0.3	4–5 × 0.2–0.3
DNA G+C content (mol%)	41.7	46	44
Temperature growth range (optimum) (°C)	30–45 (40)	20–42 (37)	20–42 (37)
pH growth range (optimum)	6.0–8.4 (7.2)	6.6–8.5 (7.3)	6.6–8.5 (7.3)
Salinity growth range (optimum) (%)	1–5 (2)	0–1.5 (0.05–0.5)	0–1.5 (0.05–0.5)
Stickland reaction	+	−	−
Electron donor:			
Cysteine (10 mM)	+	−	−
Citrate (20 mM)	+	−	−
Fumarate (20 mM)	+	−	−
α-Ketoglutarate (20 mM)	+	w	w

Table 2. Comparison of the main fatty acids of strain OTA 102^T, *Aminobacterium colombiense* and *Aminobacterium mobile*

Strains: 1, OTA 102^T; 2, *Aminobacterium colombiense* ALA-1^T (=DSM 12261^T); 3, *Aminobacterium mobile*. ILE-3^T (=DSM 12262^T) (data from Honda *et al*., 2013).

Cellular fatty acid	1	2	3
Saturated			
iso-$C_{11:0}$	7.6	5.1	6.1
iso-$C_{13:0}$	10.1	4.7	6.5
$C_{14:0}$	2.5	3.9	5.7
iso-$C_{15:0}$	58.0	53.1	43.5
$C_{16:0}$	6.3	1.5	2.8
iso-$C_{17:0}$	–	1.1	1.8
$C_{18:0}$	8.1	2.3	3.8
iso-$C_{19:0}$	3.2	3.8	4.0
$C_{20:0}$	–	2.8	2.2
Unsaturated			
$C_{20:1}\omega 7c$	–	1.3	1.1
10-Methyl			
10-Me $C_{18:0}$ (TBSA)	–	8.0	7.8
Hydroxy			
iso-$C_{13:0}$3-OH	4.2	8.1	8.0

we propose that it represents a novel species of the genus *Aminobacterium*, *Aminobacterium thunnarium* sp. nov.

Description of *Aminobacterium thunnarium* sp. nov.

Aminobacterium thunnarium [thun.na′ri.um. L. masc. adj. *thunnarium* of or belonging to a tunny (cooking tuna wastewater), where the isolate was first recovered].

Cells are Gram-staining-positive, anaerobic, motile (by means of a lateral flagellum) rods (0.6–2.5 × 0.5 μm), occurring singly or in pairs. Non-spore-forming and slightly halophilic (range: 1–5 % NaCl; optimum: 2 %). The temperature range for growth is 30–45 °C, with an optimum of 40 °C. The optimum pH is 7.2 (range pH 6.0–8.4). Yeast extract is required for growth. Does not reduce any of the electron acceptors tested (thiosulfate, sulfate, sulfite, elemental sulfur, nitrate and nitrite). The organism ferments amino acids (serine, cysteine, threonine and glycine) and organic acids (α-ketoglutarate, pyruvate, fumarate and citrate). Strain OTA 102^T performs the Stickland reaction when alanine is provided as an electron donor and glycine or serine are provided as terminal electron acceptors. Oxidizes several other amino acids (alanine, valine, leucine, isoleucine, aspartate, tyrosine, histidine, methionine, glutamate and asparagine) in the presence the hydrogenotrophic archaeon *Methanobacterium formicicum*. No growth was observed on arginine, proline, glutamine, tryptophan, lysine, phenylalanine, carbohydrates (glucose, sucrose, ribose, xylose, cellobiose, melibiose, maltose, galactose, mannose, arabinose, rhamnose, lactose or sorbose), mannitol, glycerol, ethanol, gelatin or casein. Acetate, H_2 and CO_2 are the end products of serine, cysteine, threonine, glycine, citrate and pyruvate fermentation. Succinate and propionate are produced from α-ketoglutarate fermentation, whereas succinate and malate are the end products of fumarate metabolism. The main fatty acid is iso-$C_{15:0}$.

The type strain is OTA 102^T (=DSM 27500^T=JCM 19320^T), which was isolated from an anaerobic sequencing batch reactor treating wastewater from cooking tuna. The genomic DNA G+C content of DNA of the type strain is 41.7 mol%.

References

Altschul, S. F., Madden, T. L., Schäffer, A. A., Zhang, J., Zhang, Z., Miller, W. & Lipman, D. J. (1997). Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res* **25**, 3389–3402.

Baena, S., Fardeau, M. L., Labat, M., Ollivier, B., Thomas, P., Garcia, J. L. & Patel, B. K. C. (1998). *Aminobacterium colombiense* gen. nov. sp. nov., an amino acid-degrading anaerobe isolated from anaerobic sludge. *Anaerobe* **4**, 241–250.

Baena, S., Fardeau, M. L., Ollivier, B., Labat, M., Thomas, P., Garcia, J. L. & Patel, B. K. (1999a). *Aminomonas paucivorans* gen. nov., sp. nov., a mesophilic, anaerobic, amino-acid-utilizing bacterium. *Int J Syst Bacteriol* **49**, 975–982.

Baena, S., Fardeau, M. L., Woo, T. H. S., Ollivier, B., Labat, M. & Patel, B. K. C. (1999b). Phylogenetic relationships of three amino-acid-utilizing anaerobes, *Selenomonas acidaminovorans*, '*Selenomonas acidaminophila*' and *Eubacterium acidaminophilum*, as inferred from partial 16S rDNA nucleotide sequences and proposal of *Thermanaerovibrio acidaminovorans* gen. nov., comb. nov. and *Anaeromusa acidaminophila* gen. nov., comb. nov. *Int J Syst Bacteriol* **49**, 969–974.

Baena, S., Fardeau, M.-L., Labat, M., Ollivier, B., Garcia, J.-L. & Patel, B. K. C. (2000). *Aminobacterium mobile* sp. nov., a new anaerobic amino-acid-degrading bacterium. *Int J Syst Evol Microbiol* **50**, 259–264.

Cashion, P., Holder-Franklin, M. A., McCully, J. & Franklin, M. (1977). A rapid method for the base ratio determination of bacterial DNA. *Anal Biochem* **81**, 461–466.

Chia, L.-J. & Wen, C.-K. (2002). Utilization of cooking juice young tuna processed into cannel tuna as condiments: effect of enzymatic hydrolysis and membrane treatment. *Fish Sci* **68**, 1344–1351.

Debroas, D. & Blanchart, G. (1993). Interactions between proteolytic and cellulolytic rumen bacteria during hydrolysis of plant cell wall protein. *Reprod Nutr Dev* **33**, 283–288.

Elsden, S. R. & Hilton, M. G. (1979). Amino acid utilization patterns in clostridial taxonomy. *Arch Microbiol* **123**, 137–141.

Fardeau, M.-L., Ollivier, B., Patel, B. K. C., Magot, M., Thomas, P., Rimbault, A., Rocchiccioli, F. S. & Garcia, J.-L. (1997). *Thermotoga hypogea* sp. nov., a xylanolytic, thermophilic bacterium from an oil-producing well. *Int J Syst Bacteriol* **47**, 1013–1019.

Fardeau, M.-L., Magot, M., Patel, B. K. C., Thomas, P., Garcia, J.-L. & Ollivier, B. (2000). *Thermoanaerobacter subterraneus* sp. nov., a novel thermophile isolated from oilfield water. *Int J Syst Evol Microbiol* **50**, 2141–2149.

Felsenstein, J. (1985). Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* **39**, 783–787.

Godon, J. J., Morinière, J., Moletta, M., Gaillac, M., Bru, V. & Delgènes, J. P. (2005). Rarity associated with specific ecological niches in the bacterial world: the '*Synergistes*' example. *Environ Microbiol* **7**, 213–224.

Honda, T., Fujita, T. & Tonouchi, A. (2013). *Aminivibrio pyruvatiphilus* gen. nov., sp. nov., an anaerobic, amino-acid-degrading bacterium from soil of a Japanese rice field. *Int J Syst Evol Microbiol* **63**, 3679–3686.

Hungate, R. E. (1969). A roll tube method for the cultivation of strict anaerobes. *Methods Microbiol* **3B**, 117–132.

Jumas-Bilak, E., Carlier, J. P., Jean-Pierre, H., Citron, D., Bernard, K., Damay, A., Gay, B., Teyssier, C., Campos, J. & Marchandin, H. (2007). *Jonquetella anthropi* gen. nov., sp. nov., the first member of the candidate phylum '*Synergistetes*' isolated from man. *Int J Syst Evol Microbiol* **57**, 2743–2748.

Jumas-Bilak, E., Roudière, L. & Marchandin, H. (2009). Description of '*Synergistetes*' phyl. nov. and emended description of the phylum '*Deferribacteres*' and of the family *Syntrophomonadaceae*, phylum '*Firmicutes*'. *Int J Syst Evol Microbiol* **59**, 1028–1035.

Kuykendall, L. D., Roy, M. A., O'Neill, J. J. & Devine, T. E. (1988). Fatty acids, antibiotic resistance, and desoxyribonucleic acid homology groups of *Bradyrhizobium japonicum*. *Int J Syst Bacteriol* **38**, 358–361.

Magot, M., Ravot, G., Campaignolle, X., Ollivier, B., Patel, B. K., Fardeau, M. L., Thomas, P., Crolet, J. L. & Garcia, J. L. (1997). *Dethiosulfovibrio peptidovorans* gen. nov., sp. nov., a new anaerobic, slightly halophilic, thiosulfate-reducing bacterium from corroding offshore oil wells. *Int J Syst Bacteriol* **47**, 818–824.

Maune, M. W. & Tanner, R. S. (2012). Description of *Anaerobaculum hydrogeniformans* sp. nov., an anaerobe that produces hydrogen from glucose, and emended description of the genus *Anaerobaculum*. *Int J Syst Evol Microbiol* **62**, 832–838.

Mead, G. C. (1971). The amino acid-fermenting clostridia. *J Gen Microbiol* **67**, 47–56.

Menes, R. J. & Muxí, L. (2002). *Anaerobaculum mobile* sp. nov., a novel anaerobic, moderately thermophilic, peptide-fermenting bacterium that uses crotonate as an electron acceptor, and emended description of the genus *Anaerobaculum*. *Int J Syst Evol Microbiol* **52**, 157–164.

Mesbah, M., Premachandran, U. & Whitman, W. B. (1989). Precise measurement of the G+C content of deoxyribonucleic acid by high-performance liquid chromatography. *Int J Syst Bacteriol* **39**, 159–167.

Miller, L. T. (1982). Single derivatization method for routine analysis of bacterial whole-cell fatty acid methyl esters, including hydroxy acids. *J Clin Microbiol* **16**, 584–586.

Pruesse, E., Peplies, J. & Glöckner, F. O. (2012). SINA: accurate high-throughput multiple sequence alignment of ribosomal RNA genes. *Bioinformatics* **28**, 1823–1829.

Rees, G. N., Patel, B. K., Grassia, G. S. & Sheehy, A. J. (1997). *Anaerobaculum thermoterrenum* gen. nov., sp. nov., a novel, thermophilic bacterium which ferments citrate. *Int J Syst Bacteriol* **47**, 150–154.

Saitou, N. & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406–425.

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M. & Kumar, S. (2011). MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol Biol Evol* **28**, 2731–2739.

Thabet, O. B., Fardeau, M.-L., Joulian, C., Thomas, P., Hamdi, M., Garcia, J.-L. & Ollivier, B. (2004). *Clostridium tunisiense* sp. nov., a new proteolytic, sulfur-reducing bacterium isolated from an olive mill wastewater contaminated by phosphogypse. *Anaerobe* **10**, 185–190.

Vartoukian, S. R., Palmer, R. M. & Wade, W. G. (2007). The division "*Synergistes*". *Anaerobe* **13**, 99–106.

Widdel, F. & Pfennig, N. (1982). Studies on dissimilatory sulfate-reducing bacteria that decompose fatty acids. II. Incomplete oxidation of propionate by *Desulfobulbus propionicus* gen. nov., sp. nov. *Arch Microbiol* **131**, 360–365.

Zavarzina, D. G., Zhilina, T. N., Tourova, T. P., Kuznetsov, B. B., Kostrikina, N. A. & Bonch-Osmolovskaya, E. A. (2000). *Thermanaerovibrio velox* sp. nov., a new anaerobic, thermophilic, organotrophic bacterium that reduces elemental sulfur, and emended description of the genus *Thermanaerovibrio*. *Int J Syst Evol Microbiol* **50**, 1287–1295.