International Journal of Systematic and Evolutionary Microbiology (2000), 50, 259–264 Printed in Great Britain

NOTE

Aminobacterium mobile sp. nov., a new anaerobic amino-acid-degrading bacterium

S. Baena,[1,2] M.-L. Fardeau,[1] M. Labat,[1] B. Ollivier,[1] J.-L. Garcia[1] and B. K. C. Patel[3]

Author for correspondence: B. K. C. Patel. Tel: +61 417 726671. Fax: +61 7 38757656. e-mail: bharat@genomes.sci.gu.edu.au

[1] Laboratoire ORSTOM de Microbiologie des Anaérobies, Université de Provence, CESB/ESIL Case 925, 163 Avenue de Luminy, 13288, Marseille Cedex 09, France

[2] Departamento de Biologia, Pontificia Universidad Javeriana, POB 56710, Santa Fe de Bogota, Colombia

[3] School of Biomolecular and Biomedical Sciences, Faculty of Science, Griffith University, Nathan, Brisbane 4111, Australia

A novel, curved (0·3 × 4·0–5·0 μm), Gram-negative, non-sporulating, mesophilic bacterium, designated strain ILE-3^T (T = type strain), was isolated from an anaerobic lagoon in a dairy wastewater treatment plant. Optimal growth occurred at 37 °C and pH 7·4 on a medium containing serine as an energy source and yeast extract. The strain was motile by means of one or two lateral flagella. It required yeast extract for growth on serine, glycine, threonine and pyruvate. Poor growth was obtained on cysteine, Casamino acids, biotrypcase, peptone and 2-oxoglutarate. In the presence of *Methanobacterium formicicum*, strain ILE-3^T oxidized alanine, glutamate, leucine, isoleucine, valine and aspartate to a minor extent. The G+C content of the DNA was 44 mol%. Phylogenetic analysis of the 16S rRNA gene of strain ILE-3^T indicated that it was related to *Aminobacterium colombiense* (95% similarity value). On the basis of the phenotypic and phylogenetic characteristics, strain ILE-3^T is designated as a new species of the genus *Aminobacterium*, namely *Aminobacterium mobile* sp. nov. (= DSM 12262^T).

Keywords: amino acid utilization, interspecies hydrogen transfer, *Methanobacterium formicicum*, *Aminobacterium mobile*

Saccharolytic amino-acid-degrading bacteria and the non-saccharolytic amino-acid-degrading bacteria play a significant role in protein turnover in the rumen (Smith & MacFarlane, 1997). The saccharolytic amino acid degraders have been rigorously studied in this ecosystem (Hobson & Wallace, 1982; Wallace, 1986). With the isolation of non-saccharolytic amino acid degraders (Chen & Russell, 1988, 1989; Paster *et al.*, 1993; Attwood *et al.*, 1998), much greater attention has now been paid to this bacterial community in terms of their diversity and their role in the rumen. In contrast, reports on the extent of microbial diversity of such microbes in other environments is scant (Stams & Hansen, 1984; Zindel *et al.*, 1988; Baena *et al.*, 1998a, 1999). As part of our investigations, we chose a protein-rich, anaerobic dairy wastewater lagoon to study diversity of non-saccharolytic amino-acid-degrading microbes and the role they play in protein turnover. We had previously reported the isolation and characterization of various obligate amino acid degraders from this environment, including a new species of sulfate-reducing bacteria (Baena *et al.*, 1998b) and two new genera, *Aminobacterium* and *Aminomonas* (Baena *et al.*, 1998a, 1999). In this report, we describe the isolation and characterization of a new member (strain ILE-3^T) of the genus *Aminobacterium*, which we have designated *Aminobacterium mobile* sp. nov.

The methods used for the preparation of media have been described previously (Baena *et al.*, 1998a). For initiating enrichments, a sludge sample collected from an anaerobic dairy wastewater treatment lagoon (Santa Fe de Bogota, Colombia) was serially diluted 10-fold, inoculated into a basal medium containing isoleucine (10 mM) and yeast extract (0·2%) and then incubated at 37 °C for up to 4 weeks. The same medium, fortified with agar (2%), was used to prepare roll tubes for the isolation of pure cultures. The methods used for bacterial characterization and co-culture studies with *Methanobacterium formicicum* (DSM 1535) have been described previously (Baena *et al.*, 1998a). Growth and product formation were analysed after 2 to 3 weeks of incubation at 37 °C. Light- and electron-microscopy methods were used as described by Fardeau *et al.* (1997a). Growth was measured by inserting growth tubes directly into a model UV-160A spectrophotometer (Shimadzu) and

The GenBank/EMBL/DDBJ accession number for the 16S rRNA sequence of strain ILE-3^T is AF073521.

measuring the optical density at 580 nm. The fermentation end products were determined as described by Fardeau *et al.* (1993). Amino acid concentrations were measured using HPLC (Moore *et al.*, 1958). The G+C content was determined by the DSMZ (Deutsche Sammlung von Mikroorganismen und Zellkulturen, Braunschweig, Germany) using the method of Mesbah *et al.* (1989).

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), organic acids (pyruvate, succinate, malate, fumarate, citrate, 2-oxoglutarate, lactate, acetate, propionate, butyrate) and sugars (glucose, sucrose, ribose, xylose, cellobiose, melibiose, maltose, galactose, mannose, arabinose, rhamnose, lactose, sorbose, 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), sodium fumarate (20 mM) and nitrate (10 mM). Amino acid degradation via the Stickland reaction was tested in a basal medium containing 10 mM alanine as electron donor and 20 mM glycine, 20 mM serine, 20 mM proline or 20 mM arginine as electron acceptor.

DNA extraction and amplification of the 16S rRNA gene have been described previously (Andrews & Patel, 1996; Baena *et al.*, 1998a). The new sequence data generated were aligned, checked for accuracy manually using the alignment editor, ae2 (Maidak *et al.*, 1996), and deposited in GenBank. The BLAST program (Altschul *et al.*, 1997) was used against the GenBank database to determine homology with recently released closely related sequences. For analysis, the sequences of *Anaerobaculum thermoterrenum*, *Dethiosulfovibrio peptidovorans* and *Aminobacterium colombiense* were extracted from GenBank (accession nos U50711, U52817 and AF069287, respectively) and manually aligned with the prealigned sequences obtained from the Ribosomal Database Project (Maidak *et al.*, 1996). Pairwise evolutionary distances based on 1151 unambiguous nucleotides were computed using the PHYLIP package (Felsenstein, 1993) and TREECON (Van de Peer & De Wachter, 1993).

Positive growth was observed in the 10^{-6} dilution tube after 4 weeks incubation at 37 °C in the basal medium containing isoleucine and yeast extract. 2-Methylbutyrate and acetate were detected as the major end products. Microscopic examination revealed the presence of two morphotypes, i.e. curved and straight rods. Subsequently, two distinct types of colonies developed in the highest serially diluted roll tube after 2 weeks incubation at 37 °C. One type of colony was rhizoid and the cells were spore-forming rods that grew on H_2+CO_2, producing acetate; they were tentatively identified as members of the genus *Clostridium* but were not studied further. The second type of colony was round, whitish and possessed smooth edges. Microscopic examination revealed the presence of curved cells in the latter colony; this culture was designated ILE-3^T and was characterized further.

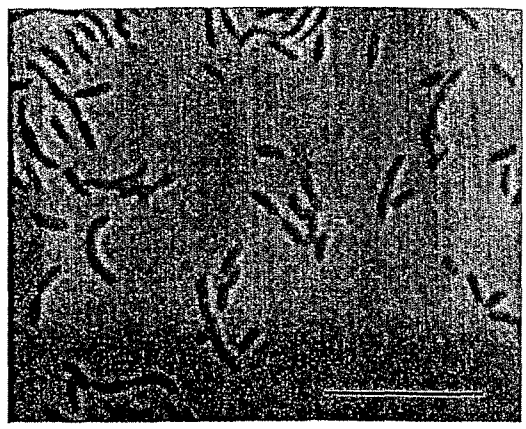

Fig. 1. Phase-contrast photomicrograph of cells, from exponential growth-phase culture of strain ILE-3^T, depicting their curved shape. Bar, 10 μm.

Although strain ILE-3^T was initially enriched on isoleucine and yeast extract, it grew poorly in this medium as a pure culture, suggesting that the hydrogenotrophic microbial partner (*Clostridium* sp.) in the initial enrichments was important for isoleucine degradation. Accordingly, co-culture experiments revealed that strain ILE-3^T grew on isoleucine only in the presence of a hydrogen scavenger, i.e. *M. formicicum* (see below). Therefore, we assume that the strain's ability to grow in initial enrichment cultures was due to the hydrogenotrophic, acetogenic *Clostridium* strain. Further studies revealed that strain ILE-3^T fermented serine (10 mM) in the presence of yeast extract (0·2%). These new culture conditions were used for growth-characterization studies.

Strain ILE-3^T cells were Gram-negative, non-sporulating, curved rods (Fig. 1) measuring 4·0–5·0 × 0·3 μm. Cells were motile by means of one or two lateral flagella. Ultra-thin sections of strain ILE-3^T revealed a multilayered, complex, Gram-negative cell wall. Strain ILE-3^T did not require NaCl for growth, grew optimally at a temperature of 37 °C (the temperature range for growth was 35–42 °C) and a pH 7·4 (the pH range for growth was 6·7–8·3).

Table 1 shows the substrates used by strain ILE-3^T and the end products. Strain ILE-3^T required yeast extract for growth. It fermented serine, glycine, threonine and pyruvate but growth was poor on Casamino acids, peptone, biotrypcase, cysteine and 2-oxoglutarate. The fermentation end products from these substrates included acetate and H_2. Propionate and acetate were the end products of 2-oxoglutarate fermentation. Acetate and alanine were produced from serine fermentation.

Table 1. Substrates used by strain ILE-3^T

Cysteine, Casamino acids, peptone and biotrypcase were poorly used. Arginine, proline, methionine, phenylalanine, histidine, asparagine, glutamine, lysine, tryptophan, gelatin, casein, succinate, malate, fumarate, citrate, lactate, sucrose, glucose, fructose, ribose, cellobiose, xylose, mannose, melibiose, maltose, galactose, lactose, arabinose, rhamnose, sorbose, mannitol, glycerol, ethanol, acetate, propionate and butyrate were not used. ND, Not determined.

Substrate	Products formed (mM):				ΔOD_{580}
	Acetate	Propionate	Alanine	H_2	
Fermented:*					
Serine	5·3	0·0	4·7	0·4	0·22
Glycine	6·0	0·0	0·0	0·0	0·18
Threonine	7·0	0·0	0·0	ND	0·15
Pyruvate	5·0	0·0	3·8	1·1	0·19
2-Oxoglutarate	1·2	1·1	0·0	ND	0·072

Oxidized in co-culture:†	Products formed (mM):					
	Acetate	Propionate	Isovalerate	2-Methyl-butyrate	Isobutyrate	CH_4
Alanine	8·5	0·0	0·0	0·0	0·0	2·3
Glutamate	2·0	4·0	0·0	0·0	0·0	1·3
Valine	0·8	0·0	0·0	0·0	8·4	1·5
Isoleucine	0·6	0·0	0·0	8·5	0·0	1·3
Leucine	0·8	0·0	4·5	0·0	0·0	1·4
Aspartate	5·5	0·0	0·0	0·0	0·0	1·3
2-Oxoglutarate	0·5	3·0	0·0	0·0	0·0	1·2

* Basal medium containing 0·2 % yeast extract was used. Results were obtained after 21 d incubation at 37 °C.

† Strain ILE-3^T was co-cultured with *Methanobacterium formicicum* in basal medium containing 0·2 % yeast extract. Results were obtained after 21 d incubation at 37 °C. Succinate, malate, fumarate and citrate were not used in co-culture.

Alanine, glutamate, valine, isoleucine, leucine and aspartate were oxidized only in the presence of the hydrogenotroph *M. formicicum*. The utilization of 2-oxoglutarate was also enhanced in the presence of *M. formicicum*. The end products resulting from the oxidation of alanine, leucine, isoleucine and valine were acetate, isobutyrate, 2-methyl-butyrate and isovalerate, respectively.

In co-culture, propionate was also produced from glutamate oxidation. In addition, the amounts of propionate produced from 2-oxoglutarate degradation were enhanced in the presence of a hydrogenotrophic partner.

Sulfate, thiosulfate, elemental sulfur, sulfite, nitrate and fumarate were not utilized as electron acceptors. Strain ILE-3^T did not perform the Stickland reaction when alanine was provided as an electron donor and glycine, serine, arginine or proline was provided as electron acceptor.

The G+C content of the DNA of strain ILE-3^T was determined as 44 mol %. Phylogenetic analysis of the almost complete 16S rRNA gene sequence (1539 nucleotides) corresponding to positions 18–1539 (*Escherichia coli* numbering according to Winker & Woese, 1991) indicated that it was a member of the low G+C, Gram-positive branch, with *Aminobacterium colombiense* its closest relative (95 % similarity value). This relationship was confidently predicted (100 %) by bootstrap analysis of 100 datasets (Fig. 2).

Asaccharolytic amino acid degraders have been isolated from different environments, such as estuary mud (*Acidaminobacter hydrogenoformans*; Stams & Hansen, 1984), black anaerobic mud (*Eubacterium acidaminophilum*; Zindel *et al.*, 1988), an anaerobic digester (*Selenomonas acidaminophila*; Nanninga *et al.*, 1987), the rumen (*Clostridium aminophilum*; Paster *et al.*, 1993), an oilfield (*Dethiosulfovibrio peptidovorans*; Magot *et al.*, 1997) and an anaerobic lagoon of dairy wastewater (*A. colombiense* and *Aminomonas paucivorans*; Baena *et al.*, 1998a, 1999). Phylogenetically, *A. colombiense* is the closest relative of strain ILE-3^T (similarities of 95 %). Similarities were observed since both species are Gram-negative, non-

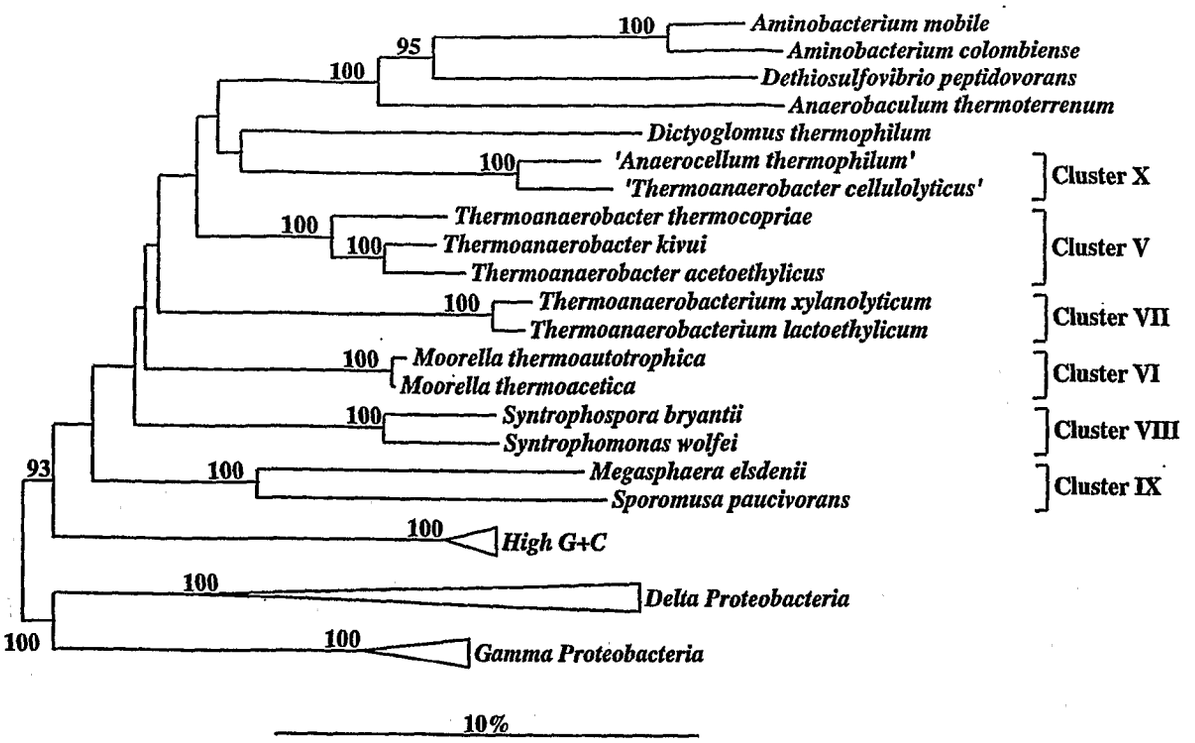

Fig. 2. Phylogenetic dendrogram, based on 16S rRNA sequence data, indicating the position of *Aminobacterium mobile* strain ILE-3^T within the radiation of representatives of the low G+C Gram-positive bacteria. All of the sequences used in the analysis, with the exception of *Anaerobaculum thermoterrenum*, *Dethiosulfovibrio peptidovorans* and *Aminobacterium colombiense* (GenBank accession nos U50711, U52817 and AF069287, respectively), were obtained from the Ribosomal Database Project, version 5.0 (Maidak *et al.*, 1996). Bootstrap values, expressed as a percentage of 100 replications, are shown at the branching points. Only values above 90% were considered significant and therefore reported. Bar, 10 nucleotide substitutions per 100 nucleotides.

Table 2. Utilization of serine by strain ILE-3^T in the presence and absence of *M. formicicum*

Results were recorded after 3 weeks incubation at 37 °C. The basal medium contained yeast extract (0·2%). Tubes containing basal medium with yeast extract (0·2%) and lacking substrates were used as controls. The final concentrations were determined by substracting values obtained from the control tubes (1·7 mM acetate) from those of utilized substrates. ND, Not determined.

Growth conditions	Serine used (mM)	Products formed (mM):					
		Acetate	Propionate	Alanine	H_2	CO_2	CH_4
Pure culture	10·0	5·3	0·0	4·7	0·4	5·2	0·0
Co-culture	7·4	8·3	0·4	0·0	0·0	ND	2·4

sporulating curved rods that use a limited number of amino acids (serine, glycine and threonine). In addition, their metabolic potential is similarly enhanced in co-culture with *M. formicicum* in comparison with pure cultures. Alanine, valine, leucine, isoleucine and aspartate are oxidized only in co-culture in both strains (Baena *et al.*, 1998a). However, strain ILE-3^T, unlike *A. colombiense*, has a slightly lower G+C DNA content (44% versus 46%), is motile and ferments serine to acetate and alanine (Table 2).

To our knowledge, this constitutes the first report on such metabolism during serine fermentation. In the case of *A. colombiense* and other aminolytic species, which include *Thermoanaerobacter brockii* (Fardeau *et al.*, 1997b), *Peptococcus prevotii* (reclassified as *Peptostreptococcus prevotii*) (Bentley & Dawes, 1974) and *Eubacterium acidaminophilum* (Zindel *et al.*, 1988), acetate and/or ethanol were the typical end products of serine fermentation.

L-Alanine production from amino acid fermentation has been rarely reported. *Caloramator proteoclasticus* (Tarlera *et al.*, 1997) was shown to produce this amino

acid during glutamate fermentation, whereas *Clostridium ultunense* (Schnürer *et al.*, 1996) produced it during cysteine degradation. L-Alanine production during saccharide fermentation has been studied in some members of the domains *Archaea* and *Bacteria* (Ravot *et al.*, 1996; Örlygsson *et al.*, 1995). In the case of *Pyrococcus furiosus* (Kengen & Stams, 1994), the electrons released from the oxidative decarboxylation of pyruvate are utilized to produce alanine and the involvement of an alanine amino-transferase has been demonstrated during this process. Because this archaeon is highly sensitive to pH_2, alanine production results in adjustment of its redox potential (Kengen & Stams, 1994). This metabolism is different from that observed in *Clostridium* strain P2 (Örlygsson *et al.*, 1995), as pyruvate utilization and alanine production are dependent upon the ammonium concentration in the growth medium. Strain ILE-3^T used pyruvate as an energy source and produced alanine and acetate, suggesting that this compound is an intermediate product of serine metabolism. Since co-culture of strain ILE-3^T with a hydrogenotrophic methanogen led only to the production of acetate and methane, we can conclude that L-alanine production from serine degradation is also sensitive to pH_2, as in *P. furiosus*. In this respect, the involvement of an alanine amino-transferase activity during serine fermentation can be now hypothesized for strain ILE-3^T.

We had previously reported the isolation and characterization of the non-saccharolytic amino acid degraders, *A. colombiense* and *A. paucivorans* from a dairy wastewater effluent sample (Baena *et al.*, 1998a, 1999). Strain ILE-3^T is the second isolate from this ecosystem to belong to the genus *Aminobacter* and is phylogenetically different from *A. colombiense*, the only species described within this genus. We are currently unsure if these bacteria dominate this ecosystem but our data clearly show that such microbes may play an important role in amino acid turnover in protein-rich dairy waste ecosystems, particularly in the presence of hydrogenotrophic partners. Further research on the degradation of amino acids and peptides should therefore take this into account.

On the basis of the phylogenetic and phenotypic traits, we propose to designate strain ILE-3^T as a new species of the genus *Aminobacterium*, namely *Aminobacterium mobile* sp. nov.

Description of *Aminobacterium mobile* sp. nov.

Aminobacterium mobile (mo'bi.le. L. masc. adj. *mobile* motile).

Slightly curved to rod-shaped bacterium 0·2–0·3 × 4·0–5·0 μm in size, occurring singly or (rarely) in chains. Gram-negative, non-spore-forming. Strictly anaerobic. Colonies (up to 1·0 mm) are round, smooth, lens-shaped and white. Mesophilic. The optimal growth temperature is 37 °C and growth occurs in the temperature range 20–42 °C. The pH range for growth is 6·6–8·5, with an optimum at pH 7·3. Strain ILE-3^T does not require NaCl for growth but tolerates up to 1·5% NaCl, with optimum growth occurring at 0·05–0·5% NaCl. Yeast extract required for growth. Ferments serine, threonine, glycine and pyruvate and uses alanine, glutamate, valine, leucine, isoleucine and aspartate only in co-culture with *Methanobacterium formicicum*. No growth observed on succinate, malate, fumarate, citrate, lactate, glucose, sucrose, ribose, xylose, cellobiose, melibiose, maltose, galactose, mannose, arabinose, rhamnose, lactose, sorbose, mannitol, acetate, propionate, butyrate, glycerol, ethanol, gelatin or casein. The G+C content of the DNA is 44 mol%. The type strain is ILE-3^T (= DSM 12262). Isolated from anaerobic sludge of a dairy wastewater treatment plant in Santa Fe de Bogota, Colombia.

Acknowledgements

We thank N. Zylber (BIP-CNRS) for amino acid analysis, and P. Thomas for electron microscopy. This work was supported by grants from the French Foreign Office (P.C.P), the Instituto Colombiano para el desarrollo de la Ciencia y la Tecnologia (COLCIENCIAS) (to S.B.) and, in part, the Australian Research Council (to B.K.C.P.)

References

Altschul, S. F., Madden, T. L., Schaffer, 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.

Andrews, K. T. & Patel, B. K. C. (1996). *Fervidobacterium gondwanense* sp. nov., a new thermophilic anaerobic bacterium isolated from nonvolcanically heated geothermal waters of the Great Artesian Basin of Australia. *Int J Syst Bacteriol* **46**, 265–269.

Attwood, G. R., Klieve, A., Ouwerkerk, D. & Patel, B. K. C. (1998). Ammonia-hyperproducing bacteria from New Zealand ruminants. *Appl Environ Microbiol* **64**, 1796–1804.

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

Baena, S., Fardeau, M.-L., Labat, M., Ollivier, B., Thomas, P., Garcia, J.-L. & Patel, B. K. C. (1998b). *Desulfovibrio aminophilus* sp. nov., a novel amino acid degrading and sulfate reducing bacterium from an anaerobic dairy wastewater lagoon. *Syst Appl Microbiol* **21**, 498–504.

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

Bentley, C. M. & Dawes, E. A. (1974). The energy-yielding reactions of *Peptococcus prevotii*, their behaviour on starvation and the role and regulation of threonine dehydratase. *Arch Microbiol* **100**, 363–387.

Chen, G. & Russell, J. M. (1988). Fermentation of peptides and amino acids by a monensin-sensitive ruminal *Peptostreptococcus*. *Appl Environ Microbiol* **54**, 2742–2749.

Chen, G. & Russell, J. M. (1989). More monensin-sensitive, ammonia-producing bacteria from the rumen. *Appl Environ Microbiol* **55**, 1052–1057.

Fardeau, M.-L., Cayol, J. L., Magot, M. & Ollivier, B. (1993). H_2 oxidation in the presence of thiosulfate by a *Thermoanaerobacter* strain isolated from a oil-producing well. *FEMS Microbiol Lett* **13**, 327–332.

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

Fardeau, M.-L., Patel, B. K. C., Magot, M. & Ollivier, B. (1997b). Utilization of serine, leucine, isoleucine, and valine by *Thermoanaerobacter brockii* in the presence of thiosulfate or *Methanobacterium* sp. as electron acceptors. *Anaerobe* **3**, 405–410.

Felsenstein, J. (1993). PHYLIP (Phylogenetic Inference Package) version 3.51c. Department of Genetics, University of Washington, Seattle, WA, USA.

Hobson, P. N. & Wallace, R. (1982). Microbial ecology and activities in the rumen. *CRC Crit Rev Microbiol* **9**, 253–320.

Kengen, S. W. M. & Stams, A. J. M. (1994). Formation of L-alanine as a reduced end product in carbohydrate fermentation by the hyperthermophilic archaeon *Pyrococcus furiosus*. *Arch Microbiol* **161**, 168–175.

Magot, M., Ravot, G., Campaignolle, X., Ollivier, B., Patel, B. K. C., 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.

Maidak, B. L., Olsen, G. J., Larsen, N., Overbeek, R., McCaughey, M. J. & Woese, C. R. (1996). The ribosomal database project (RDP). *Nucleic Acids Res* **24**, 82–85.

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.

Moore, S., Spackman, D. H. & Stein, W. H. (1958). Chromatography of amino acids on sulfonated polystyrene resins: an improved system. *Anal Chem* **30**, 1158–1190.

Nanninga, H. J., Drent, W. J. & Gottschal, J. C. (1987). Fermentation of glutamate by *Selenomonas acidaminophila* sp. nov. *Arch Microbiol* **147**, 152–157.

Örlygsson, J., Anderson, R. & Svensson, B. H. (1995). Alanine as end product during fermentation of monosaccharides by *Clostridium* strain P2. *Antonie Leeuwenhoek* **68**, 273–280.

Paster, B. J., Russell, J. M. J., Yang, C. M., Chow, J. M., Woese, C. R. & Tanner, R. (1993). Phylogeny of the ammonia-producing ruminal bacteria *Peptostreptococcus anaerobius, Clostridium sticklandii*, and *Clostridium aminophilum* sp. nov. *Int J Syst Bacteriol* **43**, 107–110.

Ravot, G., Ollivier, B., Fardeau, M.-L., Patel, B. K. C., Andrews, K., Magot, M. & Garcia, J.-L. (1996). L-Alanine production from glucose fermentation by hyperthermophilic members of the domains Bacteria and Archaea: a remnant of an ancestral metabolism? *Appl Environ Microbiol* **62**, 2657–2659.

Schnürer, A., Schink, B. & Svensson, B. H. (1996). *Clostridium ultunense* sp. nov., a mesophilic bacterium oxidizing acetate in syntrophic association with a hydrogenotrophic methanogenic bacterium. *Int J Syst Bacteriol* **46**, 1145–1152.

Smith, E. A. & MacFarlane, G. T. (1997). Dissimilatory amino acid metabolism in human colonic bacteria. *Anaerobe* **3**, 327–337.

Stams, A. J. M. & Hansen, T. A. (1984). Fermentation of glutamate and other compounds by *Acidaminobacter hydrogenoformans* gen. nov. sp. nov., an obligate anaerobe isolated from black mud. Studies with pure cultures and cocultures with sulfate-reducing and methanogenic bacteria. *Arch Microbiol* **137**, 329–337.

Tarlera, L., Muxi, L., Soubes, M. & Stams, A. J. M. (1997). *Caloramator proteoclasticus* sp. nov., a new moderately thermophilic anaerobic proteolytic bacterium. *Int J Syst Bacteriol* **47**, 651–656.

Van de Peer, Y. & De Wachter, R. (1993). TREECON: a software package for the construction and drawing of evolutionary trees. *CABIOS* **9**, 177–182.

Wallace, R. (1986). Catabolism of amino acids by *Megasphaera elsdenii* LC1. *Appl Environ Microbiol* **51**, 1141–1143.

Winker, S. & Woese, C. R. (1991). A definition of the domain *Archaea, Bacteria* and *Eucarya* in terms of small subunit ribosomal RNA characteristics. *Syst Appl Microbiol* **13**, 161–165.

Zindel, U., Freudenberg, W., Rieth, M., Andreesen, J. R., Schnell, J. & Widdel, F. (1988). *Eubacterium acidaminophilum* sp. nov., a versatile amino acid-degrading anaerobe producing or utilizing H_2 or formate. *Arch Microbiol* **150**, 254–266.