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Polyphasic taxonomic approach in the description of *Alishewanella fetalis* gen. nov., sp. nov., isolated from a human foetus

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A taxonomically unique bacterium is described on the basis of a physiological and biochemical characterization, fatty acid profiling and sequence analyses of 16S rRNA and gyrase B (*gyrB*) genes. This non-motile, non-fermentative bacterium was isolated from a human foetus in Uppsala, Sweden, and originally misidentified as a *Shewanella putrefaciens* by conventional biochemical testing. The bacterium grew well at mesophilic temperatures with optimum growth at 37 °C. It was facultatively anaerobic and utilized various electron acceptors (trimethylamine oxide, nitrate, nitrite and thiosulphate). The dominant fatty acids were 17:1B, 16:1 *cis*9, 17:0 and 16:0. Fatty acids 13:0 iso and 15:0 iso, which have been found to be typical of *Shewanella* species were not detected. The G+C content of the DNA was 50·6 mol%. Phylogenetic analysis of the 16S rRNA gene sequence revealed a clear affiliation with members of the γ subclass of the *Proteobacteria*. No relationship was seen with any of the established genera in the γ subclass of the *Proteobacteria*, although a distinct relationship with *Vibrionaceae* was observed. That the bacterium represents a novel bacterial genus distinct from *Vibrionaceae* was also supported by *gyrB* sequence analysis. Considering the source and close proximity to the genus *Shewanella*, the name *Alishewanella fetalis* gen. nov., sp. nov. is proposed, for which the type strain is strain CCUG 30811^T.

Keywords: *Alishewanella fetalis* gen. nov., sp. nov., polyphasic taxonomy, 16S rRNA, DNA gyrase, human isolate

INTRODUCTION

From an autopsy of a human foetus an unidentified strain was isolated by U. B. Stolt, Akademiska Sjukhuset, Uppsala, Sweden, on 24 November 1992. It was sent with the original strain designation Uppsala R2422019 to the Culture Collection of University of Göteborg and included in the collection as CCUG 30811^T. The strain was not identified but labelled as *Shewanella putrefaciens* due to the presence of several fatty acids typical of *S. putrefaciens*. We included this strain in a study of *S. putrefaciens* phylogeny (Fonnesbech Vogel *et al.*, 1997) and realized that CCUG 30811^T is a unique, taxonomically interesting, strain.

To our knowledge *Shewanella* strains have not been isolated from a human foetus, but strains identified as *S. putrefaciens* have been isolated from premature infants (Brink *et al.*, 1995; Pedersen *et al.*, 1970). Most human clinical isolates formerly identified as *S. putrefaciens* do belong to a separate species, *Shewanella algae* (Nozue *et al.*, 1992; Fonnesbech Vogel *et al.*, 1997). Clinical strains of *S. algae* have only been directly linked to cases of bacteraemia (Dominguez *et al.*, 1996) and ear infections (Holt *et al.*, 1997). However, strains identified as *S. putrefaciens* (formerly *Pseudomonas putrefaciens*) have sporadically been isolated from a number of clinical specimens (Gilardi, 1972; Brink *et al.*, 1995; Kim *et al.*, 1989). Probably most of these infections have been due to *S.*

Abbreviation: TMAO, trimethylamine oxide.

The GenBank accession numbers for the 16S rRNA gene and the partial *gyrB* sequences of CCUG 30811^T are AF144407 and AF205593.

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algae (Fonnesbech Vogel *et al*., 1997; Khashe & Janda, 1998) and as CCUG 30811^T reacted similarly to the marine *S. algae* in a number of biochemical tests, we suspected a close relationship. We have not been able to isolate strains like CCUG 30811^T or to obtain similar strains from other researchers or any culture collections.

Correct identification is crucial when evaluating the role of a potential human pathogen and when describing routes of infection. This is difficult when an organism is found only rarely and it is important that such organisms are carefully described. The purpose of this study is to evaluate the taxonomic position of strain CCUG 30811^T. As it was received as *S. putrefaciens*, careful attention was paid to its relationship with *S. putrefaciens* and *S. algae*.

METHODS

Bacterial strains and growth conditions. The unidentified strain, CCUG 30811^T, the type strain of *S. algae* (IAM 14159^T; Simidu *et al*., 1990), and the type strain of *S. putrefaciens* (ATCC 8071^T; Derby & Hammer, 1931) were grown in veal infusion broth (VIB; Difco), brain-heart infusion broth (BHI; Oxoid), on Long & Hammer's medium (Van Spreekens, 1974), and on iron agar (Gram *et al*., 1987). Strains of *S. putrefaciens* and *S. algae* were grown at 25 and 37 °C, respectively. Strain CCUG 30811^T was grown at 37 °C. All strains were stored frozen at −80 °C in broth with 4% (v/v) glycerol and 2% (w/v) dried skimmed milk (Gibson & Khoury, 1986).

Physiological and biochemical tests. All strains were tested for a number of key characteristics as described (Fonnesbech Vogel *et al*., 1997). Briefly, all strains were tested for Gram reaction (Gregersen, 1978), motility, cell shape, cytochrome oxidase (Kovacs, 1956), catalase reaction, reduction of trimethylamine oxide (TMAO), production of hydrogen sulphide and fermentation of glucose in the O–F medium (Merck) of Hugh & Leifson (1953). Their ability to grow at 4, 32 and 42 °C, as well as their tolerance to 6, 8 or 10% NaCl were tested. Strains were tested for haemolysis of sheep blood, growth on *Salmonella–Shigella* agar (Merck catalogue no. 7667) and acid production from D-ribose, D-glucose, L-arabinose and maltose as described previously (Fonnesbech Vogel *et al*., 1997). Strains were also examined using the API 20NE identification system (bioMérieux). Antibiotic resistance was determined on Long & Hammer agar by the disk diffusion method (Casals & Pringler, 1991) with Neo-sensitabs (Rosco A/S). Plates were incubated for 24 h and susceptibility was defined as a clearing zone with diameter larger than 15 mm (tablet diameter 14 mm) around the antibiotic tablets. The antibiotics tested were: 62·5 μg penicillin, 2·5 μg ampicillin, 30 μg amoxycillin, 115 μg carbenicillin and 10 μg tetracycline. Reduction of TMAO, nitrate, ferric iron, thiosulphate, sulphite and nitrite was determined as visible growth after 7 d at 25 °C for *S. putrefaciens*, or at 37 °C for *S. algae* and CCUG 30811^T, under anaerobic conditions in a liquid Long & Hammer's medium with final concentrations of 10 mM of various electron acceptors.

Determination of G+C content. The G+C content of DNA of the strains was determined using HPLC analysis of hydrolysed DNA according to (Fonnesbech Vogel *et al*., 1997; Mesbah *et al*., 1989).

Electron microscopy. The bacteria were cultivated over night at 37 °C on BHI-agar. A colony was scraped off the plate and the bacteria were resuspended in 50 μl sterile PBS (pH 7·4). A 5 μl drop of bacterial suspension was placed on top of a carbon-coated, glow-discharged 400 mesh nickel grid. After 2 min adsorption, the grid was washed with two drops of PBS and counter-stained with three drops of ammonium molybdate (1%, w/v, pH 7·0), and blotted dry. Electron microscopy was done as described earlier (Christiansen & Birkelund, 1998).

Cellular fatty acid profiles. The strains were grown on blood agar at 30 °C for 2 to 4 d. Harvesting, saponification, methylation, extraction, washing as well as GC conditions (column 25 m × 0·2 mm × 0·33 μm HP-5 cross-linked 5% Ph ME silicone), Hewlett Packard gas chromatograph (HP 5890A), autosampler (HP 7673) and integrator were identical to the MIDI system (MIDI). Integration and correction of the area percentage were done according to the Hewlett Packard standard.

DNA amplification and sequencing. Amplification of 16S rRNA, strand separation and purification of PCR fragment were performed as described previously (Fonnesbech Vogel *et al*., 1997). Sequencing was performed by the dideoxy chain-termination method (Sanger *et al*., 1977) using an Autoread Sequencing kit (Pharmacia Biotech) set up with 10 ng fluorescein-labelled primer using 3 U T7 DNA polymerase (Pharmacia Biotech) and 1–2 μg DNA template. Sequence reactions were analysed on the ALF Automatic DNA Sequencer (Pharmacia Biotech).

Phylogenetic analysis based on 16S rRNA gene sequence. Sequences were aligned manually to the *Escherichia coli* 16S rRNA gene sequence (Brosius *et al*., 1978) including the consensus sequence given in Lane (1991). Published sequences of *Shewanella hanedai* (Gauthier *et al*., 1995), *S. putrefaciens* (Gauthier *et al*., 1995), *S. putrefaciens* (Rossello-Mora *et al*., 1994), *Shewanella benthica* (Gauthier *et al*., 1995), *S. algae* (Rossello-Mora *et al*., 1994) and 33 other sequences of *γ Proteobacteria* found related to or selected as representatives of groups related to strain CCUG 30811^T were included in the phylogenetic analysis. Maximum-likelihood analysis was performed by fastDNAml including bootstrap analysis (Felsenstein, 1981; Olsen *et al*., 1994).

***gyrB* gene.** Primers (UP-1 and UP-2r) within the known DNA sequence (Yamamoto & Harayama, 1995) were added to the PCR mixture at a concentration of 1 μM, and the solution subjected to 30 cycles of PCR (Venkateswaran *et al*., 1998). Amplified *gyrB* fragment (1·2 kb) from strain CCUG 30811^T was sequenced directly following purification on Qiagen columns. The identity of a given PCR product was verified by sequencing using the dideoxy chain termination method with Sequenase DNA sequencing kit (USB) and with an ABI 373A automatic sequencer as recommended by the manufacturer (Perkin-Elmer Applied Biosystems). Phylogenetic analysis was performed as described before (Venkateswaran *et al*., 1999).

RESULTS AND DISCUSSION

Physiological and biochemical characteristics

Strain CCUG 30811^T is a Gram-negative, non-motile rod. Electron microscopy revealed a rod-shaped bacterium of approximately 2 μm in length and 0·5–1·0 μm in width (Fig. 1). Membrane vesicles were evident on several cells. No flagellum (corresponding to the non-

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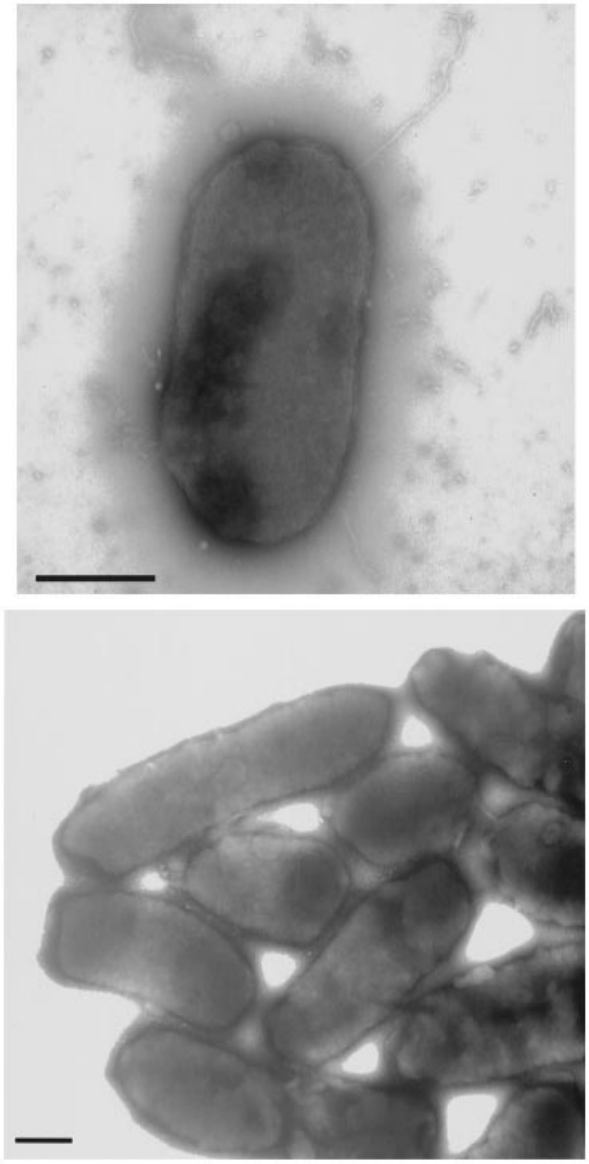

Fig. 1. Morphological characteristics of strain CCUG 30811^T as visualized by transmission electron microscopy. Bar, 0·5 μm. Cells were grown for 24 h at 37 °C on BHI agar plates.

motile phenotype) was seen but elongated structures that resemble fimbriae were occasionally observed. Some preparations showed capsular material around the cell envelope (data not shown).

Strain CCUG 30811^T had positive oxidase and catalase reactions and was unable to ferment glucose. It reduced nitrate and hydrolysed gelatin and aesculin, but was unable to produce indole and hydrolyse urease, *β*-galactosidase and arginine dihydrolase (Table 1). By these results, strain CCUG 30811^T is similar to strains of *S. putrefaciens* and *S. algae*. Strain CCUG 30811^T was unable to grow in the TMAO medium (Gram *et al.*, 1987). Strain CCUG 30811^T did not produce H_2S in iron agar or triple-sugar agar. It failed to grow at 4 °C in VIB, but showed slow and good growth at 32 and 41 °C, respectively. In general, when the tests were performed at 37 °C the results were the same as those observed for *S. algae* strains except for the inability to produce acid oxidatively from D-ribose and to grow in 10% NaCl. Strain CCUG 30811^T reduced TMAO, nitrate, thiosulphate and nitrite but not sulphite and ferric iron under anaerobic conditions.

Being a Gram-negative, non-fermentative, halotolerant, rod-shaped bacterium, with ability to use various electron acceptors in an anaerobic respiration (Stenstrøm & Molin, 1990), strain CCUG 30811^T resembles members of the genus *Shewanella*. Although several biochemical reactions match strain CCUG 30811^T with members of the genus *Shewanella* and the members of the family *Vibrionaceae*, no firm biochemical characteristic pattern was noted to classify strain CCUG 30811^T in any of the established species of these groups.

The G+C content of CCUG 30811^T was 50·6 mol% compared to 42–47 and 52–55 mol% for *S. putrefaciens* and *S. algae*, respectively. In general, the range observed for the DNA G+C content is not more than 3 mol% within a well-defined species (Stackebrandt & Liesack, 1994).

Cellular fatty acid profiles

The dominant fatty acids in strain CCUG 30811^T were 17:1B (19·5%) and 16:1 *cis*9 (19·0%). These acids also occurred in high levels in the two *Shewanella* species (Table 2). Fatty acids 17:0 and 16:0 were present at levels of 5–10% in strain CCUG 30811^T and were also detected in *S. putrefaciens* and *S. algae*. Fatty acids 15:0 iso and 13:0 iso were characteristic of the two *Shewanella* strains (CCUG 13452 and CCUG 39064) with levels of 12·2 and 7·8% (*S. putrefaciens*) and 26·8 and 5·9% (*S. algae*). Several studies have identified 15:0 iso and 13:0 iso as characteristic fatty acids of *Shewanella* (formerly some *Pseudomonas*) species (Wilkinson & Caudwell, 1980; Nichols *et al.*, 1992; Khashe & Janda, 1998) and the lack of these fatty acids clearly places strain CCUG 30811^T in a different group. Likewise, fatty acid 18:1 *cis*11/*trans*9/*trans*6 accounted for 7·2% of the fatty acids in strain CCUG 30811^T, but was only detected at a level of 1·4–1·8% in the *Shewanella* species.

Phylogenetic analysis based on 16S rRNA gene sequence

The 16S rRNA gene sequence of CCUG 30811^T was determined for the region 16–1509 (*E. coli* numbering). The phylogenetic comparison was based on the most related sequences as determined by FASTA search in GenBank and EMBL (Wisconsin Sequence Analysis Package, Genetics Computer Group, Madison, WI, USA). Representative 16S rRNA gene sequences for species of the most related genera of *Vibrio*, *Shewanella*, *Aeromonas*, *Alteromonas*, *Photobacterium*, *Pseudomonas*, *Pseudoalteromonas* and species from the

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Table 1. Phenotypic characteristics of CCUG 30811^T and the type strains of *Shewanella putrefaciens* and *Shewanella algae*

Symbols are: +, positive; −, negative; ( ), weak reaction; NG, no growth.

Reaction	Medium/method	*S. putrefaciens* ATCC 8071^T	*S. algae* IAM 14159^T	Strain CCUG 30811^T
Gram staining	Gram staining	−	−	−
Gram-string test	3 % KOH (Gregersen, 1978)	+*	+	+
Shape	Phase-contrast microscopy	Rod	Rod	Rod
Motility	Phase-contrast microscopy	+	+	−
Cytochrome oxidase	Tetramethyl-*p*-phenyl diamine hydrochloride (Kovacs, 1956)	+	+	+
Catalase	3 % H_2O_2	+	+	+
G+C mol %	HPLC	44·7 ± 0·1	53·3 ± 0·2	50·6 ± 0·2
H_2S-production	Iron agar (Gram *et al.*, 1987)	+	+	−
	Triple-sugar agar	+	+	−
Hydrolysis of DNA	DNase test agar	+	+	NG
Production of acid from:				
D-Glucose	Hugh & Leifson (1953) medium	−	−	−
Maltose	Hugh & Leifson (1953) medium	−	−	−
D-Ribose	Hugh & Leifson (1953) medium	−	+	−
L-Arabinose	Hugh & Leifson (1953) medium	+	−	−
Growth at:				
4 °C	Veal infusion broth	+	−	−
32 °C	Veal infusion broth	(+)	+	(+)
41 °C	Veal infusion broth	−	+	+
25 °C	Brain-heart infusion broth	+	+	(+)
30 °C	Brain-heart infusion broth	+	+	+
37 °C	Brain-heart infusion broth	−	+	+
Growth in:				
6 % NaCl	Veal infusion broth	−	+	+
8 % NaCl	Veal infusion broth	−	+	+
10 % NaCl	Veal infusion broth	−	+	−
Growth on *Salmonella–Shigella* agar	*Salmonella–Shigella* agar	−	+	+
Haemolysis of bovine blood	Bovine blood agar	−	+	−
Resistance to:				
Penicillin (62·5 μg)	Long & Hammer medium (Van Spreekens, 1974)	−	(+)	(+)
Ampicillin (2·5 μg)	Long & Hammer medium (Van Spreekens, 1974)	−	(+)	(+)
Amoxicillin (30 μg)	Long & Hammer medium (Van Spreekens, 1974)	−	(+)	(+)
Carbenicillin (115 μg)	Long & Hammer medium (Van Spreekens, 1974)	−	−	−
Tetracycline (10 μg)	Long & Hammer medium (Van Spreekens, 1974)	−	−	−
Reduction of:				
TMAO	TMAO medium (Gram *et al.*, 1987)	+	+	NG
	Long & Hammer broth (Van Spreekens, 1974)	+	+	+
Thiosulphate	Long & Hammer broth (Van Spreekens, 1974)	+	+	+
Sulphite	Long & Hammer broth (Van Spreekens, 1974)	+	+	−
Nitrite	Long & Hammer broth (Van Spreekens, 1974)	+	+	+
Nitrate	API 20 NE	+	+	+
Ferric iron	Long & Hammer broth (Van Spreekens, 1974)	+	+	−
Indole production	API 20NE	−	−	−
Arginine dihydrolase	API 20NE	−	−	−

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Table 1 (*cont.*)

Reaction	Medium/method	*S. putrefaciens* ATCC 8071^T	*S. algae* IAM 14159^T	Strain CCUG 30811^T
Hydrolysis of:				
Urea	API 20NE	−	−	−
Aesculin	API 20NE	+	+	+
Gelatin	API 20NE	+	+	+
β-Galactosidase	API 20NE	−	−	−
Assimilation of:				
Glucose	API 20NE	−	(+)	−
Arabinose	API 20NE	−	−	−
Mannose	API 20NE	−	−	−
Mannitol	API 20NE	−	−	−
N-Acetylglucosamine	API 20NE	+	+	−
Maltose	API 20NE	−	−	(−)
Gluconate	API 20NE	−	−	−
Caprate	API 20NE	+	+	−
Adipate	API 20NE	−	−	−
Malate	API 20NE	+	+	−
Citrate	API 20NE	−	−	−
Phenyl acetate	API 20NE	−	−	−

* Solubilization of the cell envelope (of Gram-negative bacteria) and formation of DNA string.

family *Enterobacteriaceae* were selected. The region 34–1459 (*E. coli* numbering) was aligned for 39 sequences leaving 1409 positions for phylogenetic analysis. The highest similarity, 95% was observed between CCUG 30811^T and the two uncharacterized strains OS 140 and HTB 010 (Takami *et al.*, 1999). Similarity between CCUG 30811^T and the closely related taxa of *Aeromonas*, *Erwinia*, *Shewanella*, *Klebsiella*, *Photobacterium*, *Vibrio* and *Shigella* was 88–91% but similarities of 83 and 85% were found to *Pseudomonas nitroreducens* and *Pseudomonas aeruginosa*, respectively.

The phylogenetic analysis based on maximum-likelihood is shown in Fig. 2. A relatively close relationship to strains OS 140, HTB 010, HTB 019 (Takami *et al.*, 1999) and HTB 021 (Takami *et al.*, 1999) is recognized with a more distinct relationship to representatives of the genera *Shewanella*, *Vibrio*, *Photobacterium* and *Aeromonas*, and to the family *Enterobacteriaceae*. Alignment of strain CCUG 30811^T with the closest related 16S rRNA gene sequences identified a hypervariable region in the beginning of the sequence, between 10 and 27 bases shorter than in the other bacteria, and shows the unique phylogenetic position of strain CCUG 30811^T (Table 3).

gyrB sequence analysis

The 1·2 kb nucleotide sequence of the *gyrB* gene covering base positions 274–1525 (*E. coli* numbering; accession no. AF205593), was determined for strain CCUG 30811^T. The relative phylogenetic positions occupied by the representatives of the families *Vibrionaceae* and *Enterobacteriaceae*, based on *gyrB* nucleotide sequences are given. An unrooted tree was generated by the maximum-likelihood method (Fig. 3). The results indicate that *S. putrefaciens*, *S. algae* and *Shewanella amazonensis* form separate clusters. The members of the family *Enterobacteriaceae* form a distinct clade from strain CCUG 30811^T. Although the *gyrB* sequence of strain CCUG 30811^T falls within the cluster of members of the family *Vibrionaceae*, the similarity percentage was less than 71·5% between strain CCUG 30811^T and members of this family.

The use of *gyrB* as a measure of bacterial relatedness has been established in the genera *Pseudomonas*, *Acinetobacter* (Yamamoto & Harayama, 1995, 1996), *Vibrio* (Venkateswaran *et al.*, 1998), *Bacillus* (Yamada *et al.*, 1999) and *Shewanella* (Venkateswaran *et al.*, 1999). The similarities of *gyrB* sequences between well-established genera were not greater than 75% (70–72% for *Shewanella* and *Aeromonas*, 72–74% for *Shewanella* and *Pseudoalteromonas*). The nucleotide sequence search performed with the sequences deposited in GenBank and other public databases showed that the *gyrB* sequence of CCUG 30811^T had a low similarity compared with any of the available sequences. When comparing *gyrB* nucleotide sequences between CCUG 30811^T and all known shewanellae, the percentage of nucleotide substitutions varied from 28·5 to 33·3%. The closest match was with *S. putrefaciens* (71·5%). The ten closest species had a

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Table 2. Percentage cellular fatty acid composition of strain CCUG 30811^T and related *Shewanella* species

Fatty acid	*S. putrefaciens* ATCC 8071^T	*S. algae* IAM 14159^T	CCUG 30811^T
10:0	–	–	0·5
11:0	0·4	0·4	1·5
11:0 3-OH	0·7	0·7	2·5
12:0	3·8	1·4	0·8
12:0 3-OH	2·1	1·1	2·0
13:0 iso	7·8	5·9	–
13:0	2·5	1·8	0·6
13:0 iso 2-OH	–	–	0·4
13:0 iso 3-OH	1·3	2·8	–
13:0 3-OH	2·3	2·1	2·7
14:0 iso	0·5	1·2	0·5
14:0	1·4	0·7	0·9
14:0 iso 3-OH	–	–	0·3
14:0 3-OH	1·2	0·6	1·9
15:0 iso	12·2	26·8	–
15:0 anteiso	–	0·3	0·3
15:0	9·1	9·0	6·7
15:1 A	1·2	0·6	4·9
15:1 B	0·7	0·7	0·9
15:0 iso 3-OH	0·2	0·6	–
16:0 iso	–	0·5	1·8
16:0	8·3	4·6	8·9
16:1 B	1·4	0·8	–
16:1 *cis*9	15·1	7·9	19·0
17:0 iso	0·7	1·3	0·4
17:0 anteiso	–	0·3	0·9
17:0	2·4	3·2	10·3
17:1 B	16·9	18·1	19·5
17:1 C	1·2	1·4	1·4
18:9 *cis* 9	1·6	1·7	–
18:1 *cis*11/*trans*9/*trans*6	1·8	1·4	7·2
18:2 *cis*9,12/18:0 A	1·1	0·8	0·3
18:0	0·3	0·2	–
Not identified	1·9	1·0	3·2

similarity value between 65 and 71·5 % with the CCUG 30811^T strain. These genera included: *Shewanella* (69–71·5 %), *Pseudoalteromonas* (71 %), *Citrobacter* (71 %), *Vibrio* (67–70 %), *Alteromonas* (70 %), *Pseudomonas* (69 %), *Aeromonas* (69 %) and *Acinetobacter* (65–67 %). Even though no concrete information is available on genus cut-off values based on *gyrB* sequences, the similarities of less than 75 % between any of the established genera and strain CCUG 30811^T suggested that the described strain in this study represents a new genus.

Concluding remarks

It has been recommended by Vandamme *et al.* (1996) that new genera containing only a single strain to represent a new species should be validly named only if their phenotype and genotype have been thoroughly and adequately characterized. The polyphasic approach taken in this study to characterize the taxonomic position of strain CCUG 30811^T allowed us to describe a new genus. Although the 16S rRNA gene sequences of the undescribed strains OS 140, HTB 010, HTB 019 and HTB 021 showed the closest match with our strain, phenotypic data were not available for these strains to allow further comparison. No biochemical characteristic patterns were noted to classify strain CCUG 30811^T in any of the established genera of the phylogenetic neighbours (Table 4). The genera *Photobacterium*, *Vibrio* and *Aeromonas* differed from strain CCUG 30811^T by being motile, producing acid from D-glucose, being arginine dihydrolase-positive and able to grow at 20 °C. Characteristics that distinguish *Shewanella* species from strain CCUG 30811^T were the ability to produce H_2S and growth at 20 °C or below. Strain CCUG 30811^T was distinguished

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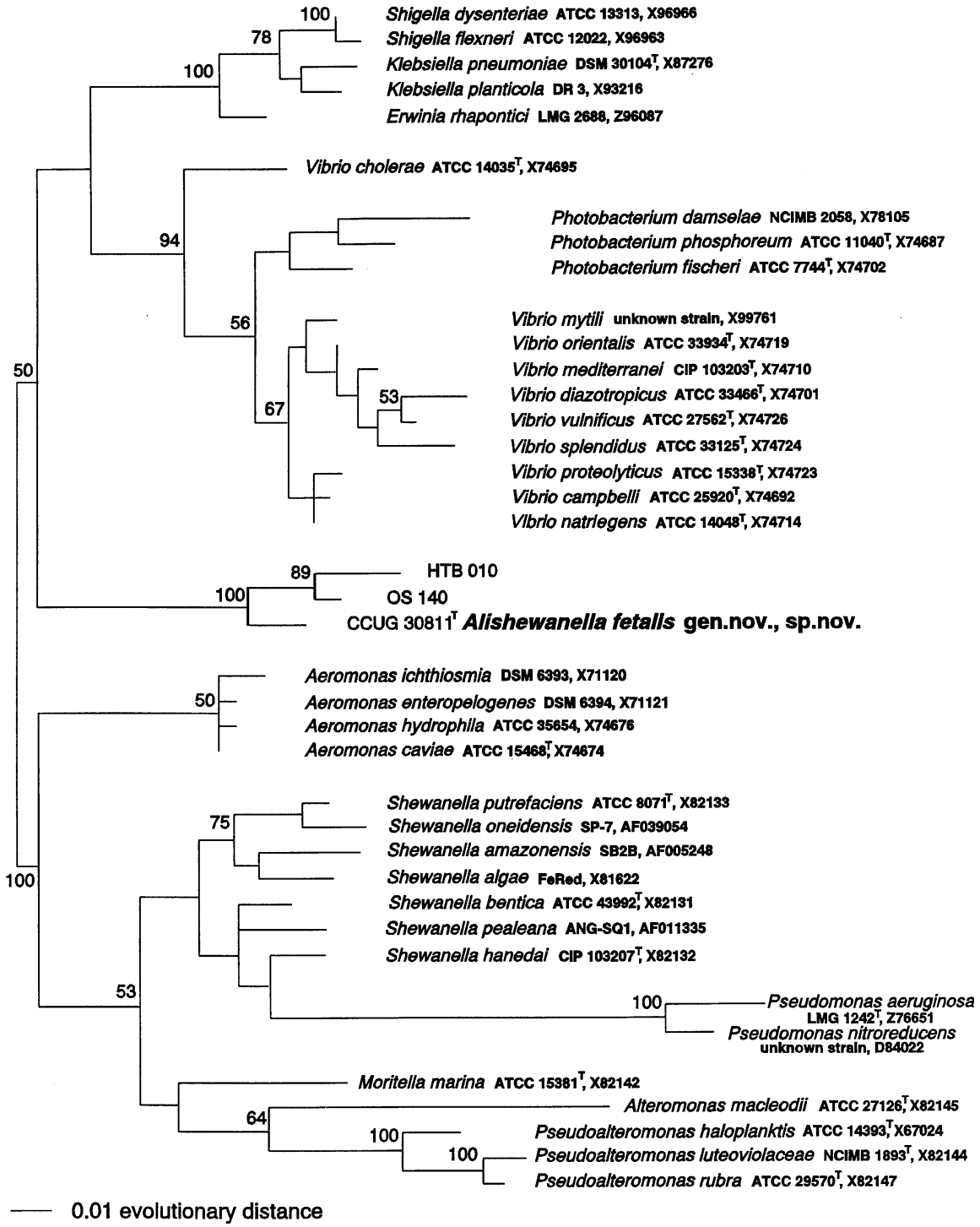

Fig. 2. Phylogenetic tree of strain CCUG 30811^T and closely related genera based on 16S rRNA sequence comparison by maximum-likelihood analysis. The significances for particular nodes were obtained by bootstrap analysis. Nodes without numbers are of low significance (< 50 %).

from the members *Erwinia*, *Klebsiella*, *Shigella* of the family *Enterobacteriaceae* by being oxidase-positive.

This paper described detailed characteristics of biochemical, fatty acid and sequence analyses of 16S rRNA and *gyrB* genes of strain CCUG 30811^T. Furthermore, as strain CCUG 30811^T exhibited a low level of similarity with any of the established species [91 % 16S rRNA (*Aeromonas*, *Erwinia*, *Shewanella*, *Klebsiella*, *Photobacterium*, *Vibrio* and *Shigella*) and

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Table 3. Alignment of *Alishewanella fetalis*, gen. nov., sp. nov., strain CCUG 30811^T, for the hypervariable region 66 to 103 (*E. coli* numberings) with representative bacteria to which high similarity was observed in 16S rRNA sequence comparison

Dots indicate identical nucleotides to the *E. coli* sequence, and gaps are shown by hyphens.

Strain	*E. coli* numbering	
	66	103
Escherichia coli K-12	ACGGTAACAGG-AAGAAGCTTGCTTCTTTGCTGA	----------CGAGT
Vibrio parahaemolyticus ATCC 17802^T	G...A...----G..TTATC..AAC...C.GG..ACGATAACGGCGT	C
Aeromonas hydrophila ATCC 35654	G...C.G.G..A...T.....A...	----------TTGCCGG....C
Vibrio cholerae ATCC 14035^T	G...C.G.-ACAG..G.A....-...C.	----------TGGGTGG....C
Pseudomonas aeruginosa LMG 1242^T	G...ATG----A.G.G.....-C..C.	----------GGATT---C..C
HTB010, HTB019, HTB020	G..AATG----ARG.G.....-C.-CY	----------GGATT---T..C
OS140	G..AATG----AGG.T.....-C.ACC	----------TGATT---T..C
CCUG 30811^T	G..A---------G.T--T..C-GGACC	----------.T..C

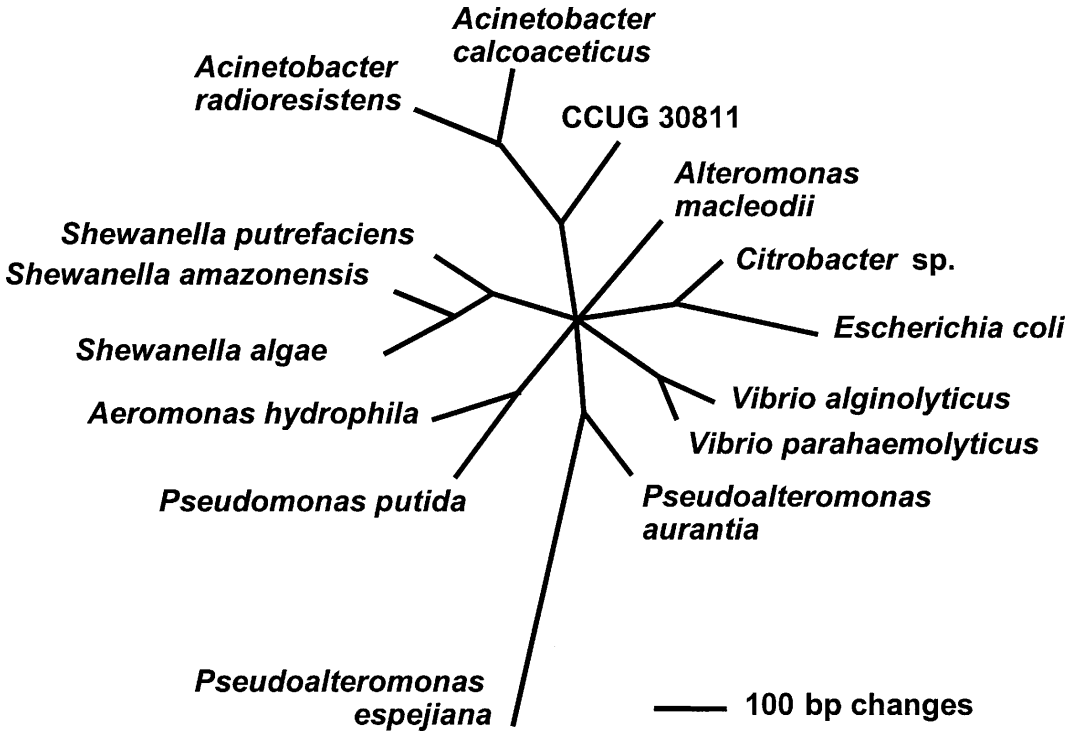

Fig. 3. Phylogenetic tree of strain CCUG 30811^T and closely related genera based on *gyrB* sequences comparison performed by maximum-likelihood analysis. 500 bootstrap analyses were performed. Accession numbers: *Alteromonas macleodii*, AF074917; *Citrobacter* sp., AF005699; *E. coli*, AF000417; *Vibrio alginolyticus*, AF007288; *Vibrio parahaemolyticus*, AF007287; *Pseudoalteromonas aurantia*, AF007275; *Pseudoalteromonas espejiana*, AF007278; *Pseudomonas putida*, D37923; *Aeromonas hydrophila*, AF074917; *S. algae*, AF005686; *S. amazonensis*, AF005257; *S. putrefaciens*, AF005669; *Acinetobacter radioresistens*, AB008732; *Acinetobacter calcoaceticus*, D73427; and CCUG 30811^T, AF144407.

71·5% *gyrB* (*S. putrefaciens*)], a new genus status is warranted for this strain. As strain CCUG 30811^T cannot be assigned to any known bacterial genera, we propose that it be designated *Alishewanella fetalis* gen. nov., sp. nov.

Description of *Alishewanella* gen. nov.

Alishewanella (A.li.she.wa.nel′la. L. pronoun. *alius* the other; M.L. fem. n. *Alishewanella* the other *Shewanella*).

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Table 4. Characteristics of the phylogenetic neighbours of strain CCUG 30811^T

Unless otherwise indicated in footnotes, the meanings of symbols are: typically positive (+), typically negative (−) and differs among different species of the genus (d). Modified from *Bergey's Manual of Determinative Bacteriology* (Holt *et al.*, 1994), *Bergey's Manual of Systematic Bacteriology* (Krieg *et al.*, 1984) and Venkateswaran *et al.* (1999).

Taxon	CCUG 30811^T	*Shewanella*	*Photobacterium*	*Vibrio*	*Aeromonas*	*Erwinia*	*Klebsiella*	*Shigella*
Motility	−	+	+	+	+	+	−	−
Oxidase	+	+	d	+	+	−	−	−
Production of:								
Acid from D-glucose	−	−	+	+	+	+	+	+
H_2S	−	+	−	−	d	d	−	−
Arginine dihydrolase	−	−	+	d	+	−	−	−
Gelatinase	+	+	−*	d	+	−	−	−
Na^+ required for or stimulates growth	+	+	+	+	−	†	†	†
Growth at 20 °C	−	+‡	+	+	+	†	†	†
Optimum growth temp. (°C)	37	d	18–25	d	22–28	27–30	37	37
G+C content (mol %)	51	39–55	40–44	38–51	57–63	50–58	53–58	49–53

* Positive in 80 % of *Photobacterium angustum* strains.

† Data not available.

‡ Except *S. benthica*.

Cells are Gram-negative, non-motile rods. Facultatively anaerobic and uses TMAO, nitrate, thiosulphate and nitrite, but not sulphite or ferric iron, as electron acceptors. Oxidase- and catalase-positive. Halotolerant and requires NaCl for growth. Glucose is not catabolized with production of acid. Hydrolyses gelatin and aesculin but is unable to produce indole, urease, β-galactosidase, arginine dihydrolase and H_2S. Does not grow at temperatures of 20 °C or less. The DNA G+C content is 51 mol %. The type species is *Alishewanella fetalis*.

Description of *Alishewanella fetalis* sp. nov.

Alishewanella fetalis (fe.ta′lis. L. adj. *fetalis* pertaining to the foetus, from which the organism was isolated).

Cells are Gram-negative, non-motile rods with a length of 2 µm and a width of 0·5–1·0 µm. They occur typically as single cell. Growth occurs between 25 and 42 °C with an optimum at 37 °C. Halotolerant, requires NaCl for growth and withstand NaCl concentration up to 8 % but does not grow at 10 % NaCl. Oxidase- and catalase-positive and unable to ferment carbohydrates (glucose, maltose, ribose and arabinose). Hydrolyses gelatin and aesculin but is unable to produce indole, urease, β-galactosidase and arginine dihydrolase. Facultatively anaerobic and uses TMAO, nitrate, thiosulphate and nitrite but not sulphite or ferric iron, as electron acceptors. The DNA G+C content is 51 mol %. Isolated at an autopsy of a human foetus in 1992. The type strain is CCUG 30811^T, deposited in the Culture Collection, University of Gothenborg, Sweden.

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