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Campylobacter hominis sp. nov., from the human gastrointestinal tract

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Sequences of 16S rDNA of a novel campylobacter from faeces of healthy humans were previously shown to originate from a new taxon, '*Candidatus* Campylobacter hominis', which could not be cultured. Since phylogenetic analysis suggested that anaerobic conditions might be required for growth, an isolation strategy was developed employing initial non-selective membrane filtration onto fastidious anaerobe agar. Campylobacters were then isolated from the resulting mixed microbial flora by a dilution strategy and/or by immunomagnetic separation with genus-specific polyclonal antibody. Isolates were identified by a genus and taxon-specific PCR assay, and 16S rDNA nucleotide sequence analysis was carried out. All isolates exhibited the typical *Campylobacter* characteristics of being non-fermentative, oxidase-positive, catalase-negative and Gram-negative. Unusually, however, they were straight rods lacking flagella. The 16S rDNA nucleotide sequence analysis, DNA and mol% G+C were consistent with a new *Campylobacter* species whose nearest phylogenetic neighbours were *Campylobacter gracilis* and *Campylobacter sputorum*. The unique species status of the isolates was further confirmed by taxonomic analysis of 47 phenotypic characteristics. The name *Campylobacter hominis* sp. nov. is proposed for the new species, the type strain of which is NCTC 13146^T (= LMG 19568^T).

Keywords: *Campylobacter*, phylogenetic study, human enteric isolates

INTRODUCTION

The taxonomy of the genus *Campylobacter* has undergone extensive revision and expansion in the last decade. *Campylobacter* is assigned to rRNA superfamily VI, which consists of the family *Campylobacteraceae* (containing *Campylobacter* and *Arcobacter*), the genera *Sulfurospirillum*, *Helicobacter*, *Wolinella* and *Thiovulum*, and the generically misnamed *Bacteroides ureolyticus* (Vandamme *et al*., 1991, 1995; Vandamme & Goossens, 1992). Campylobacters can be isolated from a variety of environmental samples, but their normal habitat is considered to be the gastrointestinal tract of birds, animals and man. The pathogenic species are associated with infections of the intestinal and genital tracts and the periodontal crevice. The most noteworthy of these infections is *Campylobacter* enteritis, due to *Campylobacter jejuni*, the most common cause of bacterial gastroenteritis worldwide (Skirrow, 1994).

We previously described the detection and analysis of partial 16S rDNA sequences, obtained by genus-specific PCR from human faecal material of individuals without clinical illness (Lawson *et al*., 1998). We identified the source of these amplicons as a new taxon, '*Candidatus* Campylobacter hominis'. We were unable to isolate the organism from which the 16S rDNA sequences originated.

The present study describes the subsequent isolation and characterization of this bacterium, and its full description as *Campylobacter hominis* sp. nov.

Abbreviation: IMS, immunomagnetic separation.

The EMBL accession number for the sequence reported in this paper is AJ251584 (NCTC 13146^T).

METHODS

Reference strains and culture conditions. Reference strains of *Campylobacter*, *Arcobacter*, *Helicobacter* and *Escherichia coli* used in this study are listed in Table 1. All strains were cultured on 5% blood agar (BA; Oxoid) at 37 °C. Micro-

Table 1 Bacterial strains and/or 16S rRNA sequences

Bacteria	Source	Location	Strain designation*	Sequence accession no.†
C. hominis sp. nov	Human, faeces	London, UK	CH001A (NCTC 13146^T)	AJ251584
	Human, faeces	London, UK	CH001B	
	Human, faeces	London, UK	CH001C	
	Human, faeces	London, UK	CH002	
	Human, faeces	London, UK	CH003	
'*Candidatus* C. hominis'‡	Human, faeces	London, UK	HS-A	AF062490
	Human, faeces	London, UK	HS-B	AF062491
	Human, faeces	London, UK	HS-C	AF062492
C. coli	Porcine, faeces	Brussels, Belgium	NCTC 11366^T	L04312
	Porcine, intestine	Melbourne, Australia	CCUG 33450	
C. concisus	Human, gingival sulcus	Boston, USA	NCTC 11485^T	L04322
	Human, gingival pocket	Boston, USA	NCTC 11486	
	Human, gingival sulcus	Boston, USA	NCTC 11487	
	Human, blood	UK	CCUG 10376	
	Human, antral biopsy	Göteborg, Sweden	CCUG 14496	
	Human, infant diarrhoea	Oroboro, Sweden	CCUG 17580	
	Human, diarrhoea	Oroboro, Sweden	CCUG 18688	
	Human, oesophageal biopsy	Perth, Australia	CCUG 19219	
	Human, diarrhoea	Oroboro, Sweden	CCUG 19393	
	Human, duodenal aspirate	Ottawa, Canada	CCUG 19505	
	Human, faeces	Oroboro, Sweden	CCUG 19995	
	Human, diarrhoea	Oroboro, Sweden	CCUG 19996	
	Human, faeces	Oroboro, Sweden	CCUG 20034	
	Human, faeces	Preston, UK	CCUG 20699	
	Human, diarrhoea	Preston, UK	CCUG 20700	
C. curvus	Human, alveolar abscess	Virginia, USA	NCTC 11649^T	L04313
	Human, apical periodontitis	Umeå, Sweden	CCUG 11644	
	Human, periodontitis	Boston, USA	FDC 521	
	Human, septicaemia	Wisconsin, USA	FDC 640	
	Human	USA	ATCC 29543	
Campylobacter fetus subsp. *fetus*	Ovine, fetus brain	Paris, France	NCTC 10842^T	M65012
C. fetus subsp. *venerealis*	Bovine, vaginal mucus	Reading, UK	NCTC 10354^T	
C. gracilis	Human, periodontitis	Boston, USA	NCTC 12738^T	L04320
	Human, periodontitis	Boston, USA	CCUG 13143	
	Human, periodontitis	Boston, USA	CCUG 22762	
	Human, gingivitis	Boston, USA	FDC EM38	
	Human, gingivitis	Boston, USA	FDC EF19	
	Human, periodontitis	Boston, USA	FDC 406	
	Human, periodontitis	Boston, USA	FDC 1084	
Campylobacter helveticus	Feline, faeces	Berne, Switzerland	NCTC 12470^T	U03022
Campylobacter hyointestinalis subsp. *hyointestinalis*	Porcine, intestine	Minnesota, USA	NCTC 11608^T	M65010
C. hyointestinalis subsp. *lawsonii*	Porcine, stomach	UK	NCTC 12901^T	
C. jejuni subsp. *jejuni*	Bovine, faeces	Brussels, Belgium	NCTC 11351^T	L04315
C. jejuni subsp. *doylei*	Human, infantile diarrhoea	Adelaide, Australia	NCTC 11951^T	
Campylobacter lari	Herring gull, cloacal swab	UK	NCTC 11352^T	L04316
	River water	UK	NCTC 11845	
Campylobacter mucosalis	Porcine, intestine	Edinburgh, UK	NCTC 11000^T	L06978
	Porcine, intestine	Edinburgh, UK	NCTC 11001	
	Porcine, necrotic colitis	Lothian, UK	NCTC 11418	

Table 1 (*cont.*)

Bacteria	Source	Location	Strain designation*	Sequence accession no.†
	Porcine, adenomatous colon	Lothian, UK	NCTC 11419	
	Porcine, haemorrrhagic enteritis	Lothian, UK	NCTC 11420	
	Porcine, intestine	UK	CCUG 23201	
	Porcine, intestine	UK	CCUG 23202	
	Porcine, intestine	UK	CCUG 23203	
	Unknown	Brussels, Belgium	CCUG 23204	
	Porcine	Denmark	CCUG 24188	
C. rectus	Human, periodontal pocket	Boston, USA	NCTC 11489^T	L04317
	Human, apical periodontitis	Umeå, Sweden	CCUG 11640	
	Human, apical periodontitis	Umeå, Sweden	CCUG 11642	
	Human, apical periodontitis	Umeå, Sweden	CCUG 11643	
	Human, apical periodontitis	Umeå, Sweden	CCUG 11645	
C. showae	Human, gingival crevice	Showa, Japan	NCTC 12843^T	L06974
	Human, apical periodontitis	Umeå, Sweden	NCTC 12843	
C. sputorum bv. *faecalis*	Ovine, faeces	Los Angeles, USA	NCTC 11415^T	
	Ovine, faeces	Canada	CCUG 12015	
C. sputorum bv. *paraureolyticus*	Bovine, faeces	UK	BU 86C	
C. sputorum bv. *sputorum*	Human, oral cavity	USA	NCTC 11528^T	X67775
	Human, pus	Belgium	LMG 14261	
Campylobacter upsaliensis	Canine, faeces	Malmo, Sweden	NCTC 11541^T	L14628
[*Bacteroides*] *ureolyticus*§	Human, amniotic fluid	Edmonton, Canada	NCTC 10941^T	L04321
	Human, vagina	Newcastle, UK	NCTC 10948	
	Human, vagina	Newcastle, UK	NCTC 10949	
	Human, urethra	Harrow, UK	NCTC 12014	
	Human, urethra	Harrow, UK	NCTC 12015	
	Human, perianal lesion	Sheffield, UK	NCTC 12016	
	Human, penile wound	Göteborg, Sweden	CCUG 9510D	
	Human, penile wound	Göteborg, Sweden	CCUG 9596	
	Human, urine	Göteborg, Sweden	CCUG 18470	
	Human	Göteborg, Sweden	CCUG EF-11762	
Arcobacter skirrowii	Ovine	Göteborg, Sweden	NCTC 12713^T	L16625
Helicobacter pylori	Human	Australia	NCTC 11637^T	M88157
Escherichia coli	Human	Denmark	NCTC 9001^T	J01695

* NCTC, National Collection of Type Cultures; CCUG, Culture Collection of the University of Göteborg; FDC, Forsyth Dental Centre; ATCC, American Type Culture Collection; BU, University of Berne; LMG, Laboratorium voor Microbiology en Microbielle Genetica. ^{T}, Type strain.

† 16S rRNA sequences for these strains are available for electronic retrieval from EMBL under the indicated accession numbers. Through cross-distribution of databases, these sequences should also be available from the GenBank and DDBJ databases.

‡ 16S rDNA sequence only (Lawson *et al.*, 1998).

§ Species *incertae sedis* which is genotypically *Campylobacter* (Vandamme *et al.*, 1995).

aerophilic species were incubated in an atmosphere of 5% O_2, 5% CO_2, 2% H_2 and 88% N_2 (by volume). Anaerobic species were incubated in an atmosphere of 5% CO_2, 5% H_2 and 90% N_2.

Faecal samples. Fresh faecal samples were collected from 18 human subjects with no current or recent gastrointestinal symptoms. They were diluted 1:10 in brucella broth (BB; Life Technologies) and vortexed briefly to produce a

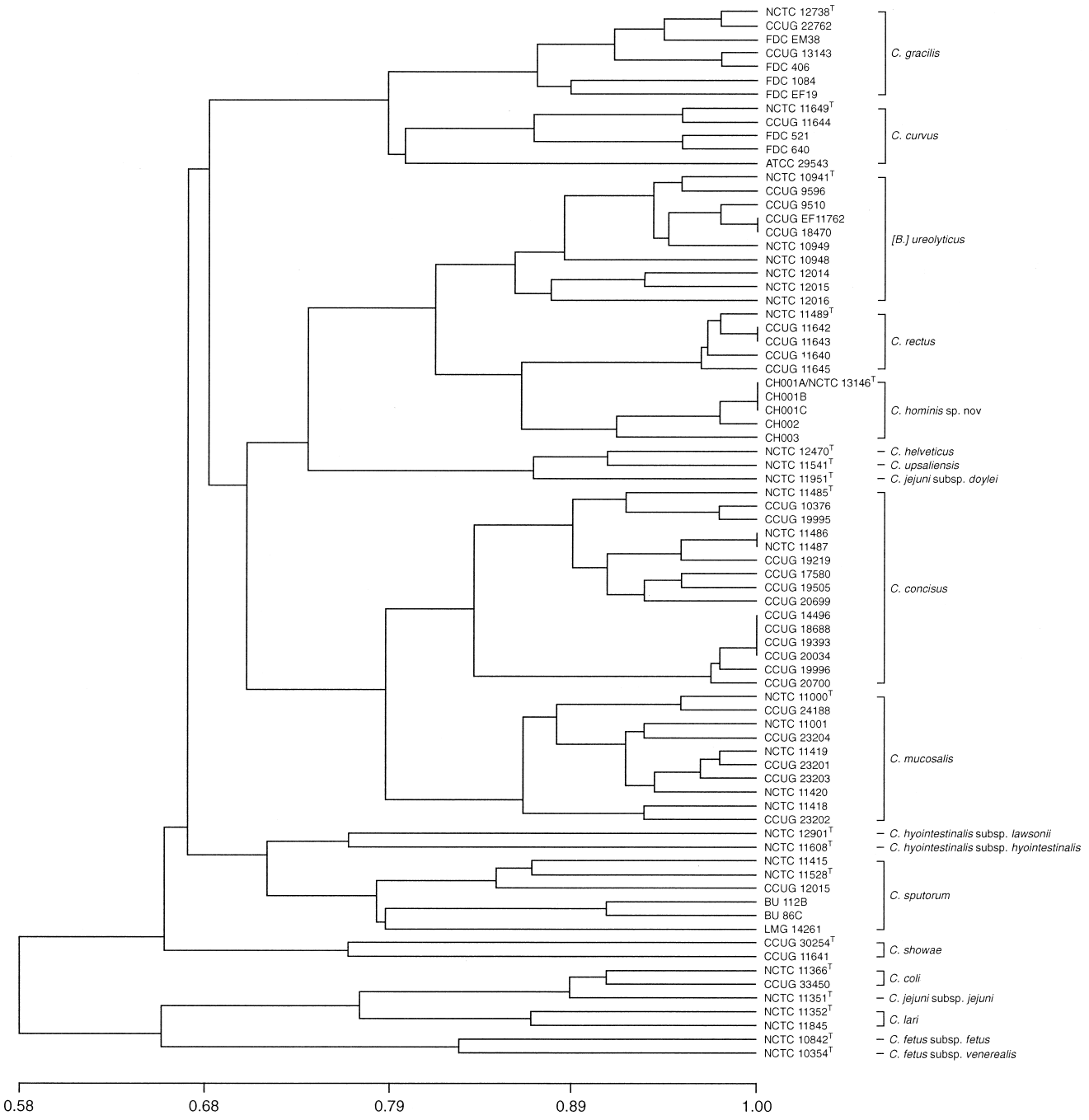

Fig. 1. Dendrogram of cluster analysis based on 47 phenotypic characteristics of *Campylobacter* strains. Strain numbers on the vertical axis correspond with those in Table 1. The numbers on the horizontal axis indicate the percentage similarities as determined by the simple matching coefficient and unweighted pair group average linkage clustering.

homogeneous suspension. Large particulate matter was allowed to settle out (10 min) and the supernatant was used for culture and PCR (see below).

PCR assay. The faecal supernatants were examined by a PCR assay specific for '*Candidatus* C. hominis' (Lawson *et al.*, 1998) and a *Campylobacter* genus-specific assay (Linton *et al.*, 1996). A subset of DNA samples extracted from diarrhoeic faecal samples in a previous study (Lawson *et al.*, 1999) were also examined. Among these samples, 114 were positive for *C. jejuni* by culture and *C. jejuni*-specific PCRs (Linton *et al.*, 1997; Metherell *et al.*, 1999), while 95 contained no enteropathogenic *Campylobacter* species by either culture or PCR.

Isolation procedures. Faecal samples were examined for *Campylobacter* species by culture on modified charcoal cefoperazone deoxycholate agar (CCDA; Oxoid) and by the membrane filter method (Bolton *et al.*, 1988; Steele & McDermott, 1984). Cellulose acetate membrane filters of

0·65 μm pore size (Sartorius) were applied to BA or fastidious anaerobe agar (FAA; Lab M). Plates were incubated for up to 21 d at 37 °C under either microaerobic or anaerobic conditions (as above) and examined at regular intervals. Mixtures of bacterial growth or campylobacter-like colonies were screened by PCR assays for the genus *Campylobacter* and the *Candidatus* status taxon.

Where a mixed growth was '*Candidatus* C. hominis' PCR-positive, a pure culture of campylobacter-like colonies was obtained by either dilution or immunomagnetic separation (IMS) (see below). In the former case, a colony sweep using a 10 μl loop was resuspended in 1 ml BB and diluted 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4} in this medium. The 10^{-3} and 10^{-4} dilutions were inoculated on FAA and incubated anaerobically for 10–20 d.

Immunomagnetic separation (IMS). Two commercially prepared antibodies specific for the genus *Campylobacter* were employed. These were an anti-flagella mouse monoclonal (Chemicon International) and a goat polyclonal antibody specific for cell wall components (Kirkegard and Perry Labs). For IMS of '*Candidatus* C. hominis', a bacterial suspension was made in 50 μl BB and mixed with 100 μl of an antibody preparation pre-diluted to a working concentration of 20 μg ml^{-1}. This was incubated at 40 °C with occasional mixing for 30 min. Cells were pelleted by centrifugation (13 000 ***g*** for 1 min). The supernatant was discarded and the pellet washed three times with 1 ml BB. The pellet was resuspended in 900 μl BB and mixed with 100 μl of a 5 mg ml^{-1} suspension of BioMag, protein-G-coated, magnetic beads (PerSeptive Diagnostics). This was incubated at room temperature for 15 min with gentle mixing. Beads were recovered with a magnetic particle concentrator, and washed three times in BB. The bound bacterial cells were then plated out and incubated anaerobically (see Results). The positive and negative controls were *C. jejuni* NCTC 11351^T and *E. coli* NCTC 9001^T.

Phenotypic characterization and numerical analysis. Isolates were initially characterized by oxidase, catalase, Gram stain and hanging drop motility as previously described (Barrow & Feltham, 1993). A more extensive analysis of the phenotypic characteristics of the isolates and control strains was undertaken using 47 phenotypic tests with media and methodologies as recommended previously (On & Holmes, 1991a, b, 1992). All tests were performed on two separate occasions using freshly prepared media whose quality was assured by appropriate control strains. Numerical analysis of the phenotypic data was performed as previously described (On & Holmes, 1995). Briefly, the simple matching coefficient was used to calculate the similarity between strains and a dendrogram which reflected these levels of similarity was constructed. Strains were clustered by the unweighted pair group with the mathematical average (UPGMA) method.

Electron microscopy. Cells were resuspended in 1% (v/v) formalin solution. A Formvar-coated grid was placed on a drop of the bacterial suspension for 2 min, and transferred to a drop of 2% (w/v) ammonium molybdate solution for a further 2 min. Grids were dried at the end of each step by touching their edge to filter paper. Grids were examined at ×13 500 magnification in a Phillips EM420 electron microscope at 80 kV.

Sequencing of 16S rDNA and phylogenetic analysis. *Campylobacter* genus-specific 16S rDNA amplified from DNA extracted from one of the putative *C. hominis* isolates (strain CH001A) was sequenced as previously described (Stanley *et al.*, 1993) using an ABI PRISM dye terminator cycle sequencing kit (Perkin Elmer). Sequences were aligned by the CLUSTAL method with the three 16S rDNA sequences of '*Candidatus* C. hominis' (Lawson *et al.*, 1998) and species type strain reference sequences obtained from the EMBL database (Table 1), using Megalign from the Lasergene suite of programs (DNAstar). The data were used to create a phylogenetic tree (Fig. 2) using the neighbour-joining method (Saitou & Nei, 1987) as implemented in the TREECON package (van de Peer & de Wachter, 1993). Ambiguous bases were removed and the remaining 1375 bases were analysed using the method of Jukes & Cantor (1969). Bootstrap analysis was performed with 1000 resampled data sets.

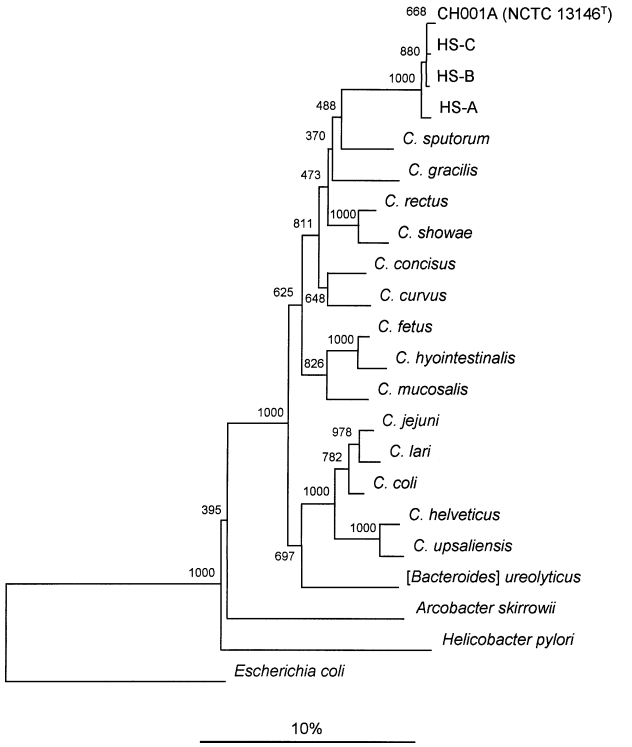

Fig. 2. Phylogenetic tree of the 16S rDNA sequences of *Campylobacter* species determined by neighbour-joining analysis. The sequence of *E. coli* was used as the outgroup. The strain numbers and sequence accession numbers are given in Table 1.

Nucleic acid techniques. Genomic DNA was prepared from representative species as previously described (Stanley *et al.*, 1992). DNA base composition (mol% G+C content) was estimated from the thermal denaturation temperature (Owen & Pitcher, 1985). DNA–DNA slot-blot hybridization was performed on genomic DNA isolated from the species type strains listed in Table 1, using DNA of CH001A (see below) as the probe. The method was as previously described (Stanley *et al.*, 1992), except that a Digoxigenin (DIG) High Prime labelling and detection kit was used (Roche) and hybridization was performed under optimal renaturation conditions: 2× SSC (0·3 M NaCl, 0·3 M sodium citrate) at 62 °C. Density analysis was performed using an Agfa scanner and Scan Analysis software (ver. 2.21; Biosoft) to determine homology values relative to self-hybridization of the probe with target DNA of CH001A.

RESULTS

Detection and isolation of the bacterium

Amplicons specific for '*Candidatus* C. hominis' were found in five out of 18 (26·8%) healthy faecal samples examined directly by DNA extraction and specific PCR assay. Fresh faecal samples were obtained from these five individuals for culture examination (see Methods). After 21 d microaerobic incubation, plates showed little or no growth and all such growth was negative by *Campylobacter* genus-specific and by '*Candidatus* C. hominis'-specific PCR assays. CCDA incubated anaerobically was similarly negative. Plates inoculated using the membrane filter method produced confluent mixed bacterial growth, when incubated anaerobically. Four out of five samples from anaerobic FAA colony sweeps were positive by both *Campylobacter* genus-specific PCR and '*Candidatus* C. hominis'-specific PCR. They were further examined by the dilution and IMS methods described above. All anaerobic BA plates were PCR-negative by both assays.

The dilution approach was successful in one of the five samples. In this case, three distinct colony types were detected from a single faecal sample, all '*Candidatus* C. hominis'-specific PCR-positive. These were termed CH001A (1·0 mm diameter, grey, flat, spreading), CH001B (0·5 mm diameter, grey, convex, entire) and CH001C (1·0 mm diameter, grey, convex, entire).

'*Candidatus* C. hominis'-specific PCR-positive material was also investigated by IMS. One millilitre of bacterial suspension was mixed with biomagnetic beads coated with either flagella-specific or cell-wall-specific antibody. Using this approach, the cell-wall-specific IMS yielded '*Candidatus* C. hominis' PCR-positive colonies, CH002 and CH003, each from two further individual samples. These isolates were both similar in appearance to colony type A of CH001. IMS with flagellin-specific antibody was unsuccessful.

The above combination of strategies yielded '*Candidatus* C. hominis' PCR-positive colonies from three of the five individuals whose faecal samples were positive by the same assay performed directly on DNA extracted from the faeces.

Phenotypic characterization and numerical analysis

We observed that strains could be successfully grown on ordinary 5% BA media after a few subcultures on FAA, although anaerobic conditions were essential. Growth was either not obtained, or was of a notably poor quality, under microaerobic conditions.

Isolates of *C. hominis* were phenotypically homogeneous, with infraspecific variance detected in only

Table 2 Characteristics differentiating *C. hominis* sp. nov. from other *Campylobacter* species

Phenotypic data on *C. hominis* were as described in this study. The characteristics of the other taxa were from previous studies and were determined by the same methods (On *et al.*, 1996, 1998). +, All strains positive; −, all strains negative; (+), 70–97% strains positive; (−), 5–30% strains positive; v, 38–66% strains positive.

	Production of:					Hydrolysis of:		Tolerance to:					Presence of flagella
	Oxidase	Catalase	Urease	Alkaline phosphatase	H_2S (TSI)	Hippurate	Indoxyl acetate	TTC (0·04%)	Metronidazole (4 mg l^{-1})	5-Fluorouracil (100 U l^{-1})	Crystal violet	Sodium fluoride	
Campylobacter hominis sp. nov	+	−	−	−	−	−	−	−	−	+	−	+	−
C. coli	+	+	−	−	v	−	+	+	(+)	+	v	+	+
C. concisus	v	−	−	(+)	(−)	−	−	−	(−)	(−)	v	(+)	+
C. curvus	+	−	−	v	(−)	(−)	v	+	−	(−)	+	−	+
C. fetus subsp. *fetus*	+	+	−	−	−	−	−	−	(+)	+	+	(+)	+
C. fetus subsp. *venerealis*	+	(+)	−	−	−	−	−	−	v	(+)	(+)	(+)	+
C. gracilis	−	(−)	−	−	−	−	(+)	−	−	(−)	+	(+)	−
C. helveticus	+	−	−	−	−	−	+	−	v	+	−	−	+
C. hyointestinalis subsp. *hyointestinalis*	+	+	−	−	(+)	−	−	(−)	(−)	(−)	(+)	v	+
C. hyointestinalis subsp. *lawsonii*	+	+	−	(−)	(+)*	−	−	−	v	v	v	−	+
C. jejuni subsp. *doylei*	+	(+)	−	−	−	+	+	v	(−)	+	−	(−)	+
C. jejuni subsp. *jejuni*	+	+	−	−	−	+	+	(+)	(+)	(+)	v	+	+
C. lari	+	+	v†	(−)	−	−	(−)	(+)	+	+	v	+	+
C. mucosalis	+	−	−	(+)	+	−	−	−	(+)	−	(+)	−	+
C. rectus	+	(−)	−	−	−	−	+	−	−	−	−	−	+
C. showae	v	+	−	−	v	−	v	−	+	−	+	+	+
C. sputorum	+	v†	v†	−	(+)*	−	−	−	(−)	−	v	(+)	+
C. upsaliensis	+	−	−	−	−	−	+	−	(+)	+	−	−	+
[*B.*] *ureolyticus*‡	+	(−)	+	−	−	−	(−)	−	−	−	(−)	+	+

* Strains of *C. sputorum* and *C. hyointestinalis* subsp. *lawsonii* normally produce copious amounts of H_2S in TSI agar slopes.

† Results for strains in these tests are biovar-dependent (On *et al.*, 1996, 1998).

‡ Phylogenetically closely related to *Campylobacter* and generically misnamed, but considered a species *incertae sedis* (Vandamme *et al.*, 1995).

4/47 test results. These were nitrate reduction (CH004 and CH005 positive), tolerance to 1·0 and 2·0 % bile (CH004 positive), and resistance to nalidixic acid (CH004 sensitive). The phenotypic characteristics of *C. hominis* are listed in the formal description.

Table 2 lists the most useful characteristics differentiating *C. hominis* from other *Campylobacter* species. Biochemically, *C. hominis* is most similar to the principally anaerobic hydrogen-requiring species *Campylobacter concisus*, *Campylobacter curvus*, *Campylobacter gracilis*, *Campylobacter rectus*, *Campylobacter showae* and *Campylobacter sputorum*, to which a close phylogenetic relationship is evident (Fig. 1). Nonetheless, the dendrogram derived from a numerical analysis of the 47 phenotypic tests used shows that the *C. hominis* strains form a distinct cluster at the 92·0 % similarity level and are readily distinguished from all other *Campylobacter* species (Fig. 2).

16S rRNA gene sequence

The 16S rRNA gene sequence (1414 nucleotides) was obtained from genomic DNA extracted from CH001A. Phylogenetic analysis showed that this sequence clustered among the sequences of '*Candidatus* C. hominis' previously determined directly from faecal material (Fig. 2). It was 0·7 % divergent from HS-A, and 0·1 % from HS-B and HS-C. The overall phylogenetic position for CH001A was the same as that for '*Candidatus* C. hominis' (Lawson *et al.*, 1998). Dissimilarities between CH001A and the most closely related species were as follows: *C. sputorum*, 6·2 %; *C. gracilis*, 6·7 %; *C. rectus*, 7·0 %; *C. concisus*, 7·4 %; *C. showae*, 7·4 %; and *C. curvus*, 8·3 %. Other dissimilarities included: *C. jejuni*, 10·5 %; *Arcobacter skirrowii*, 15·7 %; *Helicobacter pylori*, 18·1 %; and *E. coli*, 28·7 %.

DNA base composition and DNA–DNA hybridization

DNAs of strains CH001A, CH002 and CH003 were subjected to further analysis. The DNA base composition of these strains was determined as 32–33 mol % G+C.

The relative homology values obtained by DNA–DNA hybridization at 62 °C using CH001A DNA as probe were as follows: CH001A, 100 %; CH002, 91·4 %; and CH003, 98·7 %. All other *Campylobacter* type species, *H. pylori*, *A. skirrowii* and *E. coli* showed no detectable homology.

Electron microscopy

Examples of electron micrographs are presented in Fig. 3(a, b). Cells were typically blunt-ended bacilli or coccobacilli, 0·25–0·5 μm wide and 0·5–1·8 μm long. There was no evidence of spiral morphology or flagella. Cells of colony types that exhibited a spreading morphology (CH001A, CH002 and CH003) were found to possess numerous irregular fimbriae-like structures 4–8 nm wide and > 1·0 μm long (Fig. 3b). Fimbriae-like structures were not found in the non-spreading colony types (CH001B and CH001C) of the first strain.

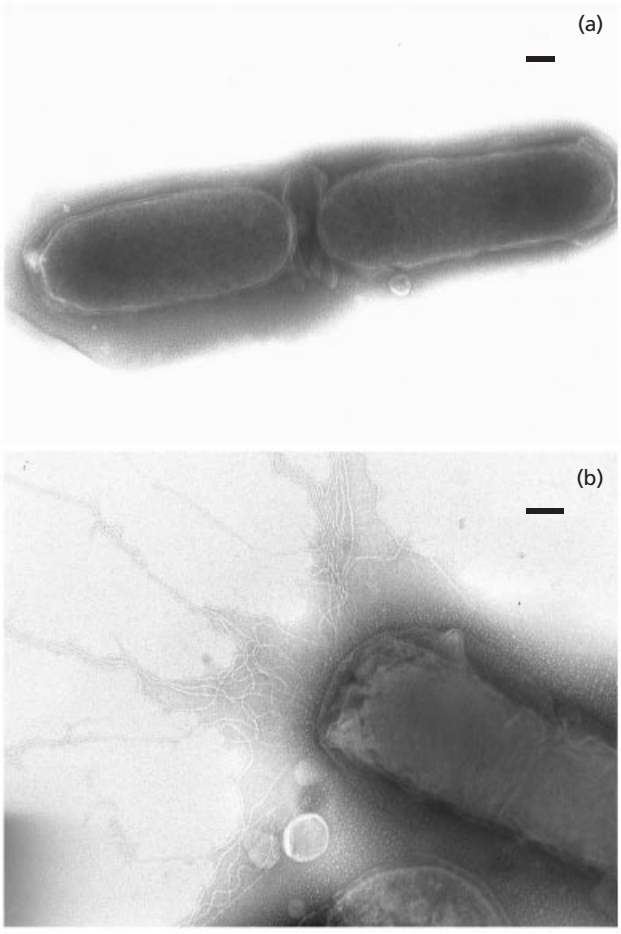

Fig. 3. Electron micrograph of *C. hominis* sp. nov. Bar, 0·1 μm. (a) CH001 (colony type B); (b) CH001 (colony type A) showing fimbriae-like structures.

Taxonomic status of *C. hominis* sp. nov.

The results described provide evidence that the strains isolated and examined in this study (CH001, CH002 and CH003) constitute a distinct species within the genus *Campylobacter*, for which we propose the name *C. hominis* sp. nov. The type strain is CH001A^T and has been deposited in the National Collection of Type Cultures and designated NCTC 13146^T.

Prevalence of *C. hominis* sp. nov. in diarrhoeic faeces

The species-specific PCR was positive in 10 of 114 (8·8 %) diarrhoeic faecal samples positive for *C. jejuni*. It was also positive in eight of 95 (8·4 %) diarrhoeic faecal samples which contained no enteropathogenic *Campylobacter* species (see Methods). Thus there was no statistical difference in the detection rate for *C.*

hominis sp. nov. in these two groups (McNemars test, $P > 0{\cdot}5$; Swinscow, 1996).

DISCUSSION

We previously described the presence in faecal samples from healthy humans of novel 16S rDNA sequences of *Campylobacter* origin. These were non-chimeric, folded to a rRNA secondary structure typical of the genus, and corresponded to a novel, uncultured taxon of *Campylobacter*. In accordance with recommendations for the description of incompletely described prokaryotes (Murray & Schleifer, 1994; Murray & Stackebrandt, 1995), the provisional taxon was named '*Candidatus* C. hominis' (Lawson *et al*., 1998).

We were unable to isolate *C. hominis* sp. nov. by conventional *Campylobacter* isolation methods; this was most likely related to the fastidious growth requirements of the organism, as reported herein. Culture from PCR-positive faecal samples was achieved only after incubation under anaerobic conditions on FAA (a rich medium which contains vitamin K, haemin, cysteine hydrochloride and L-arginine) in combination with dilution or IMS to isolate bacteria from a mixed culture. It is noteworthy that 16S rDNA sequence analysis of *C. hominis* identified its closest phylogenetic relatives as *C. gracilis*, *C. rectus*, *C. curvus*, *C. showae*, *C. concisus* and *C. sputorum*. These species form a distinctive clade, including *C. hominis*, within the genus *Campylobacter* (Fig. 2). They all show a requirement for growth in an anaerobic environment, and/or require H_2 for optimum growth (Etoh *et al*., 1993; Tanner *et al*., 1981; Vandamme & Goossens, 1992). Furthermore, most of these species are associated with the periodontal crevice of humans and are rarely associated with gastroenteritis. Several share similar cell morphologies: for example, both *C. hominis* sp. nov. and *C. gracilis* are aflagellate straight rods, whilst *C. showae* and *C. concisus* are flagellate straight rods (Etoh *et al*., 1993; Tanner *et al*., 1981). Thus overall phylogenetic, physiological, morphological and ecological properties of these species suggest that they may have a common ancestor within the genus *Campylobacter*. Furthermore, the relatively deep branching of *C. hominis* sp. nov. and *C. gracilis* observed in the phylogenetic tree, as well as their unusual aflagellate rod-like cell structure, may indicate that these two species represent a distinct lineage within *Campylobacter*. Other similar such species may await description. However, despite the similarity of *C. hominis* sp. nov. to *C. gracilis*, its mol% G+C value and 16S rDNA sequence are closer to that of *C. sputorum*, while the bootstrap value for the branching order to its two nearest neighbours (*C. sputorum* and *C. gracilis*) by 16S rDNA analysis was low (488 and 370, respectively, see Fig. 2). Also DNA–DNA hybridization showed no relative homology with other *Campylobacter* species. Thus the relative importance of these inferred phylogenetic relationships must remain speculative. The numerous irregular fimbriae-like structures produced by certain colony types of *C. hominis* sp. nov. are also distinct from the few examples of fimbriae reported for *Campylobacter* species (Dolg *et al*., 1996).

We previously demonstrated that '*Candidatus* C. hominis' 16S rDNA sequences were present in 50% of faecal samples but absent in saliva samples of healthy individuals (Lawson *et al*., 1998). In the present study, *C. hominis*-specific PCR was positive in 27·8% of healthy human faecal samples but only in 8·6% of diarrhoeic faeces. These findings may be explained if *C. hominis* sp. nov. is a commensal of the human gastrointestinal tract which is either displaced or drops below the threshold of detection during episodes of acute gastroenteritis. Carriage of a commensal *Campylobacter* species in the lower gastrointestinal tract might confer protective immunity, or resistance to colonization by pathogenic campylobacters such as *C. jejuni* and *Campylobacter coli*. However, there was no significant difference between detection rate for *C. hominis* sp. nov. in cases of *Campylobacter* enteritis or non-*Campylobacter* enteritis. We note that some isolates of the new species were unable to tolerate 1·0% bile in laboratory conditions. In contrast, other enteric *Campylobacter* species, such as *C. jejuni*, tolerate bile concentrations of between 1·5 and 2·0% (On *et al*., 1996). In humans, the initial site of infection of *C. jejuni* is the jejunum and upper ileum (Skirrow, 1994), where bile salt concentrations are high. Bile salts are largely absorbed in the terminal ileum (Percy-Robb & Collee, 1972), suggesting that the primary niche of *C. hominis* sp. nov. is the large intestine.

Description of *Campylobacter hominis* sp. nov.

Campylobacter hominis (hom.in′is. L. gen. n. *hominis* of man, from which the bacterium was first isolated).

Gram-negative, non-spore-forming rods 0·25–0·5 μm wide and 0·5–1·8 μm long after 10 d incubation (first subculture following initial isolation) on FAA in an anaerobic atmosphere. Colonies pinpoint to 1·0 mm in diameter, grey, some convex and entire, others flat and spreading. Cells are straight, blunt-ended bacilli or coccobacilli. Non-motile: flagella are not evident but certain isolates produce numerous irregular fimbriae-like structures (4–8 nm wide and $> 1{\cdot}0$ μm long). Optimal growth achieved on FAA under anaerobic conditions at 37 °C, but strains will grow on standard 5% blood agar media after adaptation to laboratory conditions. Neither green or blue–green pigmented growth is observed on BA. Pitting of the agar growth medium is absent. Non-haemolytic. No, or extremely poor, growth is attained on either FAA or BA when incubated microaerobically at 37 °C. No growth under aerobic conditions at either 25 or 37 °C. Oxidase-positive. Catalase, hippuricase, urease and alkaline phosphatase are not produced. Hydrogen sulphide is not produced in triple-sugar iron medium. Indoxyl acetate is not hydrolysed. Neither triphenyltetrazolium chloride (TTC) nor selenite is reduced. All strains grow under anaerobic conditions on media

containing 1·0 % glycine, 5-fluorouracil (100 U l^{-1}), 0·1 % sodium fluoride and 0·1 % trimethylamine M-oxide (TMAO). No growth observed under anaerobic conditions at room temperature (18–22 °C), 25 °C or 42 °C, or on media containing 4·0 % NaCl, 0·04 % TTC, 0·1 % potassium permanganate, 0·001 % sodium arsenite, 32 mg cephalothin l^{-1}, 4 mg metronidazole l^{-1}, 32 mg carbenicillin l^{-1} (nutrient and blood agar bases), 0·005 % basic fuchsin, 0·0005 % crystal violet, 0·1 % janus green, 0·1 % sodium deoxycholate or 0·02 % pyronin. No growth similarly observed on a minimal medium, or MacConkey, casein or tyrosine media. Strains may differ in their ability to reduce nitrate, and grow on media containing 1·0 % and 2·0 % bile, and nalidixic acid (32 mg l^{-1}). NCTC 13146^T (= LMG 19568^T) is the type strain of *C. hominis* sp. nov. This strain conforms to the species description given above. The G+C content of NCTC 13146^T DNA was 32·5 mol %. The strain was isolated from the faeces of a healthy adult human male (London, UK) in 1998.

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REFERENCES

Barrow, G. I. & Feltham, R. K. A. (1993). *Cowan and Steeles Manual for the Identification of Medical Bacteria*. Cambridge: Cambridge University Press.

Bolton, F. J., Hutchinson, D. N. & Parker, G. (1988). Reassessment of selective agars and filtration techniques for isolation of *Campylobacter* species from faeces. *Eur J Clin Microbiol Infect Dis* **7**, 155–160.

Dolg, P., Yao, R., Burr, D. H., Guerry, P. & Trust, T. J. (1996). An environmentally regulated pilus-like appendage involved in *Campylobacter* pathogenesis. *Mol Microbiol* **20**, 885–894.

Etoh, Y., Dewhirst, F. E., Paster, B. J., Yamamoto, A. & Goto, N. (1993). *Campylobacter showae* sp. nov., isolated from the human oral cavity. *Int J Syst Bacteriol* **43**, 631–639.

Jukes, T. H. & Cantor, C. R. (1969). Evolution of protein molecules. In *Mammalian Protein Metabolism*, pp. 21–132. Edited by H. N. Munro. New York: Academic Press.

Lawson, A. J., Linton, D. & Stanley, J. (1998). 16S rRNA gene sequences of '*Candidatus* Campylobacter hominis', a novel uncultivated species, are found in the gastrointestinal tract of healthy humans. *Microbiology* **144**, 2063–2071.

Lawson, A. J., Logan, J. M., O'Neill, G. L., Desai, M. & Stanley, J. (1999). Large-scale survey of *Campylobacter* species in human gastroenteritis by PCR and PCR-enzyme-linked immunosorbent assay. *J Clin Microbiol* **37**, 3860–3864.

Linton, D., Owen, R. J. & Stanley, J. (1996). Rapid identification by PCR of the genus *Campylobacter* and of five *Campylobacter* species enteropathogenic for man and animals. *Res Microbiol* **147**, 707–718.

Linton, D., Lawson, A. J., Owen, R. J. & Stanley, J. (1997). PCR detection, identification to species level, and fingerprinting of *Campylobacter jejuni* and *Campylobacter coli* direct from diarrheic samples. *J Clin Microbiol* **35**, 2568–2572.

Metherell, L. A., Logan, J. M. & Stanley, J. (1999). PCR-enzyme-linked immunosorbent assay for detection and identification of *Campylobacter* species: application to isolates and stool samples. *J Clin Microbiol* **37**, 433–435.

Murray, R. G. E. & Schleifer, K. H. (1994). Taxonomic notes: a proposal for recording the properties of putative taxa of procaryotes. *Int J Syst Bacteriol* **44**, 174–176.

Murray, R. G. E. & Stackebrandt, E. (1995). Taxonomic note: implementation of the provisional status Candidatus for incompletely described procaryotes. *Int J Syst Bacteriol* **45**, 186–187.

On, S. L. W. & Holmes, B. (1991a). Effect of inoculum size on the phenotypic characterization of *Campylobacter* species. *J Clin Microbiol* **29**, 923–926.

On, S. L. W. & Holmes, B. (1991b). Reproducibility of tolerance tests that are useful in the identification of campylobacteria. *J Clin Microbiol* **29**, 1785–1788.

On, S. L. W. & Holmes, B. (1992). Assessment of enzyme detection tests useful in identification of campylobacteria. *J Clin Microbiol* **30**, 746–749.

On, S. L. W. & Holmes, B. (1995). Classification and identification of campylobacter, helicobacters and allied taxa by numerical analysis of phenotypic characters. *Syst Appl Microbiol* **18**, 374–390.

On, S. L. W., Holmes, B. & Sackin, M. J. (1996). A probability matrix for the identification of campylobacters, helicobacters and allied taxa. *J Appl Bacteriol* **81**, 425–432.

On, S. L. W., Atabay, H. I., Corry, J. E., Harrington, C. S. & Vandamme, P. (1998). Emended description of *Campylobacter sputorum* and revision of its infrasubspecific (biovar) divisions, including *C. sputorum* biovar *paraureolyticus*, a urease-producing variant from cattle and humans. *Int J Syst Bacteriol* **48**, 195–206.

Owen, R. J. & Pitcher, D. G. (1985). Chemical methods for estimating DNA base compositions and levels of DNA-DNA hybridization. In *Chemical Methods in Bacterial Systematics*, pp. 67–93. Edited by M. Goodfellow & D. E. Minnikin. London: Academic Press.

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

Percy-Robb, I. W. & Collee, J. G. (1972). Bile acids: a pH dependent antibacterial system in the gut? *Br Med J* **3**, 813–815.

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

Skirrow, M. B. (1994). Diseases due to *Campylobacter*, *Helicobacter* and related bacteria. *J Comp Pathol* **111**, 113–149.

Stanley, J., Burnens, A. P., Linton, D., On, S. L., Costas, M. & Owen, R. J. (1992). *Campylobacter helveticus* sp. nov., a new thermophilic species from domestic animals: characterization, and cloning of a species-specific DNA probe. *J Gen Microbiol* **138**, 2293–2303.

Stanley, J., Linton, D., Burnens, A. P., Dewhirst, F. E., Owen, R. J., Porter, A., On, S. L. & Costas, M. (1993). *Helicobacter canis* sp. nov., a new species from dogs: an integrated study of phenotype and genotype. *J Gen Microbiol* **139**, 2495–2504.

Steele, T. W. & McDermott, S. N. (1984). The use of membrane filters applied directly to the surface of agar plates for the isolation of *Campylobacter jejuni* from faeces. *Pathology* **16**, 263–265.

Swinscow, S. D. V. (1996). *Statistics at Square One*, 9th edn. London: BMJ Publishing Group.

Tanner, A. C. R., Badger, S., Lai, C. H., Listgarten, M. A., Visconti, R. A. & Socransky, S. S. (1981). *Wolinella* gen. nov., *Wolinella succinogenes* (*Vibrio succinogenes* Wolin *et al*.) comb. nov., and description of *Bacteroides gracilis* sp. nov., *Wolinella recta* sp. nov., *Campylobacter concisus* sp. nov., and *Eikenella corrodens* from humans with periodontal disease. *Int J Syst Bacteriol* **31**, 432–435.

Vandamme, P. & Goossens, H. (1992). Taxonomy of *Campylobacter*, *Arcobacter*, and *Helicobacter*: a review. *Zentbl Bakteriol* **276**, 447–472.

Vandamme, P., Falsen, E., Rossau, R., Hoste, B., Segers, P., Tytgat, R. & de Ley, J. (1991). Revision of *Campylobacter*, *Helicobacter*, and *Wolinella* taxonomy: emendation of generic descriptions and proposal of *Arcobacter* gen. nov. *Int J Syst Bacteriol* **41**, 88–103.

Vandamme, P., Daneshvar, M. I., Dewhirst, F. E., Paster, B. J., Kersters, K., Goossens, H. & Moss, C. W. (1995). Chemotaxonomic analyses of *Bacteroides gracilis* and *Bacteroides ureolyticus* and reclassification of *B. gracilis* as *Campylobacter gracilis* comb. nov. *Int J Syst Bacteriol* **45**, 145–152.