Jonquetella anthropi gen. nov., sp. nov., the first member of the candidate phylum 'Synergistetes' isolated from man

Estelle Jumas-Bilak,¹ Jean-Philippe Carlier,² Hélène Jean-Pierre,³ Diane Citron,⁴ Kathryn Bernard,⁵ Audrey Damay,³ Bernard Gay,³ Corinne Teyssier,¹ Josiane Campos³ and Hélène Marchandin^{1,3}

Correspondence

Hélène Marchandin

h-marchandin@chu-montpellier.fr

¹Université Montpellier 1, EA 3755, Faculté de Pharmacie, Laboratoire de Bactériologie-Virologie, 15, Avenue Charles Flahault, BP 14491, 34093 Montpellier Cedex 5, France

²Institut Pasteur, Centre National de Référence des Bactéries Anaérobies et du Botulisme, 25 rue du Dr Roux, 75724 Paris Cedex 15, France

³Centre Hospitalier Universitaire de Montpellier, Hôpital Arnaud de Villeneuve, Laboratoire de Bactériologie, 371 Avenue du Doyen Gaston Giraud, 34295 Montpellier Cedex 5, France

⁴R. M. Alden Research Laboratory, Santa Monica Blvd, Santa Monica, CA 90404, USA

⁵National Microbiology Laboratory – Public Health Agency of Canada, 1015 Arlington St., Suite H5040, Winnipeg, MB R3E 3R2, Canada

Six clinical isolates of a hitherto unknown, strictly anaerobic, Gram-negative rod showing fastidious growth were subjected to a polyphasic taxonomic study, including phenotypic, genomic and phylogenetic feature analyses. 16S rRNA gene sequenced-based phylogeny revealed that the novel strains represent a homogeneous group distant from any recognized species in the candidate phylum 'Synergistetes'. The novel isolates were most closely related to species of the genus *Dethiosulfovibrio*, with 88.2–88.7% 16S rRNA gene sequence similarity. Large-scale chromosome structure and DNA G + C content also differentiated the novel strains from members of the genus *Dethiosulfovibrio*. The novel strains were asaccharolytic. Major metabolic end products in trypticase/glucose/yeast extract broth were acetic, lactic, succinic and isovaleric acids and the major cellular fatty acids iso-C_{15:0} and C_{16:0}. Based on the data presented here, a new genus, *Jonquetella* gen. nov., is proposed with one novel species, *Jonquetella anthropi* sp. nov. *J. anthropi* is the first characterized species of the candidate phylum 'Synergistetes' that includes human isolates. The G + C content of the DNA of the type strain of *J. anthropi* ADV 126^T (=AIP 136.05^T=CIP 109408^T=CCUG 53819^T) is 59.4 mol%.

The candidate phylum 'Synergistes' was first described in a phylogenetic study upon the global diversity of bacteria (Hugenholtz *et al.*, 1998). However, this name is illegitimate due to its prior use for a genus and we therefore suggest the name 'Synergistetes' for this candidate phylum. A comprehensive phylogenetic analysis demonstrated that this candidate phylum corresponds to a robust deep-branched clade that groups bacteria currently

classified in diverse taxa by *Bergey's Manual of Systematic Bacteriology* (Garrrity & Holt, 2001): the family *Syntrophomonadaceae* in the phylum *Firmicutes* and *Synergistes jonesii* in the phylum *Deferribacteres* (A. Damay, H. Marchandin, L. Roudière, C. Teyssier, H. Dechaud, H. Jean-Pierre, & E. Jumas-Bilak, unpublished results). To date, bacteria belonging to the phylum 'Synergistetes' have been found in a large range of ecosystems, including the guts of mammals (Godon *et al.*, 2005). Culture-independent studies have demonstrated their presence in man during endodontic or periodontic infections, in dental caries (Munson *et al.*, 2002, 2004; Siqueira & Rôças, 2005, 2007; Hutter *et al.*, 2003), in subgingival plaque (Paster *et al.*, 2001), in faeces (Horz *et al.*, 2006) and in vaginal flora (A. Damay, H. Marchandin, L. Roudière, C. Teyssier, H. Dechaud, H. Jean-Pierre, E. Jumas-Bilak, unpublished results). However, only eight human isolates

Abbreviations: EM, electron microscopy; ML, maximum-likelihood.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of *Jonquetella anthropi* ADV 126^T is EF436500.

A supplementary figure showing the PFGE analysis of strains of *Jonquetella anthropi* gen. nov., sp. nov. is available with the online version of this paper.

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have been reported to date (Munson *et al.*, 2002; Warren *et al.*, 2005; Horz *et al.*, 2006).

In this study, we characterized six strains of unknown, anaerobic Gram-negative rods with fastidious growth, including one isolate initially studied by Warren *et al.* (2005). The five strains labelled 'ADV' were isolated between February 2002 and November 2006 from different patients hospitalized in the University Hospital of Montpellier, France. Strain ADV 116 was recovered in pure culture from a peritoneal fluid sample (abdominal lymphocele due to wound slacking after stomach liposuction), whereas strains ADV 256, ADV 126^T, ADV 62 and ADV 808 were recovered in mixed cultures from wounds, cysts and abscesses. Strain RMA 10849 was obtained from the collection of the R. M. Alden Research Laboratory, Santa Monica, CA, USA. This isolate originated from a sacral wound and was previously described as an unnamed organism by Warren *et al.* (2005) and further described as belonging to 'Synergistes' group organisms by Horz *et al.* (2006). Colony morphology and the responses of the strains to special potency disks (Jousimies-Somer *et al.*, 2002) were observed on Columbia sheep blood agar plates. For further characterization, the novel strains were maintained anaerobically in trypticase/glucose/yeast extract (TGY) medium under anaerobic conditions at 37 °C, as described previously (Carlier *et al.*, 2004). Biochemical reactions were performed according to the procedures of the VPI Anaerobe Laboratory Manual (Holdeman *et al.*, 1977) by using trypticase/yeast extract (TY) medium supplemented with 1 % (w/v) of each sterilized substrate. Colonies appeared on Columbia sheep blood agar plates after 3–5 days at 37 °C in anaerobic conditions generated by the Anaerogen System (Oxoid Unipath). Colonies were very small (0.5 mm), non-haemolytic, circular, shiny and grey. Cells were non-motile. Gram stain showed polymorphic, Gram-negative rods arranged in pairs or short chains. Spores were not observed. Catalase, cytochrome oxidase and urease activities were not detected. Nitrate reductase activity was detected in only two of the six strains (ADV 126^T and RMA 10849). No gas was produced in deep TGY agar cultures. Indole production was not detected, gelatin was not liquefied and milk was not coagulated. Aesculin was not hydrolysed. Acid was not produced from arabinose, cellobiose, aesculin, fructose, galactose, glucose, glycerol, inositol, lactose, maltose, mannose, mannitol, melezitose, melibiose, raffinose, rhamnose, ribose, salicin, sorbitol, sucrose, starch, trehalose or xylose. The strains displayed susceptibility to a bile disk (1 mg) and to kanamycin (1 mg) and showed resistance to vancomycin (5 µg; Rosco). Apart from strain ADV 116, resistance was observed to the 10 µg colistin disk (Rosco). The enzymic profile determined by the Rapid ID 32 A system (bioMérieux) according to the manufacturer's recommendations, showed leucyl glycine and glycine arylamidase activities (API code 0000040400) for all strains. The combination of these phenotypic data did not result in the identification of the novel isolates.

DNAs were extracted and purified using the Aquapure DNA isolation kit (Bio-Rad) in accordance with the supplier's protocol. 16S rRNA genes were selectively amplified by PCR using primers 27f and 1492r as previously described (Carlier *et al.*, 2002). The PCR products were directly sequenced on an Applied Biosystems Automatic Sequencer (Genome Express) by using forward and reverse primers. The sequences were compared with sequences in the GenBank and EMBL databases using the BLAST program (Altschul *et al.*, 1997) and LALIGN software (www.expasy.org) and with sequences deposited in the RDPII database by using the SEQMATCH program (Cole *et al.*, 2007). The six novel isolates displayed at least 99 % 16S rRNA gene sequence similarity with each other and were most closely related to species of the genus *Dethiosulfovibrio*, but with no more than 88.7 % gene sequence similarity. The 16S rRNA gene sequences selected from the GenBank database were aligned by CLUSTAL_X, version 1.83 (Thompson *et al.*, 1997). The alignment was checked and corrected manually before reconstruction of phylogenetic trees. Phylogenetic trees were inferred by using the distance, parsimony and maximum-likelihood methods. The F84 substitution model (Felsenstein, 1993) was used to reconstruct neighbour-joining and maximum-likelihood (ML) evolutionary trees by using PHYLIP (Felsenstein, 1993) and PHYML (Guindon & Gascuel, 2003), respectively. The robustness of the trees was evaluated by bootstrap analysis of 100 resamplings. Distance and ML trees gave congruent results. The strains formed a homogeneous group together with the *Flexistipes* sp. E3_33 E1 oral isolate that was remote from species of the genus *Dethiosulfovibrio* and was supported by a high bootstrap value (Fig. 1). From 16S rRNA gene sequence analysis and phylogeny, the six strains studied can be considered to represent a new genus and a novel species. The *Flexistipes* sp. E3_33 E1 oral isolate should be considered to represent a seventh strain of the novel species. The novel strains clearly belonged to the phylum 'Synergistetes' but were distant from other bacteria classified in the family *Syntrophomonadaceae* (Fig. 1).

The six strains were studied further and compared with the type species of the closest genus, *Dethiosulfovibrio*, *Dethiosulfovibrio peptidovorans* strain DSM 11002^T (Magot *et al.*, 1997).

For electron microscopy, cells were prepared as described previously for negative staining (Marchandin *et al.*, 2003a; Jumas-Bilak *et al.*, 2005) and were observed with a Hitachi H7100 electron microscope. The morphology of the novel strains was not similar to that of species of the genus *Dethiosulfovibrio* (Table 1) as the cells were straight rods of 0.8–0.9 × 1.4–1.7 µm with an irregular surface (Fig. 2).

Metabolic end products from TGY broth were assayed by quantitative GC, as described previously (Carlier, 1985). The major metabolic end product was acetic acid (8.5–14.1 mmol l⁻¹). Smaller volumes of propionic (0.9–2.2 mmol l⁻¹), isovaleric (1.5–3.4 mmol l⁻¹), lactic (0–4.1 mmol l⁻¹) and succinic (0–2.5 mmol l⁻¹) acids

Fig. 1. Neighbour-joining phylogenetic tree, based on partial 16S rRNA gene sequences (1269 nt), showing the relationship of *Jonquetella anthropi* gen. nov., sp. nov. with representatives of the phyla 'Synergistetes' and Deferribacteres. Numbers given at the nodes are bootstrap values estimated from 100 replicates. *Anaeroglobus geminatus* CIP 106856^T was used as the outgroup. GenBank accession numbers are given in parentheses. Bar, 0.02 substitutions per nucleotide position.

and trace amounts (below 0.8 mmol l⁻¹) of isobutyric and phenylacetic acids were also detected. The production of 3-methylbutyric acid (isovaleric acid) was detected. However, under the analytical conditions used in this study, it could not be separated from anteisovaleric acid (2-methylbutyric acid) and thus, the latter may be produced. Consequently, this profile appeared relatively similar to that of *D. peptidovorans* DSM 11002^T consisting of acetate, isobutyrate, isovalerate and 2-methylbutyrate production (Table 1).

The cellular fatty acid (CFA) composition of the five French isolates and of the type strain of *D. peptidovorans* was analysed by GC according to Veys *et al.* (1989) after growth on Mueller–Hinton agar plates for 5 days. The CFA profiles obtained for the five novel strains were roughly similar, with the major CFAs being iso-C_{15:0} and C_{16:0}, whereas *D. peptidovorans* DSM 11002^T showed different CFA ratios and lacked anteiso-C_{15:0} (Table 2).

The ability to grow with or without 20 mM thiosulfate or 3% NaCl was tested at 37 °C and 42 °C using the DP medium as previously described by Magot *et al.* (1997) without NaCl. Growth of the novel strains in this medium was extremely poor and was enhanced by NaCl (Table 1). The optimum growth temperature for strains ADV 126^T,

ADV 62, ADV 256 and ADV 808 was 37 °C. No growth was observed at 42 °C (Table 1). The addition of thiosulfate did not enhance growth. In contrast, strain ADV 116 was similar to *D. peptidovorans* DSM 11002^T since its optimum growth temperature was 42 °C and thiosulfate was reduced to hydrogen sulfide. However, this strain reduced sulfite and thus differs from species of the genus *Dethiosulfovibrio* (Table 1). None of the novel strains contained desulfovibridin.

Based on leucyl glycine and glycine arylamidase activities observed with the Rapid ID 32 A kit, the effect of glycine and leucine on growth was investigated in TGY medium supplemented with 10 mM of each amino acid. In addition, the novel strains were tested in an undefined medium containing (l⁻¹, deionized H₂O): 1 g trypticase (BBL), 1 g yeast extract (Difco), 500 mg cysteine HCl, 5 mg haemin, 2 mg resazurin, 5 µg vitamin B12, 500 µg menadione, 1 mg thiamine HCl, 1 mg nicotinic acid, 500 µg riboflavin, 100 µg para-aminobenzoic acid, 50 µg biotin, 1 mg calcium pantothenate, 0.5 µg pyridoxamine and 500 µg folic acid. The pH of the medium was 6.8–7. The medium was prepared in an anaerobic chamber and dispensed as 10 ml aliquots into 30 × 60 mm bottles (Supelco). Bottles were closed with butyl rubber stoppers and fastened with crimp seals. The growth of the novel

Table 1. Characteristics that differentiate strains of *Jonquetella anthropi* gen. nov., sp. nov. from closely related species

Taxa: 1, ADV 62; 2, ADV 116; 3, ADV 126^T; 4, ADV 256; 5, ADV 808; 6, *Dethiosulfovibrio peptidovorans* DSM 11002^T (data from Magot *et al.*, 1997); 7, *Dethiosulfovibrio acidaminovorans* DSM 12590^T (Surkov *et al.*, 2001); 8, *Dethiosulfovibrio russensis* DSM 12538^T (Surkov *et al.*, 2001); 9, *Dethiosulfovibrio marinus* DSM 12537^T (Surkov *et al.*, 2001). +, Positive; –, negative; ND, not determined; A, acetate; P, propionate; IB, isobutyrate; IV, isovalerate and/or anteisovalerate; L, lactate; S, succinate; PHA, phenylacetate. Upper-case letters indicate major products.

Characteristic	1	2	3	4	5	6	7	8	9
Cell shape	Straight rods	Straight rods	Straight rods	Straight rods	Straight rods	Vibrio	Curved rods or spirals	Curved rods or spirals	Curved rods or spirals
Cell size (µm)	0.8–0.9 × 1.4–1.7	0.8–0.9 × 1.4–1.7	0.8–0.9 × 1.4–1.7	0.8–0.9 × 1.4–1.7	0.8–0.9 × 1.4–1.7	1 × 3–5	0.9 × 3–5	From 0.9 × 3–5 to 0.9 × 100–110	From 0.9 × 4–8 to 0.9 × 70
Motility	–	–	–	–	–	+	+	+	+
Temperature range (optimum, °C)	37–42 (37)	37–42 (42)	37–42 (37)	37–42 (37)	37–42 (37)	20–45 (42)	4–40 (25–30)	4–37 (28)	4–37 (28)
Thiosulfate as electron acceptor	–	+	–	–	ND	+	+	+	+
Sulfite reduction	–	+	–	–	ND	ND	–	–	–
Sulfide from thiosulfate	–	+	–	–	ND	+	+	+	+
NaCl requirement	+	+	+	+	ND	+	+	+	+
Metabolic end products	A, p, ib, iv, l, s, pha	A, p, ib, iv, l, s, pha	A, p, ib, iv, l, s, pha	A, p, ib, iv, l, s, pha	A, p, ib, iv, l, s, pha	A, ib, iv	ND	ND	ND
DNA G+C content (mol%)	ND	ND	59.4	ND	ND	56	50.6	50.8	51.8
Isolated from	Human breast abscess	Human peritoneal fluid	Human sebaceous cyst	Human wound	Human pelvic abscess	Oil well	Saline environment	Saline environment	Saline environment

*Thiosulfate improved growth with H₂S production only at 42 °C.

strains was not significantly enhanced in the presence of leucine plus glycine or with vitamins. The maximal OD₆₀₀ was similar to that of controls in TGY broth and varied between 0.4–0.6 after 5 days of incubation.

Due to the very limited growth of the strains in broth media, it was not possible to obtain sufficient biomass for efficient DNA–DNA hybridization experiments. Therefore, another genomic approach was performed using PFGE, as

described previously (Marchandin *et al.*, 2001, 2003b). Large-scale chromosome structure has previously been described as a sensitive indicator of phylogenetic relationships between bacteria (Liu *et al.*, 1999; Marchandin *et al.*, 2003a; Jumas-Bilak *et al.*, 2005). Both chromosomal size and *rrn* skeleton clearly distinguished the novel strains from *D. peptidovorans* DSM 11002^T. Three of the novel strains displayed a mean genomic size estimated at 1.940 ± 0.11 Mb with 3 *rrn* operon copies, whereas the

Fig. 2. General morphology of cells of *Jonquetella anthropi* gen. nov., sp. nov. viewed by EM after negative staining. (a) Strain ADV 256; (b) strain ADV 62. Bars, 600 nm (a) and 300 nm (b).

Table 2. Relative content (%) of cellular fatty acids of *Jonquetella anthropi* gen. nov., sp. nov. ($n=5$) and *Dethiosulfovibrio peptidovorans* DSM 11002^T.

Values in bold type represent the two major fatty acids. Fatty acid nomenclature: unsaturated fatty acids, the position of the double bond can be located by counting from the methyl (ω) end of the carbon chain; *cis* isomer is indicated by the suffix *c*.

Fatty acid	<i>Jonquetella anthropi</i> gen. nov., sp. nov.					<i>D. peptidovorans</i>
	ADV 126 ^T	ADV 256	ADV 62	ADV 116	ADV 808	DSM 11002 ^T
iso-C _{13:0}	6.1	7.0	5.7	6.1	7.8	6.9
C _{13:0}	1.5	4.0	0.95	1.4	2.8	0.8
iso-C _{14:0}	1.1	2.3	1.1	1.2	—	—
C _{14:0}	4.9	5.8	6.1	6.5	9.4	3.9
iso-C _{15:0}	42.6	24.8	40.2	38.4	32.5	59.7
anteiso-C _{15:0}	3.0	4.7	3.1	3.4	2.9	—
C _{15:0}	6.7	8.8	6.6	7.8	8.7	2.4
C _{16:0}	16.2	13.6	20.6	19.5	16.0	8.5
iso-C _{17:0}	2.1	1.1	1.4	1.3	0.9	3.5
C _{17:0}	5.9	12.6	4.3	5.1	5.8	1.8
C _{18:1} ω 9 <i>c</i>	—	—	—	—	1.1	3.0
C _{18:0}	9.7	15.2	9.5	9.1	11.7	9.0

type strain of *D. peptidovorans* showed a clearly distinct *rrn* skeleton with 5 *rrn* copies and a genome size of 3.48 Mb (see Supplementary Fig. S1 in IJSEM Online).

The DNA G+C content, as determined by HPLC at the Identification Service of the Deutsche Sammlung von Mikroorganismen und Zellkulturen (Braunschweig, Germany) for strain ADV 126^T was 59.4 mol%, a value distinct from that of members of the genus *Dethiosulfovibrio* (Table 1).

On the basis of 16S rRNA gene sequence divergence greater than 11% from the genus *Dethiosulfovibrio*, phylogenetic and genomic structure data, morphology, absence of motility, inability to reduce thiosulfate and metabolic end products, the novel strains should be classified within a new genus and novel species, for which the name *Jonquetella anthropi* gen. nov., sp. nov., is proposed. *J. anthropi* sp. nov. is the first characterized species of the candidate phylum 'Synergistetes' that includes human isolates.

Description of *Jonquetella* gen. nov.

Jonquetella (Jon.que'te.la. N.L. fem. dim. n. *Jonquetella* named in honour of Professor Jonquet, the clinician who first diagnosed infection involving this novel genus).

Cells are Gram-negative, non-motile, non-spore-forming, straight rods of 0.8–0.9 × 1.4–1.7 µm in size. Strictly anaerobic. Colonies are very small (0.5 mm), non-haemolytic, circular, shiny and grey after 3–5 days at 37 °C on Columbia sheep blood agar. Oxidase- and catalase-negative. Carbohydrates are not fermented. Positive for leucyl glycine and glycine arylamidase activities. NaCl is not required for growth, but enhances growth on DP medium (Magot *et al.*, 1997). Major cellular fatty acids are iso-C_{15:0} and C_{16:0}. The G+C content of the DNA is 59.4 mol%. Members of the

genus can be distinguished from other genera of the phylum 'Synergistetes' by 16S rRNA gene sequencing and DNA G+C content. Members of the genus can be differentiated from the closest genus, *Dethiosulfovibrio*, by morphology, absence of motility, inability to reduce thiosulfate at 37 °C, metabolic end products and genomic structure. The type species is *Jonquetella anthropi*.

Description of *Jonquetella anthropi* sp. nov.

Jonquetella anthropi (an.thro'pi. Gr. n. *anthropos* a human being; N.L. gen. n. *anthropi* of a human being, since virtually all strains thus far recovered are from human clinical specimens and since it represents the first characterized species including human clinical isolates in the phylum 'Synergistetes').

Displays the following characteristics in addition to those given in the genus description. Optimum temperature for growth is 37 or 42 °C. Gas is not produced. Unreactive in most of the conventional biochemical tests. Some strains can reduce thiosulfate and sulfite to sulfide. The metabolic end products in TGY broth are acetic acid, propionic acid and isovaleric acid. Lactic acid and succinic acid may be produced. Trace amounts (≤ 0.8 mmol l⁻¹) of isobutyric acid and phenylacetic acid are produced. Mean genomic size is 1.940 ± 0.11 Mb with 3 *rrn* operons. The DNA G+C content of the type strain is 59.4 mol%.

The type strain, ADV 126^T (=AIP 136.05^T=CIP 109408^T=CCUG 53819^T), was isolated from human clinical samples.

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