NOTE

Anaeroglobus geminatus gen. nov., sp. nov., a novel member of the family Veillonellaceae

¹ Centre National de Référence des Bactéries Anaérobies, Institut Pasteur, 25 rue du Dr Roux, F-75724 Paris Cedex 15, France

² Laboratoire de Bactériologie, Hôpital Arnaud de Villeneuve, Centre Hospitalo-Universitaire, F-34295 Montpellier Cedex 5, France

³ Laboratoire de Bactériologie-Virologie, Faculté de Pharmacie, F-34060 Montpellier Cedex 2, France

Jean-Philippe Carlier,¹ Hélène Marchandin,² Estelle Jumas-Bilak,³ Valérie Lorin,¹ Christine Henry,¹ Christian Carrière² and Hélène Jean-Pierre²

Author for correspondence: Jean-Philippe Carlier. Tel: +33 1 40 61 34 47. Fax: +33 1 40 61 31 23. e-mail: jcarlier@pasteur.fr

A hitherto unknown anaerobic coccus isolated from a post-operative fluid collection was characterized by phenotypic and phylogenetic methods. 16S rDNA sequence analysis revealed an affiliation of this isolate to the family Veillonellaceae. Also, a high level of sequence similarity was observed to some oral clone sequences of Megasphaera spp. contained in the GenBank database under designations BB166, CS025 and BS073. These clones and the unknown bacterium form a well-separated phylogenetic branch that may represent a novel lineage within the family Veillonellaceae. Based on phenotypic and phylogenetic evidence, a new genus, Anaeroglobus gen. nov., is proposed for the unknown bacterium, with one species, Anaeroglobus geminatus gen. nov., sp. nov. The type strain of Anaeroglobus geminatus is strain AIP 313.00^T (= CIP 106856^T = CCUG 44773^T). It is also suggested that the oral clones BB166, CS025 and BS073 belong to the genus Anaeroglobus.

Keywords: *Anaeroglobus geminatus* sp. nov., anaerobic cocci, *Megasphaera* sp., *Veillonellaceae*

The anaerobic Gram-negative cocci are classified in a single family, the *Veillonellaceae* (Rogosa, 1984). Three genera are currently accepted in this family, *Veillonella*, *Acidaminococcus* and *Megasphaera*. These organisms vary in diameter from 0.3–0.5 µm to 2.5 µm and are arranged in pairs, masses or short chains. Carbohydrates are weakly fermented or not fermented. Gas is produced. The metabolic end products are the principal characteristics by which the genera can be differentiated.

Recently, we have isolated an anaerobic coccoid-shaped organism, which exhibited a negative Gram-staining reaction, from a post-operative fluid collection from a 70-year-old woman. However, on the basis of phenotypic criteria, this strain could not be classified in any of the above genera. In order to determine the taxonomic position of this isolate, we studied its phenotypic and phylogenetic characteristics and compared our results with data for previously described

members of the family *Veillonellaceae*. From these results, we propose a novel genus and species, *Anaeroglobus geminatus* gen. nov., sp. nov.

Bacterial strain

Strain AIP 313.00^T was recovered from a post-operative fluid collection diagnosed 27 days after gastrectomy and oesophago-jejunal anastomosis in a 70-year-old woman hospitalized in a surgical unit from Montpellier University Hospital in June 2000. This collection was located near the anastomosis and was probably due to anastomosis suture slacking.

The fluid sample was streaked on Columbia sheep-blood agar and incubated for 4 days in an anaerobic jar with the AnaeroGen System (Oxoid Unipath). A mixed culture of two anaerobic bacteria was obtained, including a *Prevotella* sp. and an unidentified coccus with negative Gram-staining reaction. Moreover, three aerobic bacteria were recovered from aerobic cultures (coagulase-negative *Staphylococcus*, non-haemolytic and alpha-haemolytic streptococci).

The unidentified coccoid strain was maintained in trypticase/glucose/yeast extract (TGY) medium con-

Abbreviations: TGY, trypticase/glucose/yeast extract; WC, Wilkins-Chalgren.

The GenBank/EMBL/DBJ accession number for the 16S rDNA sequence of *Anaeroglobus geminatus* AIP 313.00^T is AF338413.

Fig. 1. Electron micrograph of negatively stained cells of strain AIP 313.00^T. Bar, 500 nm.

sisting of 3% (w/v) biotrypcase (bioMérieux), 0.5% glucose (Prolabo), 2% yeast extract (Difco), 0.05% L-cysteine hydrochloride (Prolabo) and 5 µg haemin ml⁻¹ (Calbiochem), under anaerobic conditions at 37 °C for 2–3 days in an anaerobic jar containing 95% H₂ and 5% CO₂ (v/v). Colony morphology and presumptive identification tests (Engelkirk *et al.*, 1992) were observed on Wilkins–Chalgren (WC) agar plates (Oxoid).

Electron microscopy

Cellular morphology was studied by examining cells grown on TGY for 48 h. Bacterial cells were adsorbed for 30 s on parlodion/carbon-coated grids. Negative staining was performed for 20 s with 2% uranyl acetate in aqueous solution. Micrographs were recorded with a JEOL 1010 electron microscope.

Colony and cell morphology

Colonies of the unknown bacterium appeared on WC blood agar after 2 days incubation. The colonies were circular, convex and translucent with a smooth surface and were about 0.5–1 mm in diameter, non-pigmented and non-haemolytic. The cells are coccoid to ellipsoidal, 0.5–1.1 µm in diameter, usually in pairs or, occasionally, in short chains (Fig. 1), Gram-negative after coloration, non-spore-forming and non-motile.

Biochemical characteristics

Biochemical reactions were performed according to the procedures described by Holdeman *et al.* (1977). Metabolic end products were assayed by quantitative gas chromatography as described previously (Carlier, 1985). A Rapid ID 32A kit (API bioMérieux) was used

for enzymic profile determination as recommended by the manufacturer. This strictly anaerobic coccus was unreactive in most of the conventional biochemical tests. No gas was produced in glucose agar deep cultures. Catalase activity and indole production were not detected. Gelatin was not liquefied and milk was not modified. Nitrate reduction was negative and lactate was not fermented. By presumptive identification tests, the strain was resistant to 5 µg vancomycin disc and susceptible to 1 mg kanamycin, 10 µg colistin, 4 µg metronidazole and bile discs. Acid was produced from galactose and mannose. Acid was not produced from aesculin, arabinose, cellobiose, fructose, glucose, glycerol, inositol, lactose, maltose, mannitol, melezitose, melibiose, raffinose, rhamnose, ribose, salicin, sorbitol, starch, sucrose, trehalose or xylose. Aesculin was not hydrolysed. The major metabolic end products (mmol l⁻¹) were acetic acid (2), propionic acid (0.6), isobutyric acid (0.6), butyric acid (1.5) and isovaleric acid (4). Lactic and succinic acids were not produced. The enzymic activity of this bacterium showed a positive reaction in the β-glucosidase test (API code 001000000). Tests that serve to distinguish the novel bacterium from other genera are given in Table 1.

DNA isolation and DNA base composition

Cells were disrupted with a French pressure cell. The DNA was purified on hydroxyapatite according to the procedure of Cashion *et al.* (1977) and hydrolysed with P1 nuclease. The nucleotides were dephosphorylated with bovine alkaline phosphatase (Mesbah *et al.*, 1989). The resulting deoxyribonucleosides were analysed by HPLC (adapted from Tamaoka & Komagata, 1984). The G + C content was calculated from the ratio of deoxyguanosine (dG) and thymidine (dT) according to the method of Mesbah *et al.* (1989).

16S rDNA sequencing and phylogenetic analysis

16S rDNA was selectively amplified from genomic DNA by PCR using 5'-GTGCTGCAGAGAGTTT-GATCCTGGCTCAG-3' (positions 8–36; *Escherichia coli* numbering) as the forward primer and 5'-CAGCGATCCTACGGGTACCTTGTTACGACTT-3' (positions 1478–1508; *E. coli* numbering) as the reverse primer. The PCR was carried out in 50 µl containing approximately 0.5 µg DNA template, 200 µM each primers, 200 µM each dNTP and 1 U *Taq* polymerase (Roche) in the appropriate reaction buffer. Temperature cycling was done by using 30 cycles of 1 min at 94 °C, 1 min at 65 °C and 2 min at 72 °C. The 1.4 kb PCR product was sequenced directly on an Applied Biosystems Automatic Sequencer (Genome Express) in both directions by using forward and reverse primers. A partial sequence of the 16S rRNA genes of strain AIP 313.00^T was determined. This almost-complete (1408 nt) 16S rRNA gene sequence was compared with known sequences by carrying out a BLAST search (Altschul *et al.*, 1997) in the GenBank and EMBL databases and in the Ribosomal Database

Table 1. Characteristics useful in differentiating the genera of the family Veillonellaceae

Data for *Megasphaera elsdenii*, *Veillonella* and *Acidaminococcus* were taken from Rogosa (1984). Data for *Megasphaera cerevisiae* are given elsewhere (Engelmann & Weiss, 1985).

Characteristic	<i>Anaeroglobus</i>	<i>Megasphaera</i>	<i>Veillonella</i>	<i>Acidaminococcus</i>
Cell diameter (µm)	0.5–1.1	1.7–2.6	0.3–0.5	0.6–1
Acid production from:				
Galactose	+	–	–	–
Mannose	+	–	–	–
Fermentation of lactate	–	+	+	–
Decarboxylation of succinate	–	–	+	–
Gas production	–	+	+	+
Metabolic end products*	A, P, IB, B, IV	A, P, B, V, C	A, P	A, B
DNA G+C content (mol %)	51.8	53.1–54.1	40.3–44.4	56.6

* A, Acetic acid; B, butyric acid; C, caproic acid; IB, isobutyric acid; IV, isovaleric acid; P, propionic acid; V, valeric acid.

Project II using SIMILARITY RANK and SUGGEST_TREE (Maidak *et al.*, 2001). The results indicated that the isolate belongs to the *Sporomusa* sub-branch of the Gram-positive bacteria, corresponding to clostridial cluster IX of Collins *et al.* (1994). However, this phyletic group contains organisms that exhibit both positive and negative Gram-staining reactions and several of them have a Gram-negative cell-wall type and a high G+C content. We have not examined the structure of the cell wall of strain AIP 313.00^T. Thus, though this bacterium was found to be Gram-negative after coloration, it is possible that it has the Gram-positive type of cell wall. However, this organism displayed resistance to vancomycin, which is characteristic of Gram-negative bacteria.

The phenotypic properties and G+C content (51.8 mol %) of strain AIP 313.00^T are consistent with those of other members of the *Sporomusa*–*Acidaminococcus*–*Megasphaera* phyletic group.

The 16S rDNA gene sequence (1408 nt) of strain AIP 313.00^T was then aligned against homologous sequences of selected type strains of the *Sporomusa* sub-branch and the best-matching sequences obtained with the BLAST program. The alignment was done using CLUSTAL W (Thompson *et al.*, 1994). A distance matrix was calculated and unrooted phylogenetic trees were constructed using the DNADIST and NEIGHBOR programs of the PHYLIP package, version 3.5c (Felsenstein, 1993). The significance of the nodes was tested by bootstrap analysis.

Sequence similarity calculations revealed that the closest known phylogenetic relatives of strain AIP 313.00^T were the Gram-negative cocci *Megasphaera* and *Veillonella*, two genera belonging to the family Veillonellaceae (approx. 87–91 % sequence similarity). However, the highest sequence identities were obtained from oral clone sequences BB166, CS025 and BS073, described by Paster *et al.* (2001) as *Megasphaera* spp.

Fig. 2. Neighbour-joining dendrogram of the 16S rRNA gene sequences of AIP 313.00^T, oral clones of *Megasphaera* spp. BB166, CS025 and BS073 and related type species of the *Sporomusa* sub-branch. The sequence of *Quinella ovalis* was used as an outgroup. Numbers above branches are bootstrap percentages from 1000 resampled datasets. Reference sequences were obtained from the GenBank/EMBL databases; accession numbers are given in parentheses. Bar, 5 % sequence difference.

(approx. 99, 93 and 93 %, respectively). These three sequences originated from cloning and sequencing of 16S rDNA from oral flora without isolation of bacterial strains.

Our phylogenetic analysis included other species from *Sporomusa* cluster IX, such as *Dialister pneumosintes*, *Sporomusa sphaeroides* and *Quinella ovalis* (Fig. 2). The topology of the phylogenetic tree showed that the closest relative of strain AIP 313.00^T is the oral clone

BB166. These taxa formed a cluster with a high bootstrap value (100%) that was related to another group consisting of oral clones CS025 and BS073. Together, they formed a well-separated branch (bootstrap value of 100%), which suggests that these sequences represent organisms that belong to the same genus but represent at least two different species.

On the basis of both phenotypic and phylogenetic characteristics, we therefore consider that the unknown bacterium should be classified as a novel genus and species, for which the name *Anaeroglobus geminatus* gen. nov., sp. nov. is proposed. We also suggest that the oral clones BB166, CS025 and BS073 belong to the genus *Anaeroglobus*. Clone BB166 probably represents another strain of *Anaeroglobus geminatus*.

Description of *Anaeroglobus* gen. nov.

Anaeroglobus (An.ae.ro.glo'bus. Gr. pref. *an* not; Gr. n. *aer* air; N.L. *anaero* not (living) in air; L. masc. n. *globus* sphere; N.L. masc. n. *Anaeroglobus* a sphere not living in air).

Cells are Gram-negative after coloration and coccoid to ellipsoidal, usually in pairs or, occasionally, in short chains, non-spore-forming, non-motile. Strictly anaerobic and weakly saccharolytic. Growth in broth media is poor. Catalase is negative. Metabolic end products in TGY are acetate, propionate, isobutyrate, butyrate and isovalerate. The DNA G+C content is 51.8 mol%. Members of the genus can be distinguished from other genera of the family *Veillonellaceae* by metabolic end products and 16S rDNA gene sequences. The type species is *Anaeroglobus geminatus*.

Description of *Anaeroglobus geminatus* sp. nov.

Anaeroglobus geminatus (ge.mi.na'tus. L. masc. adj. *geminatus* paired, double, referring to the twin cells of this organism).

Cells are Gram-negative after coloration, non-spore-forming cocci that occur in pairs or short chains. The cells are coccoid to ellipsoidal. Individual cells are about 0.5–1.1 µm in diameter. Colonies on blood agar after 2 days incubation are small, circular, convex, translucent with a smooth surface. Non-pigmented and non-haemolytic. Strictly anaerobic. Catalase, gelatinase, indole and nitrate-reduction tests are negative. With the exception of galactose and mannose, most of the usual carbohydrates are not fermented. The metabolic end products are acetic acid, propionic acid,

isobutyric acid, butyric acid and isovaleric acid. The G+C content is 51.8 mol%. Habitat: gastrointestinal tract, mouth.

The type strain is strain AIP 313.00^T (= CIP 106856^T = CCUG 44773^T).

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