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Oribacterium parvum sp. nov. and *Oribacterium asaccharolyticum* sp. nov., obligately anaerobic bacteria from the human oral cavity, and emended description of the genus *Oribacterium*

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Three strictly anaerobic, Gram-positive, non-spore-forming, rod-shaped, motile bacteria, designated strains ACB1^T, ACB7^T and ACB8, were isolated from human subgingival dental plaque. All strains required yeast extract for growth. Strains ACB1^T and ACB8 were able to grow on glucose, lactose, maltose, maltodextrin and raffinose; strain ACB7^T grew weakly on sucrose only. The growth temperature range was 30–42 °C with optimum growth at 37 °C. Major metabolic fermentation end products of strain ACB1^T were acetate and lactate; the only product of strains ACB7^T and ACB8 was acetate. Major fatty acids of strain ACB1^T were $C_{14:0}$, $C_{16:0}$, $C_{16:1}\omega7c$ dimethyl aldehyde (DMA) and $C_{18:1}\omega7c$ DMA. Major fatty acids of strain ACB7^T were $C_{12:0}$, $C_{14:0}$, $C_{16:0}$, $C_{16:1}\omega7c$ and $C_{16:1}\omega7c$ DMA. The hydrolysate of the peptidoglycan contained *meso*-diaminopimelic acid, indicating peptidoglycan type A1γ. Genomic DNA G+C content varied from 42 to 43.3 % between strains. According to 16S rRNA gene sequence phylogeny, strains ACB1^T, ACB8 and ACB7^T formed two separate branches within the genus *Oribacterium*, with 98.1–98.6 % sequence similarity to the type strain of the type species, *Oribacterium sinus*. Predicted DNA–DNA hybridization values between strains ACB1^T, ACB8, ACB7^T and *O. sinus* F0268 were $<$70 %. Based on distinct genotypic and phenotypic characteristics, strains ACB1^T and ACB8, and strain ACB7^T are considered to represent two distinct species of the genus *Oribacterium*, for which the names *Oribacterium parvum* sp. nov. and *Oribacterium asaccharolyticum* sp. nov. are proposed. The type strains are ACB1^T (=DSM 24637^T=HM-481^T=ATCC BAA-2638^T) and ACB7^T (=DSM 24638^T=HM-482^T=ATCC BAA-2639^T), respectively.

In this study, we report the characterization of three strictly anaerobic strains, designated ACB1^T, ACB7^T and ACB8, isolated from subgingival plaque obtained from a 25-year-old African American female.

The study protocol was approved by the Institutional Review Board of Northeastern University; informed consent was obtained from the subject. Novel isolates were enriched on liquid basic anaerobic medium (BM) supplemented with

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Abbreviations: DDH, DNA–DNA hybridization; DMA, dimethyl aldehyde; HOMD, Human Oral Microbiome Database; *meso*-Dpm, *meso*-diaminopimelic acid.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains ACB1^T, ACB7^T and ACB8 are HM120210, HM120211 and HM120212, respectively. The complete high-quality draft genome sequencing was performed as a part of the Human Microbiome Project by the Broad Institute of Harvard and MIT and available at http://www.broadinstitute.org/ and by the J. Craig Venter Institute. The GenBank/EMBL/DDBJ assembled genome sequences of strains ACB1^T, ACB7^T and ACB8 are NZ_AFZC00000000, NZ_AFZD00000000 and NZ_AJZT00000000, respectively.

One supplementary figure and one supplementary table are available with the online version of this paper.

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L-cysteine–HCl as a reducing agent and isolated in pure culture on agar-BM under an anaerobic atmosphere (2 % H_2, 1 % CO_2, 97 % N_2) (Sizova *et al.*, 2012). The Human Oral Microbiome Database (HOMD) classified the isolated strains as members of oral taxon 108 (Chen *et al.*, 2010). According to preliminary 16S rRNA gene sequence phylogeny, strains ACB1^T, ACB7^T and ACB8 belong to the genus *Oribacterium* (Carlier *et al.*, 2004) within the family *Lachnospiraceae* (Rainey, 2009).

Colony morphology assessment was performed on Wilkins-Chalgren (WC) blood agar and trypticase peptone-yeast extract (TY) agar medium. All media were supplemented with L-cysteine–HCl as a reducing agent (Sizova *et al.*, 2012). Cell morphology was observed with a Leica DMBL light microscope equipped with phase-contrast optics. For electron microscopy, cells grown on TY medium for 24–48 h were collected, fixed as described previously (Ellis, 2006; Sizova *et al.*, 2013) and observed with a Hitachi S4800 scanning electron microscope. Thin sections were stained with uranyl acetate and lead citrate and observed with a JEOL JEM 1010 transmission electron microscope. The Gram reaction was determined using the Difco Gram-stain kit. Resistance to various antibiotics and bile was tested with Oxoid 'AN-IDENT' and Remel susceptibility test discs; zones less than 10 mm were considered to indicate resistance. Oxidase, catalase and nitrate reduction activities were tested with Remel reagents. Biochemical reactions and individual carbon source utilization was assessed with API 20A tests and with cultures grown in liquid medium with glucose, lactose, maltose, sucrose, cellobiose, maltodextrin, raffinose or starch supplemented with yeast extract. The effect of temperature was assessed with cultures grown on TY medium. All experiments were conducted under anaerobic conditions; growth was scored as visible turbidity. Fermentation products were determined by HPLC in acidified supernatant of cultures grown on glucose-yeast extract and TY media before and after distillation [Agilent 1200 series HPLC; Poroshell 120 SB-C18 column 2.7 m, 3.0 × 100 mm with guard column (Agilent Technologies); 10 μM H_2SO_4 was used as the mobile phase]. Cell biomass grown in trypticase peptone-glucose-yeast extract (TYG) for 48 h was used for the whole-cell fatty acid and peptidoglycan analyses. Fatty acids were methylated, extracted and analysed by GC using the Sherlock Microbial Identification System at Microbial ID. The peptidoglycan structure was analysed in the hydrolysates (4 M HCl, 100 °C, 16 h) according to the method of Rhuland *et al.* (1955) and by GC/MS analysis after isolation of the peptidoglycan and its hydrolysis (Schumann, 2011) at the Identification Service of the German Collection of Microorganisms and Cell Cultures. 16S rRNA gene sequences were compared with those available from GenBank; phylogenetic analysis was performed as described previously (Sizova *et al.*, 2012). Whole genome sequencing of strains ACB1^T and ACB7^T was carried out by the Broad Institute of Harvard and MIT, and the data are available at http://www.broadinstitute.org/. The whole genome of strain ACB8 was sequenced by the J. Craig Venter Institute. The genome sequences of strains ACB1^T, ACB7^T and ACB8 were compared with available genome sequences of other members of the genus *Oribacterium*. Individual coding sequences were submitted to the Rapid Annotation Subsystem Technology (RAST) server (Aziz *et al.*, 2008) for subsystem annotations. DNA base content (mol% G+C) was calculated from the whole genome sequences. DNA–DNA hybridization (DDH) values were predicted by the Genome-to-Genome Distance calculator 2.0, formula 3, which is available online at http://ggdc.dsmz.de/ (Auch *et al.*, 2010a, b; Meier-Kolthoff *et al.*, 2013).

Cells of the three novel three strains were non-spore-forming, highly motile, oval rods, sometimes appearing swollen. Cells of strains ACB1^T and ACB8 were 1.2 ± 0.4 μm long (average ± SD) and 0.45 ± 0.09 μm wide; cells of strain ACB7^T were 1.6 ± 0.6 μm long and 0.50 ± 0.08 μm wide [Fig. 1, Fig. S1 (available in the online Supplementary Material), Table 1]. About 5 % of strain ACB7^T cells were curved rods up to 5–7 μm long. Cells appeared Gram-variable after staining, but were structurally Gram-positive (Fig. 1). Three to five RAST-annotated genes associated with synthesis of teichoic and lipoteichoic acids were present in the genome (Table 2). Surprisingly, we detected seven genes associated with synthesis of lipooligosaccharides in strain

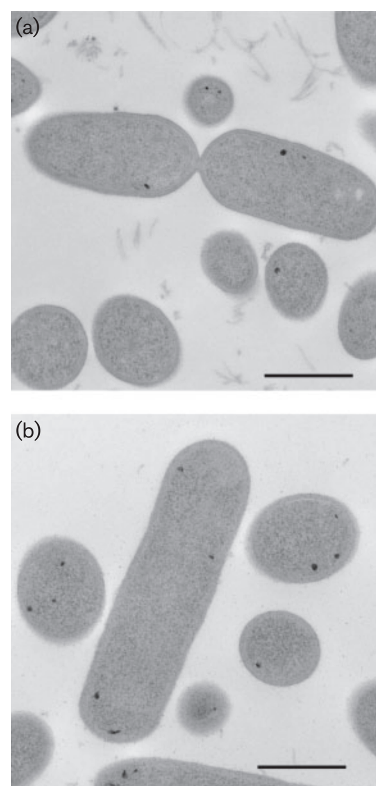

Fig. 1. Transmission electron micrograph of cells of strain ACB1^T (a) and strain ACB7^T (b). General morphology and Gram-positive cell structure of ultrathin sections is shown. Bars, 500 nm.

Table 1. Characteristics that differentiate strains ACB1^{T}and ACB8 and strain ACB7^T from *O. sinus* AIP 354.02^T (Carlier *et al.*, 2004)

Strains: 1, ACB1^T; 2, ACB8; 3, ACB7^T; 4, *O. sinus* AIP 354.02^T. ND, No data available.

Characteristic	1	2	3	4
Cell shape	Short ovoid rods in chains or aggregates, often swollen	Short ovoid rods in chains or aggregates, often swollen	Short ovoid to long curved rods occurring singly, in pairs or chains, often swollen	Ovoid rods occurring singly, in pairs or in chains
Cell size (μm)	$1.2 \pm 0.4 \times 0.45 \pm 0.09$	$1.2 \pm 0.4 \times 0.45 \pm 0.09$	$1.6 \pm 0.6 \times 0.5 \pm 0.08$	$1.7–2.2 \times 0.8–1.0$
Motility/flagella	+/Sublateral	+/Sublateral	+/Sublateral	+/Laterally inserted
Colony shape	Round, convex, beige	Round, convex, beige	Round, transparent, petite	Circular, convex, non-pigmented
Colony size after 48 h (mm)	1.5	1.5	0.5	1.5
Gram-stain	Variable	Variable	Variable	Negative
Temperature range (°C)	30–42	30–42	30–42	37
Assimilation of:				
D-Glucose	+	+	−	+
Lactose	+	+	−	−
Sucrose	−	+	+	+
Maltose	+	+	−	−
Maltodextrin	+	+	−	ND
Raffinose	+	+	−	+
Indole formation	−	ND	−	+
Urease	−	ND	−	ND
Oxidase	−	−	−	ND
Aesculin hydrolysis	−	ND	+	−
Gas formation from TGY medium	+	+	−	+
Black pigment on TY medium	+	+	+	ND
Resistance to:				
Kanamycin (1 mg)	−	ND	−	+
Erythromycin (60 µg)	−	ND	−	+
Metabolic end products*	A, L	A	A	A, L
DNA G+C content (mol%)	42.1	42	43.3	42.4
Peptidoglycan type	A1γ	ND	A1γ	ND
Major fatty acid methyl esters	$C_{14:0}$ $C_{16:0}$ $C_{16:1}\omega7c$ DMA $C_{18:1}\omega7c$ DMA	ND	$C_{12:0}$ $C_{14:0}$ $C_{16:0}$ $C_{16:1}\omega7c$ $C_{16:1}\omega7c$ DMA	$C_{14:0}$ anteiso-$C_{15:0}$ $C_{15:0}$ $C_{16:1}\omega9c$ $C_{16:0}$
Isolation source	Human subgingival plaque	Human subgingival plaque	Human subgingival plaque	Human maxillary sinus

*A, acetate; L, lactate.

ACB1^T, but not in strain ACB7^T, ACB8 or other *Oribacterium* strains with available genomes (Table 2). After 48 h of incubation on TY agar plates at 37 °C, strain ACB1^T formed beige, round, convex colonies 1–1.5 mm in diameter. Colonies of strain ACB7^T were round, non-pigmented and 0.5 mm in diameter after 48 h and umbonate and 2–3 mm in diameter with irregular wavy edges after 168 h. All three strains were non-haemolytic. After 1–2 days of incubation, strains ACB1^T, ACB7^T and ACB8 produced a diffusible black pigment in BM and TY liquid media supplemented with L-cysteine–HCl. The pigment was visible as a grainy substance surrounding cells (Fig. S1b). Most cells of strain ACB7^T and some cells of strain ACB1^T contained intracellular nanometre-sized particles (Fig. 1), probably of ferrous sulfide (Sizova *et al.*, 2013). Upon inspection of the genomes of strains ACB1^T, ACB8 and ACB7^T, we identified five to six genes putatively annotated as encoding the cysteine desulfurase enzyme EC 2.8.1.7 (Table S1). The activity of these genes probably explains the production of black precipitate, which is presumably FeS (Frazzon & Dean, 2003; Mihara & Esaki, 2002). Similar particles were previously observed in cells of another member of the family *Lachnospiraceae*, *Stomatobaculum longum*, which also contained a single gene

Table 2. Number of genes identified in the biosynthetic pathway from whole genome sequences in different members of the genus *Oribacterium* identified by the RAST server

Strains: 1, ACB1^T; 2, ACB8; 3, ACB7^T; 4, *O. sinus* F0268; 5, *Oribacterium* sp. OT 108 strain F0425; 6, *Oribacterium* sp. OT 078 strain F0262. OT, oral taxon as defined by HOMD. The number of genes identified for benzoquinones, naphthoquinones, mycolic acids and lipopolysaccharides was zero for all taxa studied.

Genes responsible for biosynthesis	1	2	3	4	5	6
Accession number	NZ_AFZC00000000	NZ_AJZT00000000	NZ_AFZD00000000	NZ_ACKX00000000	NZ_AFIH00000000	NZ_ACIQ00000000
Teichoic and lipoteichoic acids	3	4	5	4	5	4
Polar lipids	15	19	19	20	19	16
Polyamines	9	9	13	8	12	11
Diaminopimelic acid	6	6	7	7	7	7
Lipooligosaccharides	7	0	0	0	0	0

encoding a cysteine desulfurase enzyme in its genome (Sizova *et al.*, 2013).

The isolated strains grew only under strictly anaerobic conditions. Growth occurred at 30–42 °C, with optimum growth at 37 °C. Isolates ACB1^T and ACB7^T were susceptible to discs containing 1 mg kanamycin, 5 μg vancomycin, 50 μg metronidazole, 2 U penicillin, 15 mg rifampicin and 15 mg bile but resistant to 10 μg colistin. Catalase, oxidase and urease activities were negative; nitrate reduction was not detected. Gelatin was not liquefied and indole was not produced. Strain ACB7^T hydrolysed aesculin while strain ACB1^T did not. All strains were able to grow on yeast extract and Bacto proteose peptone No. 3 but not on Casamino acids or trypticase alone. Strain ACB1^T produced acid on API 20A media containing glucose, maltose and lactose, but not sucrose, arabinose, cellobiose, mannose, melezitose, raffinose, rhamnose, trehalose, xylose, glycerol, mannitol, salicin or sorbitol. In liquid medium supplemented with yeast extract at 0.5–2.0 g l^{-1}, strains ACB1^T and ACB8 weakly fermented glucose, lactose, maltose, maltodextrin and raffinose but not cellobiose or starch; strain ACB8 grew weakly on sucrose. Strain ACB7^T did not produce acid on API 20A media, and did not grow in liquid medium with any of the tested carbon sources with the exception of weak growth on sucrose (i.e. OD_{600} reached ~ 0.1 units after 7–10 days of incubation). No visible biomass was formed in medium with 0.5–2.0 g yeast extract l^{-1} only; poor growth was observed in liquid medium with 1 g yeast extract l^{-1} and 0.5 g L-cysteine–HCl l^{-1}. Strains ACB1^T and ACB8 produced gas in TY or TGY liquid media. The major metabolic end products of strain ACB1^T were acetate and lactate. Acetate was the only end product of strains ACB7^T and ACB8.

The whole-cell hydrolysate of strain ACB7^T contained *meso*-diaminopimelic acid (*meso*-Dpm); *meso*-Dpm was also detected in strains ACB1^T and ACB7^T after isolation and hydrolysis of the peptidoglycan. The occurrence of *meso*-Dpm in the novel strains indicated peptidoglycan type A1γ (or A1γ'; A31 or A32.1) according to http://www.peptidoglycan-types.info. Six and seven RAST-annotated genes associated with diaminopimelic acid synthesis were present in the genome of strains ACB1^T and ACB8, and ACB7^T, respectively (Table 2).

The genomic DNA G+C content of strains ACB1^T, ACB8 and ACB7^T was 42.1, 42.0 and 43.3 mol%, respectively. The fatty acid methyl ester profile showed that strain ACB1^T contained $C_{14:0}$ (18.6 %), $C_{16:0}$ (24.1 %), $C_{16:1}\omega7c$ dimethyl aldehyde (DMA) (18.1 %) and $C_{18:1}\omega7c$ DMA (6.1 %) as major fatty acids, minor amounts of $C_{14:0}$ DMA (4.8 %), $C_{15:0}$ (3.4 %), $C_{16:1}\omega7c$ (3.3 %) and $C_{16:0}$ DMA (3.7 %), and trace amounts of $C_{14:1}\omega7c$ DMA (1.65 %), anteiso-$C_{15:0}$ (0.6 %), $C_{15:1}\omega6c$ (0.3 %), $C_{16:0}$ aldehyde (1.1 %), $C_{16:1}\omega9c$ (0.2 %), $C_{16:1}\omega5c$ (0.55 %), $C_{17:1}\omega6c$ (0.4 %), $C_{17:0}$ (0.15 %), $C_{18:1}$ at 17.254 DMA (0.3 %), $C_{18:0}$ (0.1 %) and $C_{18:1}\omega9c$ DMA (0.1 %). Strain ACB7^T contained $C_{12:0}$ (5.4 %), $C_{14:0}$ (22.4 %), $C_{16:0}$ (15.7 %),

$C_{16:1}\omega7c$ (8.5 %) and $C_{16:1}\omega7c$ DMA (7.7 %) as major fatty acids, minor amounts of $C_{10:0}$ DMA (4.0 %), $C_{14:0}$ DMA (3.3 %), $C_{15:0}$ (4.2 %) and $C_{18:0}$ (3.1 %), and trace amounts of $C_{11:0}$ DMA (1.8 %), $C_{13:0}$ (1.3 %), $C_{14:1}\omega7c$ DMA (0.85 %), anteiso-$C_{15:0}$ (0.5 %), $C_{16:0}$ aldehyde (1.2 %), $C_{16:0}$ DMA (1.2 %), $C_{18:1}\omega9c$ (1.4 %), $C_{18:1}\omega9c$ DMA (1.1 %) and $C_{18:1}\omega7c$ DMA (0.8 %).

In whole genomes of strains ACB1^T, ACB7^T and ACB8 we found no predicted gene sequences with recognizable homology to biosynthesis of lipoquinones, mycolic acids or lipopolysaccharides. Nine to 13 RAST-annotated genes associated with metabolism of polyamines, and 15–19 genes associated with metabolism of polar lipids, were present in the genomes (Table 2).

The 16S rRNA gene-based phylogenetic tree showed that strain ACB1^T, together with ACB8, and strain ACB7^T formed two separate branches within the genus *Oribacterium* (Fig. 2). The genus *Oribacterium* comprises, at the time of writing, a single recognized species *Oribacterium sinus* (Carlier *et al.*, 2004; Rainey, 2009). Other known strains (Tables 2, 3 and S1) were reported in GenBank and HOMD (http://www.homd.org/index.php). Strains ACB1^T and ACB8 were closely related to each other at 99.7 % 16S rRNA gene sequence similarity and shared 98.5–98.6 % similarity with *O. sinus* AIP 354.02^T (Carlier *et al.*, 2004) and 98.71–98.85 % with *O. sinus* strain F0268 (Dewhirst *et al.*, 2010). Strain ACB7^T shared 98.1 % similarity with *O. sinus* AIP 354.02^T and 98.7 % with strain F0268 (Table 3). The levels of 16S rRNA gene sequence similarity between strains ACB1^T, ACB7^T and *O. sinus* AIP 354.02^T were below the 98.7 and 98.65 % cut-off values proposed for species demarcation by Stackebrandt & Ebers (2006) and Kim *et al.* (2014), respectively. The predicted DDH value between strains ACB1^T and ACB8 was 98.2 % (Table 3). The predicted DDH value between strains ACB1^T and ACB7^T was 22.6 % and between strains ACB8 and ACB7^T was 23.1 %, much less than the threshold of 70 %, the widely accepted value of relatedness between different species (Gevers *et al.*, 2005; Tindall *et al.*, 2010; Yarza *et al.*, 2008). Predicted DDH values suggested that strains ACB1^T and ACB8 represent the same species, whereas strain ACB7^T belongs to a different species. Predicted DDH values between strains ACB1^T, ACB8 and *O. sinus* F0268 and between strain ACB7^T and *O. sinus* F0268 were only 15.6 and 15.7 %, respectively, clearly indicating three separate species (Table 3). Pair-wise comparison of 16S rRNA gene sequence similarities with predicted DDH values of six *Oribacterium* strains revealed that 16S rRNA gene sequence similarity values less than 99.5 % corresponded to DDH values less then 22.3 % (Table 3). The 16S rRNA gene sequence similarity value of 99.5 % between species within a genus is higher than the reported range of 98.2–99.0 % (Kim *et al.*, 2014; Meier-Kolthoff *et al.*, 2013; Stackebrandt & Ebers, 2006; Yarza *et al.*, 2008). However, there are genera such as *Brucella*, *Burkholderia*, *Bacillus*, *Brevundimonas*, *Escherichia*, *Salmonella* and *Shigella* that contain separate species that share 100 % 16S rRNA gene sequence similarity (Ash *et al.*, 1991; Fukushima *et al.*, 2002; Gee *et al.*, 2004; Gevers *et al.*, 2005; Jaspers & Overmann, 2004).

Tables 1–3 summarize physiological and genomic properties that can be used to differentiate strains ACB1^T, ACB7^T and ACB8 from *O. sinus* and other members of the genus. The type strain of *O. sinus* (Carlier *et al.*, 2004) was isolated from sinus pus of a 6-year-old child with bilateral

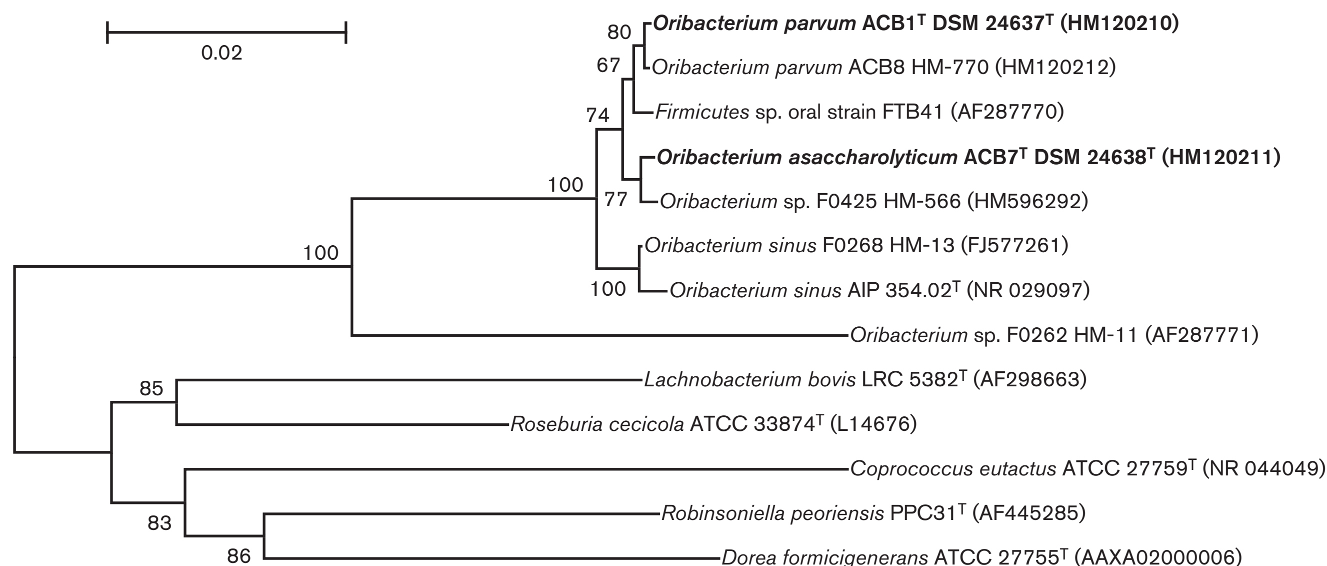

Fig. 2. Minimum-evolution phylogenetic tree based on 16S rRNA gene sequence comparisons of strains ACB1^{T}and ACB8, strain ACB7^T, and other members of the genus *Oribacterium* and the family *Lachnospiraceae*. Bootstrap values >50 % calculated for 1000 subsets are shown at branch points. Bar, 0.02 substitutions per position.

Table 3. Levels of 16S rRNA gene sequence similarity (above) and predicted DDH values (below) between different members of the genus *Oribacterium*

Strains: *Oribacterium parvum* strains ACB1^{T} and ACB8; *O. asaccharolyticum* strain ACB7^{T}; *O. sinus* strain F0268; *O. sinus* strain AIP 354.02^{T}; *Oribacterium* sp. OT 108 strain FTB41; *Oribacterium* sp. OT 078 strain F0262; *Oribacterium* sp. OT 108 strain F0425. OT, oral taxon as defined by HOMD. ND, Not determined.

Strain	Accession no.	Number of bases (top)/Genome size (Mb) (bottom)	ACB1^{T}	ACB7^{T}	ACB8	AIP 354.02^{T}	F0268	FTB41	F0425
16S rRNA gene sequence similarity (%)									
ACB1^{T}	HM120210	1395							
ACB7^{T}	HM120211	1436	98.9						
ACB8	HM120212	1397	99.7	99					
AIP 354.02^{T}	NR_029097	1374	98.6	98.1	98.5				
F0268	FJ577261	1497	98.9	98.7	99	99.4			
FTB41	AF287770	1444	99.4	98.9	98.7	98.2	98.5		
F0425	HM596292	1497	99.3	99.5	99.4	98.2	98.6	99.2	
F0262	FJ577249	1498	93.8	93.5	93.6	93.7	93.6	93.6	93.4
Predicted DDH value (%)									
ACB1^{T}	NZ_AFZC00000000	2.47							
ACB7^{T}	NZ_AFZD00000000	2.52	22.6						
ACB8	NZ_AJZT00000000	2.48	98.2	23.1					
AIP 354.02^{T}	ND	ND	ND	ND	ND				
F0268	NZ_ACKX0000000	2.71	15.6	15.7	15.6	ND			
FTB41	ND	ND	ND	ND	ND	ND	ND		
F0425	NZ_AFIH00000000	2.52	23.1	80.5	23.2	ND	15.8	ND	
F0262	NZ_ACIQ00000000	2.68	12.9	12.9	12.9	ND	12.9	ND	12.9

maxillary sinusitis, while strains ACB1^{T}, ACB7^{T} and ACB8 were enriched from a non-infectious subgingival plaque sampled from a generally healthy 25-year-old adult. Cells of *O. sinus* were 0.8–1.0 μm wide compared with 0.45–0.5 μm for cells of strains ACB1^{T}, ACB8 and ACB7^{T}. In contrast to *O. sinus*, the three novel strains did not produce indole. Strains ACB1^{T} and ACB8 fermented lactose and maltose while the type strain of *O. sinus* and strain ACB7^{T} did not. *O. sinus* and strains ACB1^{T} and ACB8 used glucose and raffinose as carbon sources, while strain ACB7^{T} did not. The only strain that hydrolysed aesculin was ACB7^{T}. Major metabolic end products of *O. sinus* and strain ACB1^{T} were acetate and lactate; strains ACB7^{T} and ACB8 produced acetate only.

The fatty acid methyl ester profile, spectrum of utilized carbon sources, DNA G+C content, metabolic end products, as well as the number of annotated genes responsible for biosynthesis of teichoic and lipoteichoic acids, polar lipids, polyamines, diaminopimelic acid and lypooligosaccharides distinguish strains ACB1^{T}, ACB8 and ACB7^{T} from *O. sinus*.

On the basis of physiological, biochemical and molecular properties, we suggest that the strains described in this study represent two novel species, for which we propose the names *Oribacterium parvum* sp. nov. to accommodate strains ACB1^{T} and ACB8 and *Oribacterium asaccharolyticum* sp. nov. to accommodate strain ACB7^{T}.

Emended description of *Oribacterium* (Carlier *et al.*, 2004)

Elongated ovoid rods, about 1.2–2.2 μm long and 0.45–1 μm wide, usually occurring singly, in pairs or, occasionally, in short chains. Motile with laterally inserted flagella. Gram-positive but may appear Gram-negative after staining. Strictly anaerobic. Do not form spores. Weakly fermentative. Major metabolic end products are acetate and lactate or acetate only. Major (>10 %) fatty acids are $C_{14:0}$, $C_{16:0}$ and anteiso-$C_{15:0}$ or $C_{16:1}\omega7c$ DMA. DNA G+C content is 42.1–43.3 mol%. Phylogenetically related to members of the family *Lachnospiraceae*. The type species is *Oribacterium sinus*.

Description of *Oribacterium parvum* sp. nov.

Oribacterium parvum (par′vum. L. neut. adj. *parvum* small, little).

Cells are Gram-variable after staining but structurally Gram-positive, short, motile, ovoid rods, 1.2 × 0.45 μm, sometimes swollen, occurring singly, in chains or as aggregates. Colonies are round, convex, beige, 1.5 mm in diameter and non-haemolytic on WC agar. Black pigment

is produced in TY or TGY liquid medium supplemented with L-cysteine–HCl. Yeast extract is required for growth on glucose, lactose, maltose, maltodextrin and raffinose liquid media; gas is produced. Indole is not produced. Gelatin is not liquefied. Aesculin is not hydrolysed. Catalase, oxidase and urease are negative. Nitrate is not reduced. Susceptible to kanamycin, vancomycin, metronidazole, penicillin, rifampicin and bile but resistant to colistin. The DNA G+C content is 42.0–42.1 mol%. The major metabolic end products are acetate and lactate. Growth temperature range is 30–42 °C. Major fatty acids are $C_{14:0}$, $C_{16:0}$, $C_{16:1}\omega7c$ DMA and $C_{18:1}\omega7c$ DMA. The peptidoglycan type is A1γ.

The type strain, ACB1^T (=DSM 24637^T=HM-481^T=ATCC BAA-2638^T), was isolated from human subgingival dental plaque. ACB8, isolated from a similar source, is a second strain of the species.

Description of *Oribacterium asaccharolyticum* sp. nov.

Oribacterium asaccharolyticum [a.sac.cha.ro.ly′ti.cum. Gr. pref. *a* not; Gr. n. *saccharon* sugar; N.L. neut. adj. *lyticum* able to lyse (from Gr. adj. *lytikos* able to lose); N.L. neut. adj. *asaccharolyticum* not digesting sugar].

Cells are Gram-variable after staining but structurally Gram-positive, motile rods 1.6 × 0.5 μm, some cells up to 5–7 μm long, often swollen, occurring singly, in pairs or in chains. Colonies are non-haemolytic, round, non-pigmented and 0.5 mm in diameter after 48 h and umbonate and 2–3 mm in diameter with irregular wavy edges after 168 h. Growth is supported by yeast extract and Bacto proteose peptone. Black pigment is produced in liquid medium supplemented with L-cysteine–HCl; gas is not produced. The lowest concentrations of yeast extract and L-cysteine–HCl required for visible growth are 1 and 0.5 g l^{-1}, respectively. In liquid medium growth is not supported by glucose, maltose, lactose, cellobiose, maltodextrin, raffinose or starch; sucrose supports poor growth. Gelatin is not liquefied. Aesculin is hydrolysed. Catalase, oxidase and urease are negative. Nitrate is not reduced. Susceptible to kanamycin, vancomycin, metronidazole, penicillin, rifampicin and bile but resistant to colistin. The major metabolic end product is acetate. Growth temperature range is 30–42 °C. Major fatty acids are $C_{12:0}$, $C_{14:0}$, $C_{16:0}$, $C_{16:1}\omega7c$ and $C_{16:1}\omega7c$ DMA. The peptidoglycan type is A1γ.

The type strain, ACB7^T (=DSM 24638^T=HM-482^T=ATCC BAA-2639^T), was isolated from human subgingival dental plaque. The DNA G+C content of the type strain is 43.3 mol%.

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