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Characterization of novel human oral isolates and cloned 16S rDNA sequences that fall in the family *Coriobacteriaceae*: description of *Olsenella* gen. nov., reclassification of *Lactobacillus uli* as *Olsenella uli* comb. nov. and description of *Olsenella profusa* sp. nov.

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The diversity of organisms present in the subgingival pockets of patients with periodontitis and acute necrotizing ulcerative gingivitis (ANUG) were examined previously. The 16S rRNA genes of subgingival plaque bacteria were amplified using PCR with a universal forward primer and a spirochaete-selective reverse primer. The amplified DNA was cloned into *Escherichia coli*. In one subject with ANUG, 70 clones were sequenced. Seventy-five per cent of the clones were spirochaetal, as expected. Twelve of the remaining clones fell into two clusters that represent novel phylotypes in the family *Coriobacteriaceae*. The first novel phylotype was most closely related to *Atopobium rimae* (98% similarity). The phylotype probably represents a novel *Atopobium* species, but will not be named until cultivable strains are obtained. The second novel phylotype was only 91% similar to described *Atopobium* species and 84% similar to *Coriobacterium glomerans*. The 16S rRNA sequences of the type strain of *Lactobacillus uli* and a strain representing the Moores' *Eubacterium* group D52 were determined as part of on ongoing sequence analysis of oral bacteria. The sequence for *L. uli* was more than 99·8% similar to sequences for the second clone phylotype. It therefore appears that the second clone phylotype and *L. uli* represent the same species. The sequence for the *Eubacterium* D52 strain was 95·6% similar to that of *L. uli*. The G+C content of the DNA of *L. uli and Eubacterium* D52 is 63–64 mol%. These organisms are thus distinct from the neighbouring genus *Atopobium*, which has a DNA G+C content of 35–46 mol%. A new genus, *Olsenella* gen. nov., is proposed for these two species on the basis of phenotypic characteristics and 16S rRNA sequence analysis to include *Olsenella uli* comb. nov. and *Olsenella profusa* sp. nov.

Keywords: *Atopobium*, *Coriobacteriaceae*, *Olsenella*, rRNA, phylogeny

INTRODUCTION

This study is part of our ongoing efforts to determine the 16S rRNA sequences of essentially all of the 500–600 cultivable and uncultivable species found in the human oral cavity. In a previous study, we examined the diversity of cultivable and uncultivable oral spirochaetes in patients with severe periodontal disease by cloning and sequencing 16S rRNA genes that were amplified by PCR from DNA extracted from subgingival plaque samples (Dewhirst *et al.*, 2000).

Abbreviations: FAA, fastidious anaerobe agar; PYG, peptone/yeast extract/glucose.

The GenBank accession numbers for the 16S rDNA sequences determined in this study are AF287760 and AF292371–AF292375.

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Using a 16S rRNA universal forward primer and a spirochaete-selective reverse primer, approximately 75% of the clones obtained were found to represent *Treponema* species, on the basis of sequence analysis. The majority of the non-spirochaete clones were closely related to the genus *Atopobium* in the family *Coriobacteriaceae*. The purpose of this study was to clarify the taxonomy and phylogeny of human oral microbes in the family *Coriobacteriaceae*.

The genus *Atopobium* was created by Collins & Wallbanks (1992) for the phylogenetically misplaced species *Lactobacillus minutus*, *Lactobacillus rimae* and *Streptococcus parvulus*. 16S rRNA sequence analysis demonstrated that *Atopobium minutum*, *Atopobium rimae* and *Atopobium parvulum* were closely related to one another, and they were considered a distinct line of descent within the lactic acid bacteria. While the G+C content of these species ranged from 35 to 46 mol%, suggesting that the genus is part of the low-G+C Gram-positive bacteria, they actually represent a deep branch within the high-G+C Gram-positive bacteria, the *Actinobacteria* (Stackebrandt *et al*., 1997; Stackebrandt & Ludwig, 1994). The family *Coriobacteriaceae* contains several genera: *Atopobium* (Collins & Wallbanks, 1992), *Coriobacterium* (Haas & König, 1988), *Collinsella* (Kageyama *et al*., 1999a), *Cryptobacterium* (Nakazawa *et al*., 1999), *Slackia*, *Eggerthella* (Wade *et al*., 1999) and *Denitrobacterium* (Anderson *et al*., 2000). As part of our analysis of cultivable human oral bacteria, we have found that the type strain of *Lactobacillus uli* falls into the family *Coriobacteriaceae* on the basis of 16S rRNA sequence analysis.

In the course of their careers at the Virginia Polytechnic Institute (VPI), W.E.C. Moore and L.V.H. Moore established a large collection of gut and oral bacteria. They placed their isolates into hundreds of groups on the basis of a large range of phenotypic tests including fatty acid methyl ester (FAME) analysis (Holdeman *et al*., 1977). Many of the groups were named formally before they retired. However, strains representing over 200 unnamed taxa remain in the collection, which was distributed to a few colleagues. One of these groups, *Eubacterium* D52, was also found to fall within the *Coriobacteriaceae* and was therefore included in this study.

METHODS

Sample collection. Plaque samples were obtained from a patient with acute necrotizing ulcerative gingivitis (ANUG). After removal of supragingival plaque using a sterile Gracey curette, subgingival plaque samples were removed from four lower anterior sites using individual sterile Gracey curettes and suspended directly in 100 µl of 250 mM Tris/HCl, pH 7. The four samples were examined immediately by phase microscopy to identify the sites with the highest proportion and diversity of spirochaetes.

Bacterial strains. *L. uli* ATCC 49627^{T} was obtained from the ATCC. *Eubacterium* D52 strains D315A-29 and D288A-6, originally isolated from subgingival plaque of subjects with adult periodontitis and characterized by W.E.C. and L.V.H. Moore, were obtained from Anne Tanner (Forsyth Institute). Bacteria were maintained on fastidious anaerobe agar (FAA; LabM) supplemented with 5% horse blood incubated under anaerobic conditions.

Biochemical tests. Fermentation tests were performed using pre-reduced, anaerobically sterilized sugars according to the methods of Holdeman *et al*. (1977) except that the pre-reduced, anaerobically sterilized media were prepared in an anaerobic workstation using pre-reduced distilled water. Other biochemical tests were performed as described by Holdeman *et al*. (1977) and Summanen *et al*. (1993).

Metabolic end-product analysis. Bacterial strains were grown in peptone/yeast extract/glucose (PYG) broth and short-chain volatile and non-volatile fatty acids were extracted by standard methods (Holdeman *et al*., 1977). Analysis was performed by GC with a capillary column coated with CP-Wax 58 solid phase.

Enzyme profiles. Enzyme profiles were generated with the Rapid ID32A anaerobe identification kit (bioMérieux) according to the manufacturer's instructions. Bacteria were harvested from blood agar plates [blood agar base no. 2 (LabM) plus 5% horse blood] incubated anaerobically at 37 °C for 72 h.

DNA isolation and PCR amplification. DNA was released from the plaque samples by adding 5 µl plaque suspension to 15 µl Gene Releaser (BioVentures) and following the manufacturer's microwave protocol. PCR was performed in thin-walled tubes with a Perkin-Elmer 480 thermal cycler, GeneAmp PCR reagent kit and AmpliWax PCR Gem 100s. Forward primer C75 (*Escherichia coli* positions 7–27; 5′-GAGAGTTTGATYCTGGCTCAG-3′) and reverse primer C90 (1501–1483; 5′-GTTACGACTTCACCCTCCT-3′) were used. The 20 µl from the Gene Releaser step was combined with 1 µM primers and other reagents in the Hot Start protocol suggested by Perkin-Elmer. The following conditions were used for amplification: denaturation at 72 °C for 45 s, annealing at 60 °C for 45 s and elongation at 72 °C for 45 s, with 5 s added for each elongation step. Thirty cycles were performed, followed by a final elongation step at 72 °C for 15 min. The purity of the product was determined by electrophoresis in a 1% agarose gel. DNA was stained with ethidium bromide and viewed under long-wavelength UV light.

Cloning. Cloning of PCR-amplified DNA was performed using either a TA cloning kit (Invitrogen) or Prime PCR Cloner cloning system (5 Prime–3 Prime) following the manufacturers' instructions.

Library screening. Up to 100 white clones were picked for each reaction. The sizes of inserts were determined by PCR using flanking vector primers followed by electrophoresis on a 1·5% agarose gel.

Purification of PCR products. The amplified DNA was purified by precipitation with PEG 8000 (Kusukawa *et al*., 1990). After removal of Ampliwax, 0·6 vols 20% PEG 8000 (Sigma) in 2·5 M NaCl was added and the mixture was incubated at 37 °C for 10 min. The sample was centrifuged for 15 min at 15000 ***g*** and the pellet was washed with 80% ethanol and pelleted as before. The pellet was air-dried and dissolved in 30 µl distilled water and used for cycle sequencing as described below.

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Table 1. Strains included in analysis of the family *Coriobacteriaceae*

Species	Collection no.	16S rDNA GenBank accession no.	Reference
Atopobium fossor	ATCC 43386^T	L34620	Kageyama *et al.* (1999b)
Atopobium minutum	ATCC 33267^T	X67148	Collins & Wallbanks (1992)
Atopobium parvulum	ATCC 33793^T	AF292372	This paper
Atopobium rimae	ATCC 49626^T	AF292371	This paper
Atopobium sp. ANUG-C19	Clone	AF287760	This paper
Atopobium vaginae	CCUG 38953^T	Y17195	Rodriguez Jovita *et al.* (1999)
Collinsella aerofaciens	JCM 10188^T	AB011816	Kageyama *et al.* (1999a)
Collinsella aerofaciens	JCM 7791	AB011815	Kageyama *et al.* (1999a)
Coriobacterium glomerans	ATCC 49209^T	X79048	Haas & König (1988)
Cryptobacterium curtum	ATCC 700683^T	AB019260	Nakazawa *et al.* (1999)
Denitrobacterium detoxificans	ATCC 700546^T	U43492	Anderson *et al.* (2000)
Eggerthella lenta	ATCC 25559^T	AF292375	This paper
Olsenella uli	ATCC 49627^T	AF292373	This paper
Olsenella uli ANUG-D15	Clone	Similar to above	This paper
Olsenella profusa D315A-29	DSM 13989^T	AF292374	This paper
Olsenella profusa D288A-6	None	Similar to above	This paper
Olsenella sp.	Clone, cow rumen	AB034002	GenBank, unpublished
Olsenella sp.	Clone, cow rumen	AB034096	GenBank, unpublished
'*Olsenella oviles*' A2	None	AJ251324	GenBank, unpublished
Slackia exigua	ATCC 700122^T	AF101240	Wade *et al.* (1999)
Slackia heliotrinireducens	NCTC 11029^T	AF101241	Wade *et al.* (1999)

Sequencing methods. The DNA sample from PCR was sequenced directly using a cycle-sequencing kit (*fmol* DNA Sequencing System; Promega). The manufacturer's protocol was followed. The eight sequencing primers used have been reported previously (Mendes *et al.*, 1996). Primers were end-labelled with ^{33}P (NEN/Dupont) using the manufacturer's protocol. Approximately 100 ng purified DNA from the PCR was used for sequencing. Reaction products were loaded onto 8 % polyacrylamide/urea gels, electrophoresed and detected by exposure to X-ray film for 24 h.

16S rRNA data analysis. A program set for data entry, editing, sequence alignment, secondary structure comparison, similarity matrix generation and dendrogram construction for 16S rRNA data was written in Microsoft Quick BASIC for use on PC-compatible computers (Paster & Dewhirst, 1988). RNA sequences were entered and aligned as described previously (Paster & Dewhirst, 1988). Our sequence database contains approximately 1000 sequences determined in our laboratory and another 500 obtained from GenBank or the Ribosomal Database Project (Maidak *et al.*, 2000). Strains used in the 16S rRNA analysis are given in Table 1. Similarity matrices were constructed from the aligned sequences by using only those sequence positions for which 90 % of the strains had data. The similarity matrices were corrected for multiple base changes at single positions by the method of Jukes & Cantor (1969). Phylogenetic trees were constructed using the neighbour-joining method of Saitou & Nei (1987). PHYLIP version 3.5 was used for maximum-likelihood analysis (Felsenstein, 1993).

Estimation of G+C content of DNA. The G+C content of DNA was estimated by an HPLC method, as described previously (Wade *et al.*, 1999).

Nucleic acid sequence accession numbers. The sequences for each of the strains examined in this report have been deposited in GenBank under the accession numbers listed in Table 1.

RESULTS AND DISCUSSION

Essentially complete 16S rRNA sequences were determined for those species listed in Table 1. Corrections and additions were made to the sequences of the type strains of *A. rimae* and *A. parvulum* and were deposited in GenBank (see Table 1). A phylogenetic tree generated by the neighbour-joining method is presented in Fig. 1. When the species with short sequences, *Atopobium vaginae*, *Spherobacter thermophilus*, *Bifidobacterium longum* and *Bifidobacterium infantis*, were excluded, trees of the same topology were obtained using both the neighbour-joining and maximum-likelihood methods. Examination of the target sequence for the spirochaete-selective reverse 16S rRNA primer shows that the clones, as well as all members of the family *Coriobacteriaceae*, have a sequence identical to spirochaetes at the primer site and should therefore amplify with primer C90.

Analysis of clones

The clone sequences fell into two tight clusters. Representative of the first group is clone ANUG-C19, which had 98 % sequence similarity to the type strain of *A. rimae*. This sequence difference is at a level that probably indicates a novel species. However, since we currently have no cultivable strains representing this group, we will not formally name it, but rather give it

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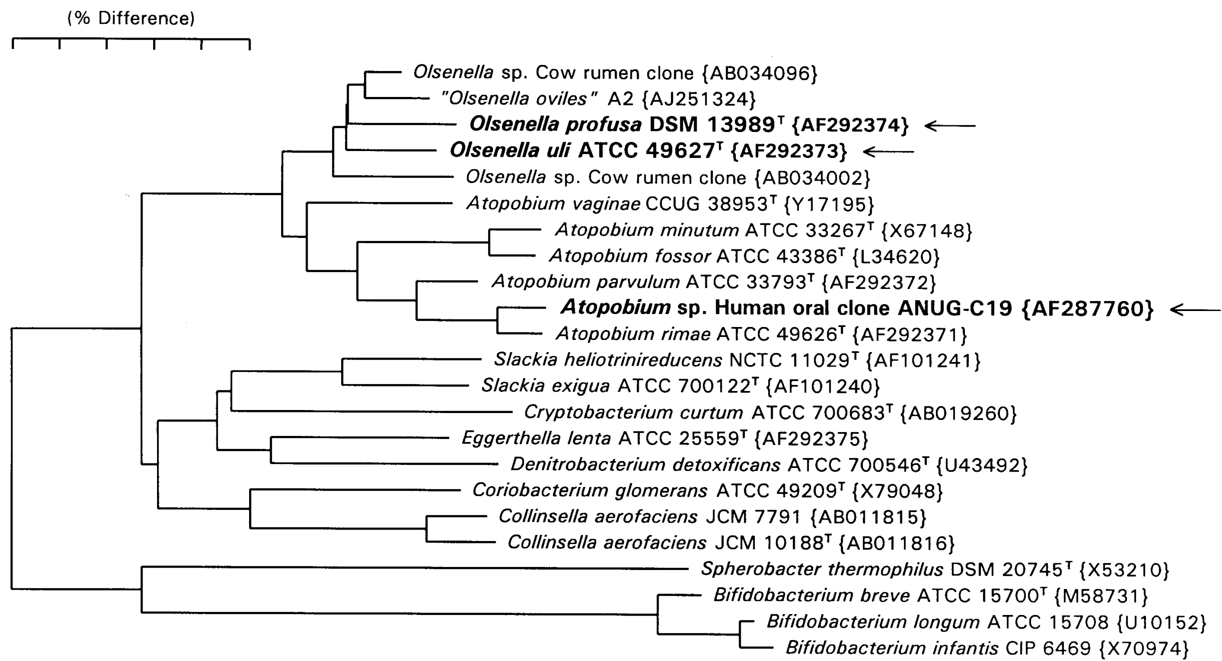

Fig. 1. 16S rDNA-based phylogenetic tree for the family *Coriobacteriaceae*. Bar, 5% difference in nucleotide sequences as estimated using the correction of Jukes & Cantor (1969). Distances are determined by measuring the lengths of the horizontal lines connecting two sequences. Sequence accession numbers are given in brackets.

the informal designation *Atopobium* sp. clone ANUG-C19.

The second clone group, represented by clone ANUG-D15, had more than 99·8% sequence similarity to the type strain of *L. uli*. The three-base difference is within species variation for 16S rRNA sequences. The G+C content of the DNA of *L. uli* was redetermined in this study and is 63–64 mol%. This is substantially above the 53 mol% reported previously (Olsen *et al*., 1991). The higher G+C content clearly places *L. uli* outside the genus *Atopobium*, species of which have G+C contents ranging from 35 to 46 mol%. In order to accommodate this species, we propose the genus *Olsenella* gen. nov. and rename *L. uli* as *Olsenella uli* comb. nov. The type species of the two genera, *A. minutum* and *O. uli*, share only 91% 16S rDNA sequence similarity (Fig. 1).

Spirochaete libraries from three other patients have subsequently been analysed and they contained clones identified as *A. rimae* (96 clones) and *A. parvulum* (3 clones). We have not detected additional clones for group ANUG-C19.

VPI *Eubacterium* D52

The genus *Eubacterium* was long a taxonomic dumping ground for Gram-positive, obligately anaerobic, chemo-organotrophic rods that produce large amounts of butyric, acetic or formic acids or no major fatty acids and that don't fit into other genera. The VPI *Eubacterium* D52 group was established on the basis of isolates having the general phenotypic characteristics of the genus *Eubacterium*, and isolates were then subdivided into groups on the basis of FAME analysis (Moore *et al*., 1994). Many species placed in the genus, such as *Eubacterium exiguum*, have been removed and placed in other genera (in this case, as *Slackia exigua*; Wade *et al*., 1999). Full sequencing of 16S rDNA from strain D315A-29 indicated that it is unrelated to *Eubacterium limosum*, but rather 96% similar to *O. uli* (Fig. 1). Strain D288A-6 had a sequence identical to that of strain D315A-29 over 500 bases at the 5′ end of the gene. Phenotypic analysis confirmed that group D52 strains represent a novel species related to *O. uli*. We propose the name *Olsenella profusa* sp. nov. for VPI *Eubacterium* group D52. The phenotypic traits that differentiate *O. uli* from *O. profusa* are given in Table 2.

GenBank sequences of organisms related to the genus *Olsenella*

A search of GenBank using the *O. uli* sequence revealed three sequences from rumen clones or isolates that may represent additional species in the genus *Olsenella*: AB034002, AB034096 and AJ251324. The first two sequences are from rumen clones and the last sequence is from a rumen isolate that has been given the informal name '*Atopobium oviles*'. The sequence from '*Atopobium oviles*' has 97·3% mean similarity to those of *O. uli* and *O. profusa*, but only 93·5% mean similarity to sequences of *Atopobium* species. The informal name of this organism should be changed to

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Table 2. Differential characteristics of *Olsenella uli* and *Olsenella profusa*

Data for *O. uli* were taken from Olsen *et al.* (1991). Colony morphology was determined after 7 d on FAA. d, 5–30 % strains positive.

Test	*O. uli* (48 strains)	*O. profusa* (2 strains)
Rapid ID 32A profile	2012033705	4516053705
Growth in PYG broth	Poor (1+)	Good (3–4+)
Stimulation of growth by Tween 80	Marked	Slight
Arginine hydrolysis	+	−
Fermentation of:		
Arabinose	−	+
Cellobiose	d	+
Lactose	d	+
Mannitol	−	+
Melibiose	−	+
Raffinose	−	+
Colony morphology	Raised, grey, semi-translucent	Pyramidal, cream, opaque

Table 3. Differentiation of genera in the family *Coriobacteriaceae*

Character	*Olsenella*	*Atopobium*	*Slackia*	*Cryptobacterium*	*Eggerthella*	*Denitrobacterium*	*Coriobacterium*	*Collinsella*
Glucose fermentation	+	+	−	−	−	−	+	+
Metabolic end-products*	a, L, (s)	a, L, (s)	(a)	None	(a, l, s)	ND	a, l	a, l
Growth stimulated by:								
Tween 80	+	+	−	−	−	ND	ND	+
Arginine	−	−	+	+	+	ND	ND	ND
Growth in 20 % bile	v	−	−	−	+	ND	ND	ND
Nitrate reduction	−	−	v	−	+	v	ND	−
Hydrogen production	v	−	ND	ND	−	+	+	+
Cell wall peptidoglycan type	ND	A3γ, A4α, A4βp	ND	ND	A3γ	ND	A4α	A4β
Major cellular fatty acids	C18:1 *cis*9†	C18:1 *cis*9	C18:1	ND	C14:0 br, C15:0 br	C14:0 br, C15:0 br	ND	ND
DNA G+C content (mol %)	63–64	35–46	60–64	50–51	62	56–60	60–61	60–61
Source	Human oral cavity	Mammals	Human oral cavity	Human oral cavity	Human intestine	Bovine rumen	Red soldier beetle intestine	Human intestine

* a, Acetic acid; l, lactic acid; s, succinic acid. Capital letters indicate major products. Products in parentheses indicate strain variation.

† Determined for *O. uli* but not for *O. profusa*.

ND, Not determined; v, variable; br, branched.

'*Olsenella oviles*'. The phylogenetic positions of these sequences are shown in Fig. 1. The taxonomy of the rumen organisms will require the isolation of additional strains and further study before formal naming is appropriate.

Differentiation of genera in the family *Coriobacteriaceae*

In recent years, five genera have been added to the family *Coriobacteriaceae* (Anderson *et al.*, 2000; Kageyama *et al.*, 1999a; Nakazawa *et al.*, 1999; Wade *et al.*, 1999). Unfortunately, because these organisms came from diverse habitats, widely differing methods were used for phenotypic characterization. As shown in Table 3, almost no phenotypic characters have been determined for all genera. As investigators extend research on each of these genera, it is hoped that all of the phenotypic tests listed in Table 3 can be determined. The menaquinone content of species in the *Coriobacteriaceae* should be examined to determine whether the unique dimethylmenaquinone present in *Eggerthella lenta* is also present in other genera (Collins *et al.*, 1985). This is a very distinct and potentially valuable chemotaxonomic marker. *Olsenella* can be differentiated from *Atopobium* by G+C content of the DNA and from *Collinsella* by site of origin. *Olsenella* can be differentiated from *Slackia* and *Eggerthella* by being fermentative and from *Cryptobacterium* by the production of acetic and lactic acids and the stimulation of growth by Tween 80 rather than by arginine. The remaining genera, *Denitrobacterium* and *Coriobacterium*, have not been recovered from humans.

16S rRNA base signatures

In their proposal for a new hierarchic classification of the *Actinobacteria*, Stackebrandt and co-workers identified signature nucleotides for each taxa. The subclass, order and family were characterized by

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Table 4. 16S rRNA base signatures

Taxa are identified as: 1, *Olsenella*; 2, *Atopobium*; 3, *Collinsella*; 4, *Coriobacterium*; 5, *Denitrobacterium*; 6, *Eggerthella*; 7, *Cryptobacterium*; 8, *Slackia*; 9, *Coriobacteriaceae*. Base signatures in bold are present in at least 95% of the *Coriobacteriaceae* and fewer than 15% of other bacteria. When two bases are listed, the bases pair with one another in a helix. Base positions are numbered relative to *E. coli*. The number of species with signature/total number of species in taxon is given. Two strains of *Collinsella aerofaciens* were included, as they differ significantly.

No.	Base(s)	Position(s)	1	2	3	4	5	6	7	8	9	Bacteria
1	C–G	113–314	5/5	6/6	2/2	1/1	0/1	0/1	0/1	0/2	14/19	< 5%
2	**G–C**	**294–303**	**5/5**	**6/6**	**2/2**	**1/1**	**0/1**	**1/1**	**1/1**	**2/2**	**18/19**	**None**
3	U–A	295–302	5/5	6/6	2/2	1/1	0/1	1/1	1/1	0/2	16/19	< 5%
4	**C–G**	**407–435***	**5/5**	**6/6**	**2/2**	**1/1**	**1/1**	**1/1**	**1/1**	**2/2**	**19/19**	**< 15%**
5	**G–C**	**613–627***	**5/5**	**6/6**	**2/2**	**1/1**	**1/1**	**1/1**	**1/1**	**2/2**	**19/19**	**< 5%**
6	G–C	670–736	5/5	6/6	2/2	1/1	1/1	1/1	1/1	2/2	19/19	> 50%
7	**U–A**	**771–808**	**5/5**	**6/6**	**2/2**	**1/1**	**0/1**	**1/1**	**1/1**	**2/2**	**18/19**	**< 15%**
8	A–U	772–807	5/5	6/6	0/2	0/1	0/1	1/1	0/1	0/2	12/19	< 15%
9	A–U	823–877	5/5	6/6	2/2	1/1	0/1	1/1	0/1	0/2	15/19	> 50%
10	**A–U**	**941–1342**	**5/5**	**6/6**	**2/2**	**1/1**	**1/1**	**1/1**	**1/1**	**2/2**	**19/19**	**< 5%†**
11	U–G	950–1231	5/5	6/6	2/2	1/1	1/1	1/1	1/1	2/2	19/19	> 90%
12	G–C	1120–1153	5/5	6/6	2/2	1/1	0/1	0/1	1/1	2/2	17/19	< 30%
13	C	1148	5/5	6/6	2/2	1/1	0/1	0/1	0/1	0/2	14/19	None
14	C–G	1165–1171	5/5	6/6	2/2	1/1	1/1	1/1	1/1	2/2	19/19	< 30%
15	G–C	1242–1295	4/5	4/6	2/2	1/1	1/1	1/1	0/1	2/2	15/19	< 5%‡
16	G–C	1313–1324	5/5	6/6	2/2	1/1	1/1	1/1	1/1	2/2	19/19	< 30%
17	**C**	**1321***	**5/5**	**5/6**	**2/2**	**1/1**	**1/1**	**1/1**	**1/1**	**2/2**	**18/19**	**< 5%**
18	A–U	1410–1490	5/5	6/6	2/2	1/1	1/1	1/1	1/1	2/2	19/19	> 50%
19	**C–G**	**1415–1485***	**5/5**	**6/6**	**2/2**	**1/1**	**1/1**	**1/1**	**1/1**	**2/2**	**19/19**	**Spirochaetes**
20	**C–G**	**1416–1484***	**5/5**	**6/6**	**2/2**	**1/1**	**1/1**	**1/1**	**1/1**	**2/2**	**19/19**	**Spirochaetes**

* Signatures not included by Stackebrandt *et al.* (1997).

† Clostridia, 50%.

‡ G at position 1242 is present in essentially all bacteria. The C present in most members of the *Coriobacteriaceae* at position 1295 is rare in other bacteria, which have a U.

possession of 15 base signatures (Stackebrandt *et al.*, 1997). Their analysis was based on sequence information for three *Atopobium* species and one *Coriobacterium* species. The base signatures have been re-examined on the basis of full sequences for 20 strains falling in the eight genera of the *Coriobacteriaceae* (Table 4). Seven of the previously identified signatures do not apply to 93% of species (signatures 1, 3, 8, 9, 12, 13, 15 in Table 4). The previously identified signatures were chosen on the basis of aligned *Actinobacteria* sequences. Our examination of the aligned sequences identified five additional base signatures for the family *Coriobacteriaceae*. Our signature analysis was performed using 60 representatives of all of the major phyla of bacteria. The previously selected signatures varied widely in their presence in *Bacteria*. Several signatures should be discarded as common to most bacteria. The signatures in bold in Table 4 are present in at least 93% of members of the *Coriobacteriaceae* and in fewer than 15% of other bacteria. The bases GG at positions 1484 and 1485 are common in the *Archaea*, but are not found in the *Bacteria* except in the spirochaetes, the *Thermotogales*, the order *Haloanaerobiales* and the family *Coriobacteriaceae*. These bases were present in the PCR primer used in our cloning experiments.

Description of *Olsenella* gen. nov.

Olsenella (Ol.sen.el′la. L. fem. dim. ending -*ella*, N.L. fem. n. *Olsenella* of Olsen, named to honour Ingar Olsen, a contemporary Norwegian microbiologist, who first described *Lactobacillus uli*).

Cells are small, elliptical, Gram-positive, non-motile rods that occur singly, in pairs and in short chains. Spores are not formed. Cells are strictly anaerobic. Fermentation products from glucose are lactic and acetic acids. Cells do not produce catalase, urease or indole or reduce nitrate. The major fatty acid of the cell membrane is C18:1 *cis*9. The G+C content of the DNA is 63–64 mol%. Found in the human oral cavity and likely in bovine rumen. The type species is *Olsenella uli*.

Description of *Olsenella uli* comb. nov.

Olsenella uli (u′li. Gr. n. *oulon* the gum; N.L. gen. n. *uli* of the gum).

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Basonym: *Lactobacillus uli* Olsen *et al*. 1991.

Characteristics of the species are as described previously by Olsen *et al*. (1991) with the following additions and corrections. The G+C content of the DNA for the type strain is 64 mol%. The Rapid ID32A profile is 2012033705. The GenBank accession number for the 16S rRNA sequence is AF292373. The type strain is strain VPI D76D-27C^{T} (=ATCC 49627^{T}).

Description of *Olsenella profusa* sp. nov.

Olsenella profusa (pro.fus′a. L. adj. *profusus* profuse, referring to the good growth of the organism).

Previously designated *Eubacterium* group D52 by W. E. C. and L. V. H. Moore. The description is based on two strains isolated from the oral cavity. Cells are obligately anaerobic, non-spore-forming, non-motile, Gram-positive, short rods, 0·6×0·8–2·0 µm. Cells occur singly or in chains. After 7 d of incubation on FAA plates, colonies are approximately 1–1·3 mm in diameter, circular, entire, pulvinate to pyramidal, cream coloured and opaque when viewed under a plate microscope. Growth in broth media is good and is stimulated only slightly by addition of Tween 80. Arabinose, cellobiose, fructose, glucose, lactose, maltose, mannitol, mannose, melibiose, raffinose, salicin, sucrose and trehalose are fermented. Melezitose and rhamnose are not fermented. Aesculin is hydrolysed but arginine and starch are not. Gelatin is not liquefied and meat is not digested. Catalase, indole and H_2S are not produced and nitrate is not reduced. Acetate and lactate are produced as end products of metabolism. The Rapid ID32A profile is 4516053705. The G+C content of the DNA of the type strain is 64 mol%.

The type strain is D315A-29^{T} (=DSM 13989^{T}). The GenBank accession number for the 16S rRNA sequence is AF292374. Isolated from human subgingival plaque in adults with periodontitis.

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