Olsenella umbonata sp. nov., a microaerotolerant anaerobic lactic acid bacterium from the sheep rumen and pig jejunum, and emended descriptions of *Olsenella*, *Olsenella uli* and *Olsenella profusa*

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Strain A2 is an anaerobic, variably Gram-stain-positive, non-spore-forming, small and irregularly rod-shaped bacterium from the ruminal fluid of a sheep that has been described informally as a representative of '*Olsenella* (basonym *Atopobium*) *oviles*'. Three phenotypically similar bacterial strains (lac15, lac16 and lac31^T) were isolated in concert with *Veillonella magna* lac18^T from the mucosal jejunum of a pig. A phylogenetic analysis based on 16S rRNA gene sequences revealed that strains A2, lac15, lac16 and lac31^T formed a genetically coherent group (100 % interstrain sequence similarity) within the bigeneric *Olsenella–Atopobium* branch of the family *Coriobacteriaceae*, class *Actinobacteria*. This group was most closely related to the type strains of the two recognized *Olsenella* species, namely *Olsenella uli* (sequence similarity of 96.85 %) and *Olsenella profusa* (sequence similarity of 97.20 %). The sequence similarity to the type strain of *Atopobium minutum*, the type species of the genus *Atopobium*, was 92.33 %. Unlike those of *O. uli* and *O. profusa*, outgrown colonies of strains A2, lac15, lac16 and lac31^T were opaque and greyish-white with an umbonate elevation on solid culture media. The four novel strains were characterized as being well-adapted and presumably indigenous to the gastrointestinal tract of homoeothermic vertebrates: they were mesophilic, microaerotolerant, neutrophilic and acidotolerant, bile-resistant, mucin-utilizing and markedly peptidolytic lactic acid bacteria. The results of DNA–DNA hybridizations, cellular fatty acid analysis and other differential phenotypic (physiological and biochemical) tests confirmed that strains A2, lac15, lac16 and lac31^T represent a novel species of the genus *Olsenella*. On the basis of the genotypic and phenotypic results, we therefore describe *Olsenella umbonata* sp. nov., with lac31^T (=CCUG 58604^T =DSM 22620^T =JCM 16156^T) as the type strain and A2 (=CCUG 58212 =DSM 22619 =JCM 16157) as an additionally available reference strain. Also, based on our data, we propose emended descriptions of the genus *Olsenella* and the species *Olsenella uli* and *Olsenella profusa*.

The genera *Olsenella* Dewhirst *et al.* 2001, *Atopobium* Collins and Wallbanks 1993 and *Bifidobacterium* Orla-Jensen 1924 constitute the high-G+C-content group of the lactic acid bacteria *sensu lato* (Inês *et al.*, 2008). The bacteria in this group are defined as members of the class *Actinobacteria* meeting the core phenotypic criteria of Orla-Jensen (1919): they are Gram-stain-positive, non-motile, non-spore-forming rods or cocci that ferment carbohydrates to predominant (*Olsenella*, *Atopobium*) or important (*Bifidobacterium*) amounts of lactic acid.

At the time of writing, the genus *Olsenella* comprises two species with validly published names: *Olsenella uli* Dewhirst *et al.* 2001 (basonym *Lactobacillus uli* Olsen *et al.* 1991) and *Olsenella profusa* Dewhirst *et al.* 2001 (previously desig-

Abbreviations: ME, minimum-evolution; MP, maximum-parsimony; NJ, neighbour-joining; URAS, unreduced aerobically sterilized.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains A2, lac15, lac16 and lac31^T are respectively AJ251324, FN178461, FN178462 and FN178463.

Four supplementary figures and two supplementary tables are available with the online version of this paper.

nated *Eubacterium* group D52 by W. E. C. Moore and L. V. H. Moore). These species were based on strains from gingival and subgingival sites in humans with periodontitis. A third species with one strain (A2) from the rumen of a sheep had been informally named '*Atopobium oviles*' (Eschenlauer *et al.*, 2002) and was renamed '*Olsenella oviles*' by Dewhirst *et al.* (2001).

According to Dewhirst *et al.* (2001), the human oral cavity is the main habitat and the bovine rumen a likely habitat of the olsenellae. Isolates of *O. uli* and *O. profusa* are regularly recovered from disease sites in the human mouth (Munson *et al.*, 2002, 2004; Hooper *et al.*, 2006) and sometimes from blood of humans with local oral or gastrointestinal infections (Lau *et al.*, 2004; Bahrani-Mougeot *et al.*, 2008). Olsenellae are found in the healthy (Ozutsumi *et al.*, 2005; Cho *et al.*, 2006; Hernandez *et al.*, 2008) and acidotic (Tajima *et al.*, 2000) bovine rumen. Molecular-genetic studies have also reported the detection of *Olsenella*-related clones in the gastrointestinal tracts of humans (Martinez-Medina *et al.*, 2006; Khachatryan *et al.*, 2008; Krogius-Kurikka *et al.*, 2009), pigs (Leser *et al.*, 2002; Tsukahara & Ushida, 2002; Dowd *et al.*, 2008), wallabies (Chhour *et al.*, 2008) and chickens (Lu *et al.*, 2003) and in diverse anaerobic environmental sites (Bowman *et al.*, 2006; Wongtanet *et al.*, 2007; Weiss *et al.*, 2008; Rivière *et al.*, 2009). This indicates that the habitats of the olsenellae generally comprise the oral cavity and gastrointestinal tract of homoeothermic vertebrates and that non-animal habitats also exist.

Strain A2 was isolated from the ruminal fluid of a sheep at the Rowett Institute of Nutrition and Health in 1994. The methods and results of isolation, 16S rRNA gene sequence-based identification and initial phenotypic characterization were described in detail by Eschenlauer *et al.* (2002). Strains lac15, lac16 and lac31^T were isolated from the mucosal jejunum of a pig that also harboured lac18^T, the type strain of *Veillonella magna*, at the Institute of Animal Nutrition in 2007. The methods of isolation and initial 16S rRNA gene sequence-based phylogenetic analysis using primers 27f and 1492r were described by Kraatz & Taras (2008).

Further genotypic studies included PCR amplification and direct sequencing of the 16S rRNA gene using the *Coriobacteriaceae*-suited primer pair C75 and C90 (Dewhirst *et al.*, 2001). DNA was extracted from cells with a NucleoSpin Tissue kit (Macherey-Nagel). PCR was performed in a T1 Thermocycler (Biometra) using a HotStarTaq Master Mix kit (Qiagen). PCR products were purified with a High Pure PCR product purification kit (Roche). Sequencing was performed commercially by primer-walking (Eurofins MWG Operon) with C75 and C90 as starting primers. Nearly full-length (1428–1434 bp) 16S rRNA gene sequences of the four novel strains and of *O. uli* DSM 7084^T, *O. profusa* strains DSM 13989^T, CCUG 45371^T and CCUG 45372 and *Atopobium minutum* DSM 20586^T were obtained and submitted to EMBL (accession nos AJ251324 and FN178461–FN178468). Phylogenetic sequence analysis was conducted using the online Ribosomal Database Project II classification algorithm (Cole *et al.*, 2009), the online NCBI BLASTN algorithm (Johnson *et al.*, 2008), MEGA software version 4.1 (Tamura *et al.*, 2007) and CONSENSE from the PHYLIP software package version 3.69 (Felsenstein, 2010). In MEGA4, alignments of the newly determined sequences and 14 related sequences retrieved from GenBank were carried out using the CLUSTAL W tool (Thompson *et al.*, 1994). Pairwise evolutionary distances were computed by using the maximum composite likelihood method (Tamura *et al.*, 2004) with the Tamura–Nei nucleotide substitution model (Tamura & Nei, 1993) after complete deletion of positions with gaps or missing data. Phylogenetic trees were inferred using maximum-parsimony (MP) (Fitch, 1971), neighbour-joining (NJ) (Saitou & Nei, 1987) and minimum-evolution (ME) (Rzhetsky & Nei, 1992) methods. The bootstrap method (Felsenstein, 1985) was always used with 1000 replicates to test the statistical reliability of the trees. Genomic DNA–DNA reassociation analysis was carried out using hybridization protocols described by Urdiain *et al.* (2008). Labelled reference DNA of strain lac31^T or *O. profusa* CCUG 45371^T was hybridized to unlabelled target DNA of strain A2, *O. profusa* CCUG 45372 and *O. uli* CCUG 31166^T (as well as to homologous unlabelled DNA). Each hybridization mixture contained 150 ng reference DNA and 15 μg target DNA in a total volume of 72 μl. The mixtures were incubated for 16 h at 71 °C (i.e. at 30 °C below the melting-point temperature of DNA with a G+C content of 63–64 mol% reported by Dewhirst *et al.*, 2001).

The results of 16S rRNA gene sequence-based phylogenetic analysis revealed that the strains lac15, lac16, lac31^T and A2 belonged to the genus *Olsenella* and formed a genetically coherent group (100 % interstrain sequence similarity) within the bigeneric *Olsenella–Atopobium* branch of the family *Coriobacteriaceae*, class *Actinobacteria* (Fig. 1). *O. uli* and *O. profusa* were the most closely related recognized species (sequence similarities of 96.85 and 97.20 % to the respective type strains), and the type strain of *A. minutum*, the type species of the genus *Atopobium*, was a more distant relative (92.33 %). The sequence similarity with respect to *Coriobacterium glomerans*, the type species of the *Coriobacteriaceae*, was 88.30–88.31 %. The genetic distinctness of the novel group within the bigeneric *Olsenella–Atopobium* branch was supported by bootstrap values of 88–92 % (mean, 90 %; n=10) in the MP consensus tree (Fig. 1). Bootstrap values were lower in the NJ and ME analyses [49–51 and 51–53 % (means, 50 and 52 %; n=10), respectively], although they still confirmed the branching pattern of the MP tree (Supplementary Fig. S1, available in IJSEM Online). The results of DNA–DNA hybridization are presented in Supplementary Table S1. Relative reassociation of DNA of strain lac31^T was maximal (102.5 %) with DNA of strain A2 and approximately 50 % with respect to DNA of strains of *O. uli* (47.3 %) and *O. profusa* (50.2 and 51.9 %). DNA of *O. profusa* CCUG

45371^T exhibited less than 50 % relative reassociation with DNA of strains lac31^T and A2 (32.7 and 40.0 %, respectively). Altogether, the DNA–DNA relatedness between the novel strains lac31^T and A2 and recognized strains of *O. uli* and *O. profusa* was clearly below the 70 % cut-off value recommended for species delineation (Wayne *et al.*, 1987; Stackebrandt *et al.*, 2002; Achtman & Wagner, 2008). As indicated by the results of 16S rRNA gene sequence-based phylogenetic analysis, the novel strains were related very closely to each other and were related more closely to *O. profusa* than to *O. uli*.

Phenotypic studies were performed using the following culture media: modified peptone-yeast extract-glucose (PYG) medium (DSMZ medium 104 including salt solution 104; http://www.dsmz.de) as the standard, unreduced PYG broth (DSMZ medium 104 without cysteine hydrochloride), peptone-yeast extract (PY) broth (DSMZ medium 104 without glucose), M2 liquid medium containing clarified rumen fluid (Hobson, 1969), fastidious anaerobe agar (FAA) [FAA (Lab M) plus 5 % defibrinated horse blood (Oxoid)], blood agar [blood agar base no. 2 (Lab M) plus 5 % defibrinated horse blood (Oxoid)], an unsupportive basal medium (UBM), porcine gastric mucin (PGM) agar, porcine gastric mucus-mucosa (PGMM) agar and porcine jejunal mucus-mucosa (PJMM) agar; the last three media contained UBM [DSMZ medium 104 without PYG and meat extract and including (l^{-1}) 3.0 g NaCl, 0.18 g $MgSO_4.7H_2O$ and 0.01 g $FeSO_4.7H_2O$, together with 1 ml trace element solution and 0.2 vitamin solution described by Kraatz & Taras (2008)] supplemented with 1 % (w/v) mucin from porcine stomach (type III; Sigma) (PGM; pH 7) or mucus and mucosa from the stomachs (PGMM; pH 5) or jejuna (PJMM; pH 6.5) of healthy pigs. Unless otherwise stated, cultivations were performed at 37 °C. Anaerobic [i.e. anoxic, CO_2-enriched (18 % v/v)], microaerobic [i.e. oxygen-reduced (5–7 % v/v) and CO_2-enriched (8–10 % v/v)] and aerobic cultivations on agar media were performed in anaerobic jars using Anaerocult A gas packs, Anaerocult C gas packs (both from Merck) and no gas packs, respectively. Anaerobic and microaerobic cultivations in broth media were respectively performed in anaerobically sterilized (nitrogen-purged) and aerobically sterilized (unpurged) rubber-stoppered glass tubes (110 × 15 mm). Growth in broth media was monitored by measuring the OD_{600} with an Ultrospec 2000 spectrophotometer (Pharmacia Biotech). Studies were usually carried out with five (analyses using API kits) or three (other analyses) repetitions for each strain.

Morphology was studied using light and scanning electron microscopy with cells grown anaerobically on FAA and PYG agar for 48 h and 7 days and on PGM, PGMM and PJMM agar for 14 days. Cell size was determined from digital scanning electron micrographs using the measurement tool of the Adobe Photoshop CS4 Extended software. Morphological studies and studies on motility, spore formation, cytochrome oxidase activity and nitrate reduction were conducted as described previously (Kraatz & Taras, 2008). The oxygen relationship was determined using anaerobic cultures on PYG agar and in PYG broth, microaerobic cultures on PYG agar and in unreduced aerobically sterilized (URAS) PYG broth and aerobic cultures on PYG agar. Oxygen levels in the culture media were assessed by adding 1 mg resazurin sodium salt (Sigma) l^{-1} as redox indicator (orange or red colour indicative of the reduced or oxidized form of resazurin in anaerobic or microaerobic and aerobic PYG medium). Referring to the approach of Karakashev *et al.* (2003), assessment of growth and changed redox status in microaerobic URAS liquid media with resazurin allows further differentiation of obligately anaerobic bacteria (Winn *et al.*, 2006) into microaerotolerant (moderately obligate) anaerobes and strictly obligate anaerobes. URAS PYG broth cultures of the novel strains and strains of *O. uli* and *O. profusa* were prepared as 7.5 ml volumes with an initial OD_{600} of 0.03–0.18 (mean, 0.09; n=24) above that of the uninoculated controls. Analyses of growth at

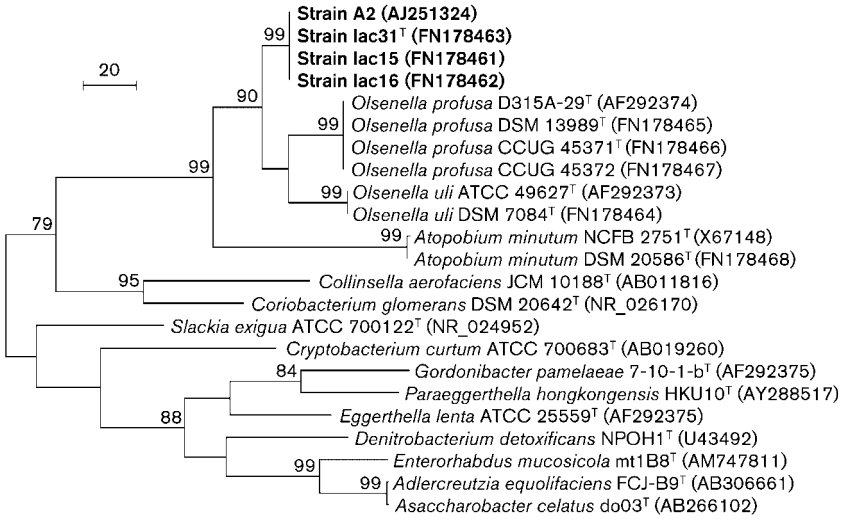

Fig. 1. Maximum-parsimony tree reconstructed using partial 16S rRNA gene sequences showing the phylogenetic relationship of strains lac15, lac16, lac31^T and A2 to other members of the bigeneric *Olsenella*–*Atopobium* branch and the family *Coriobacteriaceae*, including all type species. The tree is the unrooted consensus of 1000 bootstrap replicates. Bootstrap percentages greater than 70 % are given at branch points. Bar, 20 base changes between nodes.

different temperatures (21, 30, 37 and 45 °C) and pH (initial pH 2.0–9.5 at intervals of 0.5 pH units), growth resistance tests with 20 % bile (Jousimies-Somer *et al.*, 2002) or 6.5 % (w/v) NaCl and growth stimulation tests with 0.1 % (v/v) Tween 80 or 0.5 % (w/v) L-arginine were carried out using anaerobic cultures in PYG broth. Production of hydrogen sulfide from meat peptone or L-cysteine was studied using anaerobic cultures in UBM broth enriched with trypticase peptone and glucose and supplemented with 0.5 g ferric ammonium citrate l^{-1} and 10 g meat peptone or 0.73 g L-cysteine l^{-1}. Production of gas from 1 % (w/v) glucose was examined using anaerobic stab cultures in PYG agar deeps following the method for anaerobes of Smibert & Krieg (1994). For analysis of hydrogen peroxide production (Juárez Tomás *et al.*, 2004), cells were grown on PYG agar without resazurin and supplemented with 0.01 g horseradish peroxidase (type VI-A; Sigma) l^{-1} and 20 ml TMB (3,3′,5,5′-tetramethylbenzidine; Sigma) solution [25 mg TMB (ml $\geqslant$99.8 % ethanol)$^{-1}$] l^{-1}. Hydrogen peroxide assays were incubated anaerobically for 7 days and then exposed to air under light-protected conditions at room temperature for 24 h and evaluated as described by Otero & Nader-Macías (2006). Biochemical and enzyme profiles were determined with the API 20 A, Rapid ID 32 A and API ZYM kits (bioMérieux) according to the manufacturer's instructions. Inocula were prepared using anaerobic 24-h-old (API 20 A) or, in accordance with Dewhirst *et al.* (2001), 72-h-old (Rapid ID 32 A and API ZYM) cultures on blood agar. API 20 A tests were evaluated after 48 h of incubation. Growth on and fermentation of carbohydrates not included or with ambiguous results in API 20 A were analysed using anaerobic cultures in PY broth supplemented with 1 % (w/v) inulin or 20 mM raffinose and in UBM broth enriched with trypticase peptone and supplemented with 20 mM D-fructose, D-xylose or α-L-rhamnose or 10 mM melibiose.

Production of short-chain volatile and non-volatile fatty acids and ammonium was analysed using anaerobic cultures in PYG broth and microaerobic cultures in URAS PYG broth. Meat extract was omitted for analysis of lactic acids. Volatile fatty acids were determined using GC analysis as described previously (Schäfer, 1995). Non-volatile fatty acids were determined using HPLC analysis and (D- and L-lactic acid) /or (succinic acid) enzymic absorbance-based tests. HPLC analysis was carried out on an HP 1100 Series system (Agilent Technologies) equipped with a 150 × 4.6 mm analytical column coated with *N*,*S*-dioctyl-D-penicillamine and a 4.0 × 2.0 mm SecurityGuard C18 guard column (both from Phenomenex). The columns were operated at 35 °C. The eluents were solutions of $CuSO_4$ in (A) water (0.5 mmol l^{-1}) and (B) water with 5 % 2-propanol (2.5 mmol l^{-1}) in a gradient mode (0–35 % B over 17 min, 35 % B for 2 min, 35–100 % B over 3 min, 100 % B for 1 min, 100–35 % B over 1 min) with a flow rate of 1.0 ml min^{-1}. The total injection volume for analysis was 20 µl of 40 % (v/v) culture supernatant in eluent A. Compounds were detected by UV at 254 nm and identified by comparison against retention times using a lithium-DL-lactate standard (AppliChem). Chromeleon chromatography software (Dionex) was used for quantification. Enzymic absorbance-based tests (R-Biopharm) were applied according to the manufacturer's instructions using an Ultrospec 3300 pro UV/visible spectrophotometer (Amersham Biosciences) at 365 nm. Ammonium was measured electrometrically using a DC218-NH4 ammonium electrode in combination with a SevenMulti S80 ion meter (both from Mettler Toledo). For analysis of cellular fatty acids, bacteria were grown anaerobically overnight in 100 ml M2 liquid medium. Saponification, fatty acid methylation and analysis of fatty acid methyl esters were carried out as described previously (Devillard *et al.*, 2006). Three independent cultures, each grown in the same batch of M2, were always analysed for each strain. For some strains, the analysis was repeated using triplicate cultures in another, different batch of the medium (see Supplementary Table S2).

The results of phenotypic characterization are given in the species description. Phenotypic characteristics that serve to differentiate strains A2, lac15, lac16 and lac31^T from the type and reference strains of *O. uli* and *O. profusa* are given in Tables 1 and 2 and Supplementary Table S2. On the basis of the results of 16S rRNA gene sequencing, DNA–DNA reassociation analyses and the differential phenotypic data, strains A2 [formerly named '*Olsenella* (basonym *Atopobium*) *oviles*'], lac15, lac16 and lac31^T represent a novel species of the genus *Olsenella*, for which we propose the name *Olsenella umbonata* sp. nov. On the basis of the data from this study, we also propose emended descriptions of the genus *Olsenella* and the species *Olsenella uli* and *Olsenella profusa*.

Emended description of the genus *Olsenella* Dewhirst *et al.* 2001

The description remains as given by Dewhirst *et al.* (2001) with the following emendations. Cells are consistently or variably Gram-stain-positive. They occur singly, in pairs and in short to very long serpentine chains. Cells are microaerotolerantly (moderately obligately) anaerobic. Lactic acid is the major metabolic product from glucose. Minor products from glucose are formic and acetic acids. The cellular fatty acids consist mainly of saturated fatty acids. The major cellular fatty acid is variable, i.e. saturated unbranched or saturated branched. Found in the oral cavity and gastrointestinal tracts of humans, other mammals and probably of other homoeothermic vertebrates.

Emended description of *Olsenella uli* (Olsen *et al.* 1991) Dewhirst *et al.* 2001

The description remains as given by Dewhirst *et al.* (2001) with the following emendations. Cells are microaerotolerantly (moderately obligately) anaerobic (less than approx. 5 % O_2, v/v). They are able to grow in microaerobic URAS

Table 1. Differential phenotypic characteristics of strains lac15, lac16, lac31^T and A2 and strains of *O. uli* and *O. profusa*

Strains: 1, strains lac15, lac16, lac31^T and A2; 2, *O. uli* DSM 7084^T; 3, *O. profusa* DSM 13989^T, CCUG 45371^T and CCUG 45372. +, Positive; −, negative; w, weak. Data were obtained in the present study.

Characteristic	1	2	3
Colony and cell morphology*			
Diameter (FAA/PYG; mm)	⩽3–4/4	⩽2/2.5	⩽2/3
Elevation	Umbonate	Raised, central punctiform knob	Pulvinate
Opacity (margin/centre)	Semi-translucent/opaque	Semi-translucent/opaque	Opaque/opaque
Colour (margin/centre)	Grey/greyish white	Grey/greyish white	Cream–white/cream–white
Texture	Butyrous	Butyrous	Granular
Cell arrangement	Single, pairs, short or longer chains	Single, pairs, short or longer chains	Short to very long serpentine chains
Gram-stain variability	Marked	Slight	Slight
Microaerobic growth in URAS PYG broth			
Growth	Good	Negative to moderate	Negative to good
Change in OD_{600} [min./mean/max. (t_0)]†	1.43/1.65/1.79 (0.09)	0/0.36/1.08 (0.04)	0/1.29/1.56 (0.04)
Accumulation of hydrogen peroxide	−	−	−/w
Growth on mucin	Moderate to good	Moderate	Moderate
Colony diameter on PGM agar after 14 days (mm)	⩽1–2	⩽1	⩽1
Major cellular fatty acid(s)	14 : 0, 18 : 0	18 : 0	anteiso-14 : 0

*On FAA and PYG agar after 7 days.
†Increase (minimum/mean/maximum) in OD_{600} after 4 days. For the maximal increase, the OD_{600} at t_0 is given in parentheses. Data are from three repetitions per strain.

PYG broth. The cellular fatty acids consist mainly of saturated unbranched fatty acids. The major cellular fatty acid of cells grown in M2 liquid medium is 18 : 0 (octadecanoic acid) (Supplementary Table S2 and Table 1). Additional phenotypic characteristics of the type strain *O. uli* DSM 7084^T are given in Tables 1 and 2.

Emended description of *Olsenella profusa* Dewhirst *et al.* 2001

The description remains as given by Dewhirst *et al.* (2001) with the following emendations. Cells are microaerotolerantly (moderately obligately) anaerobic (less than approx. 5 % O_2, v/v). They are able to grow in microaerobic URAS PYG broth. The cellular fatty acids consist predominantly of saturated anteiso-branched fatty acids. The major cellular fatty acid of cells grown in M2 liquid medium is anteiso-14 : 0 (12-methyl-tetradecanoic acid) (Supplementary Table S2 and Table 1). Additional phenotypic characteristics of the type strain *O. profusa* DSM 13989^T (=CCUG 45371^T) and the reference strain CCUG 45372 are given in Tables 1 and 2.

Description of *Olsenella umbonata* sp. nov.

Olsenella umbonata [um.bo.na′ta. N.L. fem. adj. *umbonata* bossed, umbonate (from L. masc. n. *umbo*, *umbonis* a shield boss), referring to the umbonate elevations of outgrown colonies on solid culture media].

Cells grown on FAA and PYG agar plates for 48 h under anaerobic conditions at 37 °C form colonies that are circular, up to 1.5 mm in diameter, have entire margins and smooth surfaces and are raised to slightly umbonate, semi-translucent, greyish-white and butyrous. Outgrown colonies on FAA and PYG agar display opaque, greyish-white umbonate elevations (Table 1). No haemolysis occurs on FAA or blood agar. After anaerobic incubation on PGM, PGMM and PJMM agar plates at 37 °C for 14 days, colonies are circular, punctiform (PGMM) to 1 mm (PJMM) or 2 mm (PGM) in diameter, have entire margins and smooth surfaces and are flat (PGMM, PJMM) to slightly umbonate (PGM), translucent and butyrous. Cells grown under anaerobic conditions on PYG agar for 48 h and on PGM agar for 14 days are small [0.3–0.6 μm wide (mean, approx. 0.4 μm; n=78 for PYG agar and n=46 for PGM agar) by 0.6–2.2 μm long (mean, approx. 1.1 μm; n=62 for PYG agar and n=209 for PGM agar)], irregular (centrally or terminally swollen) and occasionally curved rods (Supplementary Figs S2 and S3). Cells grown on PJMM agar are slightly shorter (0.5–2.0 μm; mean, approx. 1.0 μm; n=40) and thus more often appear coccoid (Supplementary Fig. S4). Cells are variously arranged (Table 1). Very long, serpentine chains occur with cells grown for long periods on PGM agar (Supplementary Fig. S3). Cells are less (after 48 h) or more (after 7 days) variably Gram-stain-positive (Table 1). Cells are non-motile and non-spore-forming. Cytochrome oxidase activity is not detected. Nitrate is not reduced.

Table 2. Differential biochemical and enzyme characteristics of strains lac15, lac16, lac31^T and A2 and strains of *O. uli* and *O. profusa*

Strains: 1, strains lac15, lac16, lac31^T and A2; 2, *O. uli* DSM 7084^T; 3, *O. profusa* strains DSM 13989^T and CCUG 45371^T. +, Positive; −, negative; v, variable. In Rapid ID 32 A, all strains were negative for urease, α-galactosidase, α-arabinosidase, β-glucuronidase, raffinose fermentation, reduction of nitrates, indole production, pyroglutamic acid arylamidase, glutamic acid decarboxylase, α-fucosidase and glutamyl glutamic acid arylamidase and positive for arginine dihydrolase, mannose fermentation, arginine arylamidase, proline arylamidase, leucyl glycine arylamidase, phenylalanine arylamidase, leucine arylamidase, tyrosine arylamidase, alanine arylamidase, glycine arylamidase, histidine arylamidase and serine arylamidase. In API 20 A, all strains were negative for indole formation, urease and acidification of glycerol and melezitose and positive for acidification of glucose, gelatin hydrolysis (protease) and the Gram reaction. In API ZYM, all strains were negative for trypsin, α-chymotrypsin, α-galactosidase, β-glucuronidase, α-mannosidase and α-fucosidase. Values for API ZYM tests are means of the activity mark ± SD [n=20 (column 1) or n=5 (columns 2 and 3)]. The activity mark is a measure of the amount of substrate hydrolysed (nmol), scored as follows: 0, <5; 1, 5–<10; 2, 10–<20; 3, 20–<30; 4, 30–<40; 5, ⩾40. Data were obtained in the present study unless indicated. API 20 A and API ZYM data were not reported by Olsen *et al.* (1991) or Dewhirst *et al.* (2001).

Characteristic	1	2	3
Rapid ID 32 A results			
Profile	2402073705	2012073705*	2713473705*
β-Galactosidase	−	−	+
6-Phospho-β-galactosidase	−	−	+
α-Glucosidase	+	−	+
β-Glucosidase	−	+	+
N-Acetyl-β-glucosaminidase	−	−	+
Alkaline phosphatase	−	−	+
API 20 A results			
Profile	441240(2/0)2	40060002	47776632
Aesculin hydrolysis (β-glucosidase)	−	+	+
Acidification of:			
Mannitol	−	−	+
Lactose	−	−	+
Sucrose	+	−[a]†	+
Maltose	+	−[a]	+
Salicin	−	−[a]	+
Xylose	−	−	+‡
Arabinose	−	−	+
Cellobiose	−	−	+
Mannose	+	−[a]	+
Raffinose	−	−	+
Sorbitol	−	−	+‡
Rhamnose	−	−	+[b]
Trehalose	v (+)§	−	+
Other test			
Fermentation of melibiose	−	−‖	+‖
API ZYM results			
Alkaline phosphatase	0.7±0.5	1.8±0.4	2.4±0.5
Esterase (C4)	2.3±0.4	1.2±0.4	1.4±0.5
Esterase lipase (C8)	1.8±0.4	1.2±0.4	1.2±0.4
Lipase (C14)	0.6±0.5	0.0±0.0	1.0±0.0
Leucine arylamidase	3.7±0.5	3.2±0.4	3.4±0.5
Valine arylamidase	1.9±0.4	1.4±0.5	3.2±0.4
Cystine arylamidase	0.8±0.4	0.6±0.5	2.4±0.5
Acid phosphatase	3.5±0.7	3.6±0.5	3.2±0.4
Naphthol-AS-BI-phosphohydrolase	2.3±0.4	2.2±0.4	4.2±0.4
β-Galactosidase	0.0±0.0	0.0±0.0	0.4±0.5
α-Glucosidase	4.2±0.8	0.0±0.0	1.0±0.0
β-Glucosidase	0.0±0.0	3.0±0.0	0.0±0.0
N-Acetyl-β-glucosaminidase	0.0±0.0	0.0±0.0	0.2±0.4

*Profiles reported by Dewhirst *et al.* (2001) were 2012033705 (one reaction different from the present result) for *O. uli* and 4516053705 (seven reactions different) for *O. profusa*.

†Different from result reported by: *a*, Olsen *et al.* (1991); *b*, Dewhirst *et al.* (2001). In these studies, results were determined using the methods of Holdeman *et al.* (1977).
‡Not determined by Dewhirst *et al.* (2001).
§Three of four strains, including the type strain, tested positive.
||Data taken from Olsen *et al.* (1991) (*O. uli*) and Dewhirst *et al.* (2001) (*O. profusa*).

Cells are microaerotolerantly (moderately obligately) anaerobic (less than approx. 5 % O_2, v/v). They grow routinely in microaerobic URAS PYG broth (Table 1). During growth, the resazurin in URAS PYG broth is reduced (colour change of the medium from red to orange). Cells are mesophilic; growth is absent at 21 °C, moderately good at 30 °C, very good at 37 °C and good at 45 °C. Cells are neutrophilic and acidotolerant; the pH range for growth is 4.5–8.0, occasionally pH 8.5 (optimum, pH 6.0–7.0). Growth is positive in 20 % bile and is absent in 6.5 % NaCl. Growth is stimulated markedly by 0.1 % Tween 80 and is not stimulated by 0.5 % L-arginine. Hydrogen sulfide is not produced from meat peptone or L-cysteine. No gas is detected in agar deeps. Hydrogen peroxide is not accumulated. Catalase activity is negative. Biochemical and enzyme characteristics using API kits are listed in Table 2. In API 20 A, acidification of trehalose is variable (negative for strain A2). Growth on and fermentation of D-fructose is positive. Growth on and fermentation of melibiose (Table 2), inulin, raffinose, α-L-rhamnose and D-xylose is negative. Cells are able to grow on mucin from porcine stomach (Table 1). Under anaerobic conditions, glucose is metabolized predominantly to D-lactic acid (mean, approx. 39 mmol l^{-1}) and to minor amounts of formic acid (mean, approx. 4.5 mmol l^{-1}) and acetic acid (mean, approx. 3.3 mmol l^{-1}) (n=12). Under unreduced microaerobic conditions, metabolism of glucose is not decreased in terms of production of D-lactic acid (mean, approx. 39 mmol l^{-1}; n=10) and is decreased slightly in terms of production of formic and acetic acids [means, approx. 2.5 and 2.2 mmol l^{-1}, respectively (n=12)]. Glucose is not metabolized to L-lactic acid, propionic acid, butyric acid, isobutyric acid, valeric acid, isovaleric acid, caproic acid or succinic acid. Cells are presumably obligately homofermentative and produce formic and acetic acids via the anaerobic pyruvate-formate lyase system (Axelsson, 2004). Lactic and formic acids are reducing agents and contribute to the oxygen tolerance of cells (Brioukhanov & Netrusov, 2007). Ammonium is produced from peptone under anaerobic and unreduced microaerobic conditions [means, approx. 12 and 9 mmol l^{-1}, respectively (n=12)]. Cellular fatty acids consist predominantly of saturated unbranched fatty acids. The major cellular fatty acids of cells grown in M2 liquid medium are the saturated acids 14 : 0 (tetradecanoic acid) and 18 : 0 (octadecanoic acid) in varying proportions (Supplementary Table S2 and Table 1). Well-adapted and presumably indigenous to the gastrointestinal tract of homoeothermic vertebrates, as suggested by the phenotypic characteristics of mesophily, obligate anaerobiosis and microaerotolerance [appropriate to the micro-oxic conditions at the absorptive mucosae (Isolauri *et al.*, 2004; Wilson, 2005)], neutrophily and acidotolerance, bile resistance and mucin utilization.

The type strain, lac31^T (=CCUG 58604^T =DSM 22620^T =JCM 16156^T), was isolated from the mucosal jejunum of a pig in Berlin, Germany. Strain A2 (=CCUG 58212 =DSM 22619 =JCM 16157) was isolated from the ruminal fluid of a sheep in Aberdeen, UK. Strain A2 can be readily differentiated from the type strain by a negative result for acidification of trehalose in the API 20 A strip. Strains lac15 and lac16, isolated together with the type strain, are also included in the species.

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