INTERNATIONAL JOURNAL OF SYSTEMATIC AND EVOLUTIONARY MICROBIOLOGY

RESEARCH ARTICLE

Mahoney-Kurpe *et al.*, *Int. J. Syst. Evol. Microbiol.* 2023;73:005831

DOI 10.1099/ijsem.0.005831

MICROBIOLOGY SOCIETY

OPEN ACCESS

Aristaeella hokkaidonensis gen. nov. sp. nov. and *Aristaeella lactis* sp. nov., two rumen bacterial species of a novel proposed family, *Aristaeellaceae* fam. nov.

Sam C. Mahoney-Kurpe[1,2], Nikola Palevich[1], Samantha J. Noel[1]†, Dragana Gagic[2,3], Patrick J. Biggs[2,3], Priya Soni[1], Peter M. Reid[1], Satoshi Koike[4], Yasuo Kobayashi[4], Peter H. Janssen[1], Graeme T. Attwood[1] and Christina D. Moon[1,*]

Abstract

Two strains of Gram-negative, anaerobic, rod-shaped bacteria, from an abundant but uncharacterized rumen bacterial group of the order '*Christensenellales*', were phylogenetically and phenotypically characterized. These strains, designated R-7^T and WTE2008^T, shared 98.6–99.0% sequence identity between their 16S rRNA gene sequences. R-7^T and WTE2008^T clustered together on a distinct branch from other *Christensenellaceae* strains and had <88.1% sequence identity to the closest type-strain sequence from *Luoshenia tenuis* NSJ-44^T. The genome sequences of R-7^T and WTE2008^T had 83.6% average nucleotide identity to each other, and taxonomic assignment using the Genome Taxonomy Database indicates these are separate species within a novel family of the order '*Christensenellales*'. Cells of R-7^T and WTE2008^T lacked any obvious appendages and their cell wall ultra-structures were characteristic of Gram-negative bacteria. The five most abundant cellular fatty acids of both strains were $C_{16:0}$, $C_{16:0}$ iso, $C_{17:0}$ anteiso, $C_{18:0}$ and $C_{15:0}$ anteiso. The strains used a wide range of the 23 soluble carbon sources tested, and grew best on cellobiose, but not on sugar-alcohols. Xylan and pectin were fermented by both strains, but not cellulose. Acetate, hydrogen, ethanol and lactate were the major fermentation end products. R-7^T produced considerably more hydrogen than WTE2008^T, which produced more lactate. Based on these analyses, *Aristaeellaceae* fam. nov. and *Aristaeella* gen. nov., with type species *Aristaeella hokkaidonensis* sp. nov., are proposed. Strains R-7^T (=DSM 112795^T=JCM 34733^T) and WTE2008^T (=DSM 112788^T=JCM 34734^T) are the proposed type strains for *Aristaeella hokkaidonensis* sp. nov. and *Aristaeella lactis* sp. nov., respectively.

INTRODUCTION

The rumen microbiota play a pivotal role in ruminant nutrition and metabolism. This complex microbial community is primarily responsible for the breakdown and fermentation of ingested plant-based feeds to generate substrates that are a primary energy source for the host. Despite decades of research, some of the dominant microbial groups in the rumen remain unstudied [1].

Author affiliations: [1]AgResearch Ltd, Grasslands Research Centre, Palmerston North, New Zealand; [2]School of Fundamental Sciences, Massey University, Palmerston North, New Zealand; [3]School of Veterinary Science, Massey University, Palmerston North, New Zealand; [4]Research Faculty of Agriculture, Hokkaido University, Sapporo, Japan.
***Correspondence:** Christina D. Moon, christina.moon@agresearch.co.nz
Keywords: bacteria; rumen microbiology; bacterial taxonomy; phylogenetics.
Abbreviations: AAI, amino acid identity; ANI, average nucleotide identity; BY, basal medium with yeast extract; GBDP, genome BLAST distance phylogeny; GTDB, Genome Taxonomy Database; *is*DDH, *in silico* DNA-DNA hybridisation; ML, maximum likelihood; MP, maximum parsimony; NJ, neighbor-joining; POCP, percentage of conserved proteins; RGCMSA, rumen fluid-glucose-cellobiose-maltose-starch agar; SCFA, short-chain fatty acids; TEM, transmission electron microscopy.
†Present address: Department of Animal Science, Aarhus University, Aarhus, Denmark.
The GenBank accession numbers for the genome sequences of strains R-7^T and WTE2008^T are CP068393 and CP069421-22, respectively. Full-length 16S rRNA sequences of R-7^T and WTE2008^T have been deposited in GenBank under accession numbers ON706269-71 and ON706274-76, respectively. Strains R-7^T and WTE2008^T have been deposited into the Japan Collection of Microorganisms (JCM) and DSMZ-German Collection of Microorganisms and Cell Cultures culture collections as JCM 34733^T/DSM 112795^T and JCM 34734^T/DSM 112788^T, respectively. Raw sequences of genome assemblies are deposited in the Sequence Read Archive under accessions SRR15429007-08 and SRR15428980-81 for R-7^T and WTE2008^T, respectively.
One supplementary figure and four supplementary tables are available with the online Supplementary Material.

One such example is the R-7 group, which cultivation-independent studies have demonstrated is one of the most abundant rumen bacterial groups, and part of a core rumen microbiota across different host species fed different diets [2]. Taxonomic placement of the group is currently unresolved; based on 16S rRNA gene phylogeny it was predicted to fall within the order *Clostridiales* [2] and family *Christensenellaceae* [3], while more recent phylogenomic analyses using the Genome Taxonomy Database (GTDB) framework instead assigns the group as belonging to a novel family and genus within the recently proposed [4] order '*Christensenellales*' [5]. Two strains of the R-7 group are members of the Hungate1000 reference collection of rumen microbial strains for which draft genome sequences had been generated [6]. However, neither R-7 group strain had been formally classified taxonomically. This study reports the phylogenetic and phenotypic characterization of R-7^T and WTE2008^T, and formal descriptions of the new taxa that they represent.

HABITAT AND ISOLATION

Strain R-7^T was isolated in Hokkaido, Japan, from the rumen of a sheep fed a Timothy hay-concentrate diet at a 9:1 ratio of dry matter. Rumen contents were incubated with cellulose powder for 10 min at 37 °C, before harvesting cellulose and the adherent bacteria by centrifugation (250 ***g*** for 10 min). After washing the cellulose pellet five times with phosphate-buffered saline, the pellet was resuspended in anaerobic dilution solution [7]. Serial dilutions of the resuspended pellet were then used as inoculum for isolations using the anaerobic roll-tube method with rumen fluid-glucose-cellobiose-maltose-starch-agar (RGCMSA) medium, as previously described [8].

Strain WTE2008^T was isolated from rumen contents collected from a dairy cow grazing a ryegrass pasture in Waikato, New Zealand [9]. Isolation was achieved through a dilution-to-extinction approach [10], in which liquid batch cultures of RM02 media [11] were supplemented with glucose, cellobiose, xylose, L-arabinose, casamino acids, Bacto peptone and yeast extract [9], then reduced using titanium (III) nitrilotriacetic acid [12], and inoculated with serial dilutions of rumen contents.

16S rRNA GENE PHYLOGENY

Full-length 16S rRNA gene sequences for each strain were extracted from complete genome assemblies (see Genome Features section) using RNAmmer (version 1.2) [13]. Each genome possessed three copies of the 16S rRNA gene, and alignment using BLASTn (default settings) found that each copy was >99% identical to each other. Within MEGA 11 [14], R-7^T and WTE2008^T sequences were aligned with 16S rRNA sequences of other validly named *Christensenellaceae* type strains available in the GenBank non-redundant (nr) database (as of 29/08/2022), using MUSCLE (default settings) [15]. Maximum likelihood (ML), neighbor-joining (NJ) and maximum parsimony (MP) trees were built using the default settings in MEGA 11, each calculated with 500 bootstrap replicates. The resulting trees (Figs 1 and S1 available with the online version of this article) show that both R-7^T and WTE2008^T 16S rRNA gene copies fell within a distinct cluster, sharing 98.6–99.0% similarity to each other by pairwise BLASTn using default settings. Outside this cluster, the sequence of the closest type strain was from *Luoshenia tenuis* NSJ-44^T [16], which clustered with R-7^T and WTE2008^T with moderate bootstrap support (Fig. 1) and had <88.1% sequence identity to the R-7^T and WTE2008^T sequences.

GENOME FEATURES

Draft genome assemblies of R-7^T and WTE2008^T were generated as part of the Hungate1000 project [6], and they exhibited an average nucleotide identity (ANI) of 84% to each other, in contrast to their highly similar 16S rRNA gene sequence identities. To confirm this unexpected observation, complete genome sequences of R-7^T and WTE2008^T were generated using methods as described previously [5]. The genome characteristics of the closed R-7^T and WTE2008^T genomes (Table S1) were near-identical to those of the draft assemblies [6] and were similar to those of the R-7 group strains XBB3002, FE2010 and FE2011 [5]. However, WTE2008^T also possessed a ca. 44 kb linear extrachromosomal element. Alignment of this fragment against the PHASTER phage database [17] found no homology to known phage sequences, nor were any known replication origins detected using the DoriC 10 database [18]. A phylogenomic tree based on the predicted proteomes of R-7^T and WTE2008^T and other *Christensenellaceae* type strains was reconstructed using the Type Strain Genome Server [19] with default settings. Consistent with their 16S rRNA gene sequence phylogeny, R-7^T and WTE2008^T clustered on a highly distinct branch from all other *Christensenellaceae* type strains (Fig. 2). However, the two strains were clearly separated from each other at the genomic level (Fig. 2), in contrast to the close relationships between their 16S rRNA gene copies (Fig. 1).

The GTDB framework (release 05-RS95) [4] had classified the draft genomes [6] of R-7^T (sp900199385) and WTE2008^T (sp900176495) as separate species belonging to an unclassified family (CAG-74) and genus (GCA-900199385). These designations were confirmed using the complete genome sequences, using GTDB-Tk [20]. The genus GCA-900199385 also contains the currently uncharacterized R-7 group strains XBB3002, FE2010 and FE2011 [5].

To compare the genomes of R-7^T and WTE2008^T to each other and to those of other type strains of *Christensenellaceae*, available genome data in GenBank (as of 25/08/2022) were downloaded and included in ANI, *in silico* DNA–DNA hybridization (*is*DDH), average amino

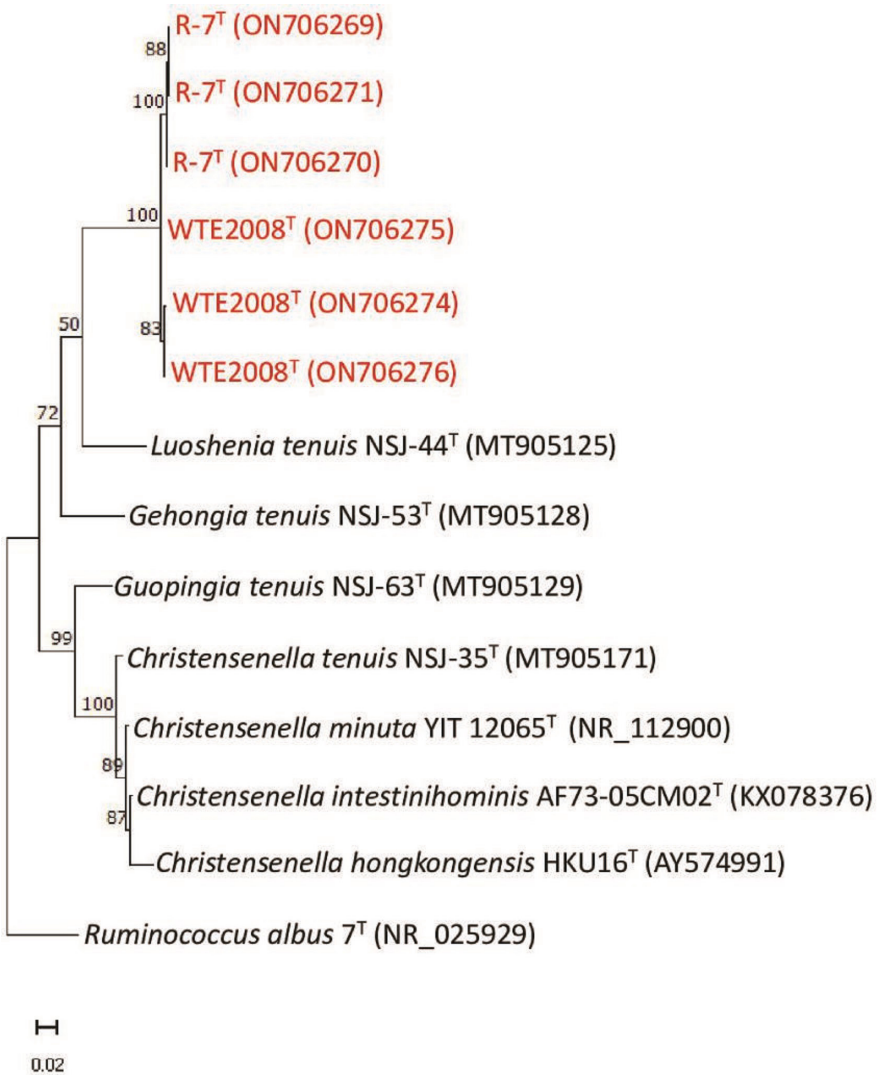

Fig. 1. Maximum likelihood phylogeny based on 16S rRNA gene sequences from R-7^T, WTE2008^T and *Christensenellaceae* type strains. GenBank accession numbers given in brackets. The sequence from *Ruminococcus albus* 7^T was used as an outgroup. The three full-length 16S rRNA sequences in each of the genomes of R-7^T and WTE2008^T are shown in red. The bootstrap values associated with branches are shown if ≥50% from 500 bootstrap replications.

acid identity (AAI) and percentage of conserved protein (POCP) comparisons (Table S2). ANI alignments were carried out using fastANI version 1.32 [21] under default settings. R-7^T and WTE2008^T shared 83.6% ANI, and alignment fractions of both R-7^T and WTE2008^T were below 20% compared with all other *Christensenellaceae* type strains and could therefore not be computed. The *is*DDH values were calculated using formula 2 of the Genome-Genome Distance Calculator (https://ggdc.dsmz.de/), which showed that R-7^T and WTE2008^T shared only 25.5% identity to each other, and ≤26.2% to all other *Christensenellaceae* type strains. Two-way AAIs of protein sequences between R-7^T and WTE2008^T, and between these and other type strains of *Christensenellaceae* were calculated using an online server (http://enve-omics.ce.gatech.edu/aai/) under default settings. R-7^T and WTE2008^T shared 80.2% AAI, and ≤44.5% to all other *Christensenellaceae* type strains. The POCP between genomes was calculated with the open-source script described in Salvetti *et al.* [22]. R-7^T and WTE2008^T shared a POCP value of 77.9%, with ≤27.7% to all other *Christensenellaceae* type strains. These results collectively highlight the genetic distinctiveness of strain R-7^T and WTE2008^T from each other and other *Christensenellaceae* type strains, thereby supporting the GTDB classifications of R-7^T and WTE2008^T as separate species of a novel genus and family.

PHYSIOLOGY AND CHEMOTAXONOMY

Cell morphology was assessed using phase-contrast microscopy and transmission electron microscopy. In both instances, visualized cells were grown anaerobically in basal medium with yeast extract (BY medium) [23] containing rumen fluid from a hay-fed

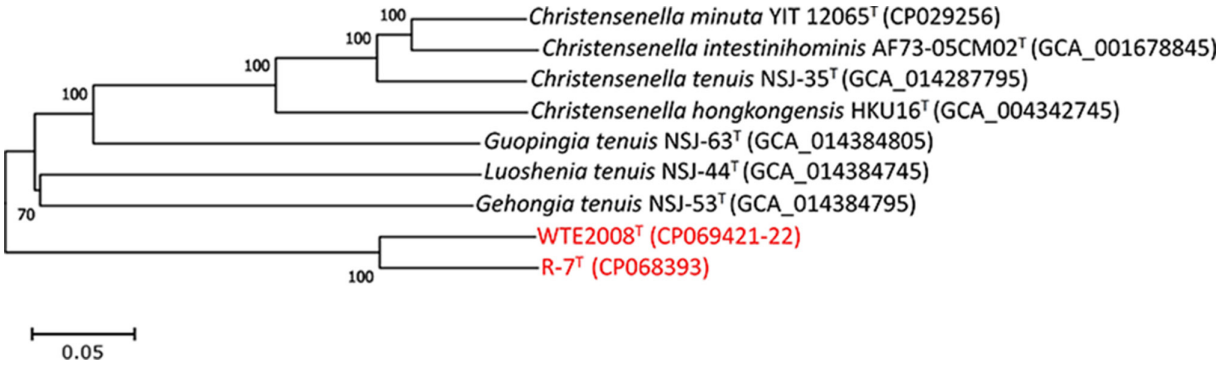

Fig. 2. Phylogenomic tree based on proteomes of R-7^T and WTE2008^T and *Christensenellaceae* type strains. The tree is inferred using FastME 2.1.6.1 [28] from whole proteome-based Genome BLAST Distance Phylogeny (GBDP) distances. Branch lengths are scaled *via* GBDP distance formula *d*5. The GBDP pseudo-bootstrap support values associated with branches are shown if >60% from 100 bootstrap replications. The tree was midpoint-rooted [29].

cow, with 0.5% (w/v) cellobiose overnight at 39 °C. For phase-contrast microscopy, cells were photographed with a DM2500 microscope (Leica Microsystems), and cells of both strains were 1–3 µm in length and 0.2–0.5 µm in width, often in pairs (Fig. 3), or occasionally forming longer chains. Cells were also assessed using the Gram staining method [24] and both strains were Gram-negative.

Transmission electron microscopy (TEM) of negatively stained cells was performed by pelleting cells by low-speed centrifugation (2800 ***g***), and resuspending in sterile water. Cell suspensions were processed and imaged at the Massey Microscopy and Imaging Centre (Palmerston North, New Zealand). Cells were fixed to a Formvar grid and stained in 2% (w/v) uranyl acetate, and then viewed using an FEI Tecnai G2 Biotwin transmission electron microscope. Cells of R-7^T and WTE2008^T appeared similar, lacking any obvious appendages (e.g. pili or flagella), and exhibited electron-dense poles. Both R-7^T and WTE2008^T had thin electron-dense rings around the mid-section of cell bodies (Fig. 3).

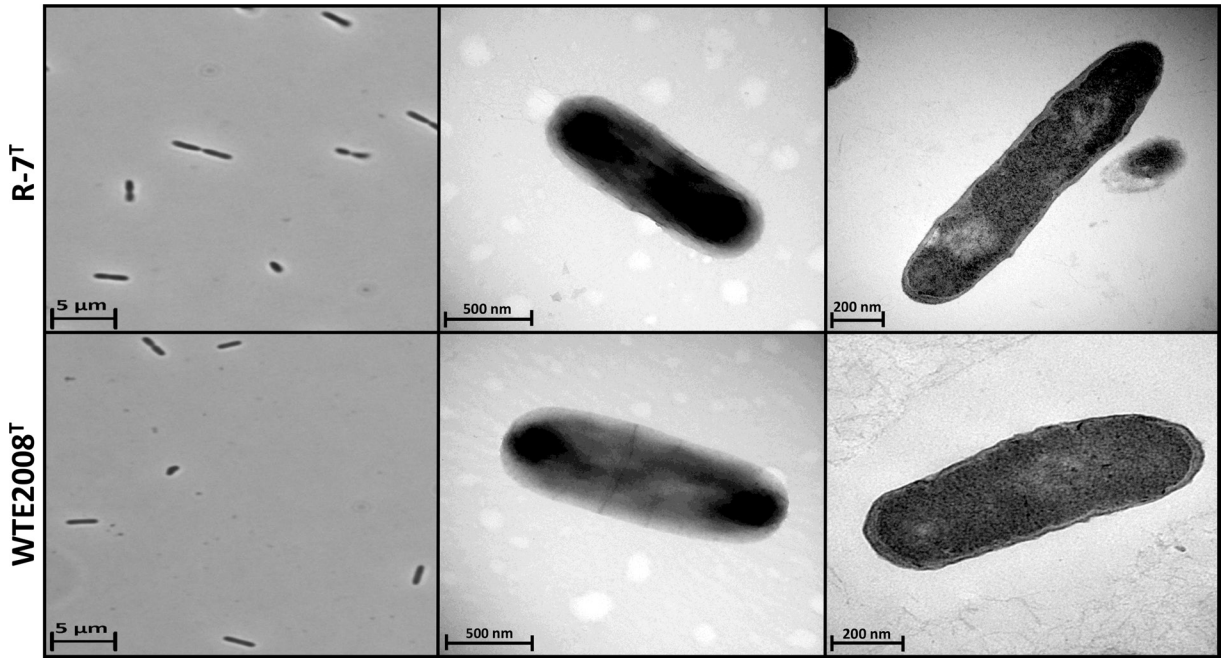

Fig. 3. Cell morphology of R-7^T and WTE2008^T. Phase-contrast images (left), transmission electron microscopy images of negatively stained cells (centre), and thin-sectioned cells (right). Scale bars are shown bottom left.

Thin cross-sections of cells were prepared for TEM by washing cell pellets three times in sterile water, then resuspending in modified Karnovsky's fixative (2% paraformaldehyde and 3% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4)) for processing and imaging. Resin-embedded thin sections of each sample were prepared using an EM UC7 ultra-microtome (Leica Microsystems, Weltzar, Germany), and viewed using an FEI Tecnai G2 Biotwin transmission electron microscope. Both R-7^T and WTE2008^T had cell wall ultra-structures characteristic of Gram-negative bacteria (Fig. 3).

To assess the cellular fatty acid compositions of strains, cultures were anaerobically grown overnight at 39 °C in 100 ml of BY medium [23] containing 0.5% (w/v) cellobiose and harvested by centrifugation. Cellular fatty acid profiles (Table S3) were determined by gas chromatography with flame-ionization detection using the MIDI Sherlock Microbial Identification System (MIS), and the Anaerobic Bacteria Library (MOORE6) for peak identification. The five most abundant cellular fatty acids of R-7^T and WTE2008^T were $C_{16:0}$, $C_{16:0}$ iso, $C_{17:0}$ anteiso, $C_{18:0}$, and $C_{15:0}$ anteiso. However, $C_{17:0}$ anteiso and $C_{16:0}$ iso were more abundant in R-7^T, whereas $C_{16:0}$ and $C_{18:0}$ were more abundant in WTE2008^T (Table 1).

Growth of R-7^T and WTE2008^T at various temperatures, pH, and NaCl concentrations were carried out over 72 h, in BY medium [23] containing 0.5% (w/v) cellobiose as substrate. Both strains grew at 30 and 45 °C, but not at 25 °C or 50 °C. Tolerance to NaCl supplementation of BY medium was tested in 0.5% increments from 0–3.0% (w/v). Both strains grew at 0.5%, but not at any greater concentrations. Tolerance to pH was measured by adjusting the pH of BY medium in increments of 0.2–0.5 over a range of pH 4.6–7.0. Both strains grew in a pH range of 5.9–7.0. WTE2008^T could grow at pH 5.6 but not R-7^T, and neither strain could grow at pH 5.1. Colonies did not form when incubated aerobically on BY agar (1.5% w/v) plates at 39 °C compared with anaerobically grown controls, suggesting R-7^T and WTE2008^T as being anaerobic.

The growth of strains on 23 different soluble carbon substrates in BY medium [23] was assessed in triplicate using a Spectronic 200 spectrophotometer (Thermo Scientific) with absorbance at 600 nm. Each strain was inoculated into anaerobic medium containing 0.5% (w/v) of the test substrate and incubated at 39 °C for 48 h. Both R-7^T and WTE2008^T grew well using L-arabinose, glucose, xylose, cellobiose, lactose, maltose, melibiose, sucrose, and trehalose as a carbon source. R-7^T also grew well on galactose, but WTE2008^T only weakly. Weak growth of both strains was observed on melezitose, raffinose, and aesculin. WTE2008^T grew on fructose and weakly on mannose, but R-7^T did not. R-7^T grew weakly on rhamnose, but WTE2008^T did not. Neither strain grew on ribose or any of the sugar-alcohols tested. Both strains grew best on cellobiose, however, WTE2008^T grew to approximately half the optical density of R-7^T.

The ability of strains to degrade a range of insoluble substrates, including major plant cell wall polysaccharides, was assessed. Each strain was incubated anaerobically in BY medium [23] with 0.5% (w/v) of each insoluble substrate in Hungate tubes fastened horizontally on a shaker, and gently shaken at 39 °C for 5 days. Fermentation end products, including short-chain fatty acids (SCFAs) and alcohols, were quantified by gas chromatography as described in Della Rosa *et al.* [25]. Overall, SCFA production was highly variable between triplicate cultures for some strain-substrate combinations, and in some instances showed net decreases in concentrations, which suggested that SCFA utilization may have occurred (Table S4). R-7^T and WTE2008^T produced acetate, but not butyrate or propionate. Ethanol was also produced by both strains, with generally less variability between triplicates than SCFAs. Using ethanol production as an indicator of microbial growth, R-7^T and WTE2008^T could degrade and ferment xylan and pectin, suggestive of a role of these organisms in ruminal fibre degradation. However, neither strain could grow on crystalline cellulose (Table 1), despite R-7^T having been originally isolated from a cellulose-adherent fraction of the rumen microbiota. Notably, R-7^T cultures yielded greater ethanol concentrations growing on dextrin and salicin than on cellobiose (Table S4).

To further characterize fermentation end product formation during growth of R-7^T and WTE2008^T, 10 ml cultures of each strain were grown in BY medium [23] containing 0.5% (w/v) cellobiose, rumen fluid from a pasture-fed sheep and 0.1% (w/v) each of Bacto peptone and casamino acids at 39 °C for 24 h. Samples were taken at various time points to determine fermentation end product concentrations. Production of hydrogen was also measured by injecting 0.1 ml of headspace of each culture into an Aerograph 660 gas chromatograph (Varian Associates) fitted with a Porapak Q80/100 mesh column (Waters Corporation) and a thermal conductivity detector. To additionally assess the production of formate, succinate and lactate, sample supernatants were derivatized using the method described by Richardson *et al.* [26]. Optical densities and production of fermentation end products over time are shown in Fig. 4. Both strains produced acetate, ethanol, hydrogen and lactate. However, R-7^T produced more hydrogen than lactate (11.8±0.4 mM l^{-1} hydrogen versus 1.5±0.2 mM lactate), whereas WTE2008^T produced considerably more lactate and less hydrogen (4.1±0.3 mM l^{-1} hydrogen versus 6.5±1.1 mM lactate). Notable was the apparent switch to copious lactate production and cessation of hydrogen production by WTE2008^T after 16 h of growth.

DESCRIPTION OF *ARISTAEELLA* GEN. NOV.

Aristaeella (A.ris.tae.el'la. N.L. fem. n. *Aristaeella* named after Aristaeus (Aristaios), a Greek god associated with animal husbandry and production of alcoholic beverages).

Cells are Gram-negative, obligately anaerobic, non-motile rods that do not form spores. Utilizes various sugars, hemicellulose and pectin to form short-chain fatty acids, lactate, hydrogen and ethanol. The type species is *Aristaeella hokkaidonensis*.

Table 1. Phenotypic characteristics of strains R-7^T and WTE2008^T compared to related type strains

Strains: 1, R-7^T; 2, WTE2008^T; 3, *Christensenella minuta* YIT 12065^T [30]; 4, *Christensenella hongkongensis* HKU16^T [31, 32]; 5, *Christensenella intestinihominis* AF73-05CM02^T [33]; 6, *Christensenella tenuis* NSJ-35^T [16]; 7, *Luoshenia tenuis* NSJ-44^T [16]; 8, *Gehongia tenuis* NSJ-53^T [16]; 9, *Guopingia tenuis* NSJ-63^T [16].

Characteristic	1	2	3*	4*	5*	6*	7*	8*	9*
Isolation source	Sheep rumen	Cow rumen	Human faeces	Human blood	Human faeces	Human faeces	Human faeces	Human faeces	Human faeces
Gram stain	–	–	+/–†	+	–	NA	NA	NA	NA
Morphology	Rods	Rods	Rods	Coccobacilli/short rods	Rods	Rods	Ovoid	Ovoid/rods	Spherical
Cell length×width (µm)	1.0–3.0×0.2–0.5	1.0–3.0×0.2–0.5	0.8–1.9×0.4	0.7–1.1×0.4–0.5	1.0–2.0×0.5	1.2–1.6×0.6–0.8	1.3–1.6×0.7–0.9	1.3–2.0×0.7–0.9	0.8–1.1×0.8–1.1
Motility	–	–	–	+	–	–	–	–	+
Major (>5%) cellular fatty acids‡	$C_{17:0}$ anteiso (19.7%), $C_{16:0}$iso (18.2%), $C_{16:0}$ (11.5%), $C_{18:0}$ (9.1%), $C_{15:0}$ anteiso (8.9%), $C_{18:0}$ iso (6.6%)	$C_{18:0}$ (19.3%), $C_{16:0}$ (15.2%), $C_{17:0}$ anteiso (8.7%), $C_{16:0}$iso (8.4%), $C_{15:0}$ anteiso (7.7%)	$C_{15:0}$ iso (37.8%), $C_{16:0}$ (31.7%), $C_{14:0}$ (14.8%)	NA	$C_{14:0}$ (46.6%), $C_{16:0}$ (9.7%), $C_{10:0}$ (7.5%), $C_{15:0}$ iso (7.4%), $C_{12:0}$ (7.2%), $C_{18:1}$ ω9*c* (6.9%), $C_{11:0}$ iso (5.6%)	NA	NA	NA	NA
Growth on soluble substrates:§									
Arabinose	+	+	+	+	+	–	–	–	–
Fructose	–	+	NA	NA	+	+	+	–	+
Galactose	+	(+)	NA	NA	+	–	+	–	+
Glucose	+	+	+	+	+	(+)	+	–	+
Mannose	–	(+)	(+)	+	+	+	+	–	+
Rhamnose	(+)	–	+	–	+	–	+	+	+
Ribose	–	–	NA	NA	+	–	–	–	–
Xylose	+	+	+	+	+	–	–	–	–
Cellobiose	+	+	–	–	–	–	+	–	–
Lactose	+	+	–	–	–	(+)	(+)	–	–
Maltose	+	+	–	–	(+)	–	+	–	–
Melibiose	+	+	NA	NA	–	–	+	–	(+)
Sucrose	+	+	–	–	+	+	–	+	–
Melezitose	(+)	(+)	–	–	(+)	–	(+)	+	–
Raffinose	(+)	(+)	–	–	(+)	–	–	–	–
Trehalose	+	+	–	–	–	–	–	–	–
Glycerol	–	–	–	+	–	–	(+)	(+)	+

Continued

Table 1. Continued

Characteristic	1	2	3*	4*	5*	6*	7*	8*	9*
Myo-inositol	–	–	NA	NA	–	–	(+)	–	–
Mannitol	–	–	–	–	–	–	(+)	–	–
Sorbitol	–	–	–	–	+	–	(+)	+	–
Xylitol	–	–	NA	NA	+	–	–	–	–
Amygdalin	–	–	NA	NA	–	+	(+)	–	–
Aesculin	(+)	(+)	–§	–§	+§	NA	NA	NA	NA
Growth on insoluble substrates:¶									
Crystalline cellulose	–	–	NA	NA	NA	NA	NA	NA	NA
Dextrin	+	+	NA	NA	NA	+	+	+	–
Glycogen	–	–	NA	NA	–	–	–	–	–
Inulin	–	–	NA	NA	–	–	–	–	–
Pectin	+	+	NA	NA	NA	–	–	–	–
Starch	+	+	NA	NA	–	NA	NA	NA	NA
Xylan	+	+	NA	NA	NA	NA	NA	NA	NA
Rutin	–	–	NA	NA	NA	NA	NA	NA	NA
Salicin	+	+	+	–	+	–	(+)	–	–
Fermentation end products**	A,L, H,E	A,L, H,E	A,B††	NA	A,F,B,L††	NA	NA	NA	NA

*Values showing NA were not assessed in previous reports of these strains.
†Described originally as Gram-negative [30], but later reports have shown Gram-positive staining of cells [32, 34].
‡Listed in order of abundance, with abundances (%) in brackets beside each cellular fatty acid. Full profiles are shown in Table S3.
§These are the results of aesculin hydrolysis tests, not growth on aesculin.
¶Growth was assessed by fermentation end product formation. +, Production of >1 mM ethanol.
**A, acetate; L, lactate; H, hydrogen; E, ethanol; B, butyrate; F, formate.
††These studies do not report testing for the production of alcohols.

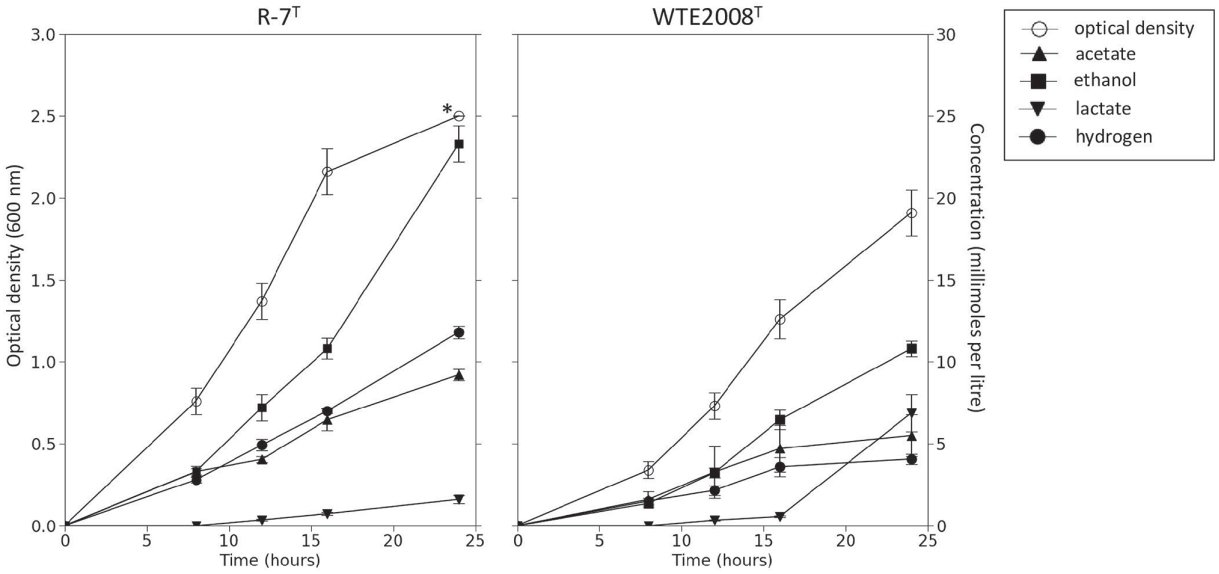

Fig. 4. Optical density and production of fermentation end products of R-7^T and WTE2008^T over 24 h of growth. Error bars denote SEM (n=3). *This data point represents the maximum absorbance limit of the spectrophotometer.

DESCRIPTION OF *ARISTAEELLA HOKKAIDONENSIS* SP. NOV.

Aristaeella hokkaidonensis (hok.kai.do.nen'sis. N.L. fem. adj. *hokkaidonensis* pertaining to Hokkaido, Japan, where the type strain was isolated).

Cells are Gram-negative, obligately anaerobic non-motile rods ranging from approximately 1 to 3 µm long and 0.2 to 0.5 µm wide. Cells possess no obvious appendages and have cell walls characteristic of Gram-negative bacteria. Dominant cellular fatty acids are $C_{15:0}$ anteiso, $C_{16:0}$, $C_{16:0}$ iso, $C_{17:0}$ anteiso, $C_{18:0}$ and $C_{18:0}$ iso. Cells use arabinose, galactose, glucose, xylose, cellobiose, lactose, maltose, melibiose, sucrose and trehalose, with weak growth on rhamnose, melezitose, raffinose and aesculin, and no growth on fructose, mannose, ribose, glycerol, *myo*-inositol, mannitol, sorbitol, xylitol and amygdalin. Cells degrade and use the breakdown products of dextrin, pectin, starch, xylan and salicin, but do not degrade crystalline cellulose, glycogen, inulin and rutin. The major fermentation end products are ethanol, hydrogen, acetate and lactate.

The type strain, R-7^T (=JCM 34733^T=DSM 112795^T), was isolated from sheep rumen contents in Hokkaido, Japan. The genome was determined to be 3.39 Mb with a G+C content of 53.0 mol%.

DESCRIPTION OF *ARISTAEELLA LACTIS* SP. NOV.

Aristaeella lactis (lac'tis. L. gen. n. *lactis* of milk, pertaining to lactate, due to the significant lactate production by the type strain).

Cells are Gram-negative, obligately anaerobic non-motile rods ranging from approximately 1 to 3 µm long and 0.2 to 0.5 µm wide. Cells possess no obvious appendages and have cell walls characteristic of Gram-negative bacteria. Dominant cellular fatty acids are $C_{15:0}$ anteiso, $C_{16:0}$, $C_{16:0}$ iso, $C_{17:0}$ anteiso and $C_{18:0}$. Cells use arabinose, fructose, glucose, xylose, cellobiose, lactose, maltose, melibiose, sucrose and trehalose, with weak growth on galactose, mannose, melezitose, raffinose and aesculin, and no growth on amygdalin, glycerol, *myo*-inositol, mannitol, rhamnose, ribose, sorbitol or xylitol. Cells degrade and use the breakdown products of dextrin, pectin, starch, xylan, and salicin, but do not degrade crystalline cellulose, glycogen, inulin or rutin. The major fermentation end products are ethanol, lactate, acetate and hydrogen.

The type strain is WTE2008^T (=JCM 34734^T=DSM 112788^T), which was isolated from bovine rumen contents from Waikato, New Zealand. The genome of the type strain is characterized by a size of 3.45 Mb and G+C content of 53.5 mol%.

DESCRIPTION OF *ARISTAEELLACEAE* FAM. NOV.

Aristaeellaceae (A.ris.tae.el.la.ce'ae. N.L. fem. n. *Aristaeella* type genus of the family; *-aceae* ending to denote a family; N.L. fem. pl. n. *Aristaeellaceae* the family whose nomenclatural type is the genus *Aristaeella*).

The family is described on the basis of phylogenetic analyses of 16S rRNA gene sequences and whole genome analyses. Cells are rod-shaped, Gram-negative and anaerobic. Belongs to the order '*Christensenellales*' [4] of the phylum *Bacillota* [27].

Funding information
This work was funded by the New Zealand Ministry of Business, Innovation and Employment Strategic Science Investment Fund (AgResearch contract C10X1702), Microbiomes from soil to plate programme. The funders had no role in the study design, data collection and interpretation, or the decision to submit the work for the publication.

Acknowledgements
Thanks to Dr Sandeep Kumar for assistance with the GTDB-Tk analyses, and to Prof. Aharon Oren and Prof. Bernhard Schink for their guidance on the etymology of the genus name. We also thank Dr Laureen Crouzet, Dr Ryan Chanyi and two anonymous reviewers for critical review of this manuscript.

Author contributions
S.C.M.: Conceptualization, data curation, formal analysis, investigation, validation, visualization, writing – original draft. N.P.: Conceptualization, supervision, writing – review and editing. S.J.N.: Resources, writing – review and editing. D.G.: Conceptualization, supervision, writing – review and editing. P.J.B.: Conceptualization, supervision, writing – review and editing. P.S.: Investigation. P.R.: Investigation. S.K.: Resources, formal analysis, investigation, writing – review and editing. Y.K.: Resources, writing – review and editing. P.H.J.: Resources, writing – review and editing. G.T.A.: Conceptualization, supervision, writing – review and editing. C.D.M.: Conceptualization, funding acquisition, project administration, supervision, writing – review and editing.

Conflicts of interest
The authors declare that there are no conflicts of interest.

Ethical statement
The rumen sample from which WTE2008^T was isolated was obtained with AgResearch Grasslands Animal Ethics Committee approval (AE12174). The rumen sample from which R-7^T was isolated was collected in accordance with the Guidelines for Animal Experiments and the Act on Welfare and Management of Animals, Hokkaido University, and all experimental procedures were approved by the Animal Care and Use Committee of Hokkaido University (No.15–0122).

References

1. Creevey CJ, Kelly WJ, Henderson G, Leahy SC. Determining the culturability of the rumen bacterial microbiome. *Microb Biotechnol* 2014;7:467–479.
2. Henderson G, Cox F, Ganesh S, Jonker A, Young W, *et al*. Rumen microbial community composition varies with diet and host, but a core microbiome is found across a wide geographical range. *Sci Rep* 2015;5:14567.
3. Henderson G, Yilmaz P, Kumar S, Forster RJ, Kelly WJ, *et al*. Improved taxonomic assignment of rumen bacterial 16S rRNA sequences using a revised SILVA taxonomic framework. *PeerJ* 2019;7:e6496.
4. Parks DH, Chuvochina M, Waite DW, Rinke C, Skarshewski A, *et al*. A standardized bacterial taxonomy based on genome phylogeny substantially revises the tree of life. *Nat Biotechnol* 2018;36:996–1004.
5. Mahoney-Kurpe SC, Palevich N, Noel SJ, Kumar S, Gagic D, *et al*. Complete genome sequences of three *Clostridiales* R-7 group strains isolated from the bovine rumen in New Zealand. *Microbiol Resour Announc* 2021;10:e0031021.
6. Seshadri R, Leahy SC, Attwood GT, Teh KH, Lambie SC, *et al*. Cultivation and sequencing of rumen microbiome members from the Hungate1000 Collection. *Nat Biotechnol* 2018;36:359–367.
7. Bryant MP, Burkey LA. Cultural methods and some characteristics of some of the more numerous groups of bacteria in the bovine rumen. *J Dairy Sci* 1953;36:205–217.
8. Minato H, Otsuka M, Shirasaka S, Itabashi H, Mitsumori M. Colonization of microorganisms in the rumen of young calves. *J Gen Appl Microbiol* 1992;38:447–456.
9. Noel S. *Cultivation and community composition analysis of plant-adherent rumen bacteria*, PhD thesis. Palmerston North, New Zealand: Massey University, 2013.
10. Button DK, Schut F, Quang P, Martin R, Robertson BR. Viability and isolation of marine bacteria by dilution culture: theory, procedures, and initial results. *Appl Environ Microbiol* 1993;59:881–891.
11. Kenters N, Henderson G, Jeyanathan J, Kittelmann S, Janssen PH. Isolation of previously uncultured rumen bacteria by dilution to extinction using a new liquid culture medium. *J Microbiol Methods* 2011;84:52–60.
12. Moench TT, Zeikus JG. An improved preparation method for a titanium (III) media reductant. *J Microbiol Methods* 1983;1:199–202.
13. Lagesen K, Hallin P, Rødland EA, Staerfeldt H-H, Rognes T, *et al*. RNAmmer: consistent and rapid annotation of ribosomal RNA genes. *Nucleic Acids Res* 2007;35:3100–3108.
14. Tamura K, Stecher G, Kumar S. MEGA11: Molecular Evolutionary Genetics Analysis version 11. *Mol Biol Evol* 2021;38:3022–3027.
15. Edgar RC. MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Res* 2004;32:1792–1797.
16. Liu C, Du M-X, Abuduaini R, Yu H-Y, Li D-H, *et al*. Enlightening the taxonomy darkness of human gut microbiomes with a cultured biobank. *Microbiome* 2021;9:119.
17. Arndt D, Grant JR, Marcu A, Sajed T, Pon A, *et al*. PHASTER: a better, faster version of the PHAST phage search tool. *Nucleic Acids Res* 2016;44:W16–21.
18. Luo H, Gao F. DoriC 10.0: an updated database of replication origins in prokaryotic genomes including chromosomes and plasmids. *Nucleic Acids Res* 2019;47:D74–D77.
19. Meier-Kolthoff JP, Carbasse JS, Peinado-Olarte RL, Göker M. TYGS and LPSN: a database tandem for fast and reliable genome-based classification and nomenclature of prokaryotes. *Nucleic Acids Res* 2022;50:D801–D807.
20. Chaumeil P-A, Mussig AJ, Hugenholtz P, Parks DH. GTDB-Tk: a toolkit to classify genomes with the genome taxonomy database. *Bioinformatics* 2019;36:1925–1927.
21. Jain C, Rodriguez-R LM, Phillippy AM, Konstantinidis KT, Aluru S. High throughput ANI analysis of 90K prokaryotic genomes reveals clear species boundaries. *Nat Commun* 2018;9:5114.
22. Salvetti E, Harris HMB, Felis GE, O'Toole PW. Comparative genomics of the genus *Lactobacillus* reveals robust phylogroups that provide the basis for reclassification. *Appl Environ Microbiol* 2018;84:e00993-18.
23. Joblin KN. Methanogenic archaea. In: Makkar HPS and McSweeney CS (eds). *Methods in Gut Microbial Ecology for Ruminants*. Dordrecht: Springer Netherlands; 2005. pp. 47–53.
24. Coico R. Gram staining. *Curr Protoc Microbiol* 2005:A.3C.1-A.3C.2.
25. Della Rosa MM, Sandoval E, Reid P, Luo D, Pacheco D, *et al*. Substituting ryegrass-based pasture with graded levels of forage rape in the diet of lambs decreases methane emissions and increases propionate, succinate, and primary alcohols in the rumen. *J Anim Sci* 2022;100:skac223.

26. Richardson AJ, Calder AG, Stewart CS, Smith A. Simultaneous determination of volatile and non-volatile acidic fermentation products of anaerobes by capillary gas chromatography. *Lett Appl Microbiol* 1989;9:5–8.
27. Oren A, Garrity GM. Valid publication of the names of forty-two phyla of prokaryotes. *Int J Syst Evol Microbiol* 2021;71:005056.
28. Lefort V, Desper R, Gascuel O. FastME 2.0: A comprehensive, accurate, and fast distance-based phylogeny inference program. *Mol Biol Evol* 2015;32:2798–2800.
29. Farris JS. Estimating phylogenetic trees from distance matrices. *Am Nat* 1972;106:645–668.
30. Morotomi M, Nagai F, Watanabe Y. Description of *Christensenella minuta* gen. nov., sp. nov., isolated from human faeces, which forms a distinct branch in the order *Clostridiales*, and proposal of *Christensenellaceae* fam. nov. *Int J Syst Evol Microbiol* 2012;62:144–149.
31. Lau SKP, McNabb A, Woo GKS, Hoang L, Fung AMY, *et al*. *Catabacter hongkongensis* gen. nov., sp. nov., isolated from blood cultures of patients from Hong Kong and Canada. *J Clin Microbiol* 2007;45:395–401.
32. Liu X, Sutter JL, de la Cuesta-Zuluaga J, Waters JL, Youngblut ND, *et al*. Reclassification of *Catabacter hongkongensis* as *Christensenella hongkongensis* comb. nov. based on whole genome analysis. *Int J Syst Evol Microbiol* 2021;71:004774.
33. Zou Y, Xue W, Lin X, Hu T, Liu S-W, *et al*. Taxonomic description and genome sequence of *Christensenella intestinihominis* sp. nov., a novel cholesterol-lowering bacterium isolated from human gut. *Front Microbiol* 2021;12:632361.
34. Alonso BL, Irigoyen von Sierakowski A, Sáez Nieto JA, Rosel AB. First report of human infection by *Christensenella minuta*, a Gram-negative, strickly anaerobic rod that inhabits the human intestine. *Anaerobe* 2017;44:124–125.