Oscillibacter ruminantium sp. nov., isolated from the rumen of Korean native cattle

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A strictly anaerobic, Gram-negative, non-spore-forming bacterium, designated GH1T, was isolated from the rumen of Korean native cattle (HanWoo). Cells were straight to slightly curved rods (2.0–4.5 μm long) and were motile by peritrichous flagella. The isolate grew at 30–45 °C (optimum 40 °C), at pH 5.5–6.5 (optimum pH 6.0) and with up to 3.5 % (w/v) NaCl. Strain GH1T produced acid from D-glucose, D-ribose and D-xylose, with butyric acid being the major end product. The genomic DNA G+C content was 54.6 mol%. Based on comparative 16S rRNA gene sequence analysis, strain GH1T was most closely related to Oscillibacter valericigenes Sjm18-20T (97.3 % 16S rRNA gene sequence similarity). DNA–DNA hybridization between strain GH1T and O. valericigenes DSM 18026T showed 24 % reassociation. The major fatty acids were iso-C13:0 (13.0 %), iso-C15:0 (17.6 %), anteiso-C15:0 (8.4 %) and C14:0 (4.1 %), and the cellular fatty acid methyl esters as dimethylacetics (DMAs) were C16:0 DMA (17.8 %), iso-C15:0 DMA (15.2 %) and C14:0 DMA (4.52 %). The cell-wall peptidoglycan of strain GH1T contained meso-diaminopimelic acid and the major cell-wall sugar was galactose. Based on 16S rRNA gene sequence similarity, phylogenetic analysis, DNA G+C content, DNA–DNA relatedness and distinct phenotypic characteristics, strain GH1T is most closely related to Oscillibacter valericigenes Sjm18-20T (97.3 % 16S rRNA gene sequence similarity). DNA–DNA hybridization between strain GH1T and O. valericigenes DSM 18026T showed 24 % reassociation. The major fatty acids were iso-C13:0 (13.0 %), iso-C15:0 (17.6 %), anteiso-C15:0 (8.4 %) and C14:0 (4.1 %), and the cellular fatty acid methyl esters as dimethylacetics (DMAs) were C16:0 DMA (17.8 %), iso-C15:0 DMA (15.2 %) and C14:0 DMA (4.52 %). The cell-wall peptidoglycan of strain GH1T contained meso-diaminopimelic acid and the major cell-wall sugar was galactose. Based on 16S rRNA gene sequence similarity, phylogenetic analysis, DNA G+C content, DNA–DNA relatedness and distinct phenotypic characteristics, strain GH1T is most closely related to Oscillibacter valericigenes Sjm18-20T (97.3 % 16S rRNA gene sequence similarity). DNA–DNA hybridization between strain GH1T and O. valericigenes DSM 18026T showed 24 % reassociation. The major fatty acids were iso-C13:0 (13.0 %), iso-C15:0 (17.6 %), anteiso-C15:0 (8.4 %) and C14:0 (4.1 %), and the cellular fatty acid methyl esters as dimethylacetics (DMAs) were C16:0 DMA (17.8 %), iso-C15:0 DMA (15.2 %) and C14:0 DMA (4.52 %). The cell-wall peptidoglycan of strain GH1T contained meso-diaminopimelic acid and the major cell-wall sugar was galactose. Based on 16S rRNA gene sequence similarity, phylogenetic analysis, DNA G+C content, DNA–DNA relatedness and distinct phenotypic characteristics, strain GH1T is most closely related to Oscillibacter valericigenes Sjm18-20T (97.3 % 16S rRNA gene sequence similarity). DNA–DNA hybridization between strain GH1T and O. valericigenes DSM 18026T showed 24 % reassociation. The major fatty acids were iso-C13:0 (13.0 %), iso-C15:0 (17.6 %), anteiso-C15:0 (8.4 %) and C14:0 (4.1 %), and the cellular fatty acid methyl esters as dimethylacetics (DMAs) were C16:0 DMA (17.8 %), iso-C15:0 DMA (15.2 %) and C14:0 DMA (4.52 %). The cell-wall peptidoglycan of strain GH1T contained meso-diaminopimelic acid and the major cell-wall sugar was galactose. Based on 16S rRNA gene sequence similarity, phylogenetic analysis, DNA G+C content, DNA–DNA relatedness and distinct phenotypic characteristics, strain GH1T is most closely related to Oscillibacter valericigenes Sjm18-20T (97.3 % 16S rRNA gene sequence similarity). DNA–DNA hybridization between strain GH1T and O. valericigenes DSM 18026T showed 24 % reassociation.

Diverse and competitive micro-organisms inhabit the rumen and belong to three domains: Bacteria, Archaea (methanogens) and Eucarya (protozoa and fungi) (Miron et al., 2001; Pers-Kamczyc et al., 2011). This complex community plays a very important role in the degradation of cellulose feeds and the supply of nutrients to the host in the form of volatile fatty acids (Kamra, 2005; Van Soest, 1994). The structure and ecology of the whole microbial community in rumen should be more completely understood in order to manipulate rumen fermentation or control the level of bio-emission of methane from ruminants. A number of micro-organisms in rumen are still non-culturab and their individual function could be critical to the ruminal ecosystem (Pers-Kamczyc et al., 2011). Therefore, isolation and characterization of novel rumen micro-organisms should be performed for complete understanding of ruminal ecology.

Korean native cattle, ‘HanWoo’ (Bos taurus coreanae), inhabit in the region of Korea peninsula and produce good-quality meat. Understanding their physiology, nutrition, metabolism and reproduction is of prime importance for the Korean native cattle industry as it is a significant contributor to farming households, the national economy and the security of food stuffs in Korea (Hwang et al., 2000).

In our study, we tried to isolate diverse bacteria from the rumen of HanWoo using strictly anaerobic cultivation technique. Fresh rumen fluid (900 ml) was collected 4 h after morning feeding from a ruminally cannulated 1-year-old HanWoo steer fed 4 kg concentrate and 0.5 kg rice straw (dry matter) twice daily at a research farm of Seoul

Abbreviation: DMA, dimethylacetal.
The GenBank/EMBL/DDJB accession number for the 16S rRNA gene sequence of strain GH1T is JF750939.

Four supplementary figures and two supplementary tables are available with the online version of this paper.
National University near Suwon city, Republic of Korea. The GH medium was used for enrichment and isolation of rumen micro-organisms. GH medium was prepared and sterilized using the anaerobic technique with N₂ as the gas phase as described by Hungate (1969). GH medium contained (l⁻¹ deionized water) 5 g yeast extract (Becton Dickinson), 5 g polypeptide (Becton Dickinson), 0.5 g MgCl₂, 0.05 g CaCl₂, 0.03 g Tween 80, 10 ml vitamin solution (Wolin et al., 1963) and 1 ml trace element solution SL-10 (Widdel et al., 1983). The pH was adjusted to pH 6 with 1 M HCl. Rumen fluid (4 %) was inoculated into GH medium and incubated at 37 °C to pH 6 with 1 M HCl. Rumen fluid (4 %) was inoculated into GH medium and incubated at 37 °C to provide an enrichment culture. Diluted enrichments were spread onto solid GH medium (containing 0.8 % gellan gum; Sigma) in a modified serum bottle (Agar Bottle; Bellco) and incubated at 37 °C. Single colonies were picked and transferred to fresh medium and the purification procedure was repeated several times until the cultures were deemed pure. Several cultures were obtained, of which one, designated GH1T, was selected for further study. For routine incubation, growth tests and biochemical analysis, modified GH medium (without MgCl₂ and CaCl₂ including a monosaccharide such as D-glucose or D-xylose) was used. Strain GH1T was stored at −80 °C in 6 % DMSO.

Cells of strain GH1T were straight to slightly curved rods (0.4–0.6 μm wide and 2–5 μm long) with rounded and tapered ends. Transmission electron microscopy (S4300N; Hitachi) and phase-contrast microscopy (DS-Ri1; Nikon) demonstrated the presence of peritrichous flagella (Fig. S1, available in IJSEM Online) and oscillating motility. Gram-staining was performed using a Difco Gram-stain kits according to standard procedures (Gerhardt et al., 1994) and the KOH test (3 %, w/v) (Wallace & Gates, 1986). Spore formation was determined by microscopy after staining with malachite green (Gerhardt et al., 1994). The Gram-staining and KOH tests were negative and spore formation was not observed.

Catalase activity was tested by placing drops of 3 % (v/v) H₂O₂ onto plate-grown cultures and observing the production of oxygen bubbles. Strain GH1T was strictly anaerobic and catalase-negative. Turbidity was measured at 600 nm by inserting anaerobic Hungate tubes directly into the cuvette holder of a ThermoFisher Scientific Spec 20. All experiments were performed in triplicate. The temperature range for growth was determined in GH medium at 10–50 °C. Growth was observed between 30 and 45 °C, with the optimum being 40 °C; no growth was observed at 20 or 50 °C. To determine growth at different pH values, the following buffers were used (50 mM): trisodium citrate (pH 3.0–5.5), MES sodium salt (pH 5.5–6.5), Tris (pH 6.5–7.5) and sodium carbonate (pH 7.5–9.5). The isolate grew at pH 5.5–8.5, with the optimum being pH 6.0; no growth was observed at pH 5.0 or 9.0. Growth occurred with up to 3.5 % (w/v) NaCl; no growth was observed with 4 % (w/v) NaCl. The generation time of strain GH1T was determined as 1.1 h by monitoring OD 600 at 2 h intervals for 24 h under optimal growth conditions.

Substrate utilization was tested by growing the isolate in GH medium containing each of the substrates. Substrates (0.5 %, w/v) were added from filter-sterilized anaerobic stock solutions. Utilization was defined as an increase of OD 600 by 0.1. Strain GH1T was able to utilize D-glucose, D-ribose and D-xylose. Growth was not observed on D-arabinose, D-mannose, D-galactose, D-fructose, L-rhamnose, maltose, cellobiose, melibiose, trehalose, L-arabinose, α-lactose, sucrose, raffinose, melezitose, starch, D-sorbitol, D-mannitol, myo-inositol or sodium gluconate. Strain GH1T grew fermentatively and produced acids from D-glucose, D-ribose and D-xylose. The fermentation end products were analysed using GC with a flame ionization detector (model 6890N) equipped with a HP-INNOWax column (Agilent). Helium was used as the carrier gas. The major end product from D-glucose, D-ribose and D-xylose was butyric acid. Minor end products such as acetic acid, ethanol and butanol were also detected (Table S1). Sulfite, nitrite, fumarate, elemental sulfur, sulfate, thiosulfate and nitrate were tested as electron acceptors in GH medium containing (0.5 % each) yeast extract and polypeptide as carbon and energy sources. Sulfite (10 mM), nitrite (10 mM) and fumarate (20 mM) could not be utilized as an electron acceptor, but elemental sulfur (1 %), sulfate (20 mM), thiosulfate (20 mM) and nitrate (10 mM) could be utilized.

Respiratory quinones were extracted from lyophilized cells and the samples were purified and analysed by HPLC (Hu et al., 2001) using a YMC-Triat C18 (5 μl) column with methanol/isopropyl ether (3:1, v/v) as the mobile phase (1 ml min⁻¹). No quinone was detected in either strain GH1T or Oscillibacter valericigenes DSM 18026T (Fig. S2). The isomers of diaminopimelic acid in whole-cell hydrolysates were analysed by TLC (Lee & Hvang, 2002). Strain GH1T contained meso-diaminopimelic acid as the diagnostic diamino acid of the cell-wall peptidoglycan (Fig. S3). The cell-wall sugars were determined by the method of Schleifer & Kandler (1972) and analysed by TLC (cellulose glass plate 20 × 20 cm; Merck). Galactose, glucose, mannose, arabinose, xylose, ribose, fucose and rhamnose (0.1 %, w/v) were used as standard sugars (Schön & Groth, 2006). The main cell-wall sugar of strain GH1T was determined to be galactose (Fig. S4). The cell wall of O. valericigenes DSM 18026T contained meso-diaminopimelic acid and the cell-wall sugars were galactose and xylose (Figs. S3 and S4).

The cellular fatty acid composition of stationary-phase cells grown on GH medium was determined by extracting and analysing the fatty acid methyl esters according to the Sherlock Microbial Identification System (MIDI). The methyl esters were separated with an automated GC system (model 6890N and 7683 autosampler; Agilent) and identified using the MIDI software (Moore library). The whole-cell fatty acid analysis of strain GH1T revealed iso-C₁₃:0 (13.0 %), iso-C₁₅:0 (17.6 %), anteiso-C₁₅:0 (8.4 %).
and C₁₄:₀ (4.1 %) as the primary fatty acids. Fatty aldehydes were also found as dimethylacetals (DMAs) such as C₁₆:₀ DMA (17.8 %), iso-C₁₅:₀ DMA (15.2 %) and C₁₄:₀ DMA (4.5 %). (Table S2). The major fatty acids for strain GH₁ᵀ were similar to those of *O. valericigenes* DSM 18026ᵀ but the isolate differed from *O. valericigenes* DSM 18026ᵀ by the major amount of iso-C₁₃:₀ (13.0 %). DNA G+C content was determined by HPLC (Mesbah *et al.*, 1989; Tamaoka & Komagata, 1984) using a YMC-Triat C18 column (150 × 4.6 mm, Japan) at 30 °C with a mobile phase of 0.5 M NH₄H₂PO₄:acetonitrile (20 : 1). DNA of *Escherichia coli* KCTC 2441ᵀ was used as a standard. The genomic DNA G+C content of strain GH₁ᵀ was 54.6 mol %. DNA–DNA hybridization was carried out using the micro-dilution technique with photobiotin-labelled DNA (Ezaki *et al.*, 1989). DNA–DNA relatedness between strain GH₁ᵀ and *O. valericigenes* DSM 18026ᵀ was 24 %.

An almost-complete (1409 bp) 16S rRNA gene sequence of strain GH₁ᵀ was obtained using the universal primers 27F (5'–AGAGTTTGATCCTGGCTCAG–3') and 1492R (5'-GGTACCTTGTTACGACTT-3') (Madrid *et al.*, 2001). Sequences of the closest known relatives of the isolate were determined by performing database searches and were retrieved from the EzTaxon server 2.1 (http://www.eztaxon.org) or GenBank database (Benson *et al.*, 1994). DNA–DNA hybridization was carried out using the micro-dilution technique with photobiotin-labelled DNA (Ezaki *et al.*, 1989). DNA–DNA relatedness between strain GH₁ᵀ and *O. valericigenes* DSM 18026ᵀ was 24 %.

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The morphological, biochemical and physiological characteristics of strain GH₁ᵀ and its closest relative are summarized in Table 1. With respect to non-sporulation and Gram-negative staining, the isolate was similar to *O. valericigenes* DSM 18026ᵀ. However, strain GH₁ᵀ did not produce acid from L-arabinose (Table 1). In addition, *O. valericigenes* DSM 18026ᵀ was not able to grow at 40 °C, but strain GH₁ᵀ was able to grow optimally at 40 °C and up to 45 °C. Growth of the isolate strain GH₁ᵀ was not observed in 4 % (w/v) NaCl, but growth of *O. valericigenes* DSM 18026ᵀ was observed. These results would be well in accordance with the different isolation origins of *O. valericigenes* DSM 18026ᵀ (from the alimentary canal of Japanese corbicula clams in a salt environment) (Iino *et al.*, 2007) and strain GH₁ᵀ (from rumen). Another important difference is that one of the major fatty acids of strain GH₁ᵀ was iso-C₁₃:₀ (13 %), which was not detected (Iino *et al.*, 2007) or was minor in *O. valericigenes* DSM 18026ᵀ (Table S2). These characteristics obviously distinguished strain GH₁ᵀ from *O. valericigenes* DSM 18026ᵀ. *Oscillospira guilliermondii* OSC5 shared 91.6 % similarity with strain GH₁ᵀ, but the separation of this strain from the genus *Oscillibacter* was supported by a bootstrap value of 99 % (Fig. 1). *Oscillospira* is the closest genus to *Oscillibacter* and was isolated from similar environmental sources, such as rumen contents of sheep and cattle (Mackie *et al.*, 2003; Yanagita *et al.*, 2003). In addition, when public databases were searched for environmental sequences similar to the 16S rRNA gene sequence of strain GH₁ᵀ, the most similar ones (e.g. JQ608266.1, JX048496 and JN688157) originated from cow and goat faecal samples. These facts well correspond with the ruminal source of strain GH₁ᵀ, indicating it may play an important role in the ruminal ecology of herbivorous animals.

On the basis of phenotypic, chemotaxonomic and phylogenetic analysis, strain GH₁ᵀ should be assigned to a novel species of the genus *Oscillibacter*, for which the name *Oscillibacter ruminantium* sp. nov. is proposed.

**Description of Oscillibacter ruminantium sp. nov.**


Cells are Gram-negative, straight to slightly curved rods, non-spor-forming and motile. Obligately anaerobic, mesophilic and catalase-negative. Cells are 0.4–0.6 m in size. Grows at 30–45 °C (optimum 40 °C), at pH 5.5–8.5 (optimum pH 6) and with up to 3.5 % (w/v) NaCl. Utilizes D-glucose, D-ribose and D-xylose, but not D-arabinose, D-mannose, D-galactose, D-fructose, L-rhamnose, maltose, cellobiose, melibiose, trehalose, L-arabinose, α-lactose, sucrose, raffinose, melezitose, starch, D-sorbitol,

![Fig. 1. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing the relationships between strain GH₁ᵀ and its closest relatives. Bootstrap values (>50 %) based on 1000 resamplings are shown at branch nodes. *Aquifex pyrophilus* Kol5aᵀ was used as an out-group. Bar, 0.05 substitutions per site.](image-url)
Table 1. Comparison of morphological, biochemical and physiological characteristics of strain GH1\(^\text{T}\) and its closest phylogenetic neighbour

All data were taken from this study, unless otherwise indicated. Both strains are straight or slightly curved rods which are motile by peritrichous flagella, utilize \(\text{d-ribose, d-xylene}\) and \(\text{d-glucose}\) and produce butyric acid as a major end product from these sugars. Both strains are negative for Gram staining, catalase sporulation and utilization of \(\text{l-arabinose}\). B, Butyric acid; +, positive; −, negative; ND, not detected

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Oscillibacter ruminantium sp. nov.</th>
<th>Oscillibacter valericigenes DSM 18026(^\text{T})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell size ((\mu\text{m}))</td>
<td>0.4–0.6 × 2.0–5.0</td>
<td>0.5 × 2.0–5.0*</td>
</tr>
<tr>
<td>Temperature for growth ((^\circ\text{C}))</td>
<td>30–45</td>
<td>15–35</td>
</tr>
<tr>
<td>Optimum</td>
<td>40</td>
<td>30</td>
</tr>
<tr>
<td>pH for growth</td>
<td>5.5–8.5</td>
<td>5.0–8.5</td>
</tr>
<tr>
<td>Optimum</td>
<td>6.0</td>
<td>6.0–6.5</td>
</tr>
<tr>
<td>NaCl for growth (%)</td>
<td>0–3.5</td>
<td>0–4</td>
</tr>
<tr>
<td>Optimum</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Acid produced from fermentation of (\text{l-arabinose})</td>
<td>ND</td>
<td>B</td>
</tr>
<tr>
<td>DNA G+C content (mol%)</td>
<td>54.6</td>
<td>52.7</td>
</tr>
</tbody>
</table>

*Data from Iino et al. (2007).

\(\text{D-mannitol, myo-inositol or sodium gluconate. Elemental sulfur, sulfate, thiosulfate and nitrate are used as electron acceptors, but sulfite, nitrite and fumarate are not. The diagnostic diamino acid in the cell wall is meso-diamino-pimelic acid and the major cell-wall sugar is galactose. The cellular fatty acids and fatty aldehydes are mainly iso-C13:0, iso-C15:0, anteiso-C15:0, C14:0, C16:0 DMA, iso-C15:0 DMA and C14:0 DMA. The type strain is GH1\(^\text{T}\) (=KCTC 15176\(^\text{T}\)=NBRC 108824\(^\text{T}\)=JCM 18333\(^\text{T}\)), isolated from the rumen fluid of a Korean native cattle (HanWoo) steer. The G+C content of the type strain is 54.6 mol\% (HPLC).}

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References


