Description of Proteiniphilum saccharofermentans sp. nov., Petrimonas mucosa sp. nov. and Fermentimonas caenicola gen. nov., sp. nov., isolated from mesophilic laboratory-scale biogas reactors, and emended description of the genus Proteiniphilum

Sarah Hahnke,¹ Thomas Langer,¹ Daniela E. Koeck² and Michael Klocke¹

¹Department of Bioengineering, Leibniz Institute for Agricultural Engineering Potsdam-Bornim, D-14469 Potsdam, Germany
²Institute for Microbiology, Technische Universität München, D-85350 Freising-Weihenstephan, Germany

Three novel, facultatively anaerobic bacteria of the family Porphyromonadaceae (phylum Bacteroidetes) were isolated from mesophilic laboratory-scale biogas reactors. The strains were Gram-negative rods. Optimal growth occurred between 35 and 45 °C and at pH 7.1–7.8. The main fermentation products were acetic and propionic acids. The predominant fatty acid in all strains was anteiso-C15 : 0 , and the only respiratory quinone detected was menaquinone MK-8. 16S rRNA gene sequence comparison indicated that strains M3/6⁰ and ING2-E5B⁰ were most closely related to the type strain of Proteiniphilum acetatigenes, with sequence similarities of 97.3 and 94.5 %. Strain ING2-E5A⁰ showed the closest affiliation to the type strain of Petrimonas sulfuriphila, with 97 % sequence identity. DNA–DNA hybridization of strain M3/6⁰ and ING2-E5A⁰ with the most closely related type strains showed 43.3–45.6 and 23.8–25.7 % relatedness, respectively, which supports the conclusion that both isolates represent novel species. Phylogenetic analysis and comparison of cellular fatty acid patterns indicated that strain ING2-E5B⁰ cannot be classified as a member of any previously described genus. Therefore, because of the physiological, genotypic and chemotaxonomic differences, it is proposed to designate novel species within the genera Proteiniphilum and Petrimonas, Proteiniphilum saccharofermentans sp. nov. (type strain M3/6⁰ = DSM 28694⁰ = CECT 8610⁰ = LMG 28299⁰) and Petrimonas mucosa sp. nov. (type strain ING2-E5A⁰ = DSM 28695⁰ = CECT 8611⁰), and a novel species of a new genus, Fermentimonas caenicola gen. nov., sp. nov. (type strain of Fermentimonas caenicola is ING2-E5B⁰ = DSM 28696⁰ = CECT 8609⁰ = LMG 28429⁰). In addition, an emended description of the genus Proteiniphilum is provided.

Members of the phylum Bacteroidetes often comprise the second most abundant group of the microbial community in biogas reactors, as indicated by cultivation-independent community analyses (Hanreich et al., 2013; Kröber et al., 2009; Rademacher et al., 2012; Schlüter et al., 2008; St-Pierre & Wright, 2014; Zakrzewski et al., 2012; Ziganshin et al., 2013). The phylum Bacteroidetes represents a metabolically heterogeneous group that includes species with a broad range of capabilities such as hydrolysis of polysaccharides and proteins, fermentation of sugars and production of acids such as acetic, propionic, succinic and butyric acids (Krieg et al., 2010). Regarding their metabolic properties, members of this group seem to play a major role during anaerobic digestion of plant biomass, namely the primary decomposition of long-chain polymers.

Abbreviations: PASC, phosphoric acid-swollen cellulose; CM-cellulose, carboxymethyl cellulose.

The GenBank/EMBL/DBJ accession numbers for the 16S rRNA gene sequences of strains ING2-E5A⁰, M3/6⁰ and ING2-E5B⁰ are respectively KP233808–KP233810.

Two supplementary figures are available with the online Supplementary Material.
(hydrolysis) and the subsequent production of volatile fatty acids (acidogenesis). Hence, members of the Bacteroidetes may represent important members of the community in biomethanation processes, similar to the Firmicutes.

The family Porphyromonadaceae within the Bacteroidetes includes many (mostly uncultured) bacteria from different biogas reactors and other methanogenic environments showing close affiliation to Proteiniphilum acetatigenes and Petrimonas sulfuriphila (Kampmann et al., 2012, 2014; Kleng et al., 2015; Krause et al., 2008; Li et al., 2009; Liu et al., 2009; Theuerl et al., 2015; Zakrzewski et al., 2012). Several of these bacteria formed clusters with three strains that we isolated from laboratory-scale biogas reactors. The novel strains are currently the only cultivated representatives of these clusters and can be assigned to novel species. In this study, we propose the description of the novel isolates as representatives of novel species within the genera Proteiniphilum and Petrimonas and as a novel species of a new genus.

The three strains, designated M3/6T, ING2-E5A T and ING2-E5B T, were isolated from mesophilic laboratory-scale biogas reactors. Strain M3/6T was obtained from a two-phase biogas reactor, i.e. an upflow anaerobic solid-state (UASS) reactor (Mumme et al., 2010), supplied continuously with a mixture of 95 % maize silage and 5 % wheat straw. The reactor was operated at an organic loading rate of 3 g VScorr l−1 day−1, where VScorr was defined as the sum of volatile solids, volatile fatty acids, lactic acid and alcohols. Strain M3/6T was isolated from the reactor fluid, which was diluted 10-fold in Ringer’s solution and spread on BBL Columbia agar base medium (Becton Dickinson) supplemented with 5 % horse blood. Strains ING2-E5A T and ING2-E5B T were obtained from a completely stirred tank reactor (CSTR) fed continuously with a mixture of maize silage [10 % organic dry matter (ODM)], pig manure (45 % ODM) and cattle manure (45 % ODM). The reactor was operated with an organic loading rate of 8.5 g VScorr l−1 day−1. Reactor material was diluted 106-fold in Ringer’s solution and spread on Columbia agar supplemented with 5 % horse blood and 1.7 g sodium propionate l−1, which corresponded to the propionic acid concentration in the reactor. The media were prepared under oxic conditions, and agar plates were transferred to an anoxic glovebox (Whitley MG 500; Meintrup DWS Laborgeräte) and stored under these conditions for at least 24 h before use. For purification, single colonies were picked and restreaked; incubation occurred at 37 °C.

Routine cultivation of the novel strains was carried out in anoxic basal medium consisting of (I−1): 0.8 g NH4Cl, 0.3 g KH2PO4, 0.6 g NaCl, 0.1 g CaCl2 · 2H2O, 0.2 g MgCl2 · 6H2O, 0.1 g KCl, 10 ml trace element solution (DSMZ medium 318), 0.5 g cysteine hydrochloride monohydrate and 1 mg resazurin. The medium was adjusted to pH 7 with NaOH and dispensed into Hungate tubes under a 100 % N2 gas atmosphere. After autoclaving, the medium was supplemented with a vitamin solution (according to DSMZ medium 141), vitamin K1 and haemin solution (according to DSMZ medium 104). Concentrations of added substrates varied depending on the purpose of cultivation and are indicated below.

Amplification and sequencing of the almost complete 16S rRNA genes of strains M3/6T and ING2-E5A T were carried out using the primer pair 27F and 1492R as described by Hahnke et al. (2014). The complete 16S rRNA gene sequence of strain ING2-E5B T was obtained from its genome sequence (Hahnke et al., 2015). To analyse the occurrence of closely affiliated strains, the 16S rRNA gene sequences as well as fragments of the 16S rRNA gene usually obtained from DGGE gels (sequence from Escherichia coli position 341 to 907) were compared with those of the NCBI GenBank database using the BLAST tool (http://www.ncbi.nlm.nih.gov/). Reconstruction of phylogenetic trees was performed using the phylogeny program ARB [http://www.arb-home.de (Ludwig et al., 2004)]. Sequences were aligned with the automatic alignment tool implemented in the ARB program, and afterwards the alignment was checked manually. Calculation of pairwise similarity values was performed with no distance correction using the ARB DISTANCE MATRIX tool. Phylogenetic trees were reconstructed by using the neighbour-joining method with bootstrap calculations based on 1000 replicates (sequence gaps were excluded and no phylogenetic distance correction was applied) and the maximum-likelihood method using the ARB PhyML tool with the Hasegawa–Kishino–Yano (HKY) model of nucleotide substitution (Hasegawa et al., 1985). DNA–DNA hybridization was carried out by the Identification Service of the Leibniz-Institut DSMZ – Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (Braunschweig, Germany). Cells were disrupted using a Constant Systems TS 0.75 kW (IUL Instruments), and DNA was purified as described by Cashion et al. (1977). DNA–DNA hybridization was carried out as described by De Ley et al. (1970) under consideration of the modifications described by Huss et al. (1983) using a model Cary 100 Bio UV/Vis spectrophotometer equipped with a Peltier-thermostatted 6 × 6 multicell changer and a temperature controller with in-situ temperature probe (Varian).

16S rRNA gene sequence analyses placed the three novel strains within the family Porphyromonadaceae (phylum Bacteroidetes). Strain M3/6T shared 97.3 % sequence similarity with Proteiniphilum acetatigenes TB107T as the most closely related strain. DNA–DNA hybridization revealed a reassociation value of 43.3–45.6 % between the two strains. Strain ING2-E5A T showed the closest affiliation to Petrimonas sulfuriphila BN3T, with 97 % 16S rRNA gene sequence identity and DNA–DNA relatedness of 23.8–25.7 %. In phylogenetic trees, these two novel isolates formed clusters with their closest relative. Strain ING2-E5B T showed 94.5 % 16S rRNA gene sequence similarity to Proteiniphilum acetatigenes TB107T as the
closest relative (and 94.1 % similarity to *Petrimonas sulfuriphila* BN3$^T$). In phylogenetic trees, strain ING2-E5B$^T$ formed a subcluster of the genus *Proteiniphilum*, different treeing methods (neighbour-joining and maximum-likelihood) confirmed this divergence (Fig. 1). Furthermore, comparison of the 16S rRNA gene sequences of the novel strains with those contained in the GenBank database revealed the presence of closely related sequences ($\geq 99$ % similarity) retrieved from different biogas reactors or similar environments. Nine of these sequences were related to strain M3/6$^T$ retrieved from different biogas reactors or similar environments such as agricultural biogas plants, different solid-waste landfill leachate, compost samples and a types of laboratory-scale biogas reactors treating different environments. Uncultured bacterial clones from different methanogenic environments such as agricultural biogas plants, different solid-waste landfill leachate, compost samples and a methanogenic corn-stalk-degrading enrichment culture.

The novel strains are currently the only cultivated and described representatives of these clusters.

To determine the temperature range and optimum of the novel isolates, cells were grown anaerobically in basal medium with 0.2 % yeast extract and 20 mM glucose at 10, 15, 20, 25, 30, 35, 37, 40, 45, 50 and 55 °C. The pH$^{37}$ °C range and optimum were examined at pH 3.5, 5.4, 5.7, 6.3, 6.6, 7.1, 7.5, 7.8, 8.6, 9.1 and 9.9 using the same medium supplemented with a buffer mixture of MES, HEPES, TAPS and CHES (10 mM each) based on Lee *et al.* (2007). The pH was adjusted with NaOH or HCl solutions and was checked at 37 °C after autoclaving. To determine the NaCl tolerance, anoxic basal medium with 0.2 % yeast extract and 20 mM glucose was used with varying NaCl concentrations (0, 0.55, 1, 2, 3, 4 and 5 %, w/v) while the concentrations of the other medium components were maintained.

Temperature profiles of the isolates showed that strains M3/6$^T$ and ING2-E5B$^T$ grew at 15–45 °C, with optimum growth at 35–40 and 37–40 °C, respectively. Strain ING2-E5A$^T$ grew at 20–50 °C with an optimum at 45 °C. All strains tolerated pH$^{37}$ °C 6.3–9.1, with optima at pH 7.5–7.8 (strains M3/6$^T$ and ING2-E5A$^T$) and 7.1 (ING2-E5B$^T$) (Table 1). Strain M3/6$^T$ tolerated NaCl concentrations from 0–5 % with optimum growth at 0.55–1 %, while strains ING2-E5A$^T$ and ING2-E5B$^T$ grew only at lower NaCl concentrations (0–2 and 0–1 %, respectively), with optimum growth in the absence of added NaCl. On Columbia blood agar, all three isolates grew under

---

**Fig. 1.** Neighbour-joining tree based on 16S rRNA gene sequences showing the relationships of strains M3/6$^T$, ING2-E5A$^T$ and ING2-E5B$^T$ within the family *Porphyromonadaceae*. Bootstrap values at nodes are percentages derived from 1000 replicates. Filled circles indicate nodes that were also recovered reproducibly with the maximum-likelihood method. The sequence of *Bacteroides fragilis* NCTC 9343$^T$ (GenBank accession no. CR628927) was used as an outgroup (not shown). Bar, 5 % sequence divergence.
strictly anoxic conditions as well as during exposure to air oxygen; thus, they are facultative anaerobes. Growth of *Proteiniphilum acetatigenes* DSM 18083\(^T\) and *Petrimonas sulfuriphila* DSM 16547\(^T\) also occurred on Columbia blood agar under oxic conditions.

Gram staining, cytochrome oxidase and catalase tests and determination of cell morphology were performed with cells grown in anoxic basal medium with yeast extract and proteose peptone (0.5 % each) and 20 mM glucose. Cells were observed at a magnification of \( \times 630 \) using an Optiphot-2 (Nikon) phase-contrast microscope. Gram staining was performed according to Claus (1992). The cytochrome oxidase reaction was tested with 20 \( \mu \)g protein sol. All three novel strains showed weak extracellular enzyme activities against cellobiose, starch, arabinogalactan, CM-cellulose and phosphoric acid-swollen cellulose [PASC; prepared from Avicel according to Wood (1988)]. None of the strains showed cellulolytic activity against crystalline cellulose (Avicel). Strain M3/6\(^T\) showed slightly enhanced activity against \( \beta \)-glucan, strain ING2-E5A\(^T\) against lactose and strain ING2-E5B\(^T\) against xylan (hardwood) and arabinoxylan (medium viscosity); the other strains showed only weak activity against these substrates.

Substrate utilization was tested in anoxic basal medium with addition of 0.2 % yeast extract. Single substrates were added from sterile-filtered stock solutions to the following final concentrations: monosaccharides (20 mM), disaccharides and ethanol (10 mM), soluble starch with the Bradford Protein Assay kit (Thermo Scientific). To measure enzyme activities, assays with 10 \( \mu \)l protein solution (0.1–0.4 \( \mu \)g protein \( \mu \)l\(^{-1}\)) were prepared as described by Koeck et al. (2014). Hydrolytic reactions were set up in triplicate and incubated at 37\(^\circ\)C for 24 h. All tested substrates are listed in the species descriptions. Enzymic hydrolysis of substrates was analysed by measurement of reducing sugars using the DNSA assay according to Miller (1959). To analyse the enzymic degradation of cellubiose, lactose and starch, reaction assays were prepared and incubated as described above. Free D-glucose, which is released during enzymic degradation, was quantified using a D-glucose assay kit (Megazyme).
(0.16 g l⁻¹), proteose peptone (Carl Roth) and tryptone (peptone from casein; Merck) (2 g l⁻¹). Growth on protease peptone and tryptone was also tested without addition of yeast extract. Growth on amino acids was tested with a mixture of 20 amino acids (2.5 mM each). All tested substrates are listed in the species descriptions. For each substrate, duplicates were inoculated with cells precultured on Columbia blood agar and suspended in basal medium without addition of substrates. Cell cultures with 0.2 % yeast extract were used as a negative control. Growth was determined after incubation at 37 °C by measuring an increase in OD₆₀₀ with a UV/Vis spectrophotometer. Growth was scored as negative when the OD₆₀₀ was equal to or less than that in the negative control. Cell-free assays with the respective substrate were used as additional controls.

The three novel isolates exhibited very similar substrate utilization spectra. Growth was supported by mono- and disaccharides, by starch and by complex proteinaceous substrates. For all three strains, growth on single substrates was weak and was strongly supported by the addition of yeast extract. Strains M₃/₆ᵀ and ING₂-E₅Aᵀ did not show growth on peptone or tryptone as single substrates, but they grew weakly when both substrates were supplied. Strain ING₂-E₅Bᵀ showed weak growth on both peptone and tryptone and on a mixture of the two. A requirement for tryptone, as described for Petrimonas sulfuriphila BN₃ᵀ (Grabowski et al., 2005), was not observed for the novel strains. For Proteiniphilum acetatigenes TB₁₀₇ᵀ, it was reported that the strain was not able to use any of a variety of tested carbohydrates (Chen & Dong, 2005); however, in our experiments, all tested mono- and disaccharides (arabinose, cellobiose, fructose, galactose, glucose, lactose, maltose, mannose, rhamnose, sucrose and trehalose) supported cell growth of Proteiniphilum acetatigenes DSM 1₈₀₈₃ᵀ. This striking discrepancy could be explained by differences in the experimental approach: we tested substrate utilization by addition of single substrates to medium containing yeast extract, whereas Chen & Dong (2005) tested growth on single substrates. Possibly, single carbohydrates cannot serve as single carbon sources but are co-metabolized with other compounds, or certain growth factors such as vitamins or trace elements are needed, which can be supplied by complex substrates.

For analysis of fermentation products, strains were grown in anoxic basal medium with yeast extract and proteose peptone (0.5 % each) with and without addition of 20 mM glucose. Each assay was carried out in duplicate. Medium without addition of cells was used as a control. Hydrogen and CO₂ were analysed with a thermal conductivity detector and a flame ionization detector, respectively. Temperatures for the injection port, the columns and the detectors were 150, 50 and 250 °C, respectively. Methanol, ethanol and propanol as well as the carboxylic acids propionic acid, acetic acid, butyric acid, isobutyric acid, valeric acid, isovaleric acid and caproic acid were analysed in the medium residue after acidification with phosphoric acid (0.85 % final) with an Agilent 7890A gas chromatograph equipped with a flame ionization detector and a Permabond FFAP column (30 m x 0.32 mm, 0.5 μm; Macherey-Nagel). Temperatures for the injection port and detector were 180 and 220 °C, respectively. The carrier gas was helium at a flow rate of 3.4 ml min⁻¹; for elution, the column temperature was increased from 80 to 210 °C at a rate of 15 °C min⁻¹. Formic acid and lactic acid were analysed with a Dionex UltiMate 3000 HPLC equipped with an Eurokat H column (300 x 8 mm, 10 μm; Knauer) and a Shodex RI-101 refractive index detector (column temperature, 35 °C; eluant, 0.01 M sulfuric acid; flow rate, 0.8 ml min⁻¹).

When grown on yeast extract and peptone, strains M₃/₆ᵀ and ING₂-E₅Aᵀ produced propionic acid, acetic acid and CO₂ and lower concentrations of isovaleric acid and H₂; for strain ING₂-E₅Aᵀ, only traces of H₂ were detected. Strain ING₂-E₅Bᵀ produced acetic acid, smaller amounts of propionic acid and CO₂ and traces of isovaleric acid and H₂. After addition of glucose, strain M₃/₆ᵀ produced acetic acid, propionic acid and CO₂ and traces of H₂, whereas strain ING₂-E₅Aᵀ produced acetic acid, small amounts of propionic acid, isovaleric acid and CO₂ and traces of butyric acid. For strain ING₂-E₅Bᵀ, acetic acid, propionic acid and CO₂ and traces of H₂ were detected (Table 1).

For analyses of cellular fatty acids, biomass of the novel strains and Proteiniphilum acetatigenes DSM 1₈₀₈₃ᵀ and Petrimonas sulfuriphila DSM 1₆₅₄₇ᵀ was obtained from exponentially growing cells cultivated at 37 °C in anoxic basal medium with yeast extract and proteose peptone (0.5 % each). To analyse respiratory quinones, the isolates were grown in the same medium, whereas Proteiniphilum acetatigenes DSM 1₈₀₈₃ᵀ was cultivated in anoxic Caso bouillon (Merck) supplemented with 0.4 % yeast extract. Analyses were carried out by the Identification Service of the DSMZ. Respiratory quinones were analysed as described by Grabowski et al. (2005). Fatty acid methyl esters were obtained by saponification, methylation and extraction based on Miller (1982) and Kuykendall et al. (1988). Identification of the fatty acids relied on the use of the Sherlock Microbial Identification System with fatty acids being identified against the TSB4A.0 database.

In the three novel strains, as well as in Proteiniphilum acetatigenes DSM 1₈₀₈₃ᵀ, the only detected respiratory lipoquinone was menaquinone MK-8, which was also reported to be the predominant quinone in Petrimonas sulfuriphila BN₃ᵀ (Grabowski et al., 2005). Cellular fatty acid profiles of all strains showed a complex mixture of straight-chain, branched (iso- and anteiso-) and hydroxylated fatty acids (Table 2), which is a collective
Table 2. Cellular fatty acid composition of the novel strains and the most closely related type strains

Strains: 1, M3/6T; 2, Proteiniphilum acetatigenes DSM 18083T; 3, ING2-E5BT; 4, ING2-E5AT; 5, Petrimonas sulfuriphila DSM 16547T.
All data are from this study. TR, Trace amount (<0.5 %); –, not detected. Values are percentages of total fatty acids. Only fatty acids that accounted for >1 % in at least one of the strains are shown. Fatty acids present at >5 % are highlighted in bold.

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saturated straight-chain</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C14 : 0</td>
<td>1.0</td>
<td>TR</td>
<td>6.3</td>
<td>3.6</td>
<td>1.2</td>
</tr>
<tr>
<td>C15 : 0</td>
<td>6.8</td>
<td>9.4</td>
<td>2.5</td>
<td>1.3</td>
<td>2.5</td>
</tr>
<tr>
<td>C16 : 0</td>
<td>2.7</td>
<td>3.2</td>
<td>1.7</td>
<td>1.0</td>
<td>1.5</td>
</tr>
<tr>
<td>C17 : 0</td>
<td>TR</td>
<td>2.3</td>
<td>–</td>
<td>TR</td>
<td>TR</td>
</tr>
<tr>
<td>Saturated branched-chain</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>anteiso-C15 : 0</td>
<td>TR</td>
<td>TR</td>
<td>1.9</td>
<td>1.8</td>
<td>TR</td>
</tr>
<tr>
<td>iso-C12 : 0</td>
<td>TR</td>
<td>TR</td>
<td>0.5</td>
<td>2.0</td>
<td>0.6</td>
</tr>
<tr>
<td>iso-C13 : 0</td>
<td>0.6</td>
<td>TR</td>
<td>6.4</td>
<td>2.0</td>
<td>2.2</td>
</tr>
<tr>
<td>anteiso-C13 : 0</td>
<td>2.3</td>
<td>1.6</td>
<td>8.3</td>
<td>5.5</td>
<td>5.7</td>
</tr>
<tr>
<td>iso-C14 : 0</td>
<td>4.7</td>
<td>4.0</td>
<td>7.2</td>
<td>9.2</td>
<td>6.4</td>
</tr>
<tr>
<td>iso-C15 : 0</td>
<td>9.6</td>
<td>5.5</td>
<td>21.1</td>
<td>18.1</td>
<td>15.0</td>
</tr>
<tr>
<td>anteiso-C15 : 0</td>
<td>49.5</td>
<td>46.6</td>
<td>24.5</td>
<td>45.9</td>
<td>47.4</td>
</tr>
<tr>
<td>iso-C16 : 0</td>
<td>1.7</td>
<td>TR</td>
<td>1.9</td>
<td>0.6</td>
<td>0.7</td>
</tr>
<tr>
<td>iso-C17 : 0</td>
<td>TR</td>
<td>1.2</td>
<td>TR</td>
<td>1.4</td>
<td></td>
</tr>
<tr>
<td>anteiso-C17 : 0</td>
<td>1.2</td>
<td>0.9</td>
<td>–</td>
<td>TR</td>
<td>1.1</td>
</tr>
<tr>
<td>Hydroxy</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>iso-C15 : 0 3-OH</td>
<td>1.0</td>
<td>1.5</td>
<td>3.7</td>
<td>1.8</td>
<td>2.7</td>
</tr>
<tr>
<td>C15 : 0 3-OH</td>
<td>0.9</td>
<td>1.4</td>
<td>1.3</td>
<td>TR</td>
<td>TR</td>
</tr>
<tr>
<td>iso-C16 : 0 3-OH</td>
<td>4.3</td>
<td>5.4</td>
<td>1.9</td>
<td>1.8</td>
<td>1.6</td>
</tr>
<tr>
<td>C16 : 0 3-OH</td>
<td>1.9</td>
<td>0.8</td>
<td>3.0</td>
<td>1.3</td>
<td>0.8</td>
</tr>
<tr>
<td>iso-C17 : 0 3-OH</td>
<td>4.0</td>
<td>4.9</td>
<td>2.1</td>
<td>1.0</td>
<td>2.4</td>
</tr>
<tr>
<td>C17 : 0 2-OH</td>
<td>4.6</td>
<td>3.4</td>
<td>0.6</td>
<td>0.5</td>
<td>2.1</td>
</tr>
<tr>
<td>Summed features</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Summed feature 3*</td>
<td>TR</td>
<td>2.1</td>
<td>0.7</td>
<td>TR</td>
<td>0.6</td>
</tr>
</tbody>
</table>

*Summed feature 3 contains C16 : 1ω7c and/or iso-C15 : 0 2-OH.

feature for all species of the family Porphyromonadaceae. In our novel strains, the predominant fatty acid was anteiso-C15 : 0, a characteristic that they share with species of several other genera within this family, whereas members of some other genera contain iso-C15 : 0 as the major fatty acid. The fatty acid profile of strain M3/6T resembled that of its closest relative Proteiniphilum acetatigenes DSM 18083T (Table 2). Besides the fatty acid anteiso-C15 : 0 (48.1 ± 1.5 %), they contained substantial proportions of iso-C15 : 0 (7.6 ± 2 %) and C15 : 0 (8.1 ± 1.3 %), and the major hydroxy fatty acids were iso-C16 : 0 3-OH (4.9 ± 0.5 %) and C17 : 0 2-OH (4.0 ± 0.6 %). The fatty acid pattern of strain ING2-E5AT was very similar to that of its closest relative Proteiniphilum sulfuriphila DSM 16547T. The major fatty acids of both strains were anteiso-C15 : 0 (mean ± SD for the two strains 46.7 ± 0.8 %), iso-C15 : 0 (16.6 ± 1.6 %), iso-C14 : 0 (7.8 ± 1.4 %) and anteiso-C13 : 0 (5.6 ± 0.1 %). Compared with isolates M3/6T and ING2-E5BT and Proteiniphilum acetatigenes DSM 18083T, they contained relatively smaller proportions of hydroxy fatty acids (Table 2). Strain ING2-E5BT exhibited a significantly different fatty acid pattern from those of the other strains. The fatty acids anteiso-C15 : 0 and iso-C15 : 0 were present in almost equal proportions (24.5 and 21.1 %). Furthermore, fatty acids that were also present in substantial amounts were anteiso-C13 : 0 (8.3 %), iso-C14 : 0 (7.2 %), iso-C13 : 0 (6.4 %) and C14 : 0 (6.3 %), and the predominant hydroxy fatty acids were iso-C15 : 0 3-OH (3.7 %) and C16 : 0 3-OH (3.0 %) (Table 2).

Analysis of the DNA G+C content was carried out by the Identification Service of the DSMZ. Cells were disrupted using a Constant Systems TS 0.75 kW (IUL Instruments), and DNA was purified as described by Cashion et al. (1977). The DNA G+C content was determined after enzymic hydrolysis of the genomic DNA and subsequent analysis of the deoxyribonucleosides by HPLC (Mesbah et al., 1989; Tamaoka & Komagata, 1984). The DNA G+C content of strains M3/6T, ING2-E5A and ING2-E5B was 45.1, 48.2 and 38.2 mol%.

In summary, on the basis of 16S rRNA gene sequence comparison, strains M3/6T and ING2-E5AT showed the closest affiliation to the type strains of Proteiniphilum acetatigenes and Petrimonas sulfuriphila (97.3 and 97.0 % sequence similarity). DNA–DNA hybridization of the strains with the respective closest related type species showed values considerably lower than 70 %, which was recommended as a suitable threshold for the delineation of different species (Wayne et al., 1987). In conjunction with physiological dissimilarities, minor differences in the fatty acid composition and differences in the DNA G+C content (Tables 1 and 2), our results support the assignment of the two strains to novel species of the genera Proteiniphilum and Petrimonas, for which we propose the names Proteiniphilum saccharofermentans sp. nov. and Petrimonas mucosa sp. nov. Our results also necessitate an emended description of the genus Proteiniphilum.

Based on 16S rRNA gene sequence analysis, strain ING2-E5BT is only distantly related to its closest characterized relative Proteiniphilum acetatigenes TB107T, showing 5.5 % sequence divergence. A 16S rRNA gene sequence identity of <95 % was reported to be a reasonable threshold as a ‘lower cut-off window’ for genera (Yarza et al., 2008); however, in order to describe a novel genus, in addition to phylogenetic evidence, clear separation on the basis of chemotaxonomic data is also needed. Within the family Porphyromonadaceae, important genus-specific chemotaxonomic features are the predominant menaquinone(s) and the cellular fatty acids, including the ratio of the fatty acids anteiso-C15 : 0 and iso-C15 : 0 (Table 3). Comparing strain ING2-E5BT with the most closely related genera, Proteiniphilum and Petrimonas, it contained the same predominant fatty acid and menaquinone, but strain ING2-E5BT showed a slightly lower ratio of...
Table 3. Comparison of strain ING2-E5B<sup>T</sup> with different genera of the family Porphyromonadaceae showing characters used for genus delineation

Data for the genus *Proteiniphilum* include results for strain M3/6<sup>T</sup> and data for the genus *Petrimonas* include results for strain ING2-E5A<sup>T</sup> obtained in this study. Other data for related genera were taken from Chen & Dong (2005), Grabowski et al. (2005), Kodama et al. (2012), Pramono et al. (2015), Sakamoto et al. (2002), Sánchez-Andrea et al. (2014), Ueki et al. (2006), Wagener et al. (2014) and Yang et al. (2014); fatty acid data for *Proteiniphilum* and *Petrimonas* were obtained in this study. ND, No data available.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>ING2-E5B&lt;sup&gt;T&lt;/sup&gt;</th>
<th><em>Proteiniphilum</em></th>
<th><em>Petrimonas</em></th>
<th><em>Dysgonomonas</em></th>
<th><em>Microbacter</em></th>
<th><em>Paludibacter</em></th>
<th><em>Tannerella</em></th>
<th><em>Porphyromonas</em></th>
<th><em>Falsiporphyromonas</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>DNA G+C content (mol%)</td>
<td>38.2</td>
<td>45.1–48.9</td>
<td>40.8–48.2</td>
<td>37.5–41.8</td>
<td>39.9</td>
<td>39.3</td>
<td>44–48</td>
<td>40–55</td>
<td>40.7</td>
</tr>
<tr>
<td>Predominant fatty acid*</td>
<td>ai-C&lt;sub&gt;15&lt;/sub&gt;:&lt;sub&gt;0&lt;/sub&gt;</td>
<td>ai-C&lt;sub&gt;15&lt;/sub&gt;:&lt;sub&gt;0&lt;/sub&gt;</td>
<td>ai-C&lt;sub&gt;15&lt;/sub&gt;:&lt;sub&gt;0&lt;/sub&gt;</td>
<td>ai-C&lt;sub&gt;15&lt;/sub&gt;:&lt;sub&gt;0&lt;/sub&gt;</td>
<td>ai-C&lt;sub&gt;15&lt;/sub&gt;:&lt;sub&gt;0&lt;/sub&gt;</td>
<td>ai-C&lt;sub&gt;15&lt;/sub&gt;:&lt;sub&gt;0&lt;/sub&gt;</td>
<td>ai-C&lt;sub&gt;15&lt;/sub&gt;:&lt;sub&gt;0&lt;/sub&gt;</td>
<td>i-C&lt;sub&gt;15&lt;/sub&gt;:&lt;sub&gt;0&lt;/sub&gt;</td>
<td>i-C&lt;sub&gt;15&lt;/sub&gt;:&lt;sub&gt;0&lt;/sub&gt;</td>
</tr>
<tr>
<td>Other major fatty acids (&gt;5 %)*</td>
<td>i-C&lt;sub&gt;15&lt;/sub&gt;:&lt;sub&gt;0&lt;/sub&gt;, ai-C&lt;sub&gt;13&lt;/sub&gt;:&lt;sub&gt;0&lt;/sub&gt;, i-C&lt;sub&gt;14&lt;/sub&gt;:&lt;sub&gt;0&lt;/sub&gt;, i-C&lt;sub&gt;13&lt;/sub&gt;:&lt;sub&gt;0&lt;/sub&gt;, i-C&lt;sub&gt;14&lt;/sub&gt;:&lt;sub&gt;0&lt;/sub&gt;, C&lt;sub&gt;14&lt;/sub&gt;:&lt;sub&gt;0&lt;/sub&gt;</td>
<td>i-C&lt;sub&gt;15&lt;/sub&gt;:&lt;sub&gt;0&lt;/sub&gt;, i-C&lt;sub&gt;14&lt;/sub&gt;:&lt;sub&gt;0&lt;/sub&gt;, ai-C&lt;sub&gt;13&lt;/sub&gt;:&lt;sub&gt;0&lt;/sub&gt;, i-C&lt;sub&gt;14&lt;/sub&gt;:&lt;sub&gt;0&lt;/sub&gt;, C&lt;sub&gt;14&lt;/sub&gt;:&lt;sub&gt;0&lt;/sub&gt;</td>
<td>NA‡</td>
<td>i-C&lt;sub&gt;15&lt;/sub&gt;:&lt;sub&gt;0&lt;/sub&gt;, i-C&lt;sub&gt;17&lt;/sub&gt;:&lt;sub&gt;0&lt;/sub&gt;, 3-OH, ai-C&lt;sub&gt;17&lt;/sub&gt;:&lt;sub&gt;0&lt;/sub&gt;, ai-C&lt;sub&gt;17&lt;/sub&gt;:&lt;sub&gt;0&lt;/sub&gt;, 3-OH</td>
<td>C&lt;sub&gt;16&lt;/sub&gt;:&lt;sub&gt;0&lt;/sub&gt;, 3-OH, C&lt;sub&gt;16&lt;/sub&gt;:&lt;sub&gt;0&lt;/sub&gt;, 3-OH, i-C&lt;sub&gt;17&lt;/sub&gt;:&lt;sub&gt;0&lt;/sub&gt;, 3-OH</td>
<td>NA‡</td>
<td>ai-C&lt;sub&gt;15&lt;/sub&gt;:&lt;sub&gt;0&lt;/sub&gt;, i-C&lt;sub&gt;17&lt;/sub&gt;:&lt;sub&gt;0&lt;/sub&gt;, 3-OH, C&lt;sub&gt;16&lt;/sub&gt;:&lt;sub&gt;0&lt;/sub&gt;, 3-OH, i-C&lt;sub&gt;17&lt;/sub&gt;:&lt;sub&gt;0&lt;/sub&gt;, 3-OH</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ratio of anteiso-C&lt;sub&gt;15&lt;/sub&gt;:&lt;sub&gt;0&lt;/sub&gt; to iso-C&lt;sub&gt;15&lt;/sub&gt;:&lt;sub&gt;0&lt;/sub&gt;</td>
<td>1.2</td>
<td>5.2–8.5</td>
<td>2.5–3.2</td>
<td>4.7–17.4</td>
<td>1.04</td>
<td>28</td>
<td>22.8–95.2</td>
<td>&lt;1</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Predominant menaquinone(s)</td>
<td>MK-8</td>
<td>MK-8</td>
<td>MK-8</td>
<td>ND</td>
<td>MK-8, MK-9</td>
<td>MK-8</td>
<td>MK-10, MK-11</td>
<td>MK-9, MK-10</td>
<td>MK-8, MK-9</td>
</tr>
<tr>
<td>Isolation source(s)</td>
<td>Anaerobic digesters treating maize silage and pig and cattle manure</td>
<td>Anaerobic digesters treating brewery wastewater or maize silage and wheat straw</td>
<td>Oil reservoir and anaerobic digesters treating maize silage and pig and cattle manure</td>
<td>Human clinical specimens and stools, microbial fuel cell, termite gut, hindgut</td>
<td>Sediment of acid rock drainage pond</td>
<td>Rice plant residue (rice straw) from irrigated rice-field soil</td>
<td>Periodontal pockets</td>
<td>Oral infections and other clinical specimens of human and animal origin</td>
<td>Post-partum bovine uterus</td>
</tr>
</tbody>
</table>

*ai, anteiso-branched; i, iso-branched.

†*Porphyromonas catoniae* contains approximately equal amounts of iso-C<sub>15</sub>:<sub>0</sub> and anteiso-C<sub>15</sub>:<sub>0</sub> as the predominant fatty acids and, in *Porphyromonas bennonis*, iso-C<sub>15</sub>:<sub>0</sub> and anteiso-C<sub>15</sub>:<sub>0</sub> were only detected at <1%.

‡Not given because the reported fatty acid patterns of members of the genus show significant differences from each other, which indicate that the genus includes members of different taxa.
antiso-C\textsubscript{15:0} to iso-C\textsubscript{15:0} as well as differences in the overall fatty acid composition. A closer look at the fatty acid patterns revealed that the pattern of strain ING2-E5B\textsuperscript{T} exhibited considerable differences from those of the species of both Proteiniphilum and Petrimonas, whereas the patterns of species within a genus showed high similarities (Table 2). As fatty acid patterns generally do not fluctuate significantly within a taxonomic group (Tindall \textit{et al.}, 2010), the distinctly different pattern of strain ING2-E5B\textsuperscript{T} indicates a clear delineation of this organism from the genera Proteiniphilum and Petrimonas. Furthermore, strain ING2-E5B\textsuperscript{T} showed a lower DNA G+C content compared with species of the most closely related genera. Based on a combination of phylogenetic, genotypic and chemotaxonomic differences, strain ING2-E5B\textsuperscript{T} can be clearly distinguished from the most closely affiliated genera; consequently, we assign strain ING2-E5B\textsuperscript{T} to a novel species of a new genus, for which the name \textit{Fermentimonas caenicola} gen. nov., sp. nov. is proposed.

**Description of Proteiniphilum saccharofermentans sp. nov.**


Anaerobically grown cells are straight rods, 1.1–4.4 \textmu m long and 0.6–0.7 \textmu m wide; filamentous cells (up to 20 \textmu m long) occur sporadically. After 2 days of anaerobic growth at 37 °C on Columbia blood agar, colonies are 0.5–0.8 mm in diameter (older colonies reach up to 1.8 mm), white, opaque, circular, convex and shiny with an entire margin. The temperature range for growth is 15–45 °C with an optimum at 35–40 °C. Grows at pH 6.3–9.1; optimum pH 7.5–7.8. The NaCl concentration range is 0–5 %, with an optimum at 0.51 mol% (HPLC).

The type strain, DSM 28695\textsuperscript{T} (= CECT 8611\textsuperscript{T}), was isolated from a mesophilic laboratory-scale completely stirred tank reactor fed with maize silage and pig and cattle manure. The DNA G+C content of the type strain is 45.1 mol% (HPLC).

**Description of Petrimonas mucosa sp. nov.**

\textit{Petrimonas mucosa} (mu.co’sa. L. fem. adj. mucosa mucous, slimy, a property of the colonies).

Anaerobically grown cells are irregular rods, 0.9–3.4 × 0.7–1.2 \textmu m; filamentous cells (up to 19.5 \textmu m long) occur sporadically. After 2 days of anaerobic incubation at 37 °C on Columbia blood agar, colonies are point-shaped (approx. 0.1 mm in diameter), cream-coloured, opaque, circular, convex and shiny with an entire margin; after 3 weeks, colonies appear white, mucoid and 0.5–1.0 mm in diameter. The temperature range for growth is 20–50 °C, with an optimum at 45 °C. Grows at pH 6.3–9.1; optimum pH 7.5–7.8. The NaCl concentration range is 0–2 %, with optimum growth in the absence of added NaCl. Gram-negative, facultative anaerobe. Catalase-positive and weakly oxidase-positive. Anaerobic growth on single substrates is very weak; yeast extract stimulates growth. Peptone, glucose, cellobiose, fructose, galactose, lactose, maltose, rhamnose, sucrose, trehalose and starch support anaerobic growth. No growth occurs on arabinose, amino acids or ethanol. Shows slightly enhanced extracellular enzyme activity against lactose and weak activity against cellobiose, starch, arabinogalactan, arabinoxylan (medium viscosity), xylan (hardwood), CM-cellulose, PASC and \(\beta\)-glucan. Crystalline cellulose (Avicel) is not degraded. After anaerobic growth on yeast extract and peptone, forms acetic acid and propionic acid, small amounts of isovaleric acid and CO\textsubscript{2} and traces of H\textsubscript{2}; with the addition of glucose, traces of butyric acid are also detected. Major cellular fatty acids are anteiso-C\textsubscript{15:0}, iso-C\textsubscript{15:0} and iso-C\textsubscript{14:0} and anteiso-C\textsubscript{13:0}, iso-C\textsubscript{14:0} and anteiso-C\textsubscript{13:0}. The only detected respiratory quinone is menaquinone MK-8.

The type strain, ING2-E5A\textsuperscript{T} (= DSM 28694\textsuperscript{T} = CECT 8611\textsuperscript{T}), was isolated from a mesophilic laboratory-scale completely stirred tank reactor fed with maize silage and pig and cattle manure. The DNA G+C content of the type strain is 48.2 mol% (HPLC).

**Description of Fermentimonas gen. nov.**

\textit{Fermentimonas} (Fer.men.ti.mo’nas. L. v. fermento to ferment; L. fem. n. monas a unit, a monad; N.L. fem. n. Fermentimonas a fermenting monad).

Facultative anaerobes. Anaerobically grown cells are straight, Gram-negative rods. Carbohydrates and complex proteinaceous substrates are fermented. The major respiratory quinone is menaquinone MK-8. The cellular fatty acids comprise both straight-chain and branched fatty acids; in addition, both 2- and 3-acyl fatty acids are present. Major fatty acids are anteiso-C\textsubscript{15:0} and iso-C\textsubscript{15:0}; anteiso-C\textsubscript{13:0} and iso-C\textsubscript{14:0} and iso-C\textsubscript{13:0} and C\textsubscript{14:0}. Predominant hydroxy fatty acids are iso-C\textsubscript{15:0} 3-acyl and C\textsubscript{16:0} 3-acyl. On the basis of 16S rRNA gene sequence analysis, the genus is most closely related to...
the genus Proteiniphilum within the phylum Bacteroidetes. The type species is Fermentimonas caenicola.

Description of Fermentimonas caenicola sp. nov.

Fermentimonas caenicola [cae.ni’co.la. L. n. caenum mud, sludge; L. suff. -cola (from L. n. incola) inhabitant, dweller; N.L. n. caenicola sludge dweller].

Anaerobically grown cells are straight rods, 0.9–8.8 × 0.7–1.0 µm; filamentous cells up to 15.5 µm long occur sporadically. After 2 days of anaerobic incubation at 37 °C on Columbia blood agar, colonies are approx. 0.8 mm in diameter, light cream-coloured, slightly translucent, circular, convex and shiny with an entire margin; after 3 weeks, colonies are white and approx. 1 mm in diameter. The temperature range for growth is 15–45 °C, with an optimum at 37–40 °C. Grows at pH 6.3–9.1; optimum pH 7.1. The NaCl concentration range is 0–1 %, with optimum growth in the absence of added NaCl. Catalase-positive and weakly oxidase-positive. Anaerobic growth on single substrates is very weak; yeast extract stimulates growth. Peptone, glucose, cellubiose, fructose, galactose, lactose, maltose, rhamnose, sucrose, trehalose and starch support anaerobic growth. No growth occurs on arabinose, amino acids or ethanol. Shows slightly enhanced extracellular enzyme activity against arabinomannan (medium viscosity) and xylan (hardwood) and weak activity against cellubiose, lactose, starch, arabinoxylan, CM-cellulose, PASC and β-glucan. Crystalline cellulose (Avicel) is not degraded. After anaerobic growth on yeast extract and peptone, forms acetic acid, propionic acid and CO2 and traces of isovaleric acid and H2.

The type strain, ING2-E5B<sup>T</sup> (=DSM 28696<sup>T</sup> = CECT 8609<sup>T</sup> = LMG 28429<sup>T</sup>), was isolated from a mesophilic laboratory-scale completely stirred tank reactor fed with maize silage and pig and cattle manure. The DNA G+C content of the type strain is 38.2 mol% (HPLC).

Emended description of the genus Proteiniphilum

Chen and Dong 2005

The description is as given by Chen & Dong (2005) with the following amendments. Facultative anaerobes. Catalase and cytochrome oxide can be present. Carbohydrates support anaerobic cell growth. The major respiratory lipoquinone is menaquinone MK-8. Major cellular fatty acids are anteiso-C<sub>15:0</sub>, iso-C<sub>15:0</sub> and C<sub>15:0</sub> among the hydroxy fatty acids, C<sub>17:0</sub> 2-OH, iso-C<sub>16:0</sub> 3-OH and iso-C<sub>17:0</sub> 3-OH predominate.

Acknowledgements

We thank Markus Schleusener, Miriam Felgentreu and Giovanna Rehde for analytical assistance. This work was supported by grants from the German Federal Ministry of Food, Agriculture and Consumer Protection (BMLFV) [grant numbers 22017111 (S. H. and T. L.) and 22007012 (D. E. K.)].

References


