Moheibacter stercoris sp. nov., isolated from an input sample of a biogas plant

Thorsten Schauss,1 Hans-Jürgen Busse,2 Jan Golke,2 Peter Kämpfer1 and Stefanie P. Glaeser1

1Institut für Angewandte Mikrobiologie, Justus-Liebig-Universität Giessen, D-35392 Giessen, Germany
2Institut für Mikrobiologie, Veterinärmedizinische Universität Wien, A-1210 Wien, Austria

A Gram-stain-negative, rod-shaped bacterium, strain 784B1_12E-CasoT, was isolated from a mixed manure sample used as input material of a German biogas plant. Phylogenetic identification based on the nearly full-length 16S rRNA gene sequence placed the strain within the family Flavobacteriaceae (Bacteroidetes) with highest sequence similarity to the type strain of Moheibacter sediminis (93.7 %) followed by Empedobacter brevis (90.7 %). Major cellular fatty acids of strain 784B1_12E-CasoT were iso-C15:0 and iso-C17:0 3-OH. The polyamine pattern contained predominantly sym-homospermidine and the quinone system consisted exclusively of menaquinone MK-6. Major polar lipids were phosphatidylethanolamine and an unidentified polar lipid only detectable after staining for total lipids. The DNA G+C content was 34.5 mol%. Based on phylogenetic, chemotaxonomic and physiological analyses, a novel species of the genus Moheibacter is proposed, Moheibacter stercoris sp. nov. (type strain 784B1_12E-CasoT=CIP 110830T=LMG 28502T).

The extraordinarily diverse family Flavobacteriaceae of the Bacteroidetes was validly published in 1992 (Reichenbach et al., 1992; effective publication Reichenbach, 1989) and the description was emended by Bernardet et al. (1996). More than 100 genera are currently assigned to the family, including Empedobacter, Wautersiella, Moheibacter, Chis-huaella and Weekella, which from a distinct phylogenetic cluster within the Flavobacteriaceae (Zhang et al., 2014a, b). The mono-specific genus Moheibacter was proposed by Zhang et al. (2014b) with the following descriptive characteristics. Cells are Gram-reaction-negative, strictly aerobic and rod-shaped. Gliding motility is not observed. Bacteria of the genus are catalase- and oxidase-positive and produce flexirubin-type pigments. Major fatty acids of the genus are iso-C15:0 and iso-C17:0 3-OH, the major respiratory quinone is menaquinone 6 (MK-6), the main polar lipid is phosphatidylethanolamine and the genomic DNA G+C content is 38.2 mol%. The type species Moheibacter sediminis was isolated from marine sediment (Zhang et al., 2014b).

During a cultivation-dependent approach of mixed manure samples used as input material of German biogas plants performed in 2012, several isolates assigned to the family Flavobacteriaceae were isolated including the recently proposed novel species Empedobacter stercoris (Schauss et al., 2015a). Here we describe strain 784B1_12E-CasoT, which was isolated from an organic waste sample composed of manure and slurry from fattening pigs, dairy cattle, and laying hens mixed in a ratio of 10:7. For cultivation, bacteria were detached from 10 g of freshly mixed material by shaking the samples for 5 min in 100 ml filter-sterilized 0.2 % (w/v) tetra-sodium-pyrophosphate (TSPP) buffer in autoclaved 250 ml Schott glass bottles on a horizontal shaker at room temperature. After 30 min of sedimentation, 40 ml of the supernatant was taken for further analysis. A serial dilution series was generated in 0.9 % (w/v) sterile sodium chloride up to a dilution of 10−8. For each dilution step, 100 µl was plated in triplicates on CASO agar (Neogen Acumedia) and incubated aerobically for 1 week at 25 °C in the dark. Strain 784B1_12E-CasoT was isolated from the 10−5 dilution plated on CASO agar. The original colonies of strain 784B1_12E-CasoT were orange-pigmented, had a diameter of 8 mm and a smooth border. The strain was purified, routinely cultured and maintained on trypticase soy agar (TSA; Becton Dickinson) at 25 °C. For long-term storage the biomass of the strain was suspended in calf serum albumin and stored at −20 °C.

For phylogenetic identification of strain 784B1_12E-CasoT, the nearly full-length 16S rRNA gene of the strain was sequenced as described in detail by Schauss et al. (2015a).
The nearly full-length 16S rRNA gene sequence of strain 784B1_12E-Caso<sup>T</sup> was used for a first phylogenetic placement by BLAST analysis in the EzTaxon type strain database (Kim et al., 2012). Detailed phylogenetic analyses were performed subsequently in ARB release 5.2 (Ludwig et al., 2004) using the 'All-Species Living Tree' Project (LTP) (Yarza et al., 2008) database LTPs115 (March 2014) according to Schauss et al. (2015a). A maximum-likelihood tree was calculated with RAxML version 7.04 (Stamatakis, 2006) using GTR-GAMMA and rapid-bootstrap analysis and a maximum-parsimony tree was calculated using DNAPARS version 3.6 (Felsenstein, 2005). Both trees were based on 100 resamplings (bootstrap analysis; Felsenstein, 1985) and sequences between gene termini 55 and 1471 (Escherichia coli numbering, Brosius et al., 1978).

The next closest related type strain to strain 784B1_12E-Caso<sup>T</sup> was M. sediminis M0116<sup>T</sup> with 93.7% 16S rRNA gene sequence similarity followed by Empedobacter brevis DSM 16922<sup>T</sup> with only 90.7% sequence similarity. In the phylogenetic trees, strain 784B1_12E-Caso<sup>T</sup> was placed within the Flavobacteriaceae subcluster containing the genera Empedobacter, Wautersiella, Moheibacter, Chishuiella and Weeksella. Within this subcluster, strain 784B1_12E-Caso<sup>T</sup> was grouped together with M. sediminis M0116<sup>T</sup>, which was supported by high bootstrap values and confirmed in both trees (Fig. 1).

The DNA G+C content was determined by the DNA melting temperature method established by Gonzales & Saiz-Jimenez (2002) with modifications as described previously (Glaser et al., 2013). High-molecular-weight genomic DNA was therefore extracted according to the method of Pitcher & Saunders (1989). The genomic G+C content of strain 784B1_12E-Caso<sup>T</sup> was 34.5 mol%, which was less than 5 mol% different from the G+C content described for M. sediminis M0116<sup>T</sup> (38.2 mol%; Zhang et al., 2014a).

Phenotypic characteristics of the novel strain were investigated as follows. Gram staining was performed by the modified Hucker method according to Gerhardt et al. (1994). Cell morphology was determined by light microscopy using an Axioshot2 microscope (Zeiss). The AxioVision Rel. 4.7 (Zeiss) software was used for cell size measurements. Gliding motility was tested by the hanging drop method as described by Bernardet et al. (2002). Catalase activity was tested by the observation of gas bubbles after the addition of a few drops of 3% (v/v) H<sub>2</sub>O<sub>2</sub> to fresh biomass grown on an agar plate. Oxidase activity was determined with

---

**Fig. 1.** Maximum-likelihood tree based on nearly full-length 16S rRNA gene sequences showing the phylogenetic relationships of strain 784B1_12E-Caso<sup>T</sup> among the type strains of closely related genera within the Flavobacteriaceae. The tree was generated via ARB with RAxML using GTR-GAMMA and rapid-bootstrap analysis and based on nucleotide sequence positions 110–1471 (according to E. coli numbering, Brosius et al., 1978). Numbers at nodes represent bootstrap values >70% (obtained from 100 resamplings). Filled circles represent nodes which were also present in the maximum-parsimony tree with bootstrap values >70%. Four type strains of the family Cryomorphaceae were used as the outgroup. The number in the outgroup cluster indicates the number of sequences included in the cluster. Bar, 0.1 substitutions per nucleotide position.
a Microbiology Bactident oxidase test strip (Merck). The production of flexirubin-type pigments was tested with the KOH method according to Fautz & Reichenbach (1980). Temperature-dependent growth was tested on TSA at 4, 10, 15, 20, 25, 28, 30, 36, 45, 50 and 55°C; salinity and pH-dependent growth was tested at 28°C in trypticase soy broth (TSB) supplemented with 1–12% (w/v) NaCl (in 1% intervals) or adjusted to pH values of 4.5–12.5 (1 pH units intervals) adjusted with 6 M HCl and 1 M NaOH after autoclaving. Further physiological properties were tested using the API 20NE, API ZYM and API 50CH test strips (bioMérieux) as described by the manufacturer. Bacterial biomass for the API 20NE and API ZYM inoculations was suspended in 0.9% (w/v) NaCl and for acid production tested with API 50CH with CHB/E media (bioMérieux). Test strips were analysed after 3, 4 and 7 days of incubation, respectively.

Cells of strain 784B1_12E-CasoT were rod shaped with a cell size of 1.21 (±0.2) µm × 0.64 (±0.2) µm. The strain showed optimal growth between 25 and 36°C on R2A

Table 1. Differential phenotypic characteristics between strain 784B1_12E-CasoT and its closest related species

| Characteristic | 1 | 2* | 3† | 4† | 5* | 6* | 7*
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Flexirubin-type pigment</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Growth at/on:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>37°C</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>MacConkey agar</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Hydrolysis of:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Starch</td>
<td>+</td>
<td>(+)</td>
<td>-</td>
<td>-</td>
<td>+‡</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Urea</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Aesculin</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Assimilation of (API 20NE):</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose</td>
<td>-</td>
<td>(+)</td>
<td>-</td>
<td>-</td>
<td>(+)</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Mannose</td>
<td>-</td>
<td>(+)</td>
<td>-</td>
<td>-</td>
<td>(+)</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>N-Acetylglucosamine</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Maltose</td>
<td>-</td>
<td>-</td>
<td>(+)</td>
<td>-</td>
<td>(+)</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Adipate</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Phenylacetate</td>
<td>-</td>
<td>-</td>
<td>(+)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Enzyme activities (API ZYM):</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trypsin</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>α-Chymotrypsin</td>
<td>+</td>
<td>(+)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>β-Glucosidase</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Acid production from (API 50CH):</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D-Arabinose</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>L-Rhamnose</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Cellobiose</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Maltose</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Starch</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Glycogen</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Potassium 5-ketogluconate</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Susceptibility to:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oxacillin</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Sulfamethoxazole/trimethoprim</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>DNA G+C content (mol%)§</td>
<td>34.5</td>
<td>38.2*</td>
<td>31.7</td>
<td>29.0</td>
<td>31.0–33.0b</td>
<td>33.8–34.4c</td>
<td>37–38</td>
</tr>
</tbody>
</table>

*Data were obtained from Zhang et al. (2014a).
†Data were obtained from Schauss et al. (2015a) (tests were performed in parallel in the same laboratory).
‡Variable data were reported by Holmes et al. (1978), Holmes et al. (1986), Hollis et al. (1995), and Kämpfer et al. (2006).
§Data from: a, Holmes et al. (1986); b, Vandamme et al. (1994); c, Kämpfer et al. (2006).
Table 2. Fatty acid profile of strain 784B1-12E-Caso\(^T\) and its closest related species

<table>
<thead>
<tr>
<th>Fatty acid profile of strain 784B1_12E-Caso(^T) and its closest related species</th>
</tr>
</thead>
</table>
| 1, 784B1_12E-Caso\(^T\); 2, M. sedominis M0116; 3, E. stercoris 990B6_12ER2A\(^T\); 4, E. stercoris 994B6_12ER2A; 5, E. brevis CCUG 7320\(^T\); 6, Empedobacter falsenii CCUG 51536\(^T\); 7, E. falsenii NF 993\(^T\); 8, W. virosa DSM 16922\(^T\). Data for taxa 3 and 4 are from Schauss et al. (2015a); tested in parallel to strain 784B1_12ECASO\(^T\), for taxa 2, 5 and 8 are from Zhang et al. (2014a) obtained under the same conditions. Data from taxon 7 are from Kämpfer et al. (2006). Tr, Trace (<1 %); –, not detected. Biomass of all strains was grown on the same medium (TSA) at 28 °C for 72 h.

<table>
<thead>
<tr>
<th>Fatty acid profile of strain 784B1_12E-Caso(^T) and its closest related species</th>
</tr>
</thead>
</table>
| Strain, CASA agar (Carl Roth), K7 [0.1 % (w/v) yeast extract, peptone and glucose, with 15 g agar l\(^{-1}\), pH 7.2], DEV agar (Merck), Luria Bertani (LB) agar (Sigma-Aldrich) and marine agar 2216 (MA; Becton Dickinson), but not on malt agar (Merck) or MacConkey agar (Merck). No growth was observed on MacConkey agar, which is in line with the type strain of M. sedominis. Comparative physiological tests revealed some differences among the strains and not all characteristics conformed to those given for the genus Moheibacter. Phenotypic properties are given in the species description and differential phenotypic characteristic are listed in Table 1.

| Susceptibility testing to 12 veterinary relevant antibiotics or combinations of antibiotic compounds was performed according to Schauss et al. (2015b). The following antibiograms were tested (concentration ranges in parentheses, mg l\(^{-1}\)): the β-lactam antibiotics amoxicillin (0.5–64), oxacillin (0.25–32), and ceftiofur and cefquinome (0.25–32), both ± clavulanic acid (4), the sulfonamides florfenicol (0.5–64) and trimethoprim/sulfamethoxazole (0.0625/1.1875–8/152), and sulfamethoxazole (4–256), the macrolide tylosin (0.125–16), tetracycline (0.25–32) and the fluoroquinolone enrofloxacin (0.0625–8). Inhibition of growth was used as a criterion to differentiate between the

---

[Table 2 continued]
categories susceptible and resistant. The strain was susceptible to all tested concentrations of amoxicillin, oxacillin, cefotiofur ± clavulanic acid, ceftiquinome ± clavulanic acid, enrofloxacin and florfenicol and resistant to tetracycline (MIC=0.5 mg l⁻¹), tylosin (MIC=0.25 mg l⁻¹), trimethoprim/sulfamethoxazole (MIC=0.25/4.75 mg l⁻¹) and sulfamethoxazole (MIC=32 mg l⁻¹).

Biomass for fatty acid analysis was grown on TSA for 72 h at 28 °C. Strain 784B1_12E-Caso⁷ was in the late exponential growth phase when the biomass was harvested. Fatty acid extraction and analysis were performed according to Kämpfer & Kroppenstedt (1996) by fatty acid separation with a S898A gas chromatograph (Hewlett Packard). Peaks were automatically integrated and fatty acid names and percentages were determined with the Sherlock MIDI version 2.1 (TSBA version 4.1). Major cellular fatty acids of strain 784B1_12E-Caso⁷ were iso-C₁₅:₀ and iso-C₁₇:₀ 3-OH as characteristic for the genus Moheibacter (Zhang et al., 2014a). Fatty acid profiles of the novel strain and the closest related type strains are listed in Table 2.

Polyamines, quinones, polar lipids and meso-diaminopimelic acid of strain 784B1_12E-Caso⁷ were extracted from biomass grown in liquid PYE medium [0.3 % (w/v) peptone from casein, 0.3 % (w/v) yeast extract, pH 7.2]. Polyamines were extracted from biomass harvested at the late exponential growth phase according to Busse & Auling (1988) and analysed according to Busse et al. (1997). The polyamine pattern consisted of [µmol (g dry weight)⁻¹]: sym-homospermidine (29.0), spermidine (0.5), spermine (0.5), putrescine (0.2), and of traces of asym-norspermidine and cadaverine (each<0.1). Quinones and polar lipids were extracted from cells harvested at the stationary growth phase and analysed as described by Tindall (1990a, b) and Altenburger et al. (1996). The HPLC apparatus applied for polyamine and quinone analyses were as reported by Stolz et al. (2007). The quinone system of strain 784B1_12E-Caso⁷ consisted exclusively of menaquinone MK-6. The polar lipid profile (Fig. 2) contained the major compounds phosphatidylethanolamine and unidentified lipid L4, moderate amounts of unidentified aminolipids AL1 and AL2, aminophospholipid APL1, and polar lipids L3 and L5 only visible after total lipid staining. Additionally, minor amounts of unidentified lipids L1, L2, L6, L7 and glycolipid GL1 were detected. The presence of meso-diaminopimelic acid in the peptidoglycan was shown after extraction and analysis according to Schumann (2011).

Based on the genotypic, phenotypic and chemotaxonomic data, strain 784B1_12E-Caso⁷ represents a novel species of the genus Moheibacter, for which the name Moheibacter stercoris sp. nov. is proposed. The type strain is 784B1_12E-Caso⁷ (=CIP 110830T=LMG 28502T).

**Description of Moheibacter stercoris sp. nov.**

*Moheibacter stercoris* (ster’co.ris.; L. gen. n. *stercoris* of faeces, referring to the isolation source).
peptidoglycan is *meso*-diaminopimelic acid. The polyamine pattern contains predominantly *sym*-homospermidine and the quinone system is exclusively composed of menaquinone MK-6. In the polar lipid profile, phosphatidylethanolamine and unidentified lipid L4 predominate. Additionally, moderate to minor amounts of unidentified aminolipids AL1 and AL2, aminophospholipid APL1, and polar lipids L1, L2 L3, L5, L6, L7 and GL1 are detected. Susceptible to amoxicillin, oxacillin, ceftiofur/± clavulanic acid, cefquinome/± clavulanic acid, enrofloxacin and florfenicol and resistant to tetracycline, tylosin trimethoprim/sulfamethoxazole and sulfamethoxazole.

The type strain, 784B1_12E-Casot (=CIP 110830T=LMG 28502T), was isolated from a mixed sample of manure and slurry from fattening pigs, dairy cattle and laying hens mixed in a ratio of 10:7 which was applied as input material to a German biogas plant. The genomic DNA G+C content of the type strain is 34.5 mol%.

Acknowledgements

We gratefully acknowledge Maria Sowinsky and Katja Grebing for their excellent technical assistance.

References


