Alistipes inops sp. nov. and Coprobacter secundus sp. nov., isolated from human faeces

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Culture-based study of the faecal microbiome in two adult female subjects revealed the presence of two obligately anaerobic, non-spore-forming, rod-shaped, non-motile, Gram-negative bacterial strains that represent novel species. The first strain, designated 627T, was a fastidious, slow-growing, indole-positive bacterium with a non-fermentative type of metabolism. The strain was characterized by the production of acetic and succinic acids as metabolic end products, the prevalence of iso-C15:0 fatty acid and the presence of menaquinones MK-10 and MK-11. The DNA G+C content was found to be 56.6 mol%. The second strain, designated 177T, was capable of fermenting a rich collection of carbohydrate substrates, producing acetic acid as a terminal product. The strain was indole-negative and resistant to bile. The major cellular fatty acids were iso-C15:0 and anteiso-C15:0 (in a 1:1 ratio) and the predominant menaquinone was MK-11. The DNA G+C content was 37.8 mol%. A phylogenomic analysis of the draft genomes of strains 627T and 177T placed these bacteria in the genera Alistipes (family Rikenellaceae) and Coprobacter (family Porphyromonadaceae), respectively.

On the basis of the phenotypic and genotypic properties of strains 627T and 177T, we conclude that these strains from human faeces represent two novel bacterial species, for which the names Alistipes inops sp. nov. (type strain 627T = DSM 28863T = VKM B-2859T) and Coprobacter secundus sp. nov. (type strain 177T = DSM 28864T = VKM B-2857T) are proposed.

The majority of Gram-negative, anaerobic, non-spore-forming, fermentative bacteria isolated from human faeces belong to the order Bacteroidales, phylum Bacteroidetes. Members of this taxonomic group account for 20–40 % of the total bacterial load in human faeces and reach absolute counts of \(10^{10}–10^{11}\) c.f.u. g\(^{-1}\) (Arumugam et al., 2011; Kulagina et al., 2012; Lagier et al., 2012a; Rajilić-Stojanović & de Vos, 2014).

Over the last two decades, the rapid evolution of molecular taxonomy has made it possible to define more accurately the taxonomic position of a number of known species of the Bacteroidales (Sakamoto & Benno, 2006; Hardham et al., 2008) and also to describe a multitude of novel taxa. This applies fully to the genus Alistipes, family Rikenellaceae, the type species of which, Alistipes putredinis (Rautio et al., 2003), was formerly known as Bacteroides putredinis (Weinberg et al., 1937). In recent years, the genus Alistipes has been expanded by the addition of a number of newly described and reclassified species, including Alistipes finegoldii (Rautio et al., 2003), Alistipes onderdonkii (Song et al., 2006), Alistipes shahii (Song et al., 2006), Alistipes indistinctus (Nagai et al., 2010), ‘Alistipes

**Abbreviations:** ANI, average nucleotide identity; NJ, neighbour-joining.

The GenBank/EMBL/DBJ accession numbers for the sequences of the contigs containing the 16S rRNA genes of strains 627T and 177T are JRGF01000044 and JTD01000039, respectively. The accession numbers for the whole-genome nucleotide sequences of strains 627T and 177T are JRGF01000000 and JTD01000000, respectively.

Two supplementary figures and three supplementary tables are available with the online Supplementary Material.
Alistipes inops sp. nov. and Coprobacter secundus sp. nov.

senegalensis’ (Mishra et al., 2012), Alistipes timonensis (Lagier et al., 2012b), ‘Alistipes obesi’ (Hugon et al., 2013) and ‘Alistipes ihumii’ (Pfeiderer et al., 2014). Most species of Alistipes were isolated from the healthy human GI tract microbiota, where they can be found in relatively high titres exceeding 10^9 c.f.u. (g faeces)^{-1} (Kulagina et al., 2012). In addition, cases have been reported of isolation of members of the genus Alistipes from blood, appendicular, abdominal, perirectal and brain abscesses, urine and intra-abdominal fluid (Song et al., 2006). Interestingly, the proposed type strain of ‘A. obesi’ was isolated from the stool of a patient suffering from morbid obesity (Hugon et al., 2013), while ‘A. ihumii’ was discovered in the faecal microbiota of an anorexia nervosa patient (Pfeiderer et al., 2014).

By contrast, the genus Coprobacter, family Rikenellaceae, is currently restricted to a single species, Coprobacter fastidiosus, isolated from faeces of a healthy infant (Shkoporov et al., 2013). The closely related genus Barnesiella comprises only two known species, Barnesiella intestinihominis (Morotomi et al., 2008), commonly found in the human faecal microbiota at concentrations exceeding 10^9 c.f.u. (g faeces)^{-1} (Kulagina et al., 2012), and Barnesiella viscericola, isolated from the chicken caecum (Sakamoto et al., 2007).

During an ongoing culture-based study of the human faecal microbiome in healthy adults and children, two strains of strictly anaerobic, Gram-negative bacteria were isolated that could not be identified to the species level by using MALDI-TOF mass spectrometry and 16S rRNA gene sequencing. The strains were designated 627^T and 177^T and respectively shared 92 % identity with A. finegoldii DSM 17242^T and 91 % identity with C. fastidiosus NSBI^T in their 16S rRNA gene sequences. The goal of the current study was to determine the taxonomic positions of these strains by using a combination of chemotaxonomic and phylogenomic approaches.

Faecal samples were collected from two female subjects and transferred immediately anaerobically. Samples were weighed, serially diluted with saline and spread onto Egggerth–Gagnon (EG) medium supplemented with 5 % (v/v) defibrinated horse blood. Plates were incubated anaerobically (in an atmosphere of 85 % N_2, 10 % H_2, 5 % CO_2) at 37 °C for 72 h in anaerobic jars (Schuett–Biotec). Grown colonies were assessed macroscopically and microscopically. Well-isolated single colonies were picked and streaked out several times to obtain pure cultures on EG blood agar. Strain 627^T was isolated from a stool of a 31-year-old healthy Russian female, where it was present at a concentration of \( \sim 1 \times 10^9 \) c.f.u. g\(^{-1}\). Interestingly, the same stool sample also contained \( 5 \times 10^9 \) c.f.u. strain 626 (=VKM B-2858) g\(^{-1}\), which was almost 100 % identical to the recently described ‘A. obesi’ in its partial 16S rRNA gene sequence and biochemical properties. Strain 177^T was isolated at a concentration of \( \sim 1 \times 10^9 \) c.f.u. g\(^{-1}\) from a stool of an 18-year-old healthy Russian female.

Upon isolation, strains 627^T and 177^T were cultured anaerobically on EG blood agar and EG broth. Cultures were incubated at 37 °C for 48–72 h. Susceptibility of the strains to bile and NaCl was tested in EG broth supplemented with 0–8 % (w/v) of either oxgall (Sigma-Aldrich) or NaCl. Media were inoculated from fresh agar cultures and growth was examined visually after 48 h. Physiological properties and enzyme profiles were determined using the API 20A and Rapid ID 32A identification systems (bio-Mérieux) according to the manufacturer’s instructions. Aesculin hydrolysis and H_2S production were tested on perfringens agar (HiMedia) supplemented with 0.02 % (w/v) cystine, 0.05 % (w/v) cystine hydrochloride, 0.4 % (w/v) Na_2HPO_4 and 0.5 % (w/v) aesculin. Growth and colony colour were monitored after 72 h of incubation.

Organic acid metabolites were determined in broth culture (48–72 h) supernatants using HPLC. Metabolites were separated on a Knauer HPLC system equipped with an InertSill ODS-3 5 μm column (4.6×250 mm; GL Sciences) at 35 °C and a flow rate of 1 ml min\(^{-1}\), with 20 mM H_2PO_4 used as the eluent. Fractions were detected by absorbance at 210 nm and identified using analytical standards (Sigma-Aldrich). Cellular fatty acids and menaquinones were determined in late-exponential-phase cultures in EG broth. Long-chain fatty acids were analysed using GC-MS. Fatty acid methyl esters were prepared by acid methanolytic of dry biomass and extracted as described before (Zhilina et al., 2012) and processed on an Agilent Technologies AT-5850/5973 GC-MS system according to Schcherbakova et al. (2005). Respiratory quinones were detected following the procedure of Collins (1985).

Genomic DNA was extracted using the ZR Fungal/Bacterial DNA MiniPrep kit (Zymo Research). Shotgun genome sequencing was performed using 454 pyrosequencing technology on a GS Junior instrument (Roche). Libraries were constructed by using GS Rapid Library reagents (Roche) with a median fragment size of 800 bp. Library concentrations were determined using the Quant-IT PicoGreen dsDNA Assay kit (Life Technologies). Emulsion PCR and sequencing were performed using GS Junior emPCR and sequencing kits (Roche). Reads were assembled using Newbler version 2.7. For strain 627^T, a total of 66 contigs (N_{50}=197 419 bp) were obtained with a combined length of 2 340 821 bp and 14.9 × coverage. Sequencing of DNA from strain 177^T produced 45 contigs (N_{50}=234 174 bp) with a combined length of 4 138 744 bp and 36 × coverage.

The genome sequences were annotated using the NCBI Prokaryotic Genome Annotation Pipeline (Angiuoli et al., 2008). Metabolic pathways were reconstructed using the KEGG automated annotation server (Moriya et al., 2007) and curated manually. Nucleotide and amino acid sequence searches were performed using BLASTN and BLASTP algorithms. Phylogenetic analysis based on partial 16S rRNA gene sequences was performed with the neighbour-joining (NJ) algorithm using MEGA 6 software (Tamura et al., 2013) after multiple alignment of the sequences by MUSCLE (Edgar, 2004). Tree topologies were evaluated by a bootstrap analysis using 1000 resamplings of the sequences (Felsenstein, 1985).
1985). Alternatively, the phylogeny based on conserved proteins predicted from the whole-genome sequences was inferred by using PhyloPhAn (Segata et al., 2013). This algorithm uses amino acid sequences of the 400 most-conserved bacterial proteins, selects the most informative sites and performs phylogeny reconstruction using an approximately maximum-likelihood approach implemented in the FastTree 2 software (Price et al., 2010). The complete amino acid sequences of 100 conserved proteins from this set, which were encoded by all studied genomes, were also concatenated and analysed using the NJ algorithm. The genome of Chlorobium limicola TLS was used as an outgroup. Evolutionary distances in the NJ algorithm were computed using the Tamura–Nei substitution model (Tamura & Nei, 1993) for nucleotide sequences and the JTT model (Jones et al., 1992) for protein sequences. All positions containing gaps and missing data were eliminated. Average nucleotide identity (ANI) was calculated using a ‘blastall’-based approach with the JSpecies software (Richter & Rosselló-Móra, 2009).

Cells of strain 627T were obligately anaerobic, non-spore-forming, non-motile, Gram-negative cocccobacilli. When grown on EG agar supplemented with 5 % (v/v) horse blood, cells were 1.01 ± 0.29 × 0.56 ± 0.15 μm (Fig. S1a, available in the online Supplementary Material) and occurred mostly singly. Colonies on EG blood agar appeared after 48 h of incubation at 37 °C and after 72 h of incubation were 0.18–0.30 mm in diameter, light grey, circular, entire, slightly convex, smooth, with a pale-brown centre and weak haemolysis zone. Growth on EG agar without blood was significantly retarded. Growth in liquid media was scanty. When grown in EG broth at 37 °C, the strain was able to produce visible turbidity within 48 h after inoculation from fresh agar culture. On EG blood agar slants, visible growth occurred after 48 h of incubation at 32–45 °C but not at 25 or 47 °C. The strain was tolerant of oxgall and NaCl in EG broth in concentrations up to 1 and 2 % (w/v), respectively. Neither aesculcinase nor cytochrome oxidase activity was observed. Nitrate was not reduced. Catalase activity was also negative. Hydrogen sulphide was not produced. The strain was positive for indole production and possessed gelatinase activity. The latter phenotypic features were supported by the genome annotation data, which revealed the presence of a tryptophanase gene and a collagenase gene. Based on API 20A and Rapid ID 32A biochemical identification tests, strain 627T was completely asaccharolytic, but was able to decarboxylate glutamic acid (Table S1). The latter property clearly separates this strain from A. finegoldii, A. shahii and A. indistinctus, which are characterized by fermentative metabolism, but not from A. putredinis, which is also non-fermentative (Rautio et al., 2003; Song et al., 2006). Homology searches in the draft genome sequence of strain 627T confirmed the absence of genes coding for z- and β-galactosidases, z- and β-glucosidases, β-glucuronidase, α-arabinosidase and α-fucosidase and the presence of a glutamate decarboxylase gene.

Similarly to other members of the genus Alistipes (Rautio et al., 2003), the metabolic end products of strain 627T grown in EG broth with glucose were mostly represented by succinic and acetic acids, with minor amounts of propionic and fumaric acids. In accordance with these results, metabolic pathways reconstructed from the draft genome sequence of strain 627T include a complete Embden–Meyerhof–Parnas (EMP) pathway, a partial pentose phosphate pathway lacking transaldolase, a partial reverse tricarboxylic acid (TCA) cycle and a partial respiratory chain, which together enable production of succinic acid. Acetic acid is probably produced in an oxidative pathway through the action of pyruvate–flavodoxin oxidoreductase or pyruvate dehydrogenase, phosphate acetylbutyryltransferase and acetate kinase. The presence of the genes coding for cytochrome bd oxidase may be indicative of the ability of the strain to utilize oxygen at low concentrations for energy production (Baughn & Malamy, 2004; Borisov et al., 2011).

Cellular fatty acids in strain 627T were predominantly represented by iso-C15 : 0 (60.0–65.4 %) and iso-C17 : 0 3-OH (10.8 %). Minor amounts of other fatty acids were also present, including anteiso-C15 : 0 (3.8–4.0 %), iso-C15 : 0 3-OH (4.3–5.0 %) and iso-pentadecanil dimethylacetate (8.5–9.9 %) (Table S2). The predominant menaquinones MK-10 and MK-11 were detected in strain 627T in comparable amounts (57 and 32 %, respectively). Similar compositions of cellular fatty acids and respiratory quinones were observed previously in other species of the genus Alistipes (Nagai et al., 2010). The most important discriminative features of strain 627T are presented in Table 1.

The draft genome sequence of strain 627T includes only 1728 protein-coding genes, 43 tRNA genes, a set of rRNA genes and three CRISPR arrays, and has a combined length of 2.30 Mb, which makes this genome the smallest available in the genus Alistipes. The second smallest available genome of a member of the genus Alistipes belongs to A. putredinis DSM 17216T (2.55 Mb, 2742 protein-coding genes), which is also related to strain 627T by its non-fermentative metabolism, scanty growth and sensitivity to bile. The DNA G + C content of strain 627T calculated from the draft genomic sequence is 56.6 mol %, and is typical of the genus Alistipes (55.0–58.8 %). Aside from the metabolic pathways discussed above, the genome of strain 627T also harbours two β-lactamase genes and a chloramphenicol acetyltransferase gene, which correlates well with resistance of the strain to penicillin G, semi-synthetic penicillins (but not to amoxicillin/clavulanic acid) and cephalosporins, as well as to chloramphenicol (Table S3).

Although only a single strain of the proposed novel species was isolated in this study, BLASTN searches in the GenBank nr database revealed that numerous 16S rRNA gene sequences with ≥99 % identity to that of strain 627T have been deposited previously. The majority of these sequences were obtained by high-throughput sequencing of 16S rRNA gene libraries from faeces of healthy infants,
Strains: 1, 627 T; 2, A. putredinis DSM 17216 T; 3, A. finegoldii DSM 17242 T; 4, A. onderdonkii DSM 19147 T; 5, A. shahii WAL 8301 T; 6, A. indistinctus YIT 12060 T. Data for reference strains were taken from Rautio et al. (2003), Song et al. (2006), Nagai et al. (2010), Mishra et al. (2012), Lagier et al. (2012b), Hugon et al. (2013) and Pfleiderer et al. (2014). V, Variable; W, weak; NA, data not available.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
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<tbody>
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<td>Human faeces</td>
<td>Appendix tissue</td>
<td>Abdominal abscess</td>
<td>Appendix tissue</td>
<td>Human faeces</td>
</tr>
<tr>
<td>Metabolism*</td>
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<td>NF</td>
<td>F</td>
<td>F</td>
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<tr>
<td>Pigment production</td>
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<td>W</td>
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<td>+</td>
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<td>Aesculin hydrolysis</td>
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<td>V</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Indole production</td>
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<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
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<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
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<tr>
<td>Resistance to 20 % bile</td>
<td>S, A, p, f (E)</td>
<td>S, H₂, a, ib, iv, p (C)</td>
<td>S, a, p, iv§, l§ (P)</td>
<td>S, a, p (P)</td>
<td>S, a, p (P)</td>
<td>S, A (P)</td>
</tr>
<tr>
<td>Major end products‡</td>
<td>S, A, p, f (E)</td>
<td>S, H₂, a, ib, iv, p (C)</td>
<td>S, a, p, iv§, l§ (P)</td>
<td>S, a, p (P)</td>
<td>S, a, p (P)</td>
<td>S, A (P)</td>
</tr>
<tr>
<td>Major cellular fatty acid(s)</td>
<td>i-C₁₅ : 0, i-C₁₅ : 0, 3-OH</td>
<td>i-C₁₅ : 0, 3-OH</td>
<td>i-C₁₅ : 0</td>
<td>i-C₁₅ : 0</td>
<td>i-C₁₅ : 0</td>
<td>i-C₁₅ : 0, 9c</td>
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<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>10, 11</td>
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<td>DNA G+C content (mol%)</td>
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<td>55</td>
<td>57</td>
<td>58</td>
<td>56</td>
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<td></td>
<td></td>
<td>2.55</td>
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<td></td>
</tr>
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<td>N-Acetyl-β-glucosaminidase</td>
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<td>+</td>
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<tr>
<td>β-Glucosidase</td>
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<td>+</td>
<td>+</td>
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<td>β-Fucosidase</td>
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<td>+</td>
<td>V</td>
<td>+</td>
</tr>
</tbody>
</table>

*F, Fermentative; NF, non-fermentative.
‡Colonies demonstrate pigmentation after 2 weeks of incubation on blood agar.
§a, Acetic acid; f, fumaric acid; ib, isobutyric acid; iv, isovaleric acid; l, lactic acid; p, propionic acid; s, succinic acid. Upper- and lower-case letters indicate major and minor products, respectively. Growth media are given in parentheses as follows: C, CMC broth; E, EG broth; P, PYG broth.
§Present in trace amounts.
‖Based on draft genome assemblies.

Adolescents and adults, Crohn disease patients (Manichanh et al., 2006; Turnbaugh et al., 2009; Ferrer et al., 2013; Sjöberg et al., 2013) and from caecal contents of chickens (unpublished data). Therefore, we conclude that strains similar to 627 T might be common to the human and chicken intestinal microbiota. Among the known species of the genus Alistipes, the 16S rRNA gene sequence of strain 627 T had high similarity to those of A. finegoldii DSM 17242 T (91.1 % identity), A. putredinis JCM 16772 T (91.8 %), A. shahii WAL 8301 T (90.6 %) and A. indistinctus JCM 16068 T (91.4 %). Searches in the wgs database revealed no previously sequenced genomes similar to that of strain 627 T.

Cells of strain 177 T were obligately anaerobic, non-spore-forming, non-motile, Gram-negative, polymorphic rods. When grown on EG agar supplemented with 5 % (v/v) horse blood, cells were 1.31 ± 0.60 × 0.70 ± 0.13 μm (Fig. S1b) and occurred mostly singly. However occasional cells were unusually long and reached up to 6 μm in length. Colonies on EG blood agar appeared after 48 h of incubation at 37 °C and after 72 h of incubation were 0.48–0.65 mm in diameter, light brown, circular, entire, slightly convex and smooth, with a weak haemolytic zone. Growth on EG agar without blood was significantly retarded. Interestingly, similarly to C. fastidiosus NSB1 T (Shkoporov et al., 2013), turbidity of EG broth inoculated with strain 177 T reached its maximum after 36 h but then dropped rapidly, presumably because of cell autolysis. On EG blood agar slants, visible growth occurred after 48 h of incubation at 32–45 °C but not at 47 °C. Weak growth was also visible at 25 °C. The strain was tolerant of oxgall and NaCl in EG broth at concentrations up to 3 and 1 % (w/v), respectively. Aesculinase activity was positive. Cytochrome oxidase and nitrate reductase were not produced. Hydrogen sulphide was not produced. Catalase and gelatinase activities were positive. The strain did not produce indole. In accordance with these results, the draft genome of 177 T harboured predicted catalase and collagenase genes, but not a tryptophanase gene. When tested using API 20A, strain 177 T was able to ferment cellobiose, D-glucose, lactose, maltose, mannose, raffinose, L-rhamnose, sucrose and trehalose. Weak acid production was observed from salicin (Table S1). According to Rapid ID 32A, the strain produced a vast collection of glycosyl hydrolases, including α- and β-galactosidases, α- and β-glucosidases, α-glucuronidase,
N-acetyl-β-glucosaminidase and α-fucosidase. This observation was further confirmed by the presence of the corresponding annotated genes along with other glycosyl hydrolase genes in the draft genome sequence of strain 177T.

The main metabolic end products produced by strain 177T grown on glucose were acetic, succinic and fumaric acids. Similarly to C. fastidiosus NSB1T (Chaplin et al., 2014), metabolic pathway reconstruction based on the draft genome sequence of strain 177T revealed the presence of a complete EMP pathway, a partial pentose phosphate pathway lacking transaldolase, an almost-complete TCA cycle lacking succinyl-CoA synthetase and a respiratory chain that includes the NADH dehydrogenase complex, succinate dehydrogenase/fumarate reductase and the cytochrome bd complex. Acetate is probably produced from pyruvate by sequential action of pyruvate–ferredoxin oxidoreductase, phosphate acetyltransferase and acetate kinase.

Identically to C. fastidiosus NSB1T, the major cellular fatty acids in strain 177T were iso-C15:0 and anteiso-C15:0 in a molar ratio of 0.9–1:1 (Table S2). Also, similarly to C. fastidiosus NSB1T, the predominant menaquinone in strain 177T was MK-11 (Table 2).

The draft genome sequence of strain 177T harbours 3064 protein-coding genes, 57 tRNA genes, an rRNA operon in a separate contig and two CRISPR arrays. The overall length of 4.14 Mb is significantly larger than the genome sizes of the most closely related species of the genera Coprobacter and Barnesiella. The DNA G+C content of strain 177T deduced from the draft genome sequence was 37.8 mol%, and thus was much closer to the DNA G+C content of the type strain of C. fastidiosus (38.5 mol%) than to those of representatives of the genus Barnesiella (43.9–52 mol%) (Sakamoto et al., 2007; Morotomi et al., 2008).

The antibiotic resistance gene repertoire in strain 177T includes genes coding for several β-lactamases, tetracycline resistance protein TetQ and a putative multidrug efflux transporter AcrAB-TolC/AdeABC (Hornsey et al., 2010; Venter et al., 2015). These genes are presumably responsible for resistance of strain 177T to penicillin G, semi-synthetic penicillins, cephalosporins, doxycycline and chloramphenicol (Table S3).

In this work, we propose the description of a novel species based on the single strain 177T. However, close homologues (99–100 % similarity) of the complete 16S rRNA

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**Table 2. Differential characteristics of strain 177T and related type strains**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
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<tbody>
<tr>
<td>Isolation source</td>
<td>Human faeces</td>
<td>Human faeces</td>
<td>Chicken caecum</td>
<td>Human faeces</td>
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<td>Aesculin hydrolysis</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Gelatin digestion</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
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<tr>
<td>Catalase</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Resistance to 20 % bile</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>NA</td>
</tr>
<tr>
<td>Major cellular fatty acids</td>
<td>i-C15:0, ai-C15:0</td>
<td>i-C15:0, ai-C15:0</td>
<td>ai-C15:0, i-C15:0</td>
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<tr>
<td>Ratio of ai-C15:0 to i-C15:0</td>
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<td>0.9–1.0</td>
<td>2.3–3.5</td>
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<tr>
<td>Predominant menaquinone(s)</td>
<td>11</td>
<td>11</td>
<td>11, 12</td>
<td>NA</td>
</tr>
<tr>
<td>DNA G+C content (mol%)</td>
<td>37.8b</td>
<td>38.5b (38.3b)</td>
<td>52.0b (51.7b)</td>
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<td>β-Gluconoridase</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>V</td>
</tr>
<tr>
<td>α-Fucosidase</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>Glutamate decarboxylase</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Acid production from:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lactose</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Sucrose</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>L-Rhamnose</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Trehalose</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
</tbody>
</table>

*a, Acetic acid; f, fumaric acid; p, propionic acid; s, succinic acid. Upper- and lower-case letters indicate major and minor products, respectively.

†Determined as follows: a, HPLC; b, from draft or completed genome sequence.

‡Based on draft genome assemblies.
gene sequence of strain 177\textsuperscript{T} were detected previously in a number of faecal and intestinal biopsy DNA libraries. For instance, such sequences were observed in colitis and control biopsy samples by Walker et al. (2011), in faecal samples from a healthy female by Bonnet et al. (2002) and in a number of unpublished studies. Therefore, we presume that bacterial species similar to 177\textsuperscript{T} could be found frequently within the human intestinal microbiota. The search for similar 16S rRNA gene sequences among cultured bacteria revealed relatively high similarity of the complete 16S rRNA gene sequence of strain 177\textsuperscript{T} to those of C. fastidiosus DSM 18177\textsuperscript{T} (91.1\%), B. viscericola DSM 17242\textsuperscript{T} (90.7\%) and Parabacteroides distasonis (86.8\%).

To establish the taxonomic positions of strains 627\textsuperscript{T} and 177\textsuperscript{T}, a phylogenetic analysis was conducted that employed the reconstruction of phylograms based on complete 16S rRNA gene sequences and amino acid sequences of 100 conserved housekeeping proteins, predicted from the whole genomes of representatives of the families Porphyromonadaceae and Porphyromonasaceae. The phylogenetic analysis based on 16S rRNA gene sequences positioned strain 627\textsuperscript{T} between the genera Alistipes and Rikenella, close to A. indistinctus. Similarly, it was shown that strain 177\textsuperscript{T} belongs to a clade comprising the genera Coprobacter and Barnesiella, and holds an intermediate position between these two genera (Fig. 1). However, it is known that, despite its ubiquity and conservation, the 16S rRNA gene sequence is highly prone to noise as a sole marker for phylogeny reconstructions (Segata et al., 2013). Therefore, we used a set of 100 highly conserved protein sequences, predicted from the whole-genome sequences, for a more robust phylogenetic analysis. After aligning the concatenated sequences, a phylogenetic tree was reconstructed using the NJ approach. In addition, the PhyloPhAn package (Segata et al., 2013) was used, which reconstructs phylogenetic trees based on highly informative sites from a predefined set of highly conserved bacterial proteins (Fig. 2). Using these approaches, strain 627\textsuperscript{T} clustered reliably with the genus Alistipes and was placed on the phylogram between A. indistinctus and A. putredinis. Similarly, strain 177\textsuperscript{T} was found to be more closely related to C. fastidiosus than to the genus Barnesiella (Fig. 2).

As an alternative to 16S rRNA gene and conserved protein phylogeny, we also employed the JSpecies package.

![Fig. 1. NJ phylogenetic tree of complete 16S rRNA gene sequences, showing the relationships between strains 627\textsuperscript{T} and 177\textsuperscript{T} and type strains of species of the order Bacteroidales. Bootstrap values from 1000 replications are given at branch nodes. Evolutionary distances were computed using the Tamura–Nei method. Bar, 0.05 substitutions per nucleotide position.](http://ijs.microbiologyresearch.org)
(Richter & Rosselló-Móra, 2009) to calculate pairwise whole-genome ANI between strains 627T and 177T and representative strains of the genera Alistipes, Coprobacter and Barnesiella (Fig. S2). The results obtained show that members of the genus Alistipes display a wide range of interspecies ANI, from 69.0 % (A. indistinctus/ A. putredinis) to 90.6 % (A. timonensis/A. senegalensis). The genome of strain 627T demonstrated a particularly low coherence with other genomes of the genus Alistipes (68.7–69.5 % ANI). Within the Coprobacter/Barnesiella cluster, the interspecies ANI ranged from 66.8 % (B. viscerocola/C. fastidiosus) to 75.7 % (B. viscerocola/ B. intestiniihominis). According to ANI, strain 177T was more closely related to C. fastidiosus (70.8 % ANI to the type strain) than to the genus Barnesiella (68.1– 68.6 %). Whereas the threshold of 95 % is a widely accepted standard for intraspecies ANI (Konstantinidis et al., 2006; Richter & Rosselló-Móra, 2009), interspecific and intergeneric comparisons usually result in huge variations in ANI. In a recent study, the interspecific ANI within different bacterial genera ranged from 62 to 100 % (Kim et al., 2014). With regard to the results obtained, we conclude that strains 627T and 177T do not belong to any known species and respectively form separate phylogenetic lineages within the genera Alistipes and Coprobacter.

In summary, strain 627T differs from phylogenetically neighbouring strains of the genus Alistipes in enzymic activity, bile tolerance, genome size and sequence. Based on the phenotypic and genotypic properties of strain 627T, we conclude that this strain represents a novel species within the genus Alistipes, for which we propose the name Alistipes inops sp. nov. Strain 177T was found in turn to be different from the phylogenetically related C. fastidiosus NSB1T in the composition of metabolic end products, the ability to produce acids from certain carbohydrate substrates, enzymic activity and nucleotide sequence of genomic DNA. Therefore, we propose to assign this strain to a novel species within the genus Coprobacter, for which the name Coprobacter secundus sp. nov. is proposed.

Fig. 2. NJ phylogenetic tree of concatenated sequences from the 100 most-conserved homologous proteins encoded by the genomes of strains 627T and 177T and the genomes of type strains of species within the order Bacteroidales. Bootstrap values from 1000 replications are given at branch nodes. Evolutionary distances were computed using the JTT matrix-based method. Asterisks (*) denote tree nodes that were confirmed by maximum-likelihood algorithm calculations based on a set of highly informative amino acid sites selected by PhyloPhlAn software (Segata et al., 2013). Bar, 0.1 substitutions per nucleotide position.
Description of Alistipes inops sp. nov.

Alistipes inops (in’ops. L. masc. adj. inops helpless, destitute, indigent, poor, referring to its small genome size, lack of fermentation potential and scanty growth).

Cells are Gram-negative, rod-shaped, obligately anaerobic, non-spore-forming, non-motile, 1.01 ± 0.29 × 0.56 ± 0.15 μm and mostly occur singly. Cultures are mesophilic, asaccharolytic and tolerant of oxgall at concentrations up to 1 % (w/v). Growth on EG blood agar is visible after 72 h of incubation at 37 °C. Colonies are 0.18–0.30 mm in diameter, light grey, circular, entire, slightly convex, smooth, with a pale brown centre and weak haemolysis zone. Growth on liquid media is scanty. Aesculin is not hydrolysed. Indole production is positive. Catalase, oxidase, nitrate reductase and urease are not produced. Gelatin is digested. Acid production is negative from all carbohydrate substrates tested. Positive reactions are obtained for glutamic acid decarboxylase, leucyl glycine arylamidase, alanine arylamidase and glutamy1 glutamic acid arylamidase. The rest of the reactions in the API 20A and Rapid ID 32A identification systems give negative results. The main metabolic end products are succinic and acetic acids. The major cellular fatty acid is iso-C₁₅:0. The major menaquinones are MK-10 and MK-11. A member of the family Rikenellaceae, phylum Bacteroidetes.

The type strain is 627^T (DSM 28863^T=VKM B-2859^T), isolated from human faeces. The DNA G+C content of the type strain is 56.6 mol%.

Description of Coprobacter secundus sp. nov.

Coprobacter secundus (se.cun’dus. L. masc. adj. secundus second, next, referring to the fact that this is the second species to be described within the genus Coprobacter).

Cells are Gram-negative, rod-shaped, obligately anaerobic, non-spore-forming, non-motile, 1.31 ± 0.60 × 0.70 ± 0.13 μm and occur singly. Cultures are mesophilic, saccharolytic and tolerant of oxgall at concentrations up to 3 % (w/v). Growth on EG blood agar is visible after 72 h of incubation at 37 °C. Colonies are 0.48–0.65 mm in diameter, light brown, circular, entire, slightly convex and smooth. Aesculin is hydrolysed. Indole and urease are not produced. Catalase is produced. Gelatin is digested. Acid is produced from cellubiose, D-glucose, lactose, maltose, mannose, raffinose, L-rhamnose, sucrose and trehalose, and produced weakly from salicin, but not from L-arabinose, glycerol, D-mannitol, melezitose, D-sorbitol or D-xyllose. Positive reactions are obtained for α-xgalactosidase, β-galactosidase, α-glucosidase, β-glucosidase, β-glucuronidase, N-acetyl-β-glucosaminidase, α-fucosidase, alkaline phosphatase, leucyl glycine arylamidase and alanine arylamidase. Raffinose and mannose are fermented. The main metabolic end product is acetic acid; succinic and fumaric acids are produced to a lesser extent. The major cellular fatty acids, iso-C₁₅:0 and anteiso-C₁₅:0 are present at a molar ratio of 1 : 1. The major menaquinone is MK-11. A member of the family Porphyromonadaceae, phylum Bacteroidetes.

The type strain is 177^T (DSM 28864^T=VKM B-2857^T), isolated from human faeces. The DNA G+C content of the type strain is 37.8 mol%.

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References


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