Gordonibacter pamelaeae gen. nov., sp. nov., a new member of the Coriobacteriaceae isolated from a patient with Crohn’s disease, and reclassification of Eggerthella hongkongensis Lau et al. 2006 as Paraeggerthella hongkongensis gen. nov., comb. nov.

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A strictly anaerobic, Gram-positive, short-rod/coccobacillus-shaped bacterial strain, designated 7-10-1-bT, was isolated from the colon of a patient suffering from acute Crohn’s disease. The isolate formed small, pale-white, semi-translucent colonies on solid cultivation media. The strain was catalase-positive and metabolized only a small number of carbon sources. Whole-cell fatty acids consisted predominantly of saturated fatty acids (89 %), of which 15:0 anteiso was the major component. The polar lipids phosphatidylglycerol and diphosphatidylglycerol as well as four glycolipids were identified. 16S rRNA gene sequence analysis revealed that the isolate represents a distinct lineage within the family Coriobacteriaceae and has 94.6 % identity to the type strain of [Eggerthella] hongkongensis, the phylogenetically closest bacterial species. On the basis of the analyses performed, the new genus and species Gordonibacter pamelaeae gen. nov., sp. nov. is described, with strain 7-10-1-bT (=DSM 19378T =CCUG 55131T) as the type and only strain of Gordonibacter pamelaeae. Also, based on the chemotaxonomic data obtained for all type strains of the neighbouring genus Eggerthella, we propose that Eggerthella hongkongensis Lau et al. 2006 be transferred to a new genus as Paraeggerthella hongkongensis gen. nov., comb. nov.; the type strain of Paraeggerthella hongkongensis is HKU10T (=DSM 16106T =CCUG 49250T).

During a study on the intestinal flora of patients with inflammatory bowel disease (IBD), one isolate from the colon of a patient with acute Crohn’s disease showed a unique BOX-PCR fingerprint and subsequently a unique 16S rRNA gene sequence pattern, with 94.6–95.2 % similarity to members of the genus Eggerthella. The genus Eggerthella belongs to the family Coriobacteriaceae, placed within the class Actinobacteria (Wade et al., 1999).
Members of this genus are anaerobic, non-sporulating, non-motile, Gram-positive bacilli that grow singly, as pairs or in short chains. They are found in the human colon and faeces and have been implicated as a cause of ulcerative colitis, liver and anal abscesses and systemic bacteraemia (Chan & Mercer, 2008; Landais et al., 2007; Lau et al., 2004a, b). The type species of the genus, *Eggerthella lenta*, was originally described as ‘*Bacteroides lentus*’ (Eggerth, 1935), but was included on the Approved Lists of Bacterial Names in the genus *Eubacterium*, as *Eubacterium lentum*.

**Growth characteristics**

Strain 7-10-1-bT was isolated from the sigmoid region of the colon of a patient with active Crohn’s disease (male, age 33, medication Azathioprine + Mutaflor + cortisone) and was obtained by colonoscopy on 30 November 2006 in the Clinic for General Internal Medicine (University-Hospital Schleswig-Holstein, Kiel, Germany). Samples were placed in standard anaerobic transport medium (Port-a-cul tube; BBL) on ice and processed for isolation of bacteria within 24 h. One biopsy specimen was transferred to a microcentrifuge tube with 100 μl sterile PBS (50 mM potassium phosphate, 150 mM NaCl, pH 7.2) and homogenized by repeated grinding with a Teflon mortar. The homogenate was diluted further in PBS and aliquots corresponding to 10% (1:10) and 1% (1:100) of the original biopsy material were plated on a variety of different media. The isolation procedure and incubation of plates was carried out under anaerobic conditions (MACS-VA500 workstation; Meintrup DWS) with an atmosphere consisting of N₂/H₂/CO₂ (80:10:10) at 37 °C. Plates were monitored for growth and single colonies were subcultivated, checked for purity and screened by BOX-PCR as described previously (Dombek et al., 2000; van Belkum et al., 1996), allowing the differentiation of taxa by targeting highly conserved repetitive DNA elements. One colony (designated here strain 7-10-1-bT), from a 1:10 dilution plated on Schaedler basal agar (Oxoid) supplemented with 5% defibrinated horse blood, exhibited a unique BOX-PCR profile (data not shown) and was subjected to further investigation using a combination of phenotypic, genotypic and chemotaxonomic methods.

Unless specified otherwise, strain 7-10-1-bT was cultivated in pre-reduced brain heart infusion (BHI) medium (Oxoid) supplemented with 1% arginine hydrochloride (w/v) in Hungate tubes.

Subcultures of strain 7-10-1-bT occurred as small, pale-white, semi-translucent colonies after 48–72 h of incubation at 37 °C under strictly anaerobic conditions on agar plates. Growth in liquid media was generally slow, with cells growing to low optical densities (data not shown). Growth could be enhanced with the addition of 1% arginine hydrochloride (w/v) (data not shown), which is consistent with the growth requirements of other anaerobic strains isolated from the human colon (Jin et al., 2007). Uematsu et al. (2006) reported on the metabolism of arginine by members of the genera *Slackia* and *Cryptobacterium*.

**Microscopic and ultrastructural analysis**

For phase-contrast microscopy, mid-exponential and stationary phase cells were visualized under phase-contrast microscopy (Zeiss). Broth cultures of strain 7-10-1-bT revealed short-rod/coccobacillus-shaped, motile, non-spor-forming small cells (data not shown).

For electron microscopy, mid-exponential phase cells grown in BHI with and without 1% arginine hydrochloride were prepared either as whole mounts or as embedded samples and were analysed by energy-filtered transmission electron microscopy as described by Yakimov et al. (1998) and Golyshina et al. (2000).

At the ultrastructural level, as revealed from whole-mount shadow-cast (Fig. 1a, b) and ultrathin-sectioned (Fig. 1c, d) samples, cells appeared rather unusual in morphology. In whole-mount samples, cells grown in BHI appeared flagellated (Fig. 1a; fl) with subpolar flagellum insertion. These short-rod/coccoid cells produced a small amount of slime, which may be seen as a tail-like feature at the cell end. Despite the presence of this slime, the cell wall is clearly outlined (Fig. 1a; cw). Cells grown in BHI supplemented with 1% arginine hydrochloride, however, were surrounded/encapsulated by a thick amorphous layer of slime, seen as a smooth greyish halo around individual cells or groups of cells (Fig. 1b; sl). These samples also showed cells to have an electron-translucent, mass-deficient centre, apparently equivalent to the bacterial chromosome. The mean cell length was 1.01 ± 0.21 μm (n=35; min=0.64 μm; max=1.46 μm; median=1.01 μm) and the mean cell diameter was 509 ± 55 nm (n=49; min=403 nm; max=644 nm; median=507 nm). A characteristic feature of whole-mount or ultrathin-sectioned cells was a marked longitudinal asymmetry, with one end of the cell exhibiting a pronounced conical form (Fig. 1a c; ae). In ultrathin-sectioned cells, the condensed chromosomal DNA is seen in the centre as electron-translucent areas (Fig. 1c, d), surrounded by a rather densely packed cytoplasm. Occasionally, bright inclusions (Fig. 1c; incl) were apparent, probably indicating low-level carbon-storage activity, perhaps as alkanoatoa, under the growth conditions employed. At higher magnification, the cell-wall architecture is typical of a classical Gram-positive cell wall. An outer murein layer of mean thickness 12.8 ± 0.9 nm (n=25; min=10.8 nm; max=14.7 nm; median=12.8 nm) was in direct contact with the cytoplasmic membrane (mean thickness=7.6 ± 1.1 nm; n=43; min=4.6 nm; max=9.6 nm; median=7.7 nm).

**Phenotypic characterization**

The type strains of all three currently recognized species of the genus *Eggerthella* (*E. lenta* DSM 2243T, [*Eggerthella*] *hongkongensis* DSM 16106T and *Eggerthella sinensis* DSM
16107T) were obtained from the DSMZ for comparative analyses and were resuscitated and maintained under the conditions specified in the DSMZ online catalogue (http://www.dsmz.de).

Routine tests (such as Gram stain and catalase activity) were carried out using standard protocols (Doetsch, 1981; McCarthy & Cross, 1984). Additional physiological tests were performed using the API Rapid ID32A and API 20A kits (bioMérieux). Conversion of a variety of different carbon sources was examined using the AN MicroPlate system (an anaerobe identification test panel) following the manufacturer’s instructions (Biolog).

The biochemical characteristics of strain 7-10-1-bT and the Eggerthella type strains are summarized in Table 1. Strain 7-10-1-bT was Gram-positive and catalase-positive, but generally negative for utilization of most substrates. Only one positive reaction (arginine dihydrolase) was observed with the API kits, the only test that gave a positive reaction for all strains used in the comparison. In addition, only six carbon sources, L-methionine, L-phenylalanine, L-valine, L-valine plus L-aspartic acid, dextrin and D-glucose 6-phosphate, were metabolized in Biolog plates. Weak reactions were observed for pyruvic acid and pyruvic acid methyl ester.

Parsimony analysis of phenotypic data presented in Table 1 was performed with the program PARS (PHYLIP package, version 3.66; Felsenstein, 1989), where positive reactions were defined as 1 and negative or variable reactions declared as 0. A dendrogram representing the phenotypic differences between strain 7-10-1-bT and the Eggerthella type strains was constructed (Supplementary Fig. S1, available in IJSEM Online). Three distinct branches were observed and differentiate strain 7-10-1-bT from the members of the genus Eggerthella. E. lenta and E. sinensis grouped together.

**Flavonoid activity**

Flavonoids are polyphenolic compounds that are widely found in fruits, vegetables, nuts, seeds, flowers, tea, wine and honey, and which are ingested in significant quantities as part of the normal diet in humans (Cushnie & Lamb, 2005). Certain intestinal bacteria have a major role in the metabolism of flavonoids, which exhibit an array of health benefits to the host (Chun et al., 2007).

Flavonoid conversion/degradation was tested (for strain 7-10-1-bT only) using the fluorescence-quenching assay as...
described by Schoefer et al. (2001) although using Schaedler anaerobic agar (Oxoid) as the basal medium. In brief, nylon membranes were soaked with individual flavonoids [quercetin (10 mM), rutin (10 mM), genistein (25 mM) or phloretin (25 mM)] and a fluorescent dye, DPH (1, 6-diphenyl-1,3,5-hexatriene), and placed onto the

Table 1. Biochemical profiles of strain 7-10-1-bT, [E.] hongkongensis DSM 16106T, E. lenta DSM 2243T and E. sinensis DSM 16107T

<table>
<thead>
<tr>
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<td>Catalase</td>
<td>+</td>
<td>+</td>
<td>V(^{a\ast})</td>
<td>+</td>
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<tr>
<td>Metabolism of:</td>
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<tr>
<td>β-Cyclodextrin</td>
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<tr>
<td>L-Fucose</td>
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<td>–</td>
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<td>–</td>
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<tr>
<td>D-Glucose 6-phosphate</td>
<td>+</td>
<td>–</td>
<td>–</td>
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<td>D-Mannose</td>
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<td>+</td>
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<td>3-Methyl D-glucose</td>
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<td>+</td>
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<td>–</td>
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<td>–</td>
<td>+</td>
<td>–</td>
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<tr>
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<td>+</td>
<td>–</td>
<td>–</td>
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<td>+</td>
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<td>+</td>
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<td>+</td>
<td>V</td>
<td>–</td>
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<td>L-Valine</td>
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<td>+</td>
<td>–</td>
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<td>L-Valine plus L-aspartic acid</td>
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<td>+</td>
<td>–</td>
<td>–</td>
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<tr>
<td>2'-Deoxyadenosine</td>
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<td>–</td>
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<td>+</td>
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<td>Uridine 5'-monophosphate</td>
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<td>Reduction of nitrate</td>
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<td>–</td>
<td>V(^{\ast})</td>
<td>–</td>
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<tr>
<td>Activity of:</td>
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<tr>
<td>α-Fucosidase</td>
<td>–</td>
<td>–</td>
<td>V(^{a\ast})</td>
<td>–</td>
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<tr>
<td>β-Glucosidase</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Alanine arylamidase</td>
<td>–</td>
<td>–</td>
<td>V(^{a\ast})</td>
<td>–</td>
</tr>
<tr>
<td>Arginine arylamidase</td>
<td>–</td>
<td>–</td>
<td>V(^{a\ast})</td>
<td>–</td>
</tr>
<tr>
<td>Glycine arylamidase</td>
<td>–</td>
<td>–</td>
<td>V(^{a\ast})</td>
<td>–</td>
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<tr>
<td>Histidine arylamidase</td>
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<td>–</td>
<td>V(^{a\ast})</td>
<td>–</td>
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<tr>
<td>Leucine arylamidase</td>
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<td>–</td>
<td>V(^{a\ast})</td>
<td>–</td>
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<tr>
<td>Phenylalanine arylamidase</td>
<td>–</td>
<td>–</td>
<td>V(^{a\ast})</td>
<td>–</td>
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<tr>
<td>Proline arylamidase</td>
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<td>–</td>
<td>V(^{a\ast})</td>
<td>–</td>
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<tr>
<td>Tyrosine arylamidase</td>
<td>–</td>
<td>–</td>
<td>V(^{a\ast})</td>
<td>–</td>
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<tr>
<td>DNA G + C content (mol%)</td>
<td>66.4</td>
<td>61.1(^{c}), 61.8</td>
<td>62.0, 63.8(^{c})</td>
<td>64.9(^{c}), 65.6</td>
</tr>
</tbody>
</table>

\(^{a}\)Data from: a, Lau et al. (2004b); b, Kageyama et al. (1999b); c, Maruo et al. (2008). These studies also included data from other strains.

\(^{\ast}\)Different from result reported by Lau et al. (2004b).
surface of the basal medium. Strain 7-10-1-bT was subsequently inoculated onto the surface of the membranes and incubated under anaerobic conditions for 48–72 h at 37 °C. Plates were subsequently checked for fluorescence using a UV lamp. No significant conversion/degradation of quercetin, rutin, genistein or phloretin was observed for strain 7-10-1-bT.

**Molecular phylogenetic analysis**

For 16S rRNA gene sequence determination and phylogenetic analysis, one colony was picked from a plate culture for DNA preparation, suspended in 60 μl TE buffer (10 mM Tris/HCl, 1 mM EDTA, pH 8.0) and boiled at 95 °C for 5 min and the lysate was centrifuged briefly. A 1 μl aliquot of the supernatant was used for PCR (Mullis & Faloona, 1987) targeting the 16S rRNA gene with primers 27F (5'-AGAGTTTGATCMTGGCTCAG-3') and 1492R (5'-TACGGYTACCTGTAGACTCT-3') (Lane, 1991). Direct sequence determination of the PCR-amplified DNA was carried out using an ABI3130xl DNA sequencer and Taq cycle-sequencing reactions according to the manufacturer’s instructions (Applied Biosystems). Sequences were compared initially with the EMBL nucleotide sequence database (Kanz et al., 2005) using the BLAST tool (Altschul et al., 1990) and were subsequently aligned with related taxa using CLUSTAL W (Thompson et al., 1994). Phylogenetic relationships were estimated for evolutionarily conserved regions in MEGA (Tamura et al., 2007) using the Jukes–Cantor correction model (Jukes & Cantor, 1969), and a dendrogram representing evolutionary distances was generated by minimum evolution (Rzhetsky & Nei, 1992) with support for evolutionary distances (Jukes–Cantor) between 16S rRNA gene sequences of strain 7-10-1-bT, the type strain of [E] hongkongensis, E. sinensis and E. lenta and other representatives of the family Coriobacteriaceae. GenBank accession numbers are presented in parentheses. Bar, 2 % nucleotide sequence difference. Numbers at nodes (≥ 70 %) indicate support for internal branches within the tree obtained by bootstrap analysis (percentages of 500 resamplings).

The closest relatives of strain 7-10-1-bT are [E] hongkongensis HKU10T (94.6 % 16S rRNA gene sequence similarity), E. lenta DSM 2243T (93.1 %) and E. sinensis HKU14T (95.2 %). Strain 7-10-1-bT formed a distinct lineage independent of the Eggerthella species which, also confirmed with maximum-likelihood (Supplementary Fig. S2), is evidenced by a stable branching point with high bootstrap values of 87, 91 and 94 % (percentages of 500 resamplings), respectively.

Also of interest was the relatively low 16S rRNA gene sequence similarity of [E] hongkongensis HKU10T to E. lenta DSM 2243T (93.5 %). This was reflected in the separation of these taxa into two distinct groups and is suggestive of the fact that the genus Eggerthella is in need of revision. On the basis of these 16S rRNA gene sequence similarity values, DNA–DNA hybridization work was not undertaken, since the work of Nakazawa & Hoshino (2004) has shown that such low values correlate with low DNA–DNA hybridization values. Nevertheless, in consideration of the revised minimum sequence identity (94.9 ± 0.4 %) required to describe a new genus (Yarza et al., 2008), it is likely that both strain 7-10-1-bT and [E] hongkongensis represent new genera.

**G+C content of DNA**

Isolation of DNA for determination of the DNA G+C content by HPLC followed described procedures (Cashion et al., 1977; Mesbah et al., 1989). The G+C content of the
DNA of strain 7-10-1-bT was 66.4 mol%. This high G+C content is generally observed for the class Actinobacteria and is marginally higher than that observed for E. lenta, E. sinensis or [E] hongkongensis (Table 1).

Chemotaxonomy

Fatty acids were analysed as methyl ester derivatives prepared from 10 mg dry cell material. Cells were subjected to differential hydrolysis in order to detect ester-linked and non-ester-linked (amide-bound) fatty acids (Labrenz et al., 1998). Fatty acid methyl esters were analysed by gas chromatography using a 0.2 μm × 25 m non-polar capillary column and flame-ionization detection. The run conditions were: injection and detector port temperature 300 °C, inlet pressure 60 kPa, split ratio 50:1, injection volume 1 μl, with a temperature program from 130 to 310 °C at a rate of 4 °C min⁻¹.

Differences in fatty acid components detected by GC analysis (≥0.5%) were compared using the Bray–Curtis similarity algorithm (Primer 6, version 6.1.6; Primer-E). A dendrogram representing the percentage similarity in fatty acid composition between strain 7-10-1-bT and the Eggerthella type strains was constructed using group average hierarchical clustering (Primer 6). The major fatty acid components that contributed to differences in fatty acid composition were assessed using the Similarity Percentages (SIMPER) tool in Primer 6. A table listing these components (≥5%) and their percentage composition in each of the strains was generated (Supplementary Table S1). Complete fatty acid compositions of all analysed strains are presented in Table 2.

An overall greater percentage of saturated (89%) rather than monounsaturated (8%) fatty acids was extracted from cells of strain 7-10-1-bT. In comparison, cells of E. sinensis DSM 16107T and E. lenta DSM 2243T contained smaller amounts of saturated fatty acids (63 and 61%, respectively) and larger quantities of monounsaturated components (33 and 36%, respectively). Almost equal proportions of saturated and monounsaturated fatty acids were observed for [E] hongkongensis DSM 16106T (45 and 51%, respectively), which was also the only strain to contain polyunsaturated fatty acids (namely 18:2 6,9).

Of particular interest was the relatively large percentage of branched-chain (saturated) fatty acids extracted from cells of strain 7-10-1-bT (41%) and their almost complete absence from [E] hongkongensis DSM 16106T (0.6%) compared with E. sinensis DSM 16107T (22%) and E. lenta DSM 2243T (13%). Analysis of the major components that contributed to these differences revealed strain 7-10-1-bT to contain larger amounts of the branched, saturated fatty acid 15:0 anteiso (the predominant component extracted) than obtained for [E] hongkongensis DSM 16106T, E. lenta DSM 2243T or E. sinensis DSM 16107T (Supplementary Table S1). In contrast, larger amounts of the unbranched saturated 16:0 dimethylacetal

<table>
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<th>Fatty acid</th>
<th>1</th>
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<tbody>
<tr>
<td>Saturated</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Iso-branched</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>13:0 iso</td>
<td>1.09</td>
<td>0</td>
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<tr>
<td>13:0 iso 3-OH</td>
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<td>14:0 iso</td>
<td>6.78</td>
<td>3.91</td>
<td>9.25</td>
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</tr>
<tr>
<td>15:0 iso DMA</td>
<td>0.67</td>
<td>0</td>
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</tr>
<tr>
<td>15:0 iso</td>
<td>2.96</td>
<td>0.55</td>
<td>0</td>
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<td>16:0 iso</td>
<td>0.66</td>
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<td>Anteiso-branched</td>
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<td>0.52</td>
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<td>19.79</td>
<td>0.63</td>
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<td>0.63</td>
<td>8.50</td>
<td>11.95</td>
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<tr>
<td>(Total branched)</td>
<td>(40.61)</td>
<td>(0.63)</td>
<td>(12.96)</td>
<td>(21.85)</td>
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<td>Unbranched</td>
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</tr>
<tr>
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<td>4.26</td>
<td>1.49</td>
<td>0.58</td>
</tr>
<tr>
<td>14:0</td>
<td>8.71</td>
<td>3.78</td>
<td>6.51</td>
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<tr>
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<tr>
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<td>3.92</td>
<td>3.50</td>
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<tr>
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<td>23.08</td>
<td>29.42</td>
<td>22.53</td>
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<tr>
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<td>0</td>
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<tr>
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<tr>
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<td>5.93</td>
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<tr>
<td>Summed feature 13*</td>
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<tr>
<td>(Total unbranched)</td>
<td>(48.77)</td>
<td>(44.19)</td>
<td>(47.81)</td>
<td>(40.85)</td>
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<tr>
<td>Total saturated</td>
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<td>44.82</td>
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<tr>
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<td>1.64</td>
<td>1.26</td>
<td>0.84</td>
</tr>
<tr>
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<td>0.78</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

*Summed features contain the following fatty acids: summed feature 5, 15:0 DMA and/or 14:0 3-OH; summed feature 10, 18:1 10:7c and/or unknown 17:834; summed feature 13, 15:0 anteiso DMA and/or 14:0 2-OH.
(DMA) and the monounsaturated fatty acid 18 : 1ω9c were observed for [E.] hongkongensis DSM 16106T, E. lenta DSM 2243T and E. sinensis DSM 16107T, consistent with results reported by Maruo et al. (2008). These differences were reflected in the hierarchical clustering of the strains and revealed the presence of at least three distinct branches representing three independent taxa (Fig. 3). A notable observation was the low similarity of strain 7-10-1-bT to all other type strains (47%) and the separation of [E.] hongkongensis DSM 16106T from E. sinensis DSM 16107T and E. lenta DSM 2243T (74% similarity), which clustered together (83% similarity). This is consistent with the findings of the phenotypic and 16S rRNA gene sequence analyses of these strains.

**Polar lipids and quinones**

Respiratory lipoquinones and polar lipids were extracted from 100 mg freeze-dried cell material using the two-stage method described by Tindall (1990a, b). Respiratory quinones were extracted using methanol/hexane (Tindall, 1990a, b) and the polar lipids were extracted by adjusting the remaining methanol/0.3% aqueous NaCl phase (containing the cell debris) to give a chloroform/methanol/0.3% aqueous NaCl mixture (1:2:0.8, by vol.). The extraction solvent was stirred overnight and the cell debris was then pelleted by centrifugation. Polar lipids were recovered into the chloroform phase by adjusting the chloroform/methanol/0.3% aqueous NaCl mixture to a ratio of 1:1:0.9 (by vol.).

Respiratory lipoquinones were separated into their different classes (menaquinones and ubiquinones) by TLC on silica gel (Macherey-Nagel art. no. 805 023), using hexane/tert-butylmethylether (9:1 v/v) as solvent. UV-absorbing bands corresponding to menaquinones or ubiquinones were removed from the plate and further analysed by HPLC. This step was carried out on an LDC Analytical HPLC (Thermo Separation Products) fitted with a reversed-phase column (Macherey-Nagel; 2 × 125 mm, 3 μm, RP18) using methanol/heptane (9:1 v/v) as the eluent. Respiratory lipoquinones were detected by absorbance at 269 nm.

Polar lipids were separated by two-dimensional silica gel TLC (Macherey-Nagel art. no. 818 135). The first direction was developed in chloroform/methanol/water (65:25:4, by vol.) and the second in chloroform/methanol/acetic acid/water (80:12:15:4, by vol.). Total lipid material and specific functional groups were detected using dodecamolybdophosphoric acid (total lipids), Zinzadze reagent (phosphate), ninhydrin (free amino groups), periodate–Schiff (α-glycols), Dragendorff reagent (quaternary nitrogen), anisaldehyde–sulphuric acid and α-naphthol (glycolipids).

The presence of DMAs in the fatty acid patterns is consistent with the presence of plasmalogens (vinyl ethers) in the polar lipids. However, it should be noted that the interpretation of the data presented here and in other publications requires some caution. While our results only indicate that DMAs are present, Maruo et al. (2008), Verhulst et al. (1987) and Anderson et al. (2000) also report the presence of the equivalent chain-length aldehydes. In contrast, Ihk et al. (1995) report only the presence of aldehydes. These differences may be due to the slightly different methods used and it should be remembered that, under certain conditions, plasmalogens hydrolyse to give first the appropriate long-chain aldehyde, which may be modified to give the equivalent DMA. The presence of aldehydes and absence of DMAs would suggest that the methods used have not converted the aldehydes to the corresponding DMA, whereas the presence of both (aldehydes and DMAs) would suggest that the conversion of the aldehydes to DMAs is not complete, while the presence of only DMAs would suggest that the reaction has gone to completion. This would need to be investigated further, but is significant in the evaluation of the data. Irrespective of which method is used, a particular aldehyde and its equivalent DMA should be treated as being derived from the same parent plasmalogen. In some cases, neither aldehydes nor DMAs are reported in members of the family *Coriobacteriaceae*, although it is not clear whether they were present, but simply not recorded in the results (Minamida et al., 2008; Lawson et al., 2005).

The two predominant menaquinones detected in all strains were menaquinone 6 (MK-6) and dimethylmenaquinone 6 (MMK-6) (Supplementary Table S2). Dimethylmenaquinones, in particular dimethylmenaquinone 6 (DMMK-6), were not detected in any of the strains, but this may have been due to the failure to detect this compound by HPLC, since only relatively small amounts of menaquinones were observed by TLC. DMMK-6 has been reported in a number of taxa, including members of genus *Eggerthella* (Collins et al., 1985; Fernandez & Collins, 1987; Maruo et al., 2008), although the relative amounts vary, suggesting both growth conditions-related variations as well as taxon-specific differences. The unidentified quinone reported in *Asaccharobacter celatus* (Minamida et al., 2008) may also be DMMK-6. MK-6 was the major respiratory
lipoquinone in strain 7-10-1-bT\(^{T}\) (59.5 %; with 40.5 % MMK-6), [E.] \textit{hongkongensis} DSM 16106\(^{T}\) (67.8 %; with 32.2 % MMK-6) and \textit{E. lenta} DSM 2243\(^{T}\) (63.7 %), whereas MMK-6 was predominant in \textit{E. sinensis} DSM 16107\(^{T}\) (60.4 %) (Supplementary Table S2). These results also suggest that routine determination of the menaquinone composition within the family \textit{Coriobacteriaceae} should not be neglected.

To our knowledge, the polar lipid composition of members of the genus \textit{Eggerthella}, or even other taxa within the family \textit{Coriobacteriaceae}, has not been reported previously. In all strains examined, two major phospholipids were detected, phoshatidylglycerol and diphosphatidylglycerol, as well as up to four glycolipids. All four glycolipids ran with similar, but not identical, \(R_f\) values, suggesting structural differences that may be the result of differences in the sugars present and/or the configuration of their linkages, rather than the number of sugars present. This pattern of glycolipids appears to be unique within this group of organisms. This is particularly evident given the relatively conserved polar lipid profiles of strain 7-10-1-b\(^{T}\), \textit{E. lenta} DSM 2243\(^{T}\) and \textit{E. sinensis} DSM 16107\(^{T}\), which were characterized by the presence of two phospholipids, phosphatidylglycerol and diphosphatidylglycerol, and four glycolipids, GL1–GL4 (Supplementary Fig. S3). [E.] \textit{hongkongensis} DSM 16106\(^{T}\) displayed a similar pattern but lacked glycolipid GL3, further supporting its separation from the genus \textit{Eggerthella}.

It is evident from the results presented here, which record for the first time the polar lipid, fatty acid (including plasmalogen-derived aldehydes and DMAs) and respiratory lipoquinone composition, that these three datasets are of value in the differentiation of organisms within the family \textit{Coriobacteriaceae}. The presence of menaquinones and methylated menaquinones with six isoprenoid units is a feature that these organisms also share with certain members of the \textit{Epsilonproteobacteria} (Carlone & Anet, 1983; Moss \textit{et al.}, 1984; Collins \textit{et al.}, 1984; Collins & Widdel, 1986), but they may be easily distinguished based on their fatty acid patterns. The presence of dimethylme-

nenaquinones is, however, unique to this group of organisms. Clearly, such data may be of value in delineating higher taxa within this taxon. To date, the subclass \textit{Coriobacteridae}, the order \textit{Coriobacterales} and the family \textit{Coriobacteriaceae} are defined only in terms of 16S rRNA gene signature nucleotides (Stackebrandt \textit{et al.}, 1997). The definition of these taxa is such that the signatures that define the subclass also define the order and family. It would seem appropriate to examine the value of chemotaxonomy in improving the definition of the order and family, or even re-examining the taxonomic infrastructure within the subclass. It is worthwhile noting that the papers of Dewhirst \textit{et al.} (2001), Rodriguez Jovita \textit{et al.} (1999), Collins & Wallbanks (1992), Lau \textit{et al.} (2004b), Nakazawa \textit{et al.} (1999), Wade \textit{et al.} (1999), Kageyama & Benno (2000) and Kageyama \textit{et al.} (1999a, b, c) do not include any fatty acid, polar lipid or quinone data. Such work may also have consequences for the definition of taxon-specific gene probes (Harmsen \textit{et al.}, 2000).

In the present study, we investigated the phylogenetic, physiological/biochemical and chemotaxonomic properties of strain 7-10-1-b\(^{T}\) (an isolate from the colon of a patient with active Crohn’s disease) in comparison with all type strains of the genus \textit{Eggerthella}. Based on the strain’s unique 16S rRNA gene sequence, its phenotypic and chemotaxonomic properties and the data obtained for its nearest neighbours, two new genera, one novel species and an emended description of the genus \textit{Eggerthella} are proposed within the family \textit{Coriobacteriaceae}. Thus, we propose that strain 7-10-1-b\(^{T}\) should be assigned as the type strain of a novel species within a new genus, for which we propose the name \textit{Gordonibacter pamelaeae} gen. nov., sp. nov., and that [Eggerthella] \textit{hongkongensis} Lau \textit{et al.} 2006 should be transferred to a novel genus, \textit{Paraeggerthella} gen. nov., as \textit{Paraeggerthella hongkongensis} gen. nov., comb. nov. The characteristics that discriminate the newly proposed taxa are presented in Tables 1 and 2.

The prevalence of strain 7-10-1-b\(^{T}\) amongst individuals with Crohn’s disease and its association with the mucosa as an opportunistic pathogen or a member of the normal flora requires further investigation.

\textbf{Description of Gordonibacter gen. nov.}

\textit{Gordonibacter} (Gor.do’ni.bac.’ter. N.L. masc. n. \textit{Gordon} named after Jeffrey I. Gordon, MD, the Dr Robert J. Glaser Distinguished University Professor and Director of the Center for Genome Sciences at Washington University School of Medicine, St. Louis, MO, USA; N.L. masc. n. \textit{bacter} a rod; N.L. masc. n. \textit{Gordonibacter} a rod named after Jeffrey I. Gordon).

Gram-positive, motile, non-spore-forming coccobacilli (0.5–0.6 \(\times\) 0.8–1.2 \(\mu\)m). Obligately anaerobic. Cellular fatty acids consist mainly (~90 %) of saturated fatty acids (predominantly \(\text{C}_{12}\) and \(\text{C}_{13}\)) and include \(\text{C}_{12}:0\), \(\text{C}_{13}:0\) \(\Delta 3\)-OH, \(\text{C}_{14}:0\), \(\text{C}_{14}:0\) DMA, \(\text{C}_{15}:0\) iso DMA, \(\text{C}_{15}:0\) iso, \(\text{C}_{16}:0\) DMA, \(\text{C}_{16}:1\) \(\Delta 7\)c, \(16:1\) \(\Delta 7\)c DMA, \(\text{C}_{17}:0\) anteiso, \(\text{C}_{17}:0\) anteiso DMA, \(\text{C}_{16}:0\) iso, \(\text{C}_{16}:0\) DMA, \(\text{C}_{16}:1\) \(\Delta 7\)c DMA, \(\text{C}_{18}:0\) DMA, \(\text{C}_{18}:1\) \(\Delta 9\)c DMA and \(\text{C}_{18}:1\) \(\Delta 9\)c. The major respiratory lipoquinone present is menaquinone MK-6; MMK-6 is a minor component. Major polar lipids are phosphatidylglycerol, diphosphatidylglycerol and four glycolipids (GL1–GL4). Oxidation/fermentation of arabinose, glucose, mannose, raffinose, trehalose and xylose is not observed. Nitrate is not reduced. The G+C content of the genomic DNA of the single known strain is 66.4 mol\%. The type and only species of the genus is \textit{Gordonibacter pamelaeae}.

\textbf{Description of Gordonibacter pamelaeae sp. nov.}

\textit{Gordonibacter pamelaeae} [pa.me’la.eae. N.L. fem. n. \textit{pamelaeae} named after Dr Pamela Lee Oxley (née Fredericks), biochemist, environmentalist, teacher, mentor and mother].
Morphology and general characteristics are as described for the genus, with cells displaying a conical cell apex. Growth is generally slow on BH1 and Schaedler anaerobic media (Oxoid) supplemented with 5% defibrinated horse blood, with pale-white, semi-translucent colonies forming after 48–72 h of incubation at 37 °C in an anaerobic environment. Growth is enhanced by the supplementation of the basal medium with 1% arginine hydrochloride (w/v). Subpolarly inserted flagella are apparent when cells are grown in BH1 medium. Cells are catalase-positive and show hydrolysis of arginine. The carbon sources, fatty acids, and monounsaturated fatty acids (45:1 in the type strain of E. sinensis 2008). In addition, cellular fatty acids consist of saturated l-methionine, l-phenylalanine, l-valine, l-valine plus l-aspartic acid, dextrin and D-glucose 6-phosphate are metabolized. Only weak conversion of pyruvic acid and pyruvic acid methyl ester is observed. All other organic substrates included in the Biolog AN MicroPlate are not metabolized. Chemotaxonomy is as given for the genus.

The type strain is 7-10-1-bT (=DSM 19378T =CCUG 55131T), isolated from the sigmoid region of the colon of a patient suffering from active Crohn’s disease.

Description of Paraeggerthella gen. nov.

Paraeggerthella (Pa’ra.eg.ger.ther’la. L. prep. para beside; N.L. fem. n. Eggerthella a bacterial genus name; N.L. fem. n. Paraeggerthella beside Eggerthella, named in recognition of the close relationship to the genus Eggerthella).

Characteristics of the genus are as described previously for [Eggerthella] hongkongensis (Lau et al., 2004b; Maruo et al., 2008). In addition, cellular fatty acids consist of saturated and monounsaturated fatty acids (45:1 in the type strain of the type species) (predominantly C16 and C18) and include 12:0, 14:0, 14:0 DMA, 14:1o5c, 15:0 anteiso, 16:0, 16:0 DMA, 16:1o7c, 16:1o7c DMA, 18:0, 18:0 DMA, 18:1o7c DMA, 18:1o9c DMA, 18:1o9c and 18:2o6,9c the major component is 18:1o9c. The major respiratory lipoquinone present is menaquinone MK-6; MMK-6 is a minor component. Polar lipids consist of two phospholipids, phosphatidylglycerol and diphosphatidylglycerol, and four glycolipids, GL1–GL4. The G+C content of the genomic DNA of the known species is 62.0–63.8 mol% (E. lenta) and 64.9–65.6 mol% (E. sinensis). The genus comprises two known species, Eggerthella lenta (the type species) and E. sinensis.

Description of Paraeggerthella hongkongensis (Lau et al. 2006) comb. nov.

Basonym: Eggerthella hongkongensis Lau et al. 2006.

Characteristics of the species are as described previously by Lau et al. (2004b) and Maruo et al. (2008). In additional, physiological testing using the two API kits Rapid ID32A and API 20A reveals just one positive reaction, for arginine dihydrolase. Results obtained with Biolog AN MicroPlates indicate that the following substrates are metabolized: 3-methyl D-glucose, palatinose, urocnic acid, l-methionine, l-threonine, l-valine, l-valine plus l-aspartic acid and uridine 5’-monophosphate. Additionally, weak conversion of rhamnose is observed. The other organic substrates included in the Biolog AN MicroPlate are not metabolized. Chemotaxonomy is as given for the genus.

The type strain is HKU10T (DSM 16106T =CCUG 49250T).

Emended description of the genus Eggerthella

Wade et al. 1999

When Wade et al. (1999) transferred Eubacterium lentum to the genus Eggerthella, the authors referred to the description of Eubacterium lentum published by Moore et al. (1971). However, the Approved Lists of Bacterial Names (Skerman et al., 1980, 1989) refer to Holdeman et al. (1977) as the source of the description for Eubacterium lentum. In emending the description of the genus Eggerthella Wade et al. 1999, Maruo et al. (2008) also make reference to the publication of Moore et al. (1971) and not to Holdeman et al. (1977). It is important to remember that, when a name was included on the Approved Lists of Bacterial Names (Skerman et al., 1980, 1989), the type of the name and the description which accompanies the name are those referenced on the Approved Lists of Bacterial Names.

The description is as given previously (Moore et al., 1971; Holdeman et al., 1977; Kageyama et al., 1999b; Wade et al., 1999; Maruo et al., 2008) with the following modifications. Respiratory lipoquinones consist of MK-6 and MMK-6; MK-6 is the major component in E. lenta and MMK-6 in E. sinensis. Polar lipids consist of two phospholipids, phosphatidylglycerol and diphosphatidylglycerol, and four glycolipids, GL1–GL4. The G+C content of the genomic DNA of the known species is 62.0–63.8 mol% (E. lenta) and 64.9–65.6 mol% (E. sinensis). The genus comprises two known species, Eggerthella lenta (the type species) and E. sinensis.

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References


