**Paenalcaligenes hermetiae** sp. nov., isolated from the larval gut of *Hermetia illucens* (Diptera: Stratiomyidae), and emended description of the genus *Paenalcaligenes*

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A novel Gram-stain-negative, facultatively anaerobic, non-motile and short rod-shaped bacterium, strain KBL009T, was isolated from the larval gut of *Hermetia illucens*. Strain KBL009T grew optimally at 37 °C, at pH 6.0 and with 1–2 % (w/v) NaCl. The 16S rRNA gene sequence of strain KBL009T showed 97.6 % similarity to that of *Paenalcaligenes hominis* CCUG 53761T indicating its classification with the genus *Paenalcaligenes*. The major fatty acids were cyclo-C17:0, C16:0 and summed feature 2 (comprising C14:0 3-OH/iso-C16:1). The respiratory quinones were ubiquinone-8 (Q-8), predominating, and a minor amount of Q-7. The polar lipids were diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylglycerol, one unknown aminolipid and five unknown polar lipids. The polyamine pattern contained predominantly putrescine and relatively high amounts of spermidine. The betaproteobacterial-specific 2-hydroxyputrescine could only be detected in trace amounts. The G+C content of genomic DNA was 56.1 mol%. Results from DNA–DNA hybridization with *P. hominis* KCTC 23583T unambiguously demonstrated that strain KBL009T represents a novel species in the genus *Paenalcaligenes*. Based on phenotypic, genotypic and phylogenetic characterization, the novel species *Paenalcaligenes hermetiae* sp. nov. is proposed. The type strain is KBL009T (=KACC 16840T =JCM 18423T). An emended description of the genus *Paenalcaligenes* is also provided.

The genus *Paenalcaligenes* in the class Betaproteobacteria, which is phylogenetically closely related to the genus *Alcaligenes*, was described by Kämpfer et al. (2010). Currently, the type species of the genus, *Paenalcaligenes hominis* CCUG 53761T, which was isolated from human blood of an 85-year-old man in Göteborg, Sweden, is the only species within this genus. Here, we report on the classification of a second species of the genus *Paenalcaligenes* that was isolated from the larval gut of *Hermetia illucens* (black soldier fly; BSF). BSF larvae are known to dramatically reduce food waste and to convert the nutrients from food waste and manure into insect larval biomass (Kim et al., 2008; Diener et al., 2009). Thus, the BSF larva itself is a potential high protein and high energy feed as well as a resource for isolation of novel species of bacteria, archaea, yeasts and protists from insect guts of adults and larvae (Breznak, 1982; Brauman et al., 2001; Hongoh et al., 2003; Schmitt-Wagner et al., 2003; Ohkuma, 2008; Kudo, 2009; Jeon et al., 2011; Kim et al., 2013).

Strain KBL009T was isolated from the larval gut of *H. illucens* fed with Korean food waste. Isolation was performed using the standard dilution-plating method and incubated under aerobic conditions at 20 °C on marine agar (MA; MB cell). For purification, a single colony was repeatedly streaked on tryptic soy agar (TSA; MB cell). Gram staining was done using a Gram staining kit (Sigma) according to the manufacturer’s instructions. Cell morphology and Gram reaction of strain KBL009T

**Abbreviations:** DPG, diphosphatidylglycerol; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; AL, unknown aminolipid.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain KBL009T is JN873915.
We amplified the 16S rRNA gene of strain KBL009T by colony PCR using PCR pre-mix (iNtRon Biotech) with two universal bacterial-specific primers: forward primer 27F (5'-AGAGTTTGATCMTGGCTCAG-3') and reverse primer 1492R (5'-TACGGYTACCTTGTTACGACTT-3') (Weisburg et al., 1991). The PCR products of the 16S rRNA gene were purified by using a QIAquick PCR Purification kit (Qiagen). Sequence analysis, including analysis with intergenic region primer sets, was done by Solgent Co, Republic of Korea. The acquired sequence fragments were assembled with SeqMan software (DNASTAR) to obtain an almost full-length 16S rRNA gene sequence. The 16S rRNA gene sequence for bacterial strain identification was then compared with those of other strains using the EzTaxon-e server (Kim et al., 2012). The sequence arrangement of the 16S rRNA gene was confirmed manually using BioEdit software (Hall, 1999) and was aligned with the most closely related species using the multiple alignment program CLUSTAL W (Thompson et al., 1994). As a result, strain KBL009T had the highest level of 16S rRNA gene sequence similarity to P. hominis CCUG 53761T with 97.6% similarity.

For DNA–DNA hybridization (DDH) tests, the type strain of P. hominis was obtained for reference via the Korean Collection for Type Cultures (KCTC 23583T) from the Czech Collection of Microorganisms, where it was deposited as CCM 7698T with the proposal of this species. DDH was performed using genome-probing microarrays (Chang et al., 2008) to determine the genetic relatedness between the novel isolate and the reference species P. hominis with which it shared >97% 16S rRNA sequence similarity. DDH values were calculated based on the signal-to-noise ratio of each probe using a previously reported formula (Loy et al., 2005). Strain KBL009T and P. hominis KCTC 23583T showed 71.7% and 1.6% DDH relatedness with counter-probed DNA, respectively. Strain KBL009T was observed by light microscopy (ECLIPSE 50i, Nikon). Motility was examined using the semi-solid agar method of Tittsler & Sandholzer (1936). Strain KBL009T grew well under aerobic conditions overnight and anaerobic conditions (90% N₂, 5% H₂ and 5% CO₂) within two days of incubation. Strain KBL009T was a short rod (width ~0.5 μm and length ~1.5 μm), stained Gram-negative, was facultatively anaerobic and weakly motile. Growth at different temperatures (4, 15, 20, 30, 37, 42 and 55 °C) and different pH values (pH 4.0–11.0, at intervals of 1.0 pH unit) and tolerance of various NaCl concentrations (0, 1, 2, 3, 4, 5, 8, 10, 12, 15 and 20 %, w/v) were tested in tryptic soy broth (TSB; MB cell). The pH was adjusted by adding the following buffers: 10 mM MES (for pH 4, 5 and 6), 10 mM TAPS (for pH 7, 8 and 9) or 10 mM Na₂HPO₄ (for pH 10 and 11). All experiments were performed in triplicate at least, with the strain cultivated in TSB at 37 °C. For optimal growth conditions, the turbidity of cultures was measured as the OD₆₀₀ using a spectrophotometer (Bio-Rad) after incubation for 24 and 48 h. Strain KBL009T grew with 0–15 % (w/v) NaCl, at 15–42 °C and at pH 5.0–9.0, with optimal growth in the presence of 1–2 % (w/v) NaCl, at 37 °C and at pH 6.0. Thus, all biochemical and taxonomic tests including API and Biolog tests were carried out in the presence of 2 % NaCl (w/v).

**Fig. 1.** Phylogenetic tree based on 16S rRNA gene sequences showing the taxonomic position of strain KBL009T. GenBank accession numbers are given in parentheses. Filled diamonds indicate generic branches that were present in phylogenetic consensus trees generated by both the neighbour-joining and the maximum-parsimony algorithms. Bootstrap values based on 1000 replicates for the neighbour-joining and maximum-parsimony algorithms, respectively, are shown at the branch points. *Brucella suis* 1330T was used as an outgroup. Bar, 0.02 substitutions per nucleotide.
was considered to represent a distinct species from the reference species because a DDH similarity of less than 70% indicates species distinctness (Wayne et al., 1987).

Phylogenetic relationships among strain KBL009<sup>T</sup> and closely related type strains were analysed by applying the MEGA5 software program (Tamura et al., 2011) using the maximum-parsimony (Kluge & Farris, 1969), maximum-likelihood (Felsenstein, 1981) and neighbour-joining (Saitou & Nei, 1987) methods with 1000 bootstrap replications. All phylogenetic analysis tools showed that strain KBL009<sup>T</sup> is closely related to the type strain of the genus *Paenalcaligenes*, *P. hominis* (Fig. 1).

Biochemical analyses were performed with strain KBL009<sup>T</sup> and the reference strain *P. hominis* KCTC 23583<sup>T</sup> cultivated at 37°C for 18 h in TSB or on TSA medium supplemented with a final concentration of 2% (w/v) NaCl. Catalase and oxidase activities were determined by observing bubble production with 3% (v/v) hydrogen peroxide solution and indophenol blue production with 1% (w/v) tetramethyl-p-phenylenediamine (bioMérieux), respectively. Strain KBL009<sup>T</sup> showed positive responses in both catalase and oxidase reactions.

Oxidation of sole carbon sources was examined using GN2 MicroPlates and GN/GP inoculating fluid (Biolog) supplemented with 2% NaCl (w/v). Acid production from carbohydrates was determined with API 50CH test strips and API 50CHB/E medium (bioMérieux) supplemented with 2% NaCl (w/v) according to the manufacturer’s instructions. Enzyme activities were determined using API 20NE and API ZYM test strips (bioMérieux), according to the manufacturer’s instructions. Strain KBL009<sup>T</sup> could be distinguished from the reference strain *P. hominis* KCTC 23583<sup>T</sup> based on comparison of biochemical characteristics (Table 1).

Analyses for chemotaxonomic features were performed with the cell biomass of strain KBL009<sup>T</sup> and the reference species grown on TSB supplemented with a final concentration of 2% NaCl (w/v) and harvested at the stationary growth phase. Cellular fatty acids were extracted as described for the Sherlock Microbial Identification System (MIDI, 1999), analysed by GC (Hewlett Packard 6890), and identified by the Microbial Identification software package (Sherlock software 4.0) (Sasser, 1990). Isoprenoid quinones were extracted from freeze-dried cells using chloroform/methanol (2:1, v/v) (Collins & Jones, 1981a), and identified by one-dimensional TLC on a silica gel 60 F<sub>254</sub> plate (Merck) and reversed-phase HPLC (Collins & Jones, 1981b) using a Thermo ODS HYPERSIL (250 x 4.6 mm) column. Polyamines from strain KBL009<sup>T</sup> were extracted from biomass that was grown on PYE medium (0.3% yeast extract, 0.3% peptone, pH 7.2) and harvested at the late exponential growth phase as reported by Busse & Auling (1988). Analysis by HPLC was carried out as reported by Stolz et al. (2005). Polar lipids of strain KBL009<sup>T</sup> were extracted according to the modified method described by Kamekura (1993), separated by two-dimensional TLC on silica gel plates (Merck), and identified by spraying the plates with appropriate detection reagents (Tindall, 1990), i.e. molybdatophosphoric acid for total lipids, ninhydrin reagent for aminolipids, and Zinnadze reagent for phospholipids. For identification, authentic standards of phosphatidylglycerol (PG), phosphatidylcholine, phosphatidylethanolamine (PE), and diphosphatidylglycerol (DPG) were applied.

The major fatty acids (>10% of total fatty acids) of strain KBL009<sup>T</sup> were cyclo-C<sub>17:0</sub>, C<sub>16:0</sub> and summed feature 2 (comprising C<sub>14:0</sub> 3-0H/iso-C<sub>16:1</sub>). The cellular fatty acid profile of strain KBL009<sup>T</sup> was qualitatively similar to that of *P. hominis* KCTC 23583<sup>T</sup> (Table 2). The quinone system of KBL009<sup>T</sup> was composed of the major ubiquinone Q-8 (relative amount 98%) and a minor amount of Q-7, which is most similar to that of *P. hominis* CCUG 53761<sup>T</sup> (Kämpfer et al. 2010). The polyamine pattern of strain KBL009<sup>T</sup> was composed of 52.7 µmol putrescine (g dry weight)<sup>-1</sup>, 5.4 µmol spermidine (g dry weight)<sup>-1</sup>, 1.8 µmol spermine (g dry weight)<sup>-1</sup> and 1.1 µmol cadaverine (g dry weight)<sup>-1</sup>. 2-Hydroxyputrescine was present only in trace amounts <0.1 µmol (g dry weight)<sup>-1</sup>. This polyamine pattern is well in agreement with that of the closest relative, *P. hominis*. Presence of trace amounts of 2-hydroxyputrescine in strain KBL009<sup>T</sup> and absence of this polyamine in the type strain of *P. hominis* is a trait distinguishing strain KBL009<sup>T</sup> from the majority of members of the class *Betaproteobacteria* (Busse & Auling, 1988; Hamana & Takeuchi, 1998; Hamana et al., 2000, 2006). The polar lipid profile of strain KBL009<sup>T</sup> was composed of DPG, PE, PG, one AL and five unknown polar lipids (L1–5) (Fig. 2).
Table 2. Fatty acid composition of strain KBL009<sup>T</sup> and *P. hominis* KCTC 23583<sup>T</sup>

Strains: 1, KBL009<sup>T</sup>; 2, *P. hominis* KCTC 23583<sup>T</sup>. All data were obtained from the current study. Values are percentages of the total fatty acids. Strains were cultivated under the same conditions. TR, Trace amount (<0.5%); –, not detected.

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>1</th>
<th>2</th>
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<tr>
<td>C&lt;sub&gt;10 : 0&lt;/sub&gt;</td>
<td>TR</td>
<td>TR</td>
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<tr>
<td>C&lt;sub&gt;12 : 0&lt;/sub&gt;</td>
<td>5.1</td>
<td>7.8</td>
</tr>
<tr>
<td>C&lt;sub&gt;14 : 0&lt;/sub&gt;</td>
<td>TR</td>
<td>5.9</td>
</tr>
<tr>
<td>C&lt;sub&gt;15 : 0&lt;/sub&gt;</td>
<td>TR</td>
<td>–</td>
</tr>
<tr>
<td>C&lt;sub&gt;16 : 0&lt;/sub&gt;</td>
<td>31.6</td>
<td>23.9</td>
</tr>
<tr>
<td>C&lt;sub&gt;17 : 0&lt;/sub&gt;</td>
<td>TR</td>
<td>–</td>
</tr>
<tr>
<td>C&lt;sub&gt;18 : 0&lt;/sub&gt;</td>
<td>1.6</td>
<td>TR</td>
</tr>
<tr>
<td>C&lt;sub&gt;16 : 1o5c&lt;/sub&gt;</td>
<td>TR</td>
<td>–</td>
</tr>
<tr>
<td>C&lt;sub&gt;18 : 1o7c&lt;/sub&gt;</td>
<td>1.9</td>
<td>1.2</td>
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<tr>
<td>C&lt;sub&gt;12 : 0 3-OH&lt;/sub&gt;</td>
<td>TR</td>
<td>–</td>
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<tr>
<td>iso-C&lt;sub&gt;16 : 0&lt;/sub&gt;</td>
<td>TR</td>
<td>–</td>
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<tr>
<td>cyclo-C&lt;sub&gt;17 : 0&lt;/sub&gt;</td>
<td>33.4</td>
<td>32.3</td>
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<td>–</td>
<td>TR</td>
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<tr>
<td>cyclo-C&lt;sub&gt;19 : 0o8c&lt;/sub&gt;</td>
<td>2.5</td>
<td>TR</td>
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</tr>
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<td>Unknown ECL 13.95</td>
<td>TR</td>
<td>–</td>
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</table>

*Summed features represent two or three fatty acids that cannot be separated by the Microbial Identification System. Summed feature 2 comprises C<sub>14 : 0</sub> 3-OH/iso-C<sub>16 : 1</sub>. Summed feature 3 comprises C<sub>16 ;1o7d</sub>/iso-C<sub>15</sub> 2-OH.

Figure 2. Two-dimensional thin-layer chromatogram of the polar lipids of strain KBL009<sup>T</sup>. The polar lipids of strain KBL009<sup>T</sup> were separated by two-dimensional TLC on a silica gel plate (Merck) using chloroform/acetone/methanol/water (50 : 6.8 : 1, by vol.) as the solvent, and molybdatophosphoric acid was sprayed to detect each spot of total polar lipids. L1–5, unknown polar lipids.

Extraction of the genomic DNA from strain KBL009<sup>T</sup> was performed according to the method described by Rochelle et al. (1992). The G+C content of genomic DNA was determined using HPLC as described by Mesbah & Whitman (1989). The genomic DNA G+C content of strain KBL009<sup>T</sup> was 56.1 mol%.

On the basis of phenotypic and phylogenetic characterization, and results from DNA–DNA hybridizations, the assignment of KBL009<sup>T</sup> to the genus *Paenalcaligenes* as a representative of a novel species is suggested. For this novel species the name *Paenalcaligenes hermetiae* sp. nov. is proposed.

**Emended description of the genus *Paenalcaligenes***

The description is as given by Kämpfer et al. (2010), with the following amendments. The betaproteobacterial-specific polypeptide 2-hydroxyproteusine can only be detected in trace amounts (<0.1 μmol (g dry weight)<sup>−1</sup>). The polar lipids contain DPG, PG, PE, AL and several unknown polar lipids. Grows weakly in an atmosphere consisting of 90% N<sub>2</sub>, 5% H<sub>2</sub> and 5% CO<sub>2</sub>.

**Description of *Paenalcaligenes hermetiae* sp. nov.**

*Paenalcaligenes hermetiae* [her.me.ti] a.e. N.L. n. *Hermetia* a scientific zoological generic name; N.L. gen. n. *hermetiae* of Hermetia, isolated from larval gut of *Hermetia illucens* (Diptera: Stratiomyidae).

Cells are weakly motile, Gram-stain-negative, facultatively anaerobic and short rods (mean 0.5–1.5 μm in diameter). Colonies are opaque, ivory, smooth, circular, raised and 1.0–2.0 mm in diameter after cultivation at 37°C for 3 days on TSA medium. Growth occurs at 15–42°C, in presence of 0–15% (w/v) NaCl and at pH 5.0–9.0. Optimal grow conditions are 37°C, 1–2% NaCl and pH 6.0. Oxidase-positive and catalase-positive. Oxidizes glycerogen, Tween 40, Tween 80, pyruvic acid methyl ester, succinic acid monomethyl ester, acetic acid, cis-aconitic acid, citric acid, formic acid, α-hydroxybutyric acid, β-hydroxybutyric acid, γ-hydroxybutyric acid, α-ketobutyric acid, α-keto acid, D-lactic acid, L-lactic acid, propionic acid, succinic acid, bromosuccinic acid, succinamic acid, L-alaninamide, D-alanine, L-alanine, L-asparagine, L-aspartic acid, L-glutamic acid, L-histidine, L-leucine, L-ornithine, L-phenylalanine, L-serine, L-threonine, and phenethylyamine in the Biolog GN2 MicroPlate. The following compounds are not assimilated: α-cyclodextrin, dextrin, N-acetyl-D-galactosamine, N-acetyl-D-glucosamine, adonitol, L-arabinose, D-arabitol, cellobiose, L-erythritol, D-fructose, L-fucose, D-galactose, gentiobiose, α-D-glucose, myo-inositol, α-lactose, lactulose, maltose, D-mannitol, D-mannose, melibiose, methyl β-D-glucoside, D-psicose, raffinose, L-rhamnose, D-sorbitol, sucrose, trehalose, turanose, xylitol, D-galactonic acid lactone, D-galacturonic acid, D-glucic acid, D-glucosaminic acid, D-glucuronic acid, p-hydroxyphenylacetic acid, itaconic acid, malonic acid, quinic...
acid, D-saccharic acid, sebacic acid, glucuronamide, L-α-lanyl glycine, glycy1 L-α-aspartic acid, glycy1 L-glutamic acid, hydroxy-L-proline, L-proline, L-α-pyrrol glutamic acid, D-serine, DL-carnitine, γ-αminobutyric acid, urocanic acid, inosine, uridine, thymidine, putrescine, 2-aminoethanol, 2,3-butanediol, glycerol, DL-2-glycerol phosphate, α-D-gluco 1-phosphate, or D-glucose 6-phosphate. In the API 50 CH strip, acid is produced from D-tagatose and 5-ketogluconate, but not from the other substrates. The following API ZYM test enzymes scored as positive: esterase (C4), esterase lipase (C8), leucine arylamidase and naphthol-AS-BI-phosphohydrolase. The major fatty acids are cyclo-C₁₇:0, C₁₆:0 and summed feature 2 (comprising C₁₄:0 3-OH/iso-C₁₆:1). The polar lipid profile contains DPG, PE, PG, one AL and five unknown polar lipids (L1–5). The major ubiquinone is Q-8 and Q-7 is detected in minor amounts. The polyamine pattern contains predominantly putrescine, spermidine, spermine and cadaverine. 2-Hydroxyputrescine is present only in trace amounts.

The type strain is KBL009ᵀ (=KACC 16840ᵀ=JCM 18423ᵀ), which was isolated from the larval gut of Hermetia illucens (black soldier fly) in South Korea. The DNA G+C content of the type strain is 56.1 mol%.

Acknowledgements

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