Parasediminibacterium paludis gen. nov., sp. nov., isolated from wetland

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A novel orange-pigmented bacterial strain, designated HME6815ᵀ, was isolated from wetland in Jeju Island, Republic of Korea. The cells were Gram stain-negative, non-motile, strictly aerobic and rod-shaped. Optimal growth occurred at 30 °C and pH 7.0 on R2A agar. Phylogenetic analysis based on 16S rRNA gene sequences indicated that strain HME6815ᵀ formed a distinct phylogenetic lineage within the family Chitinophagaceae and was most closely related to members of the genera Sediminibacterium, Vibrionimonas, Hydrobacter, Hydrotalea and Asinibacterium with 92.3–94.3 % 16S rRNA gene sequence similarity. The major cellular fatty acids were iso-C₁₅ : 0, iso-C₁₇ : 0 3-OH, summed feature 3 (C₁₆ : 1ω7c and/or C₁₆ : 1ω6c) and iso-C₁₃ : 0. The only respiratory quinone was MK-7. Polar lipid analysis revealed the presence of phosphatidylyethanolamine, four unidentified aminolipids, one unidentified aminophospholipid and three unidentified polar lipids. The DNA G+C content was 38.4 mol%. On the basis of the evidence presented in this study, strain HME6815ᵀ represents a novel species of a new genus in the family Chitinophagaceae, for which the name Parasediminibacterium paludis gen. nov., sp. nov. is proposed. The type strain of the type species is HME6815ᵀ (=KCTC 23736ᵀ = CECT 8010ᵀ).

The family Chitinophagaceae was first proposed by Kämpfer et al. (2011) and represented a phylogenetically diverse family within the phylum Bacteroidetes (Eder et al., 2015). At the time of writing, the family Chitinophagaceae comprises 24 genera with validly published names, including the recently identified genera Arachidicoccus (Madhaiyan et al., 2015), Asinibacterium (Lee et al., 2014), Cnuella (Zhang et al., 2014), Flaviaesturariibacter (Kang et al., 2015), Heliimonas (Landro et al., 2013), Hydrobacter (Eder et al., 2015), Parafilimonas (Kim et al., 2014), Taibaiella (Zhang et al., 2013), Thermoflavifilum (Anders et al., 2014) and Vibrionimonas (Albert et al., 2014). Members of the family Chitinophagaceae are aerobic or facultatively anaerobic, usually non-motile, rod-shaped and contain menaquinone-7 (MK-7) as the isoprenoid quinone, and iso-C₁₅ : 0, iso-C₁₇ : 0 3-OH and iso-C₁₅ : 1 as the major fatty acids (Kämpfer et al., 2011; Kim et al., 2014).

As part of a study on the microbial diversity of a water sample from wetland in Jeju Island, Republic of Korea (33° 21’ 52” N 126° 26’ 56” E), the water sample was diluted using tenfold-dilution-series methods, spread on R2A agar (Difco) plates and incubated at 30 °C for 5 days. A novel orange-coloured bacterial strain was isolated and designated HME6815ᵀ. The pure culture of strain HME6815ᵀ was routinely cultured on R2A at 30 °C and preserved at −80 °C in distilled water supplemented with 20 % (v/v) glycerol.

The 16S rRNA gene sequence (1460 bp) of strain HME6815ᵀ was obtained using sequencing primers (27F, 518R, 785F and 1492R; Lane, 1991) for phylogenetic analysis and submitted to the GenBank database. Pairwise similarity values between strain HME6815ᵀ and closely related type strains were calculated using the EzTaxon-e server (http://eztaxon-e.ezbiocloud.net/; Kim et al., 2012). The 16S rRNA gene sequence of strain HME6815ᵀ was aligned with sequences of close relatives using SINA Incremental Aligner (v1.2.11) (Pruesse et al., 2012). A phylogenetic analysis was performed using the software package MEGA version 6.0 (Tamura et al., 2013). Phylogenetic trees were reconstructed by neighbour-joining (Saitou & Nei, 1987), maximum-parsimony (Fitch, 1971) and maximum-likelihood (Felsenstein, 1981) algorithms. The topologies of the resultant trees were evaluated by using the bootstrap resampling method of Felsenstein (1985) with 1000 replicates.

The 16S rRNA gene sequence of strain HME6815ᵀ exhibited highest similarities to Sediminibacterium salmonense (94.3 %), Sediminibacterium ginsengisoli DCY13ᵀ (93.8 %), Sediminibacterium goheungense HME7863ᵀ (93.7 %), Vibrionimonas...
magnilachitabantis MU-2T (92.9 %), Hydrobacter penzbergensis EM 4T (92.8 %), Hydrotalea flava CCUG 51397T (92.7 %) and Asinibacterium lactis LCJ02T (92.3 %). Phylogenetic trees based on 16S rRNA gene sequences, generated by using all three tree-making methods showed that strain HME6815T was a member of the family Chitinophagaceae. Strain HME6815T formed a distinct phylogenetic branch and clustered with the genera Sediminibacterium, Vibrionimonas, Hydrobacter, Hydrotalea and Asinibacterium as evidenced by the high bootstrap value (Fig. 1). On the basis of 16S rRNA gene sequence similarities and phylogenetic analysis, S. salmonew KACC 15020T, S. ginsengisoli KCTC 12833T, S. goheungense HME7863T, V. magnilachitabantis KCTC 42570T Hydrotalea flava KACC 17854T and Asinibacterium lactis KCCM 90108T were obtained from our previous study (Kang et al., 2014), the Korean Collection for Type Cultures (KCTC), the Korean Agricultural Culture Collection (KACC) and the Korean Culture Center of Microorganisms (KCCM), respectively, and used as reference strains in the following tests.

Cell morphology was examined by light microscopy and transmission electron microscopy (JEM1010; JEOL) after cells had been negatively stained with 1 % (w/v) phosphotungstic acid. The hanging-drop technique was used to assess gliding motility (Bernardet et al., 2002). Gram staining was carried out as described by Hucker (1921). Oxidase activity was evaluated via the oxidation of 1 % N,N,N',N'-tetramethyl-p-phenylenediamine dihydrochloride (Sigma). Catalase activity was determined by measurements of bubble production after the application of 3 % (v/v) H2O2 solution. The growth temperature range was determined on R2A agar at 4, 10–30 (at intervals of 5 °C), 37 and 42 °C. The pH range for growth was tested in R2A broth at pH 4.0–10.0 by using 0.1 M sodium acetate buffer (for pH 4.0–6.0), 0.1 M phosphate buffer (pH 7.0 and 8.0) and 0.1 M sodium carbonate buffer (pH 9.0 and 10.0). Tolerance to NaCl was examined in R2A broth with 0.5 % and 1.0–5.0 % NaCl (w/v; at intervals of 1.0 %). Growth was tested on nutrient (NA), marine 2216 (MA), tryptic soy (TSA), blood and MacConkey agars (all Difco). Hydrolysis of casein (3 % skimmed milk (Difco), w/v), CM-cellulose (1 % CM-cellulose (Sigma), w/v), cellulose (1 % filter paper (Whatman #1) w/v), dextrin (1 % dextrin (Sigma), w/v) and starch (1 % soluble starch (Sigma), w/v) was tested using 0.1 x R2A agar as the basal medium. Anaerobic growth was tested on R2A agar at 25 °C for 4 weeks by using the GasPak EZ Anaerobic container System (BD). Flexirubin-type pigments were detected from a colour shift after exposure to 20 % (w/v) KOH solution (Bernardet et al., 2002). Additional biochemical tests were performed by using API ZYM and API 20NE kits (bioMerieux) and oxidation of carbon compounds was evaluated with GN2 MicroPlates (Biolog) according to the manufacturer's instructions. DNA was extracted and purified using a genomic DNA prep kit (Solgent) to determine the DNA G+C content of strain HME6815T. DNA was analysed using a real-time PCR thermocycler (Bio-Rad) with SYBR Green 1 (SG; Invitrogen), according to the fluorometric method (Gonzalez & Saiz-Jimenez, 2002). Fluorescent DNA melting curves were generated in triplicate.

Cells of strain HME6815T were Gram-stain-negative, non-motile, strictly aerobic and straight rods. Flexirubin-type pigments were absent. Good growth was obtained on R2A agar but not on NA, blood agar, MA, TSA or MacConkey agar. The DNA G+C content was 38.4 mol%, a value lower than those of the most closely related reference strains. The detailed morphological, physiological and biochemical characteristics of strain HME6815T are given in the species description and in Table 1.

The fatty acid profiles of strain HME6815T, V. magnilachitabantis KCTC 42570T, Hydrotalea flava KACC 17854T and Asinibacterium lactis KCCM 90108T were determined under identical conditions. The cells were grown on R2A agar at 30 °C for 3 days and collected from the third streak quadrant to obtain cells of the same physiological age. Biomasses were harvested and cellular fatty acids were saponified, methylated and extracted according to the standard protocol of MIDI (Sherlock Microbial Identification System, version 6.1). The fatty acids were analysed by GC (7890; Hewlett Packard) and identified by using the RTSSA6 database of the Microbial Identification System (Sasser, 1990). Cell biomass of strain HME6815T for the analyses of isoprenoid quinones and polar lipids was obtained from cultures grown on R2A agar at 30 °C for 3 days. Isoprenoid quinones and polar lipids of strain HME6815T were extracted and analysed from freeze-dried cells as described by Minnikin et al. (1984). Lipids were separated using two-dimensional TLC (silica-gel-coated plates, 10 x 10 cm; Merck), using chloroform/methanol/water (65 : 25 : 4, by vol.) in the first dimension and chloroform/acetic acid/methanol/water (80 : 15 : 12 : 4, by vol.) in the second dimension. The plates were sprayed with 10 % ethanolic molybdo-phosphoric acid (Sigma) to detect total polar lipids, ninhydrin (Sigma) for aminolipids, x-naphthol for glycolipids and molybdenum blue (Sigma) for phospholipids.

The fatty acid compositions of strain HME6815T and the seven reference strains are presented in Table 2. The major fatty acids of strain HME6815T were iso-C15 : 0, iso-C17 : 0 3-OH, summed feature 3 (C16 : 1ω7c and/or C16 : 1ω6c) and iso-C13 : 0 3-unsat. The overall fatty acid profile of strain HME6815T is similar to those of the seven reference strains in that iso-C15 : 0 and iso-C17 : 0 3-OH are major fatty acids. However, significant differences in respective proportions of several components, the presence of iso-C16 : 1ω6c and summed feature 9 (C16 : 0 10-methyl and/or C17 : 0 10ω9c), the smaller amount of iso-C15 : 0 3-unsat and larger amount of iso-C13 : 0 3-unsat clearly distinguished the strain from other related species. The only isoprenoid quinone of HME6815T was menaquione-7 (MK-7). Polar lipids of strain HME6815T were phosphatidylethanolamine, four unidentified
**Fig. 1.** Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences, showing the phylogenetic positions of strain HME6815<sup>T</sup> and related taxa of the family Chitinophagaceae. Bootstrap percentages >70 % are shown at nodes. Filled and open circles indicate nodes recovered by all three treeing methods or by two treeing methods, respectively. *Flavobacterium aquatile* DSM 1132<sup>T</sup> was used as an outgroup. Bar, 0.02 substitutions per nucleotide position.
Table 1. Differential characteristics of strain HME6815<sup>T</sup> and type strains of related species

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<td>45.2&lt;sup&gt;d&lt;/sup&gt;</td>
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<td>49.2&lt;sup&gt;g&lt;/sup&gt;</td>
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*Data from: a, Qu & Yuan (2008); b, Kim et al. (2013); c, Kang et al. (2014); d, Albert et al. (2014); e, Eder et al. (2015); f, Kämpfer et al. (2011); g, Lee et al. (2013).
Table 2. Cellular fatty acid contents (%) of strain HME6815<sup>T</sup> and type strain of related species

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<td>Summed features*</td>
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<td>1.4</td>
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*Summed features are groups of two or three fatty acids that could not be separated by GC with the MIDI system. Summed feature 3 comprised C<sub>16</sub> : 1<i>α</i>7c and/or C<sub>16</sub> : 1<i>α</i>6c; summed feature 9 comprised 10-methyl C<sub>16</sub> : 0 and/or C<sub>17</sub> : 1<i>α</i>9c.

aminolipids, one unidentified aminophospholipid and three unidentified polar lipids (Fig. S1, available in the online Supplementary Material). Strain HME6815<sup>T</sup> differed from the genus <i>Sediminibacterium</i> by the absence of unidentified phospholipids and pattern of unidentified aminolipids, unidentified aminophospholipids and unidentified polar lipids (Kim <i>et al.</i>, 2013; Kang <i>et al.</i>, 2014; Albert <i>et al.</i>, 2014).

On the basis of phylogenetic and phenotypic data, strain HME6815<sup>T</sup> is considered to represent a novel species of a new genus in the family <i>Chitinophagaceae</i>, for which the name <i>Parasediminibacterium paludis</i> gen. nov., sp. nov. is proposed.

**Description of <i>Parasediminibacterium gen. nov.</i>**

<i>Parasediminibacterium</i> (Pa.ra.se.di.mi.ni.bac.te’ri.um). Gr. pref. para- like, beside; N.L. neut. n. <i>Sediminibacterium</i> the name of a bacterial genus; N.L. neut. n. <i>Parasediminibacterium</i> resembling the genus <i>Sediminibacterium</i>, referring to the close relationship to the genus <i>Sediminibacterium</i>.

Cells are Gram-stain-negative, aerobic, straight rod-shaped, non-motile, oxidase- and catalase-positive. Flexirubin-type pigments are not produced. Nitrate is not reduced to nitrite. The predominant respiratory quinone is MK-7. The genomic DNA G+C content is 38.4 mol%.

Phylogenetically, the genus is a member of the family <i>Chitinophagaceae</i>, phylum <i>Bacteroidetes</i>.

The type species is <i>Parasediminibacterium paludis</i>.

**Description of <i>Parasediminibacterium paludis</i> sp. nov.**

<i>Parasediminibacterium paludis</i> (pa.lu’dis. L. gen. n. paludis of a wetland, of a swamp, of a marsh).

The characteristics are the same as those given in the genus description with the following additions. Cells are approximately 1.5–3.0 μm in length and 0.4–0.5 μm in diameter. Colonies on R2A agar are orange and approximately 1 mm in diameter after 3 days at 30 °C. Growth occurs at 25–30 °C (optimum 30 °C), at pH 7–8 (optimum pH 7.0) and in R2A broth supplemented with 0–1.0 % NaCl (optimum 0 % NaCl). Growth does not occur on NA, blood agar, MA, TSA or MacConkey agar. Casein (skimmed milk), DNA, starch, dextrin, CM-cellulose and cellulose (filter paper) are not hydrolysed. H<sub>2</sub>S is not produced. In the API 20NE strip, positive for aesculin hydrolysis and β-galactosidase activity (PNPG test). In the API ZYM strip, alkaline phosphatase, leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α-glucosidase and N-acetyl-β-glucosaminidase activities are present; esterase (C4), esterase lipase (C8), lipase (C14), α-chymotrypsin, α-galactosidase, β-galactosidase, β-glucuronidase, β-glucosidase, α-mannosidase and x-fucosidase activities are absent. The following compounds are utilized as sole carbon sources in the GN2 MicroPlate: succinic acid, monomethyl ester, α-ketobutyric acid, L-glutamic acid, glycerol L-aspartic acid, glycyl L-glutamic acid, L-serine and L-threonine; the other substrates are not utilized. The major cellular fatty acids (>10 %) are iso-C<sub>15</sub> : 0, iso-C<sub>17</sub> : 0-3-<i>OH</i>, summed feature 3 (C<sub>16</sub> : 1<i>α</i>7c and/or C<sub>16</sub> : 1<i>α</i>6c) and iso-C<sub>13</sub> : 0. MK-7 is the only respiratory quinone. The polar lipids are phosphatidylethanolamine, four
unidentified aminolipids, one unidentified aminophospholipid and three unidentified polar lipids.

The type strain is HME6815T (=KCTC 23736T = CECT 8010T), isolated from wetland in Jeju Island, Republic of Korea. The DNA G+C content of the type strain is 38.4 mol%.

Acknowledgements

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References


