**Dysgonomonas alginatilytica** sp. nov., an alginate-degrading bacterium isolated from a microbial consortium

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Gram-stain-negative, facultatively anaerobic, non-motile, non-spore-forming, rod-shaped bacterium, designated strain HUA-2T, was isolated from an alginate-degrading microbial consortium. Strain HUA-2T was related to *Dysgonomonas capnocytophagoides* JCM 16697T, *Dysgonomonas macrotermitis* JCM 19375T and *Dysgonomonas mossii* CCUG 43457T with 95.1%, 94.1% and 92.1% 16S rRNA gene sequence similarity, respectively. The optimal growth temperature and pH for strain HUA-2T were 35°C and pH 8.0, respectively. Enzyme production, major fermentation products from glucose, and the major cellular fatty acids were different from those of *D. capnocytophagoides* CCUG 17966T or other members of the genus *Dysgonomonas*. Therefore, strain HUA-2T is proposed to represent a novel species of the genus *Dysgonomonas*, for which we propose the name *Dysgonomonas alginatilytica* sp. nov. The type strain is HUA-2T (=DSM 100214T=HUT 8134T).

The members of the genus *Dysgonomonas*, established by Hofstad et al. (2000), are Gram-stain-negative, facultatively anaerobic, coccobacillus-shaped bacteria (Lawson et al., 2010). To date, seven species of the genus *Dysgonomonas* have been identified, namely *Dysgonomonas gadei* (Hofstad et al., 2000), *Dysgonomonas capnocytophagoides* (Hofstad et al., 2000), *Dysgonomonas mossii* (Lawson et al., 2002), *Dysgonomonas hofstadii* (Lawson et al., 2010), *Dysgonomonas oryzarvi* (Kodama et al., 2012), *Dysgonomonas macrotermitis* (Yang et al., 2014) and *Dysgonomonas termitidis* (Pramono et al., 2015). The first four species were isolated from human clinical specimens, whereas the remaining three species were isolated from amicrobial fuel cell, the hindgut of a fungus-growing termite and the gut of a subterranean termite, respectively. To date, the isolation of strains of species of the genus *Dysgonomonas* from marine environments has not, to our knowledge, been reported. In this study, strain HUA-2T isolated from a sea sand sample was characterized and proposed to represent a novel species of the genus *Dysgonomonas*.

The 16S rRNA gene sequence of strain HUA-2T was isolated from an alginate-degrading microbial consortium that was acclimated from sea sand of a beach in the Hiroshima Gulf, Hiroshima, Japan. The enrichment sample was serially diluted and inoculated onto peptone–yeast extract–glucose (PYG) agar medium (DSMZ medium no. 104) using the roll-tube method (Hungate, 1969). The tubes were incubated at 30°C for 3 days. The resulting eight colonies were picked and cultured in PYG medium. Analysis of the 16S rRNA gene sequences of all eight isolated strains were performed as previously described (Kita et al., 2013). The 16S rRNA gene sequences of eight isolated strains were completely identical. Hence, one strain was randomly selected and designated strain HUA-2T. This strain was further purified twice by the roll-tube method and cultured anaerobically at 30°C on PYG or EG medium (JCM medium no. 14).

The 16S rRNA gene sequence of the isolated strain, HUA-2T, and related species were aligned using CLUSTAL w (Thompson et al., 1994) and edited with BioEdit version 7.2.0 (Hall, 1995). A phylogenetic tree was reconstructed using the neighbour-joining method (Saitou & Nei, 1987)
and the Kimura two-parameter model (Kimura, 1980) with MEGA version 5.2 software (Tamura et al., 2011). Bootstrap values (Felsenstein, 1985) are shown for each node (percentage of 1000 bootstraps >50 %). To identify the optimal growth conditions, strain HUA-2T was cultured at different temperatures (15, 20, 25, 30, 35, 37 and 42 °C) and pH values (4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5, 9.0 and 9.5) on PYG medium. The pH values were adjusted with 1 M NaOH or 1 M HCl. Fermentative growth was assessed using PYG medium substituting the glucose with 1 g l⁻¹ of each other substrate as the carbon source. Alginate, glucose, L-arabinose, lactose, maltose, D-mannose, melibiose, raffinose, L-rhamnose, sucrose, D-xylene, adonitol, D-arabitol, L-arabitol, dulcitol, inositol, D-mannit, D-sorbitol and trehalose were used as carbon sources. Utilization of organic acids as carbon sources was examined using PYG medium substituting the glucose with lactate, succinate, malate, propionate, acetate, or formate (all at a concentration of 1 g l⁻¹). Utilization of different electron acceptors was tested in BP medium supplemented with 1 g glucose l⁻¹ as the sole carbon source and sodium nitrate, sodium fumarate, ferric citrate (each at 5 mM), 2 mM MnO₂ or O₂ (the head-space N₂ gas was replaced with air) as the sole electron acceptor according to the protocol of Kodama et al. (2012). To examine whether strain HUA-2T utilized H₂ for aceticogenic growth, vials containing glucose-eliminated PYG medium were flushed with a filter-sterilized H₂/CO₂ mixture (80 : 20, v/v) after inoculation to 0.15 MPa final pressure. Consumption of H₂ was analysed by gas chromatography as previously described (Miura et al., 2014). The consumption of substrates and production of organic acids were identified by HPLC as previously described (Miura et al., 2014). Growth rate was measured based on OD₆₆₀. Bile sensitivity was determined by measuring the effect of adding 0.5 % (w/v) bile powder (Wako) to the liquid PYG medium on the growth rate. All tests were performed in triplicate.

DNA G + C content (mol%), long-chain fatty acid composition, major respiratory quinones, as well as the morphological and biochemical characteristics of the bacteria were analysed at the TechnoSuruga Laboratory (Shizuoka, Japan). The G + C content (mol%) of the genomic DNA was determined by HPLC (Katayama-Fujimura et al., 1984). To identify the long-chain fatty acid components, cells were cultured on EG medium containing agar and incubated for 48 h at 30 °C (strain HUA-2T) or 37 °C (D. capnocytophagoides JCM 16697T=CCUG 17966T and D. macrotermitis JCM 19375T). The saponification, methylation, extraction and identification of the cellular fatty acid methyl esters were performed according to the manual of the Sherlock Microbial Identification System version 6 (MIDI). For the identification of the major respiratory quinones, cells were cultured anaerobically on EG medium (48 h; 30 °C). Total lipids of strain HUA-2T were extracted from freeze-dried cells, according to the modified method of Bligh & Dyer (1959). Subsequently, they were purified in hexane to extract quinones. Quinones were detected by ultraperformance liquid chromatography. Morphological analysis was performed with a BX50F4 light microscope (Olympus). Gram staining was conducted using Favor G Nissui (Nissui Pharmaceutical) and biochemical characterization was conducted using the API 20 A and API ZYM kits (bioMérieux), according to the manufacturer’s instructions. Requirements for the X and V factors for growth were examined using X and V discs (Oxoid) on EG agar plates.

The DNA G + C content of strain HUA-2T was 37.5 mol%. Results of phylogenetic analysis revealed that strain HUA-2T represented a member of the genus Dysgonomonas, which is supported by a high bootstrap value of 98 % (Fig. 1). Strain HUA-2T was related to D. capnocytophagoides JCM 16697T, D. macrotermitis JCM 19375T and D. mossii CCUG 43457T with 95.1 %, 94.1 % and 92.1 % 16S rRNA gene sequence similarity, respectively.

In PYG medium, strain HUA-2T was able to grow at 20–37 °C but not at 15 °C or 42 °C; the optimal temperature was 35 °C. The pH range supporting growth was 5.5–9.0, and the optimum pH was 8.0. Strain HUA-2T was facultatively anaerobic, growing under both aerobic and anaerobic conditions. Growth was detected with the following substrates: alginate, L-arabinose, lactose, maltose, D-mannose, melibiose, raffinose, L-rhamnose, sucrose, D-xylene, malate and lactate. Conversely, growth did not occur with adonitol, D-arabitol, L-arabitol, dulcitol, inositol, D-mannitol, D-sorbitol, trehalose, acetate, propionate, succinate or formate. Interestingly, HUA-2T was able to utilize alginate as a sole carbon source, unlike D. capnocytophagoides DSM 22835T (=CCUG 17966T=JCM 16697T). The major fermentation products of strain HUA-2T from glucose (1 g l⁻¹) in the PYG medium were lactate (6.6 ± 0.2 mM), succinate (3.8 ± 0.7 mM), acetate (1.6 ± 0.1 mM) and propionate (0.9 ± 0.2 mM). H₂ was not produced. On the other hand, the major fermentation products of D. capnocytophagoides DSM 22835T were propionate (6.6 ± 0.2 mM), acetate (3.0 ± 0.1 mM), succinate (2.6 ± 0.5 mM) and lactate (2.0 ± 0.1 mM), under the same conditions. When strain HUA-2T was cultured in glucose-eliminated PYG medium as negative control, the major products were succinate (2.3 ± 0.1 mM), acetate (1.0 ± 0.1 mM) and propionate (0.9 ± 0.1 mM). As for D. capnocytophagoides DSM 22835T, the major products were acetate (1.3 ± 0.1 mM) and propionate (4.9 ± 0.2 mM). Thus, the production order of major acids obtained by us for D. capnocytophagoides DSM 22835T was different to that for HUA-2T. Ferric citrate and oxygen served as electron acceptors in the presence of glucose in BP medium while sodium nitrate, sodium fumarate and MnO₂ did not. Acetogenic growth was not observed with glucose-eliminated PYG liquid medium supplemented with H₂/CO₂ mixture. No growth was observed with PYG liquid medium supplemented with bile powder, indicating sensitivity to bile.
Table 1 shows the long-chain fatty acid composition of strain HUA-2T, *D. capnocytophagoides* JCM 16697<sup>T</sup> and *D. macrotermitis* JCM 19375<sup>T</sup>. anteiso-C<sub>13</sub>:0 and iso-C<sub>14</sub>:0 were more abundant in strain HUA-2T than in *D. capnocytophagoides* JCM 16697<sup>T</sup> and *D. macrotermitis* JCM 19375<sup>T</sup>. anteiso-C<sub>15</sub>:0 was less abundant in strain HUA-2T than in *D. capnocytophagoides* JCM 16697<sup>T</sup> and *D. macrotermitis* JCM 19375<sup>T</sup>. The main respiratory quinones of strain HUA-2T were a ubiquinone (Q-10, 37.7 %) and menaquinones (MK-9, 8.4 % and MK-10, 53.8 %).

Strain HUA-2T is characterized as Gram-stain-negative, non-motile, non-spore-forming and rod-shaped (0.5–0.6 6.1.5–3.0 μm). Fig. 2 shows a photograph of a phase-contrast microscopic image of strain HUA-2T. On PYG agar medium, the strain formed smooth, circular, cream-coloured and opaque colonies of 2–3 mm in diameter after 48 h at 30 °C. The strain was negative for catalase and oxidase activities. The X factor but not the V factor was required for growth, indicating a growth dependency for haem. The API 20 A and API ZYM kits detected alkaline phosphatase, esterase (C-4), acid phosphatase, naphthol-AS-BI-phosphohydrolase, α-galactosidase, α-glucosidase, δ-glucosidase, N-acetyl-β-glucosaminidase and α-fucosidase activities. In contrast, enzyme reactions...
Table 1. Composition of cellular fatty acids in some species of the genus *Dysgonomonas*

<table>
<thead>
<tr>
<th>Fatty acid</th>
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<tr>
<td>Straight-chain</td>
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<tr>
<td>C_{13} : 0</td>
<td>0.4</td>
<td>0.3</td>
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<td>0.6</td>
<td>0.6</td>
<td>0.4</td>
</tr>
<tr>
<td>C_{15} : 0</td>
<td>10.6</td>
<td>8.4</td>
<td>6.5</td>
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<td>C_{16} : 0</td>
<td>2.0</td>
<td>2.1</td>
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<td>ND</td>
<td>0.8</td>
</tr>
<tr>
<td>C_{18} : 0</td>
<td>ND</td>
<td>ND</td>
<td>0.5</td>
</tr>
<tr>
<td>Branched</td>
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<tr>
<td>iso-C_{12} : 0</td>
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<td>ND</td>
</tr>
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<td>ND</td>
<td>ND</td>
<td>0.3</td>
</tr>
<tr>
<td>iso-C_{14} : 0</td>
<td>8.1</td>
<td>2.3</td>
<td>1.4</td>
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<td>1.3</td>
<td>3.4</td>
<td>2.6</td>
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<td>anteiso-C_{15} : 0</td>
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<td>41.6</td>
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<td>ND</td>
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<td>Unsaturated</td>
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<td>ND</td>
</tr>
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<td>C_{16} : 0_7c</td>
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<td>0.2</td>
<td>0.4</td>
</tr>
<tr>
<td>C_{18} : 0_9c</td>
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<td>2.2</td>
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<td>C_{18} : 0_9c</td>
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<td>1.3</td>
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<tr>
<td>Hydroxy</td>
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<tr>
<td>C_{15} : 0_3-OH</td>
<td>2.7</td>
<td>2.5</td>
<td>0.8</td>
</tr>
<tr>
<td>iso-C_{15} : 0_3-OH</td>
<td>ND</td>
<td>0.6</td>
<td>ND</td>
</tr>
<tr>
<td>C_{16} : 0_3-OH</td>
<td>1.9</td>
<td>2.4</td>
<td>1.6</td>
</tr>
<tr>
<td>C_{17} : 0_3-OH</td>
<td>1.1</td>
<td>1.7</td>
<td>1.1</td>
</tr>
<tr>
<td>anteiso-C_{17} : 0_3-OH</td>
<td>5.1</td>
<td>4.7</td>
<td>4.8</td>
</tr>
</tbody>
</table>

were not detected for esterase lipase (C-8), lipase (C-14), leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin, chymotrypsin, β-galactosidase, β-glucuronidase, α-mannosidase and urease. Strain HUA-2 T hydrolysed aesculin but not gelatin. Finally, this strain did not support the production of indole.

The characteristics of strain HUA-2 T were compared with those of the other known members of the genus *Dysgonomonas* (Table 2). Whereas strain HUA-2 T was isolated from an alginate-degrading microbial consortium acclimated from beach samples, the other strains were isolated from human clinical specimens (four strains), termite gut (two strains), or a microbial fuel cell (one strain). The 16S rRNA gene sequence of strain HUA-2 T was most similar to that of *D. capnocytophagoides* CCUG 17966 T (95.1 %). The similarities in 16S rRNA gene sequence between strain HUA-2 T and the other strains of species of the genus *Dysgonomonas* were <96 %. It has been suggested that levels of DNA–DNA hybridization which define a genomic species (Aslam *et al.*, 2005; Wayne *et al.*, 1987) are less than 70 % in bacterial strains with less than 97 % 16S rRNA gene sequence similarity (Aslam *et al.*, 2005; Stackebrandt & Goebel, 1994). Although strain HUA-2 T and *D. capnocytophagoides* CCUG 17966 T displayed similar DNA G + C contents and substrate utilization patterns, they differed in major functional characteristics, namely their enzymic profiles, major fermentation products from glucose and major cellular fatty acids. In particular, the order of major fermentation products (lactate, succinate, acetate, propionate) from glucose and the predominance of major cellular fatty acids (anteiso-C_{13} : 0 and iso-C_{14} : 0) in strain HUA-2 T was clearly distinct from those for *D. capnocytophagoides* CCUG 17966 T. Altogether, these results demonstrate that the strain HUA-2 T represents a novel species of the genus *Dysgonomonas*, for which we propose the name *Dysgonomonas alginatilytica* sp. nov.

### Description of *Dysgonomonas alginatilytica* sp. nov.

*Dysgonomonas alginatilytica* (al gi.na.ti.ly’ti.ca. N.L. neut. n. alginatum alginate; N.L. fem. adj. lytica dissolving; N.L. fem. adj. alginatilytica alginate-dissolving).

Cells are Gram-stain-negative, non-motile, non-spore-forming and rod-shaped (0.5–0.6 × 1.5–3.0 μm). Colonies on PYG agar medium are circular, cream-coloured, smooth and opaque, reaching 2–3 mm within 48 h at 30 °C. Catalase- and oxidase-negative. Facultatively anaerobic. Can grow at 20–37 °C and pH 5.5–9.0 in PYG medium. The optimal growth temperature and pH are 35 °C and pH 8.0, respectively. Utilizes alginate,
Table 2. Differential characteristics between strain HUA-2\textsuperscript{T} and members of the genus *Dysgonomonas*

Strains: 1, HUA-2\textsuperscript{T} (data from this study); 2, *D. capnocytophagoides* JCM 16697\textsuperscript{T} (unless indicated otherwise, data were taken from Hofstad et al., 2000); 3, *D. macrotermitis* JCM 19375\textsuperscript{T} (Yang et al., 2014); 4, *D. mossii* CCUG 43457\textsuperscript{T} (Lawson et al., 2002); 5, *D. oryzarvi* JCM 16859\textsuperscript{T} (Kodama et al., 2012); 6, *D. gadei* CCUG 42882\textsuperscript{T} (Hofstad et al., 2000); 7, *D. termitidis* JCM 30204\textsuperscript{T} (Pramono et al., 2015); 8, *D. hofstadii* CCUG 54731\textsuperscript{T} (Lawson et al., 2010).

\'+', Positive; ‘−’, negative; ‘+−’, may or may not be produced; W, weakly positive; ND, no data available; A, acetate; L, lactate; P, propionate; S, succinate.

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<th>Characteristic</th>
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<th>5</th>
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<tr>
<td>Isolation source</td>
<td>Sea sand sample</td>
<td>Human clinical sample</td>
<td>Termite hindgut</td>
<td>Human clinical sample</td>
<td>Microbial fuel cell</td>
<td>Human clinical sample</td>
<td>Termite gut</td>
<td>Human clinical sample</td>
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<td>16S rRNA gene sequence similarity with strain HUA-2\textsuperscript{T}</td>
<td>100</td>
<td>95.1</td>
<td>94.1</td>
<td>92.1</td>
<td>92.1</td>
<td>91.4</td>
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<td>DNA G+C content (mol%)</td>
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<td>40.0</td>
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<td>Resistance to bile</td>
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<td>Major fatty acid</td>
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<td>anteiso-C\textsubscript{15} : 0, C\textsubscript{15} : 0, C\textsubscript{18} : 0,3-OH, anteiso-C\textsubscript{17} : 0,3-OH</td>
<td>anteiso-C\textsubscript{15} : 0, anteiso-C\textsubscript{15} : 0, anteiso-C\textsubscript{15} : 0, C\textsubscript{15} : 0,3-OH</td>
<td>C\textsubscript{16} : 0, isoc 3-OH, C\textsubscript{16} : 0,3-OH</td>
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<td>C\textsubscript{16} : 0, isoc 3-OH</td>
<td>ND</td>
<td>S, A</td>
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</table>

\*Data from Kodama et al. (2012). \textsuperscript{1}Data from Yang et al. (2014). \textsuperscript{2}Determined during this study.
L-arabinose, lactose, maltose, D-mannose, melibiose, raffinose, L-rhamnose, sucrose, D-xylose, malate and lactate as substrates but not adonitol, D-arabitol, L-arabitol, dulcitol, inositol, D-mannitol, D-sorbitol, trehalose, acetate, propionate, succinate or formate. The major fermentation products from glucose (1.0 g l⁻¹) in PYG medium are lactate, succinate, acetate and propionate. Utilizes ferric citrate and oxygen as electron acceptors in the presence of glucose. Bile-sensitive. The X factor is required for growth but not the V factor. The major cellular fatty acids (>8.0 % of total) are anteiso-C₁₅:0, C₁₅:0 and iso-C₁₄:0. The predominant menaquinones are Q-10 and MK-10. Positive reactions for alkaline phosphatase, esterase, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α-galactosidase, α-glucosidase, β-glucosidase, N-acetyl-β-glucosaminidase and α-fucosidase. Negative for esterase lipase (C-8), lipase (C-14), leucine arylamidase, valine arylamidase, cysteine arylamidase, trypsin, chymotrypsin, β-galactosidase, β-glucuronidase and α-mannosidase. Aesculin is hydrolysed but not gelatin. Negative for indole production and urease activity.

The type strain, HUA-2T (=DSM 100214T = HUT 8134T), was isolated from an alginate-degrading microbial consortium in Hiroshima, Japan. The genomic DNA G+C content of this strain is 37.5 mol%.

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References


