Agarivorans aestuarii sp. nov., an agar-degrading bacterium isolated from a tidal flat

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A Gram-reaction-negative, aerobic, non-spore forming, rod-shaped bacterium motile with a single polar flagellum, designated strain hydD622T, was isolated from the sediment of a tidal flat at Asan Bay, Korea. Strain hydD622T exhibited an agarolytic activity. Comparison of 16S rRNA gene sequences revealed that strain hydD622T was closely related to Agarivorans litoreus KCTC 42116T, Agarivorans albus KCTC 22256T and Agarivorans gilvus KCTC 32555T with similarities of 98.4, 98.0 and 96.5%, respectively. Strain hydD622T was clustered distantly from the other genera in the family Alteromonadaceae but formed a unique clade within the genus Agarivorans based on the 16S rRNA gene sequence. The DNA–DNA relatedness with Agarivorans litoreus KCTC 42116T and Agarivorans albus KCTC 22256T was 39.0 and 37.8%, respectively. The major fatty acids (>10%) were C16:0, C16:1ω6c, C16:1ω7c and C18:1ω6c/C18:1ω7c. The respiratory quinone was ubiquinone-8, and the polar lipid profile consisted of phosphatidylethanolamine, diphasphatidylglycerol, phosphatidylglycerol and an unidentified lipid. The DNA G+C content was 44 mol%. On the basis of physiological, chemotaxonomic and phylogenetic analyses, strain hydD622T represents a novel species within the genus Agarivorans, for which the name Agarivorans aestuarii sp. nov. is proposed. The type strain of Agarivorans aestuarii sp. nov. is hydD622T (=KCTC 32543T=CGMCC 1.12692T).

The genus Agarivorans was first proposed by Kurahasi & Yokota (2004), who isolated bacterial strains from the marine mollusk Omphalius pfeifferi in the Kanto area, Japan. The major characteristic of the genus Agarivorans is its agarolytic activity, and members have isolated from marine environments including marine animals, seawater, and seaweeds (Kurahasi & Yokota, 2004; Du et al., 2011; Park et al., 2014). Agar is a complex polysaccharide and can be obtained from red algae. Agar has been used as a food additive in many Asian countries. It is also one of the gelling materials commonly used in preparation of solid medium for cultivation of microorganisms. On the other hand, degradation products of agar formed by the hydrolysing enzyme agarase are simple sugars that can be used as substrates for biofuel production (Chi et al., 2012). During a sample collection and bacterial biodiversity study to isolate novel bacteria in Asan Bay, Korea, one novel strain, designated strain hydD622T, with agar-degrading activity was recovered. Phylogenetic analysis of the 16S rRNA gene sequence showed that the isolate is affiliated within the genus Agarivorans by having similarity with Agarivorans litoreus GJSW-6T (98.4%), Agarivorans albus MKT 106T (98.0%) and Agarivorans gilvus WH0801T (96.5%), respectively. Through phenotypic and phylogenetic analyses, we report that strain hydD622T represents a novel species in the genus Agarivorans for which the name Agarivorans aestuarii sp. nov. is proposed.

To isolate strain hydD622T, a serially diluted seawater suspension of tidal flat sediment from Asan Bay, Korea (36° 58’ 43" N 126° 47’ 55" E) was plated onto mineral salt agar formed by the hydrolysing enzyme agarase are simple sugars that can be used as substrates for biofuel production (Chi et al., 2012). During a sample collection and bacterial biodiversity study to isolate novel bacteria in Asan Bay, Korea, one novel strain, designated strain hydD622T, with agar-degrading activity was recovered. Phylogenetic analysis of the 16S rRNA gene sequence showed that the isolate is affiliated within the genus Agarivorans by having similarity with Agarivorans litoreus GJSW-6T (98.4%), Agarivorans albus MKT 106T (98.0%) and Agarivorans gilvus WH0801T (96.5%), respectively. Through phenotypic and phylogenetic analyses, we report that strain hydD622T represents a novel species in the genus Agarivorans for which the name Agarivorans aestuarii sp. nov. is proposed.

Abbreviations: DPG, diphasphatidylglycerol; PE, phosphatidylethanolamine; PG, phosphatidylglycerol.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain hydD622T is KM203871.

Two supplementary figures are available with the online Supplementary Material.
lysed with API 20E, API 20NE and API ZYM (bioMérieux) assessed with the wet mount method and observed with light microscopy. Acid production from carbon sources was also determined with Biolog’s automated microbial identification system using cells cultured on MG plates for 24 h. Cell suspensions were made by mixing 2% NaCl with the Biolog medium following the manufacturer’s instructions. Methylamine, dimethylamine, and trimethylamine utilization was examined on minimal salt medium solidified with 1% (w/v) gellan gum (Janssen et al., 2002) with 10 mM of each substrate. All the tests were performed twice. Resistance or susceptibility to antibiotics was tested on MG plates with antibiotic discs (Liofilchem) containing the following (µg per disc): ampicillin (10), carbenicillin (100), chloramphenicol (30), erythromycin (15), gentamicin (10), kanamycin (30), lincomycin (15), nalidixic acid (30), neomycin (30), novobiocin (30), penicillin G (10), streptomycin (10), tetracycline (30) and vancomycin (30).

For cellular fatty acid analysis, strain hydD622T and three references strains, A. albus KCTC 22256T (Kurahashi & Yokota, 2004), A. gilvus KCTC 3255T (Du et al., 2011) and A. litoreus KCTC 42116T (Park et al., 2014) obtained from the Korea Collection for Type Culture (KCTC) were cultured for 2 days under the same conditions on MG plates at 25°C. Cells were harvested at the exponential phase of growth, and fatty acid methyl esters were extracted and analysed by gas chromatography with the Microbial Identification System (MIDI, version 6.0) software package (database TSBA 6.0). Isoprenoid quinones were extracted with chloroform/methanol (2:1, v/v), purified by TLC on Kieselgel 60 F254 plates (20×20 cm; Merck) with petroleum ether/diethyl ether (9:1, v/v) as the solvent, visualized by short-wavelength UV and analysed by reverse-phase HPLC at a flow rate 1 ml min⁻¹ (Kim et al., 2010). Polar lipids were extracted and analysed by two-dimensional TLC and identified with molybdenum blue, ninhydrin, α-naphthol, Dragendorff’s reagent and 5% molybdatophosphoric acid reagent spray (Minnikin et al., 1984). The DNA G+C content was determined by the HPLC method (Mesbah et al., 1989), and Escherichia coli KCTC 2441T DNA was used as the reference. DNA–DNA hybridization was performed between strain HydD622T and the reference strains A. albus KCTC 22256T and A. litoreus KCTC 42116T. DNA from all three strains was independently labelled with photobiotin (Sigma) and then hybridized to DNA from the other two strains non-covalently linked to a microplate (Greiner Bio-One) at 45°C (Ezaki et al., 1989).

Strain hydD622T formed yellowish-white colonies of 1.5–2 mm in diameter with a slight depression on MSA in 3 days. After 1 week, the colonies sank down below the surface of the solid agar. About 3 weeks after incubation, all the agar was melted to a liquid. Growth occurred at pH 5–9 and at temperatures from 10 to 30°C but not at 4°C or above 32°C, with an optimum pH and temperature of pH 7–8 and 25°C, respectively. Strain HydD622T was Gram-reaction-negative and motile with a single polar flagellum (Fig. S1, available in the online Supplementary Material). Strain hydD622T was susceptible to ampicillin, carbenicillin, chloramphenicol,
erythromycin, gentamicin, lincomycin, nalidixic acid, neomy-
cin, novobiocin, penicillin G and tetracycline but resistant to
kanamycin, streptomycin and vancomycin.

Strain HydD622<sup>T</sup> shared 98.4, 98.0 and 99.5 % 16S rRNA
gene sequence similarity with the type strains of <i>A. litoreus</i>,
<i>A. albus</i> and <i>A. gilvus</i>, respectively, calculated by the
sequence identity matrix of the BioEdit program (Hall,
1999). In a phylogenetic tree based on the 16S rRNA gene
sequences, strain hydD622<sup>T</sup> was clustered with <i>A. gilvus</i>
KCTC 32555<sup>T</sup>, forming a branch with <i>A. albus</i> KCTC
22256<sup>T</sup> and <i>A. litoreus</i> KCTC 42116<sup>T</sup> (Fig. 1).

The DNA–DNA relatedness between strain hydD622<sup>T</sup>
and <i>A. litoreus</i> KCTC 42116<sup>T</sup> and <i>A. albus</i> KCTC 22256<sup>T</sup>
was 39.0 and 37.8 %, respectively (Table 1). A summary of the
morphological, physiological and biochemical characteristics
of strain hydD622<sup>T</sup> is presented in Table 2. The major
fatty acids (>10 %) of hydD622<sup>T</sup> were C<sub>16:0</sub> (26.0 %),
summed feature 3 (C<sub>16:1ω7c/C<sub>16:1ω6c</sub></i>) (19.8 %) and
summed feature 8 (C<sub>18:1ω7c/C<sub>18:1ω7c</sub></i>) (33.5 %). A summary
of the fatty acid contents of strain hydD622<sup>T</sup> and
related species is given in Table 3. Respiratory quinone
analysis indicated that Q-8 was the isoprenoid quinone.
Phosphatidylethanolamine (PE), diphosphatidylglycerol
(DPG), phosphatidyglycerol (PG) and one unknown lipid
were detected in the polar lipid extracts of strain hydD622<sup>T</sup>
The polar lipid profile of hydD622<sup>T</sup> is shown in Fig. S2.

The results obtained from this study were sufficient to show
that strain hydD622<sup>T</sup> belongs to the genus <i>Agarivorans</i>. However, strain hydD622<sup>T</sup> could be differentiated from <i>A. albus</i>, <i>A. gilvus</i>
and <i>A. litoreus</i> based on the enzymic

![Phylogenetic tree](http://ijs.microbiologyresearch.org)

**Fig. 1.** Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing the position of strain hydD622<sup>T</sup>
among related species. Numbers at branch points represent bootstrap values >50 % for percentages of 1000 resamplings.

Filled circles indicate that the corresponding nodes were also recovered in the trees generated with the maximum-likelihood
and maximum parsimony algorithms. *Pseudomonas aeruginosa* DSM 50071<sup>T</sup> (X06684) was used as an outgroup. Bar, 0.02
substitutions per nucleotide position.

http://ijs.microbiologyresearch.org
1.0–7.0 % (w/v) NaCl, while optimum growth occurs at 25°C, pH 7.0–8.0 in 2.0–3.0 % (w/v) NaCl. Catalase and oxidase are positive. Nitrate is reduced to nitrite. Indole is not produced from tryptophan. Aesculin, DNA, starch, Tween 20, Tween 40, Tween 60 and Tween 80 are hydrolysed but gelatin and urea are not hydrolysed. Enzyme activities are positive for \(N\)-acetyl-\(b\)-glucosaminidase, acid phosphatase, alkaline phosphatase, \(a\)-fucosidase, \(a\)-galactosidase, \(b\)-galactosidase, \(b\)-glucosidase, \(b\)-glucuronidase, \(\alpha\)-mannosidase, leucine arylamidase and naphthol-AS-BI-phosphohydrolase but negative for arginine dihydrolase, \(\alpha\)-chymotrypsin, cystine arylamidase, esterase (C4), esterase lipase (C8), lipase (C14), valine arylamidase and trypsin. Acid is produced from \(N\)-acetylglucosamine, cellobiose, \(D\)-fructose, gentiobiose, \(D\)-galactose, \(D\)-glucose, glycogen, inositol, lactose, \(D\)-mannose, \(D\)-mannitol, maltose, melibiose, sucrose, \(D\)-sorbitol and starch. The following substrates are used as the sole energy source for growth: acetate, adipate, citrate, \(L\)-fucose, gluconate, glycerol, inosine, malonate, \(D\)-mannose, phenylacetate, propionate, salicin, \(L\)-serine, \(D\)-sorbitol, sucrose, dimethylamine, trimethylamine.

### Table 1. DNA–DNA relatedness determined by DNA hybridization, expressed as a percentage

<table>
<thead>
<tr>
<th>Strain providing bound DNA</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Strain hydD622(^T)</td>
<td>–</td>
<td>20.9</td>
<td>26.1</td>
</tr>
<tr>
<td>2. (A.) litoreus KCTC 42116(^T)</td>
<td>39.0</td>
<td>–</td>
<td>30.4</td>
</tr>
<tr>
<td>3. (A.) albus KCTC 22256(^T)</td>
<td>37.8</td>
<td>37.6</td>
<td>–</td>
</tr>
</tbody>
</table>

### Table 2. Differential characteristics of strain hydD622\(^T\) and the type strains of three species of the genus \(Agarivorans\)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colony colour</td>
<td>Yellowish white</td>
<td>White(^a)</td>
<td>Light yellow(^b)</td>
<td>White(^c)</td>
</tr>
<tr>
<td>Growth at 4°C</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Growth at 37°C</td>
<td>–</td>
<td>+(^a)</td>
<td>+(^b)</td>
<td>+(^c)</td>
</tr>
<tr>
<td>Esterase lipase</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Utilization of:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acetate</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Adipate</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Citrate</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>L-Fucose</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Gluconate</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Glycerol</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Inosine</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Malonate</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>D-Mannose</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Phenylacetate</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Propionate</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Salicin</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>L-Serine</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>D-Sorbitol</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Sucrose</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Dimethylamine</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Trimethylamine</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Susceptibility to:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gentamicin</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Kanamycin</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Neomycin</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>DNA G+C content (mol%)</td>
<td>44</td>
<td>49.5(^a)</td>
<td>48.5(^b)</td>
<td>45.5(^c)</td>
</tr>
</tbody>
</table>

\(^a\)Data were taken from: \(a\), Kurahasi & Yokota (2004); \(b\), Du et al. (2011); \(c\), Park et al. (2014).
and carbon sources: L-arabinose, cellobiose, dimethylamine, dextrin, D-fructose, L-fucose, D-galactose, gentiobiose, glycollate, D-histidine, itaconic acid, malonic acid, D-mannitol, 3-D-mannose, melibiose, monomethylamine, L-proline, D-sorbitol and sucrose. The following substrates are not used as sole energy and carbon sources: adonitol, alanine, L-asparagine, D-arabitol, L-ascorbic acid, L-glutamic acid, glycerol, L-erythritol, inositol, inosine, leucine, phenylethylamine, raffinose, serine, thymidine, trehalose, trimethylamine, turanose and xyitol. Q-8 is the ubiquinone. PE, DPG, PG and an unidentified lipid are the polar lipids. The major cellular fatty acids (>10%) are C₁₂:0 summed feature 3 (C₁₆:0ω6c and/or C₁₆:1ω7c) and summed feature 8 (C₁₈:1ω7c and/or C₁₈:ω6c) while the minor fatty acids (<10%) are C₁₄:0 summed feature 2 (C₁₄:0ω6c and/or C₁₄:1ω7c) and summed feature 2 (C₁₂:0 aldehyde or unknown fatty acid of ECL 10.928).

The type strain, hydD622T (=KCTC 32543T=CGMCC 1.12692T), was isolated from the tidal flat sediment of Asan Bay, Korea. The DNA G+C content of hydD622T is 44 mol%.

**Acknowledgements**

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**References**


