Catenovulum agarivorans gen. nov., sp. nov., a peritrichously flagellated, chain-forming, agar-hydrolysing gammaproteobacterium from seawater

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A novel Gram-negative, strictly aerobic, agar-hydrolysing bacterium, designated YM01T, was isolated from seawater samples collected from the Yellow Sea (coastal region of Qingdao, PR China). Cells were rod-shaped, peritrichously flagellated and formed long chains end-to-end. The isolate had an absolute requirement for Na\(^+\) ions, but not seawater, for growth and grew optimally at about 28 °C, in 2 % NaCl and at pH 8.0–9.0. The isolate could not be cultured in marine broth 2216, but grew well on marine agar 2216. YM01T was able to hydrolyse cellulose, starch, aesculin and Tween 80, but not egg yolk, gelatin, urea or casein. 16S rRNA gene sequence analysis demonstrated that this isolate was unique, showing only 88.4–91.0 % sequence similarity to its closest neighbours, including members of the genera Glaciecola (88.4–91.0 %), Alteromonas (88.7–89.6 %), Aestuariibacter (89.3–90.4 %), Salimonas (89.0 %), Bowmanella (90.1–90.3 %) and Agarivorans (88.5–89.9 %). Phylogenetic analyses demonstrated that strain YM01T formed a distinct clade closely related to species of the family Alteromonadaceae within the group of Alteromonas-like gammaproteobacteria. It contained menaquinone MK-7 as the predominant isoprenoid quinone and C\(_{16:0}\) (38.3 %), C\(_{16:1\alpha7c}\) and/or iso-C\(_{15:0}\) 2-OH (29.0 %), C\(_{18:1\alpha7c}\) (9.3 %) and C\(_{10:0}\) 3-OH (8.2 %) as major cellular fatty acids. Phosphatidylethanolamine, phosphatidylglycerol and an aminophospholipid were the major phospholipid constituents. The DNA G+C content was 44.8 mol%. Based on its phenotypic, chemotaxonomic and phylogenetic distinctiveness, strain YM01T is considered to represent a novel species in a new genus in the Gammaproteobacteria, for which the name Catenovulum agarivorans gen. nov., sp. nov. is proposed; the type strain of Catenovulum agarivorans is YM01T (=CGMCC 1.10245\(^T\) = DSM 23111\(^T\) = JCM 16580\(^T\)).

The taxonomic history of the Alteromonas-like bacteria is a reflection of changes in bacterial systematics due to the rapid development of phylogenetic analysis and molecular techniques (Ivanova et al., 2004). Since the initial creation of the genus Alteromonas, which contains Gram-negative, aerobic, heterotrophic marine bacteria with one polar flagellum (Baumann et al., 1972), several considerable revisions have been carried out: in 1995, a new genus Pseudoalteromonas was formed, with the revised genus Alteromonas containing only one species, Alteromonas macroleoidii (Gauthier et al., 1995); in 2001, on the basis of their phenotypic, genotypic and phylogenetic characteristics, Ivanova & Mikhailov (2001) combined the genera Alteromonas, Pseudoalteromonas, Idiomarina and Colwellia into a new family Alteromonadaceae, with the type genus Alteromonas; and the phylogenetic relationships among marine Alteromonas-like bacteria were further studied by Ivanova et al. (2004) and several novel families, such as Pseudoalteromonadaceae, Colwelliaceae, Shewanellaceae, Moritellaceae, Ferrimonadaceae, Idiomarinae and Psychromonadaceae, were proposed. In recent years, members of a wide variety of genera, including Agarivorans, Aliagarivorans, Bowmanella, Oceanimonas, Oceanisphaera, Zobellella, Saccharophagus, Halicea and Melitea, have been classified as showing Alteromonas-like phenotypic properties (Kurahashi & Yokota, 2004; Ekborg et al., 2005; Jean et al., 2006, 2009; Urios et al., 2008a, b).

Alteromonas-like bacteria belonging to the class Gammaproteobacteria are a large group of marine, heterotrophic, Gram-negative rods that are mainly polarly flagellated and
occur as single cells. The major isoprenoid quinone of these bacteria is ubiquinone Q-8 (Ivanova & Mikhailov, 2001; Ivanova et al., 2004). In this study, a novel peritrichously flagellated bacterial strain (designated YM01\(^T\)), which formed long chains of up to 20–60 cells and contained menaquinone MK-7 as the only isoprenoid quinone, was isolated and its taxonomic status was determined using a polyphasic approach.

Seawater samples collected from the coastal region of Qingdao, PR China, in October 2008, were diluted with sterilized seawater and spread onto plates that contained nitrobacteria specialized agar (NBS; 1.0 g NaNO\(_3\), 0.01 g MnSO\(_4\).4H\(_2\)O, 0.75 g K\(_2\)HPO\(_4\), 0.05 g MgSO\(_4\).7H\(_2\)O, 1.0 g Na\(_2\)CO\(_3\), 0.25 g NaH\(_2\)PO\(_4\), 20 g agar, 1000 ml seawater, pH 7.5). An agar-hydrolysing colony (designated YM01\(^T\)) was isolated after incubation at 28 °C for 1 week and was subsequently purified three times on marine agar 2216 (MA; Difco). Working cultures were maintained on MA at 28 °C and preserved at −80 °C in sterilized saline [0.9% (w/v) NaCl] supplemented with 15% (v/v) glycerol.

Standard protocols (Tindall et al., 2007) were used to perform Gram, endospore and flagellum staining and to assess catalase and oxidase activities, degradation of casein, starch, agar, cellulose, gelatin, Tween 80, urea and egg yolk, nitrate reduction, H\(_2\)S production from thiosulfate and indole production from l-tryptophan. The presence of flagella was determined using transmission electron microscopy (TEM) after cells had been washed twice with 50 mM phosphate buffer (pH 7.5) and negatively stained with 1% (w/v) phosphotungstic acid (Tindall et al., 2007). Colonies of YM01\(^T\) incubated on peptone-yeast extract-glucose (PYG) agar at 16 °C for 15 days were used to determine the presence of prosthecae and buds via TEM (Van Trappen et al., 2004a). The polyhydroxybutyrate (PHB) used Nile blue A as a fluorescent stain and was carried out under a fluorescence microscope (BH2; Olympus) (Tindall et al., 2007). The growth capability of YM01\(^T\) on different media was investigated by inoculating freshly activated cultures on Luria–Bertani (LB) agar (Shivaji et al., 2005), R2A seawater agar (Yi et al., 2004), nutrient agar (NA) and PYG agar (Van Trappen et al., 2004a) at 28 °C for 15 days. Growth under anaerobic conditions was determined after incubation in an anaerobic chamber using MA and MA supplemented with 0.25% (w/v) NaNO\(_3\) (both of which had been prepared anaerobically) for 15 days at 28 °C. The temperature range for growth was determined on MA plates incubated at 10–45 °C for 7 days and at 0 and 4 °C for 30 days. Salt tolerance was tested in synthetic ZoBell medium (ZoBell, 1941; 15 g Bacto agar, 5 g Bacto peptone, 1 g yeast extract, 0.1 g ferric citrate in 1000 ml distilled water) containing appropriate concentrations of NaCl [0–15% (w/v), at intervals of 0.5%]. To confirm the absolute requirement for Na\(^+\) ions for growth of YM01\(^T\), 2% NaCl was replaced by an equimolar amount of either KCl or NaNO\(_3\). The pH range for growth was determined by assessing growth at pH 3.5–13.0 (at intervals of 0.5 pH unit) using citrate/phosphate, Tris/HCl, Na\(_2\)CO\(_3\)/NaOH or KCl/NaOH buffers (Breznak & Costilow, 1994) in synthetic ZoBell medium supplemented with 2% NaCl. Susceptibility to antibiotics was investigated on MA plates by the agar diffusion method using filter discs containing different antibiotics. Oxidation or fermentation of carbohydrates (D-glucose, melibiose, sucrose, amygdalin, D-mannitol and inositol) was examined by using modified oxidation-fermentation medium (Leifson, 1963). Activities of constitutive enzymes and other physiological properties were determined by using API 20E, API 20NE and API ZYM strips (bioMérieux) and Gram-negative MicroPlates (Biolog) according to the manufacturers’ instructions except that sterile seawater was used to prepare the inocula. Glaciecola punicea DSM 14233\(^T\), obtained from the Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSMZ), Braunschweig, Germany, was used as a reference strain.

For analysis of fatty acid methyl esters, respiratory quinones and polar lipids, cell mass of strain YM01\(^T\) was harvested from marine broth 2216 (MB; Difco) containing 4% agar (added as a lump in the bottom of the broth) after incubation at 28 °C for 48 h. Fatty acid methyl esters were extracted and analysed according to the standard protocol described in the MIDI (Microbial Identification) system (Sasser, 1990). Analyses of respiratory quinones and polar lipids were carried out by the Identification Service and B. Tindall of the DSMZ. The DNA G+C content was determined by the method of Mesbah & Whitman (1989) using reversed-phase HPLC.

Cells grown on MA plates at 28 °C for 48 h were washed out with sterilized 0.9% (w/v) saline and harvested by centrifugation. Extraction and purification of total genomic DNA from the cells and PCR amplification of the 16S rRNA gene were performed according to the methods described by Jin et al. (2010). The 16S rRNA gene was amplified by PCR using two universal primers: B8F, 5'-AGAGTTTGATCCTGGCTCAG-3' and B1510, 5'-GGTTACCTTGTTACGACTT-3'. The PCR product was purified using the TaKaRa agarose gel DNA purification kit and was sequenced in both directions with primers B8F and B1510 by Shanghai Biosune (China) with an Applied Biosystems automatic sequencer (ABI 3730). The nearly complete 16S rRNA gene sequence (1380 nt) of strain YM01\(^T\) was submitted to GenBank and EMBL to search for similar sequences using the BLAST algorithm. The identification of phylogenetic neighbours and the calculation of pairwise 16S rRNA gene sequence similarities were achieved using the EzTaxon server (http://www.eztaxon.org/; Chun et al., 2007). Sequences were aligned using CLUSTAL X 1.8 (Thompson et al., 1997) and edited manually using the BioEdit Sequence Alignment Editor version 5.0.9 (Hall, 1999). Phylogenetic trees were constructed using the neighbour-joining (NJ), maximum-parsimony (MP) and maximum-likelihood (ML) methods with Kimura 2-state parameter model analyses implemented in the program MEGA version 5 (Tamura et al., 2007).
Characteristics that distinguish strain YM01<sup>T</sup> from members of the genera *Glaciecola*, *Alteromonas*, *Aestuariibacter*, *Salinimonas*, *Bowmanella* and *Agarivorans*

Taxa: 1, strain YM01<sup>T</sup> (data from this study); 2, *Glaciecola* (*G. punicea* DSM 14233<sup>T</sup> (data in parentheses are from this study; other data from Bowman et al., 1998), *G. pallidula* ACAM 615<sup>T</sup> (Bowman et al., 1998), *G. mesophila* KMM 241<sup>T</sup> (Romenenko et al., 2003), *G. agarlytica* NO2<sup>T</sup> (Yong et al., 2007), *G. chathamensis* S18K6<sup>T</sup> (Matsuyama et al., 2006), *G. nitratireducens* FR1064<sup>T</sup> (Baik et al., 2006), *G. polaris* LMG 21857<sup>T</sup> (Van Trappen et al., 2004b), *G. psychrophila* 170<sup>T</sup> (Zhang et al., 2006)); 3, *Alteromonas* (*A. macleodii* DSM 6062<sup>T</sup> (Baumann et al., 1972; Gauthier et al., 1995; Yoon et al., 2003), *A. tagae* AT1<sup>T</sup> and *A. similis* AS1<sup>T</sup> (Chiu et al., 2007), *A. marina* KCCM 41638<sup>T</sup> (Yoon et al., 2003), *A. addita* R105W13<sup>T</sup> (Ivanova et al., 2005), *A. hispanica* F-32<sup>T</sup> (Martinez-Checa et al., 2005), *A. litorea* TF-22<sup>T</sup> (Yoon et al., 2004), *A. stellipolaris* LMG 21861<sup>T</sup> (Van Trappen et al., 2004a)); 4, *Aestuariibacter* (*A. salexigens* JC2042<sup>T</sup> and *A. halophilus* JC2043<sup>T</sup> (Yi et al., 2004), *A. aggregatus* WH169<sup>T</sup> (Wang et al., 2010)); 5, *Salinimonas chungwhensis* BH030046<sup>T</sup> (Jeon et al., 2005); 6, *Bowmanella* (*B. denitrificans* BD1<sup>T</sup> (Jen et al., 2006), *B. pacifica* W3-3A<sup>T</sup> (Lai et al., 2009)); 7, *Agarivorans albus* MKT 106<sup>T</sup> (Kurahashi & Yokota, 2004); 8, *Aligcola* (*A. barioliformis* ATCC 700679<sup>T</sup> (Sawabe et al., 1998), *A. sagamiensis* B-10-31<sup>T</sup> (Kobayashi et al., 2003)). +, Positive; -, negative; w, weakly positive; v, variable; ND, no data available. Results separated by a solidus (/) indicate that the first result is more widespread in the genus than the second; results separated by the word 'or' indicate that the two results are spread equally within the genus.

**Table 1.** Characteristics that distinguish strain YM01<sup>T</sup> from members of the genera *Glaciecola*, *Alteromonas*, *Aestuariibacter*, *Salinimonas*, *Bowmanella* and *Agarivorans*

<table>
<thead>
<tr>
<th>Feature</th>
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<tbody>
<tr>
<td><strong>Pigmentation</strong></td>
<td>Pale yellow</td>
<td>None, pale pink (pink–red)</td>
<td>None, none, cream, brown</td>
<td>None, pale yellow</td>
<td>Creamy</td>
<td>None, off-white</td>
<td>White</td>
<td>Red, yellow</td>
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<tr>
<td><strong>Chain formation</strong></td>
<td>Long chains</td>
<td>Polar</td>
<td>Polar</td>
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<tr>
<td><strong>Flagellar arrangement</strong></td>
<td>Peritrichous</td>
<td>Polar/ND</td>
<td>Polar</td>
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<td><strong>Buds/prosthecae</strong></td>
<td>–</td>
<td>ND/ND</td>
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<td><strong>Growth temperature (°C)</strong></td>
<td>10–42</td>
<td>–2 to 35 (0–25)</td>
<td>4–44</td>
<td>15–45</td>
<td>10–45</td>
<td>10–42</td>
<td>Mesophilic</td>
<td>15–35</td>
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<td><strong>Growth at:</strong></td>
<td>4 °C</td>
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<td><strong>NaCl range for growth (% w/v)</strong></td>
<td>0.5–3.0</td>
<td>1–10/ND</td>
<td>0.5–15,0</td>
<td>0.5–10.0</td>
<td>2–15</td>
<td>0–10</td>
<td>ND</td>
<td>≤6</td>
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<td><strong>Hydrolysis of:</strong></td>
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<td><strong>Casein</strong></td>
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<td><strong>Agar</strong></td>
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<td><strong>Aesculin</strong></td>
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<td><strong>Starch</strong></td>
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<td><strong>TWEEN 80</strong></td>
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<td><strong>Cellulose</strong></td>
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<td><strong>Reduction of nitrate to nitrite</strong></td>
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<td><strong>Utilization of:</strong></td>
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<td><strong>Citrate</strong></td>
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<td><strong>Cellobose</strong></td>
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<td><strong>α-D-Glucose</strong></td>
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<td><strong>Lactose</strong></td>
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<td><strong>Sucrose</strong></td>
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<td><strong>D-Mannose</strong></td>
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<td><strong>D-Galactose</strong></td>
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<td><strong>Maltose</strong></td>
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of peritrichous flagella are significant in distinguishing strain YM01\textsuperscript{T} from members of the genera Glaciecola, Alteromonas, Aestuariibacter, Salinimonas, Bowmanella and Agarivorans, which all occur as single cells with a polar flagellum (Table 1; Ivanova et al., 2004). The significant phenotypic differences between YM01\textsuperscript{T} and these Alteromonas-like bacteria indicated that this isolate may represent a novel taxon of the Alteromonas-like gammaproteobacteria.

Strain YM01\textsuperscript{T}, like species belonging to the Alteromonadaeae, contained C\textsubscript{16:0} (38.3 %), C\textsubscript{16:1\textit{ω}7c} and/or iso-C\textsubscript{15:0} 2-\textit{OH} (29.0 %) and C\textsubscript{18:1\textit{ω}7c} (9.3 %) as major fatty acids (Ivanova et al., 2004; Jean et al., 2009). Other cellular fatty acids present at levels greater than 3 % included C\textsubscript{10:0} 3-\textit{OH} (8.2 %), C\textsubscript{18:0} (4.5 %) and C\textsubscript{12:0} (3.7 %). The amount of C\textsubscript{10:0} 3-\textit{OH} in strain YM01\textsuperscript{T} was significantly higher than that observed in members of the genera Glaciecola, Alteromonas, Aestuariibacter, Salinimonas, Bowmanella and Agarivorans. Other quantitative differences in the fatty acids that might serve to differentiate the novel isolate from these relevant Alteromonas-like species are listed in Supplementary Table S1.

The isolate contained menaquinone MK-7 (100 %) as the only isoprenoid quinone, which was an obvious characteristic that distinguished YM01\textsuperscript{T} from previously described species in the Alteromonas-like proteobacteria. The predominant respiratory quinone in species of the genera Glaciecola, Alteromonas, Aestuariibacter, Salinimonas, Bowmanella and Agarivorans was ubiquinone Q-8 (Gauthier et al., 1995; Ivanova & Mikhailov, 2001). Several other ubiquinones have also been detected in relatively small amounts in Alteromonas simiduii (4.3 % Q-4 and Q-6), Alteromonas hispanica (3.5 % Q-7) and Bowmanella denitrificans (11.1 % Q-9 and 7.4 % Q-10) (Chiu et al., 2007; Martinez-Checa et al., 2005; Jean et al., 2006), but MK-7 has never been detected in Alteromonas-like bacteria. Phosphatidylethanolamine (PE) and phosphatidylglycerol (PG) were the major phospholipid constituents in YM01\textsuperscript{T},

### Table 1. cont.

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<th>Feature</th>
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<tr>
<td><strong>Major fatty acids</strong></td>
<td>C\textsubscript{16:0}, C\textsubscript{16:1\textit{ω}7c/iso-C\textsubscript{15:0} 2-\textit{OH}, C\textsubscript{16:0}, C\textsubscript{16:1\textit{ω}7c}</td>
<td>C\textsubscript{16:0}, C\textsubscript{16:1\textit{ω}7c}</td>
<td>C\textsubscript{16:0}, C\textsubscript{16:1\textit{ω}7c}</td>
<td>C\textsubscript{16:0}, C\textsubscript{16:1\textit{ω}7c}</td>
<td>C\textsubscript{16:0}, C\textsubscript{16:1\textit{ω}7c}</td>
<td>C\textsubscript{16:0}, C\textsubscript{16:1\textit{ω}7c}</td>
<td>C\textsubscript{16:0}, C\textsubscript{16:1\textit{ω}7c}</td>
<td>C\textsubscript{16:0}, C\textsubscript{16:1\textit{ω}7c}</td>
</tr>
<tr>
<td><strong>Predominant phospholipids</strong></td>
<td>PE, PG, PN</td>
<td>ND/PE, PG</td>
<td>ND/PE, PG</td>
<td>ND/PE, PG</td>
<td>ND</td>
<td>PG, PE, DPG, PG, PE, UA</td>
<td>ND</td>
<td>ND</td>
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<tr>
<td><strong>Isoprenoid quinone</strong></td>
<td>MK-7</td>
<td>ND</td>
<td>Q-8</td>
<td>Q-8</td>
<td>Q-8</td>
<td>Q-8</td>
<td>Q-8</td>
<td>ND or Q-8</td>
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<tr>
<td><strong>DNA G+C content (mol%)</strong></td>
<td>44.8</td>
<td>40–46</td>
<td>43.0–46.3</td>
<td>48–54</td>
<td>48</td>
<td>49–50</td>
<td>48–50</td>
<td>42.0–46.0</td>
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*DPG, Diphosphatidylglycerol; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PN, unidentified aminophospholipid; UA, unidentified aminolipid.

Morphological, physiological and biochemical characteristics of strain YM01\textsuperscript{T} are given in the genus and species descriptions or shown in Table 1. Supplementary Fig. S1, available in IJSEM Online, shows the optical flagella stains of strain YM01\textsuperscript{T}. For most phenotypic characteristics, strain YM01\textsuperscript{T} had properties that were typical of species of the Alteromonadaeae-like gammaproteobacteria (Ivanova et al., 2004), e.g. Gram-negative, rod-shaped, non-spore-forming cells, chemo-organotrophic, strictly aerobic metabolism, positive reactions for oxidase and catalase activity and negative reactions for H\textsubscript{2}S and indole production. The isolate possessed an absolute requirement for NaCl and failed to grow when NaCl was replaced by KCl. Seawater salts were not absolutely required for growth when the medium was supplemented with 0.5–3.0 % NaCl. However, there were also many peculiar phenotypic traits that distinguished YM01\textsuperscript{T} from other Alteromonas-like bacteria. Strain YM01\textsuperscript{T} was able to hydrolyse agar during growth on MA. Three other agar-degrading species (Glaciecola agarilytica, Glaciecola mesophila and Agarivorans albus) belonging to the Alteromonadaeae-like gammaproteobacteria have been described (Romanenko et al., 2003; Kurahashi & Yokota, 2004; Yong et al., 2004), and a few isolates reported by Baumann et al. (1972). Cells of strain YM01\textsuperscript{T} were peritrichously flagellated (Fig. 1). Long chains of 20–60 cells were formed when YM01\textsuperscript{T} was cultivated on MA at 28 °C for less than 20 h, and the chains subsequently split into single cells (see Supplementary Fig. S1). The characteristic formation of long chains and the presence of peritrichous flagella are significant in distinguishing strain YM01\textsuperscript{T} from members of the genera Glaciecola, Alteromonas, Aestuariibacter, Salinimonas, Bowmanella and Agarivorans, which all occur as single cells with a polar flagellum (Table 1; Ivanova et al., 2004). The significant phenotypic differences between YM01\textsuperscript{T} and these Alteromonas-like bacteria indicated that this isolate may represent a novel taxon of the Alteromonas-like gammaproteobacteria.
followed by a small amount of an aminophospholipid (PN) and two unknown phospholipids (PL1, PL2). PE and PG have also been reported to be the major polar lipid constituents in some Alteromonas-like proteobacteria, such as Alteromonas addita, Alteromonas macroleii, B. denitrificans, G. agarilytica, G. mesophila and Glaciecola lipolytica (Ivanova et al., 2000, 2005; Romanenko et al., 2003; Jean et al., 2006; Yong et al., 2007; Chen et al., 2009). However, PN has not been detected in these Alteromonas-like bacteria. The DNA G+C content of strain YM01T was 44.8 mol%, which falls within the range for members of the genera Alteromonas (44–48 mol%; Ivanova et al., 2004) and Glaciecola (40–46 mol%; Bowman et al., 1998), but was lower than those of Aestuariibacter (48–54 mol%; Yi et al., 2004), Salinimonas (48.0 mol%; Jeon et al., 2005), Bowmanella (49–50 mol%; Jean et al., 2006) and Agarivorans (48–50 mol%; Kurahashi & Yokota, 2004).

The almost-complete 16S rRNA gene sequence of strain YM01T was determined (1380 nt). Pairwise analysis revealed that strain YM01T exhibited highest 16S rRNA gene sequence similarity with members of the genera Glaciecola (88.4–91.0%), Alteromonas (88.7–89.6%), Aestuariibacter (89.3–90.4%), Salinimonas (89.0%), Bowmanella (90.1–90.3%) and Agarivorans (88.5–89.9%), with slightly lower similarities to members of the families Pseudoalteromonadaceae (89.2–90.9%) and Colwelliaceae (87.82–90.6%). Phylogenetic analysis based on 16S rRNA gene sequences showed that strain YM01T was a member of the Alteromonas-like bacteria in the class Gammaproteobacteria. The NJ tree, showing phylogenetic relationships between strain YM01T and selected Alteromonas-like bacteria, is presented in Fig. 2. Strain YM01T formed a distinct lineage (deeply branched clade with a bootstrap value of 68% in the NJ tree) within the clade of Alteromonas, Glaciecola, Aestuariibacter, Salinimonas and Bowmanella, which separated it from the clade composed of the genera Agarivorans, Aligcola and Pseudoalteromonas. The branch pattern of these clades was identical to those seen in phylogenetic trees obtained by the MP and ML algorithms (not shown). The major branches revealed by the different tree-making methods are indicated by asterisks. The low level of sequence similarity with other bacteria indicated that strain YM01T could be assigned to a novel genus. Phylogenetically, the strain could be considered to represent a novel family, since it was clearly an outgroup with respect to species of the family Alteromonadaceae and other Alteromonas-like bacteria. Moreover, the 16S rRNA gene sequences of species of the Alteromonadaceae have been defined as possessing signature nucleotides 304(A), 734(A), 736(T), 770(T) and 809(A) (Ivanova et al., 2004), whereas strain YM01T has nucleotides T, G, C, C and G, respectively, at these sequence positions.

So far, there are no formal rules for the creation of a taxon above the species level. A genus is generally defined as a group of organisms that present distinct shared phenotypes, forming a robust phylogenetic branch (Ivanova et al., 2004). The conclusion drawn from phylogenetic analysis that strain YM01T represents a novel genus or even a novel family of the Alteromonas-like gammaproteobacteria was supported by morphological and physiological characteristics that distinguished it from other Alteromonas-like...
bacteria: (i) the presence of peritrichous flagella and long chains; (ii) marked loss of viability within 5 days; (iii) C\textsubscript{10}:0 3-OH (8.2\%) as one of the major fatty acids; (iv) MK-7 as the predominant isoprenoid quinone; and (v) different signature nucleotides from members of the family Alteromonadaceae. Other characteristics that differentiate strain YM01\textsuperscript{T} from members of related genera are shown in Table 1. By combining phenotypic, phylogenetic and genetic data, strain YM01\textsuperscript{T} should be classified as a representative of a novel species in a new genus in the Gammaproteobacteria, for which the name *Catenovulum agarivorans* gen. nov., sp. nov. is proposed.

**Description of *Catenovulum* gen. nov.**

*Catenovulum* [Ca.te.no’vu.lum. L. fem. n. catena chain; N.L. dim. neut. n. ovulum (from L. n. ovum an egg) a small egg; N.L. neut. n. *Catenovulum* chain egg].

Gram-negative, strictly aerobic and chemo-organotrophic. Cells with peritrichous flagella are rod-shaped and form long chains end-to-end. PHB accumulation is not observed. Possess an absolute requirement for Na\textsuperscript{+} ions and fail to grow when Na\textsuperscript{+} ions are replaced with K\textsuperscript{+} ions. Seawater ions are required for growth. Oxidase- and catalase-positive. For Voges–Proskauer test and H\textsubscript{2}S and indole production. Major isoprenoid quinone is MK-7. PE and PG are the major constituents of the phospholipids, with small amounts of PN and two unknown phospholipids. Predominant cellular fatty acids are C\textsubscript{16}:0, C\textsubscript{15}:1\text{\textit{o}7c} and/or iso-C\textsubscript{15}:0 2-OH, C\textsubscript{18}:1\text{\textit{o}7c} and C\textsubscript{10}:0 3-OH. Phylogenetically, the genus belongs to the Gammaproteobacteria. The type species is *Catenovulum agarivorans*.

**Description of *Catenovulum agarivorans* sp. nov.**

*Catenovulum agarivorans* (a.ga.ri.vo’rans. N.L. neut. n. *agarum* agar; L. part. adj. *vorans* devouring, destroying; N.L. part. adj. *agarivorans* agar-devouring). In addition to the characteristics that define the genus, the following are observed. Forms colonies with smooth surfaces that are thin, circular in shape and pale yellow in colour when grown on MA for 3 days at 28°C. Cells form long chains end-to-end that split into single cells after 24 h of cultivation. Marked viability loss occurs within 5 days on MA at 28°C accompanied by fading of the pale yellow colour of the colonies, indicating that cells need to be transferred every 3 days. Cells are approximately 0.8–1.5 \(\mu\text{m}\) long and 0.4–0.6 \(\mu\text{m}\) wide. Spores are not formed. Buds and prosthecae are not observed when the isolate is grown at 16°C for 5 days on PYG agar. Abundant growth occurs on MA and marine agar R2A, but not on MB, LB agar or NA. Growth occurs in 0.5–3.0 % (w/v) NaCl and occurs on MA and marine agar ATCC 11170\textsuperscript{T} (D30778) and *Rhodospirillum sulfur-exigens* JA143\textsuperscript{T} (AM710622) were used as the outgroup. Bar, 2 substitutions per 100 nucleotide positions.

![Fig. 2. NJ dendrogram based on 16S rRNA gene sequences showing the phylogenetic relationship between strain YM01\textsuperscript{T} and species of the Alteromonadaceae and other families and genera of Alteromonas-like gammaproteobacteria. GenBank accession numbers are given in parentheses. Bootstrap values (percentages of 1000 replicates) greater than 50\% are shown at branch points. Asterisks indicate that the corresponding generic branches were also recovered in MP and ML trees. In each case, bootstrap values were calculated based on 1000 replicates. *Marispirillum indicum* B142\textsuperscript{T} (EU642410), *Rhodospirillum rubrum* ATCC 11170\textsuperscript{T} (D30778) and *Rhodospirillum sulfur-e-xigens* JA143\textsuperscript{T} (AM710622) were used as the outgroup. Bar, 2 substitutions per 100 nucleotide positions.](http://ijs.sgmjournals.org)
β-glucosidase; negative for lysine decarboxylase, ornithine decarboxylase, urease, lipase (C14), leucine aminopeptidase, valine aminopeptidase, cystine aminopeptidase, trypsin, α-chymotrypsin, β-glucuronidase, N-acetyl-β-glucosaminidase, z-mannosidase and z-fucosidase (API ZYM and API 20NE).

Utilizes the following substrates as carbon and energy sources: α-cyclodextrin, dextrin, Tween 40 and 80, N-acetyl-D-glucosamine, cellulose, D-fructose, D-galactose, gentiobiose, α-D-glucose, maltose, D-mannose, melibiose, raffinose, sucrose, acetic acid, D-galacturonic acid, D-glucuronic acid and capric acid. The following substrates are not utilized as carbon and energy sources: L-arabinose, D-mannitol, potassium gluconate, adipic acid, malic acid, citrate, glycolc, N-acetyl-D-galactosamine, adonitol, D-arabitol, L-erythritol, L-fucose, myo-inositol, lactose, lactulose, methyl β-D-glucoside, D-psicose, L-rhamnose, D-sorbitol, trehalose, turanose, xyitol, methyl pyruvate, monomethyl succinate, cis-aconitic acid, citric acid, formic acid, D-galactonic acid lactone, D-gluconic acid, D-glucosaminic acid, x- and β- and γ-hydroxybutyric acids, p-hydroxyphenylacetic acid, itaconic acid, α-ketobutyric acid, α-ketogluconic acid, α-ketovaleric acid, D-lactic acid, alonic acid, propionic acid, quinic acid, D-saccharic acid, sebacic acid, succinic acid, bromosuccinic acid, succinic acid, glucuronamide, L-alaninamide, D- and L-alanine, L-alanyl glycine, L-asparagine, L-aspartic acid, L-glutamic acid, glycyl L-aspartic acid, glycyl L-glutamic acid, L-histidine, hydroxy-L-proline, L-leucine, L-ornithine, L-phenylalanine, L-proline, L-pyrroglyutaric acid, D- and L-serine, L-threonine, D,L-carnitine, γ-aminoxybutyric acid, urocanic acid, inosine, uridine, thymidine, phenylethylamine, putrescine, 2-aminoethanol, 2,3-butanediol, glycerol, DL-2-glycerol phosphate, glucose 1-phosphate and glucose 6-phosphate (Biolog). Susceptible to norfloraxcin (10 μg), chloramphenicol (30 μg), neomycin (30 μg), polymyxin B (300 U), ciprofloxacin (5 μg), gentamicin (10 μg), kanamycin (30 μg), ampicillin (10 μg), erythromycin (15 μg), vancomycin (30 μg), piperacillin (100 μg), amikacin (30 μg), rocepin (30 μg) and fortum (30 μg) and intermediate susceptible to cefazolin V (30 μg). Resistant to furaxone (300 μg), tetracycline (30 μg), carbencillin (100 μg), oxacillin (1 μg), doxycyclin (30 μg), minocin (30 μg) and penicillin (10 μg). Distinctive traits are given in Table 1.

The type strain is YM01<sup>T</sup> (=CGMCC 1,10245<sup>T</sup> =DSM 23111<sup>T</sup> =JCM 16580<sup>T</sup>), isolated from seawater of the Yellow Sea in China. The DNA G+C content of the type strain is 44.8 mol%.

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References


