**Agarivorans gilvus** sp. nov. isolated from seaweed

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A novel agarase-producing, non-endospore-forming marine bacterium, WH0801T, was isolated from a fresh seaweed sample collected from the coast of Weihai, China. Preliminary characterization based on 16S rRNA gene sequence analysis showed that WH0801T shared 96.1 % similarity with *Agarivorans albus* MKT 106T, the type species of the genus *Agarivorans*. A polyphasic taxonomic study was conducted and confirmed the phylogenetic affiliation of strain WH0801T to the genus *Agarivorans*. Isolate WH0801T produces light-yellow-pigmented colonies; cells are Gram-stain-negative, straight or curved rods, which are motile with a single polar flagellum. Strain WH0801T grew in 0.5–5 % NaCl, with optimum growth at 3 % NaCl, and its optimal pH and cultivation temperature were 8.4–8.6 and 28–32 °C, respectively. Data from biochemical tests, whole-cell fatty acid profiling, 16S rRNA gene sequence studies and DNA–DNA hybridization clearly indicated that isolate WH0801T represented a novel species within the genus *Agarivorans*, for which the name *Agarivorans gilvus* sp. nov. is proposed. The type strain of *Agarivorans gilvus* sp. nov. is WH0801T (≡NRRL B-59247T =CGMCC 1.10131T).

The genus *Agarivorans* was created by Kurahashi & Yokota (2004) to accommodate Gram-negative, strictly aerobic and agar-hydrolysing species. The genus is affiliated with the class *Gammaproteobacteria* and presently contains only the type species, *Agarivorans albus*. The *Agarivorans albus* strains studied by Kurahashi & Yokota were agarolytic and isolated from healthy marine organisms collected from the coast of the Kanto area in Japan. Many novel agar-degrading bacterial species have been isolated from marine environments, where they occur as part of the indigenous flora. For example, *Simiduia agarivorans*, *Aliagarivorans marinus*, *Aliagarivorans taiwanensis* and *Tamlana agarivorans* were isolated from seawater samples (Shieh et al., 2008; Jean et al., 2009; Yoon et al., 2008); *Psychromonas agarivorans*, *Microbulbifer agarilyticus* and *Marinimicrobium agarilyticum* were found in marine sediment samples (Hosoya et al., 2009; Miyazaki et al., 2008; Lim et al., 2006); and *Salegentibacter agarivorans* was recovered from a species of sponge (Nedashkovskaya et al., 2006). The widespread occurrence of agar-degrading bacteria is probably best explained by their likely role in the carbon cycle involving the breakdown of agar and other sulfated galactans (Armisen & Galactas, 1987; Pomin, 2010), which form a significant component of the cell walls of red and green algae, the egg jelly coating of certain sea urchin species, and the outer tunics of ascidians (Pomin, 2010). We examined the diversity of agarolytic marine bacteria isolated from various marine samples such as seawater, sediment, seaweed and some marine animal samples that were taken from waters off various locations across coastal China. As a result of these efforts, we have already described one new genus, *Gilvimarinus* (Du et al., 2009). Here, we report the taxonomic characteristics of a novel species of the genus *Agarivorans* that was isolated from seaweed sampled off the coast of Weihai, China.

Strain WH0801T was isolated from the surface of seaweed collected from the shallow coastal region of Weihai, China. The seaweed samples were washed several times with sterile seawater and subsequently put into a centrifuge tube with sterile seawater and shaken vigorously. Aliquots (0.1 ml each) of the dilution were spread onto marine agar 2216 (MA; BD). Plates were then incubated at 25 °C for 3 to 7 days. Single colonies that formed pits or craters were picked and were checked for purity by repeated streaking on the same medium. One of the colonies exhibiting agarolytic activities was chosen as a representative strain for further study and was given the designation WH0801T. Cultures were maintained on MA slants at room temperature, and stock cultures were kept in tryptone soya broth (Oxoid) supplemented with 1 % (w/v) NaCl (TNB) and 20 % (v/v) glycerol at −70 °C.
Phenotypic features of the isolate (i.e. colony morphology, pigment production and agar hydrolysis) were determined by cultivating the isolate on MA at 25 °C. Motility was assessed in a semi-solid medium prepared according to Barrow & Feltham (2004). The tube was incubated at 25 °C for 5 days. Flagellation was observed with a light microscope after staining with Ruy staining solution (West et al., 1977). Growth with 0, 0.5, 1, 3, 5, 7 and 9 % (w/v) NaCl was assessed on appropriately modified plate count agar (PCA; Oxoid). Inoculated plates were incubated at 25 °C for up to 5 days. The effects of different temperatures on growth were assessed on tryptone soya agar (TSA; Oxoid) plates supplemented with 2.0 % (w/v) NaCl and incubated at 4, 10, 15, 28, 30, 37, 42 and 50 °C. The pH range for growth was determined for the culture in marine broth 2216 at various pH, adjusted with HCl or NaOH (1 M).

Catalase and oxidase activity were examined by standard methods as described by Lányi (1987). The API 20E, API 20NE and API 50CH tests (bioMérieux) were used according to the manufacturer’s instructions, except that the inoculum was prepared by suspending cells in a 2 % (w/v) NaCl solution. The API 50 CH strips were read after 7 days of incubation at 25 °C. Enzyme activities were examined by using the API ZYM test (bioMérieux). All API tests were performed in duplicate and Agarivorans albus MKT 106T was used for comparison under the same cultural conditions.

Antibiotic sensitivity was assessed as follows: a cell suspension (~10^7 cells ml⁻¹) was swabbed over the surface of Iso-Sensitest agar (Oxoid) plates supplemented with 3 % (w/v) NaCl to create a uniform lawn before aseptic placement of antibiotic discs onto the agar surface. The inoculated plates were incubated overnight at 28 °C.

DNA was extracted and purified by using a genomic DNA extraction kit (Sangon, China). The gene encoding 16S rRNA was amplified by PCR with two universal primers, 27f and 1492r (Jordan et al., 2007). The 16S rRNA gene sequence of strain WH0801T was submitted to GenBank and similar sequences were searched in public databases using the BLAST algorithm. The phylogenetic placement of strain WH0801T was obtained and used to determine the almost-complete 16S rRNA gene sequence (1455 nt) of strain WH0801T was obtained and used to determine the phylogenetic placement of strain WH0801T (Fig. 1). The 16S rRNA gene sequence comparison showed clearly that strain WH0801T is a member of the genus Agarivorans. The organism showed the highest sequence similarity (99.1 %) with Agarivorans sp. LQ48 (Long et al., 2010). However, the sequence similarity observed between the new isolate and its closest relative, Agarivorans albus, was 96.1 %, which is below the threshold value (97 %) used for defining bacterial species as proposed by Stackebrandt & Goebel (1994). On the other hand, the similarity between strain WH0801T and the type strains of Aliagarivorans marinus and Aliagarivorans taiwanensis were 93.6 % and 93.2 %, respectively. Significantly lower levels of similarity were shown with species in other genera (less than 93 % similarity). The DNA–DNA relatedness value between strain WH0801T and Agarivorans albus MKT 106T was 33.9 ± 0.85 % (experiment repeated twice). Therefore these two strains are not related at the species level, since the

Table 1. Comparison of strain WH0801T with Agarivorans albus MKT 106T

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
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<tbody>
<tr>
<td>Pigment of colony</td>
<td>Light yellow</td>
<td>White</td>
</tr>
<tr>
<td>Gelatin hydrolysis</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Utilization of:</td>
<td></td>
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</tr>
<tr>
<td>Mannitol</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>N-Acetylglucosamine</td>
<td>–</td>
<td>+</td>
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<tr>
<td>Maltose</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Acid from:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sucrose</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Amygdalin</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>l-Arabinose</td>
<td>–</td>
<td>–</td>
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<tr>
<td>Production of:</td>
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<td></td>
</tr>
<tr>
<td>Esterase (C4)</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Esterase lipase (C8)</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>β-Glucosidase</td>
<td>+</td>
<td>–</td>
</tr>
</tbody>
</table>

Strains: 1, strain WH0801T, 2, Agarivorans albus MKT 106T. +, Positive; –, negative. Data from this study.
value falls below the threshold (70%) recommended for species definition by Wayne et al. (1987).

Therefore, on the basis of phylogenetic, chemotaxonomic and other taxonomic data resulting from this study, we found that strain WH0801^T is clearly different from Agarivorans albus MKT 106^T. Thus, the novel species Agarivorans gilvus sp. nov. is proposed with strain WH0801^T as the type strain.

Description of Agarivorans gilvus sp. nov.

Agarivorans gilvus (gil’vus. L. masc. adj. gilvus pale yellow, referring to the pale yellow pigmentation of the bacterium).

Cells are Gram-stain-negative, non-spore-forming and rod-shaped, motile by single polar flagella. Colonies are pale yellow, smooth and 2.5–3.5 mm in diameter after 48 h of incubation at 28 °C on MA. Strictly aerobic and agar-hydrolysing; shallow craters with a clear zone (6–8 mm in diameter) are observed surrounding the colonies due to agar hydrolysis. Grows at pH 6.5–9.5, optimum at pH 8.4–8.6. Cells grow in the presence of 0.5–5 % (w/v) NaCl and at 10–45 °C. Prolific growth occurs at 28–32 °C in media containing 3 % (w/v) NaCl. No growth occurs without NaCl. Cells are oxidase, catalase, ONPG and Voges–Proskauer positive. No citrate utilization, H2S production, arginine dihydrolase or indole production. Aesculin and starch are hydrolysed, but urea and gelatin are not. Nitrate is not reduced to nitrite. Activity is detected for alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, acidic phosphatase, naphthol-AS-BI-phosphohydrolase, β-galactosidase and β-glucosidase. Lipase (C14), cystine arylamidase, trypsin, chymotrypsin, α-galactosidase, β-glucuronidase, α-glucosidase, N-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase are not produced. The following substrates are utilized as sole carbon sources: L-arabinose, D-xylose, D-galactose, D-mannose, arbutin, aesculin ferric citrate, salicin, lactose, starch, glycogen and gentiobiose. The following substrates are not utilized as sole carbon sources: glycerol, erythritol, D-arabinose, D-ribose, L-lyxose, D-adonitol, methyl β-D-xylopyranoside, D-glucose, D-fructose, L-sorbose, L-rhamnose, dulcitol, inositol, D-mannitol, D-sorbitol, methyl α-D-mannopyranoside, methyl α-D-glucopyranoside, N-acetylglucosamine, amygdalin, cellobiose, maltose, melibiose, sucrose, trehalose, inulin, melezitose, raffinose, xylitol, turanose, D-mannitol, D-sorbitol, L-fucose, L-arabinose, D-arabinose, D-glucose, D-fructose, D-mannose, D-mannitol, N-acetylglucosamine, aesculin ferric citrate, salicin, cellobiose, maltose, lactose, sucrose, starch, glycogen and gentiobiose. Resistant to sulfamethoxazole/trimethoprim (23.75/1.25 mg), tetracycline (30 mg), clindamycin (30 µg), carbenicillin (100 µg), acetylsalicylamycin (30 µg), tobramycin (30 µg) and midecamycin (30 µg), but sensitive to nalidixic acid (30 µg), gentamicin (120 µg), chloramphenicol (30 µg) and streptomycin (25 µg). Major whole-cell fatty acids are C16:1^ω7c/iso-C15:0 2-OH, C16:0, C18:1^ω9c, C12:0, C14:0, 3-OH/iso-C16:1^ω7c and C14:0 (Supplementary Table S1 available in IJSEM Online). The genomic DNA G+C content of the type strain is 48.5 mol%.

The type strain is WH0801^T (=NRRL B-59247^T =CGMCC 1.10131^T), isolated from the surface of seaweed collected in the shallow coastal region of Weihai, China.
Acknowledgements

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References


