**Neiella marina** gen. nov., sp. nov., isolated from the sea cucumber *Apostichopus japonicus*

Zong-Jun Du,1,2 Ting-Ting Miao,1 Alejandro P. Rooney,3 Qian-Qian Liu1 and Guan-Jun Chen1,2

1College of Marine Science, Shandong University at Weihai, Weihai 264209, PR China
2State Key Laboratory of Microbial Technology, Shandong University, Jinan 250100, PR China
3National Center for Agricultural Utilization Research, Agricultural Research Service, US Department of Agriculture, Peoria, IL 61604, USA

A novel strain, designated J221T, was isolated from the intestine of a sea cucumber, *Apostichopus japonicus*, collected from earthen ponds in Qingdao, China. The strain was Gram-negative, oxidase-positive, aerobic, rod-shaped and motile by means of one to several polar flagella. Growth of strain J221T was observed at temperatures between 10 and 40 °C with optimum growth between 25 and 28 °C. The pH range for growth was 5.0–9.0 with optimum growth at pH 7.5–8.0. The dominant fatty acids were summed feature 3 (comprising C16:1ω7c and/or C16:1ω6c, 29.04 %), C16:0 (28.93 %) and C18:1ω7c (26.15 %). The major polar lipids were phosphatidylglycerol and phosphatidylethanolamine. Diphosphatidylglycerol, an unknown aminolipid and an unknown aminophospholipid were present in moderate to minor amounts in the polar lipid profile. Strain J221T had Q-8 as the major respiratory quinone. The DNA G+C content of strain J221T was 46.8 mol%. Phylogenetic analysis based on 16S rRNA gene sequences showed that strain J221T is a member of the *Gammaproteobacteria*. It formed a distinct phylogenetic line with less than 91 % sequence similarity to any species within previously recognized genera. On the basis of this polyphasic taxonomic study, strain J221T should be classified as a representative of a novel species of a new genus, for which the name *Neiella marina* gen. nov., sp. nov. is proposed. The type strain of *Neiella marina* is J221T (≡CGMCC 1.10130T=NRRRL B-51319T).

Numerous marine animals live in permanent and close associations with microbes, and many of them host phylogenetically diverse populations of bacteria. These microbes have noticeable beneficial and detrimental impacts on their host (Neulinger et al., 2008). In the last few years, many novel bacteria associated with various marine animals in the Echinodermata have been isolated and described. *Bizonia echini*, *Halomonas zhanjiangensis*, *Jotgalicoccus marinus* and *Pontibacillus halophilus* were isolated from sea urchin (Nedashkovskaya et al., 2007), and *Colwellia asteriadiis* and *Jeotgalicoccus marinus* were identified from starfish (Romanenko et al., 2004, 2010; Choi et al., 2010). Here, we report the taxonomic characteristics of a novel marine bacterium, designated strain J221T, which originated from the intestine of a sea cucumber, *Apostichopus japonicus*. The holothurian samples of the species *Apostichopus japonicus* were collected from earthen ponds in Qingdao, China. The animal’s body wall was cut away to expose the intestine, which was taken aseptically, weighed and homogenized in a mortar. The homogenate (1 ml) was transferred to a tube containing 10 ml of sterile 0.85 % (w/v) NaCl prepared in deionized water. One millilitre of the solution was serially diluted to 10⁻³ v. Volumes (0.1 ml) of the dilutions (10⁻³, 10⁻⁴, 10⁻⁵) were spread on marine agar 2216 (MA; BD Biosciences), in duplicate. Plates were incubated aerobically at 28 °C for 5 days. After recording morphological characteristics and pigmentation, three to five representatives of each colony type were streaked and then re-streaked on fresh media to obtain pure cultures. An aerobic, Gram-negative and rod-shaped bacterial strain (J221T) was isolated and stored at −80 °C in 20 % (v/v) glycerol.

For phenotypic tests, the strain was grown on MA for 48 h at 28 °C and cells were re-suspended in saline for use as an inoculum. Cell morphology was examined under a light microscope (Olympus BX51). Flagellation was examined by tannin staining (Dong & Cai, 2001). Colony morphology

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain J221T is EU513001.

A supplementary figure is available with the online version of this paper.
was observed on MA after incubation at 28 °C for 2 days. Tolerance of 0, 1, 2, 3, 4, 5, 6, 8 and 10% (w/v) NaCl was assessed on appropriately modified plate count agar. Inoculated plates were incubated at 28 °C for up to 5 days. The effects of different temperatures on growth were assessed on MA plates incubated at 4, 10, 15, 28, 30, 37, 40 and 45 °C. The pH range for growth was determined for the culture in marine broth 2216 at various pH values adjusted with HCl or NaOH (1 mol l⁻¹). Oxidase and catalase activities were determined by using standard methods. The API 20E, API 20NE and API 50CH tests (bioMérieux) were used according to the manufacturer’s instructions, except that the inocula were 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. All the API tests were performed in duplicate. General physiological tests were performed using conventional methods (Dong & Cai, 2001). Antibiotic sensitivity was assessed as follows: a cell suspension (~10⁷ cells ml⁻¹) was swabbed over the surface of Iso-Sensitest agar (Oxoid) plates supplemented with 2% (w/v) NaCl to create a uniform lawn before aseptic placement of antibiotic discs (Tianhe) onto the agar surface. The inoculated plates were incubated overnight at 28 °C.

Fatty acids from whole cells grown on MA at 28 °C for 48 h (at the end of the logarithmic phase) were extracted, saponified and esterified; this was followed by GC analysis of the fatty acid methyl esters according to the instructions of the MIDI system (Sasser, 1990). Isoprenoid quinones were determined as described by Komagata & Suzuki (1987), using reversed-phase HPLC. Polar lipid analyses were carried out by the Identification Service of the DSMZ, Braunschweig, Germany.

DNA was extracted and purified by using a bacterial genomic DNA extraction kit (Sangon). 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 J221ᵀ was submitted to GenBank and similar sequences were searched for in public databases using the BLAST algorithm. The phylogenetic position of strain J221ᵀ and several closely related species was determined through an analysis of 16S rRNA gene sequence data; the tree was reconstructed using the
neighbour-joining method (Saitou and Nei, 1987) as implemented in the computer program MEGA version 4.1 (Tamura et al., 2007), and statistical reliability was assessed from 1000 bootstrap pseudoreplicates. The G+C content of DNA isolated from strain J221T was determined by HPLC according to a method described by Tamaoka & Komagata (1984) and Mesbah et al. (1989).

The almost-complete 16S rRNA gene sequence (1501 nt) of strain J221T was obtained and used in the phylogenetic analysis. The resultant tree shows clearly that strain J221T is a member of the Gammaproteobacteria (Fig. 1). The organism showed highest sequence similarity (99.3 %) with gammaproteobacterial strain r61 isolated from the massive coral Montipora sp. collected from Okinawa, Japan. However, 16S rRNA gene sequence similarity between the new isolate and the type strains of recognized species was below 91 %. Levels of similarity between strain J221T and the type strains of Pseudoalteromonas haloplanktis, Shewanella putrefaciens and Colwellia psychrerythraea were 90.2, 89.9 and 89.6 %, respectively. Strain J221T and the type strains of recognized species were 90.2, 89.9 and 89.6 %, respectively. Strain J221T could be distinguished from other related members of the order Alteromonadales on the basis of its ability to hydrolyse agar and its inability to hydrolyse gelatin. Strain J221T was also different from members of the genus Colwellia notably in the inability of the latter to grow at 35 °C. These findings warrant the creation of a new genus to encompass this strain, as the most closely related species that have been validly published are quite distant from it in terms of overall 16S rRNA gene sequence divergence and its subsequent phylogenetic placement among these strains.

The main cellular fatty acids were C16:1ω7c/C16:1ω6c (29.0 %), C16:0 (28.9 %), C18:1ω7c (26.2 %), C18:0 (4.4 %), C12:0 3-OH (3.6 %), C14:0 (1.6 %) and C14:0 3-OH/iso-C16:1 I (1.3 %). Complete morphological and biochemical data for strain J221T are given in the species description. The phenotypic features that differentiate strain J221T from its closest phylogenetic relatives are provided in Table 1.

On the basis of phylogenetic, chemotaxonomic and other taxonomic data from this study, strain J221T is considered to represent a novel species of a new genus within the class Gammaproteobacteria, for which the name Neiella marina gen. nov., sp. nov. is proposed.

**Description of Neiella gen. nov.**

Neiella (Ne.i’el.la. N.L. fem. n. Neiella in honour of Masatoshi Nei for the development of the neighbour-joining method (Saitou and Nei, 1987) as implemented in the computer program MEGA version 4.1 (Tamura et al., 2007), and statistical reliability was assessed from 1000 bootstrap pseudoreplicates. The G+C content of DNA isolated from strain J221T was determined by HPLC according to a method described by Tamaoka & Komagata (1984) and Mesbah et al. (1989).

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### Table 1. Differential characteristics of strain J221T and representatives of closely related genera within the Gammaproteobacteria

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cell size (μm)</strong></td>
<td>0.4–0.6 × 1.4–1.8</td>
<td>0.4–1.0 × 1.5–5.0</td>
<td>0.3–0.75 × 0.7–3.5</td>
<td>0.4–0.8 × 1.0–2.3</td>
<td>0.2–1.5 × 1.8–3.0</td>
<td>0.7–1.0 × 2.0–3.0</td>
</tr>
<tr>
<td><strong>Metabolism</strong></td>
<td>Aerobic</td>
<td>Aerobic facultatively anaerobic</td>
<td>Aerobic</td>
<td>Aerobic</td>
<td>Aerobic</td>
<td>Aerobic</td>
</tr>
<tr>
<td><strong>Catalase</strong></td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>V</td>
<td>V</td>
</tr>
<tr>
<td><strong>Nitrate reduction</strong></td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>ND</td>
<td>−*</td>
</tr>
<tr>
<td><strong>Growth at/with:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>NaCl (%)</strong></td>
<td>1–5</td>
<td>&gt;2.5</td>
<td>0.6–15</td>
<td>2–4</td>
<td>1–9</td>
<td>1–6</td>
</tr>
<tr>
<td><strong>4 °C</strong></td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td><strong>35 °C</strong></td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td><strong>Hydrolysis of:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Gelatin</strong></td>
<td>−</td>
<td>V</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><strong>Starch</strong></td>
<td>+</td>
<td>V</td>
<td>−</td>
<td>−</td>
<td>ND</td>
<td>V</td>
</tr>
<tr>
<td><strong>Agar</strong></td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>V</td>
<td>−</td>
</tr>
<tr>
<td><strong>Utilization of:</strong></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td><strong>D-Glucose</strong></td>
<td>−</td>
<td>+</td>
<td>ND</td>
<td>V</td>
<td>+</td>
<td>+*</td>
</tr>
<tr>
<td><strong>Sucrose</strong></td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>V</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><strong>Mannitol</strong></td>
<td>V</td>
<td>−</td>
<td>V</td>
<td>−</td>
<td>V</td>
<td>V</td>
</tr>
<tr>
<td><strong>Lactose</strong></td>
<td>−</td>
<td>ND</td>
<td>−</td>
<td>V</td>
<td>−</td>
<td>+*</td>
</tr>
<tr>
<td><strong>Maltose</strong></td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>V</td>
<td>ND</td>
</tr>
<tr>
<td><strong>DNA G+C content (mol%)</strong></td>
<td>46.8</td>
<td>40–46</td>
<td>45–53.9</td>
<td>39.3–50</td>
<td>37–50</td>
<td>43–47</td>
</tr>
</tbody>
</table>

*Alteromonas simiduii was positive.

†Alteromonas hispanica was negative.
method of phylogenetic tree reconstruction widely used in bacterial systematics and taxonomy).

Cells are Gram-stain-negative, aerobic, mesophilic, non-spore-forming rods, motile by means of one to several polar flagella. Positive for oxidase and negative for catalase. NaCl is required for growth. The major respiratory quinone is Q-8. The major polar lipids are phosphatidylglycerol and phosphatidylethanolamine. Diphosphatidylglycerol, an unknown aminolipid and an unknown aminophospholipid are present in moderate to minor amounts in the polar lipid profile. The type species is Neiella marina.

**Description of Neiella marina sp. nov.**

*Neiella marina* (ma.ri’na. L. fem. adj. marina of the sea, marine).

In addition to properties given in the genus description, the species is characterized as follows. Cells are 1.4–1.8 μm in length and 0.4–0.6 μm in width. They form translucent to opaque, low convex, non-swarming, smooth-rounded colonies with entire margins that are beige in colour and about 2–3 mm in diameter on MA after 48 h incubation at 28 ºC. Growth occurs at 10–40 ºC, with optimum growth at 25–28 ºC. Growth occurs in the presence of 1–5 % (w/v) NaCl and the optimum salt concentration is 2–3 %. The pH range for growth is 5.0–9.0 with optimum growth at pH 7.5–8.0. Nitrate is reduced to nitrite. Indole production, Simmons’ citrate, Voges–Proskauer test, arginine dihydrolase, urease and gelatinase are negative. Agarase, alginase, cellulase, amylase and esterase are positive. The following substrates are utilized as sole carbon sources: L-arabinose, D-xylene, D-mannose, D-mannitol, arbutin, aesculin ferric citrate, inulin and gentiobiose. The following substrates are not utilized as sole carbon sources: glycerol, ethyl alcohol, D-xylose, D-ribose, D-lactose, D-glucose, D-fructose, l-sorbosé, l-rhamnose, dulcitol, inositol, D-sorbitol, methyl α-D-mannopyranoside, methyl α-D-glucopyranoside, N-acetylglucosamine, amygdalin, salicin, cellulobiose, maltose, lactose, melibiose, sucrose, trehalose, melezitose, raffinose, starch, glycogen, xylitol, turanose, D-lyxose, D-tagatose, D-fucose, L-fucose, D-arabitol, L-arabitol, potassium gluconate, potassium 2-ketogluconate and potassium 5-ketogluconate. With API 50CHE strips, acid is produced from D-xylose, D-glucose, aesculin ferric citrate, maltose, melibiose, starch, glycogen, gentiobiose, D-tagatose and potassium 5-ketogluconate. Resistant to kanamycin (30 μg), oxacillin (1 μg), tetracycline (30 μg), doxycycline (30 μg), clindamycin (30 μg) and tobramycin (30 μg), but sensitive to cephaloridine (30 μg), sulfamethoxazole/trimethoprim (23.75/1.25 μg), erythromycin (15 μg), ofloxacin (5 μg), spectinomycin (100 μg), rifampicin (5 μg), ciprofloxacin (5 μg), cefaclor (30 μg), norfloxacin (10 μg) and gentamicin (10 μg). The dominant fatty acids are summed feature 3 (comprising C₁₆:₁ω7c and/or C₁₆:₁ω6c), C₁₆:₀ and C₁₈:₁ω7c.

The type strain, J221<sup>T</sup> (=CGMCC 1.10130<sup>T</sup>=NRRL B-51319<sup>T</sup>), was isolated from the intestine of a sea cucumber. The DNA G+C content of the type strain is 46.8 mol%.

**Acknowledgements**

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