The genus *Shewanella*, which was first described by MacDonell & Colwell (1985), is a member of the class *Gammaproteobacteria* (Anzai et al., 2000). Established species in the genus are facultatively anaerobic, aquatic marine bacteria that are Gram-negative, motile, rod-shaped and oxidase-positive and have genomic DNA G+C contents of 42–55 mol% (Bowman, 2005; Gauthier et al., 1995; MacDonell & Colwell, 1985; Venkateswaran et al., 1999). At the time of writing, the taxonomic position of this strain, designated UDC329^T, was investigated using a polyphasic approach.

A novel bacterial strain, designated UDC329^T, was isolated from a sample of seawater collected at Dong-do, on the coast of Dokdo Island, in the East Sea of the Republic of Korea. The Gram-staining-negative, motile, facultatively anaerobic, non-spore-forming rods of the strain developed into dark orange–yellow colonies. The strain grew optimally between 25 and 30 °C, with 1% (w/v) NaCl and at pH 7. It grew in the absence of NaCl, but not with NaCl at >7% (w/v). The predominant menaquinone was MK-7, the predominant ubiquinones were Q-7 and Q-8, and the major fatty acids were iso-C15:0 (33.52%) and C17:1ω8c (11.73%). The genomic DNA G+C content of strain UDC329^T was 50.2 mol%. In phylogenetic analyses based on 16S rRNA and gyrB gene sequences, strain UDC329^T was grouped with members of the genus *Shewanella* and appeared most closely related to *Shewanella fodinae* JC15^T* (97.9% 16S rRNA gene sequence similarity), *Shewanella indica* KJW27^T* (95.0%), *Shewanella algae* ATCC 51192^T* (94.8%), *Shewanella halioitis* DW01^T* (94.5%) and *Shewanella chilikensis* JC5^T* (93.9%). The level of DNA–DNA relatedness between strain UDC329^T* and S. fodinae* JC15^T* was, however, only 27.4%. On the basis of phenotypic, genotypic and DNA–DNA relatedness data, strain UDC329^T* represents a novel species in the genus *Shewanella*, for which the name *Shewanella dokdonensis* sp. nov. is proposed. The type strain is UDC329^T* (=KCTC 22898^T=DSM 23626^T)*.
In September 2006, strain UDC329\textsuperscript{T} was isolated from a sample of seawater collected at Dong-do (37°14′12″N 131°52′07″E) on the coast of Dokdo Island, in the East Sea of the Republic of Korea. The strain was isolated on 10-fold-diluted marine agar 2216 (MA; Difco) (Yang et al., 2006), by using a standard dilution plating technique and incubation at 25 °C for 7 days. The isolate was routinely cultured on MA, and preserved at 70 °C on marine broth (MB; Difco) containing 15 % (v/v) glycerol. Cell morphology was explored by both light microscopy (Sw 804255; Samwon) and transmission electron microscopy (H-7100; Hitachi) using cells that had been grown on MA at 25 °C for 5 days. Cell motility was investigated by observing cells grown in motility test agar (BBL Becton Dickinson). To check for the presence of flagella, cells from exponentially growing cultures were examined by transmission electron microscopy after being negatively stained with 2 % uranyl acetate and air-dried. Gram staining was determined by using a commercial Gram staining kit (bioMérieux) according to the manufacturer’s instructions. Growth at various temperatures (4–45 °C) was measured on MA. The pH range for growth was determined in trypticase soy broth (TSB; Difco) which was adjusted to pH 4.5–10 (at intervals of 0.5 pH unit), prior to sterilization, by the addition of HCl or Na\textsubscript{2}CO\textsubscript{3}. Growth in the absence of NaCl was investigated in TSB without NaCl. Growth in TSB with 1–10 % (w/v) NaCl (at intervals of 1 %) was also investigated. Anaerobic growth in an atmosphere of CO\textsubscript{2}/H\textsubscript{2}/N\textsubscript{2} (10:10:80, v/v/v) was explored in an anaerobic chamber (Sheldon Manufacturing) on standard MA and on MA supplemented with 0.1 % (w/v) KNO\textsubscript{3}, with incubation times of up to 1 week. Catalase activity was determined by bubble production in 3 % (v/v) H\textsubscript{2}O\textsubscript{2} and oxidase activity was determined by the oxidation of 1 % (w/v) p-aminodimethylaniline oxalate. Hydrolysis of casein, starch, and Tween 20, 40, 60 and 80 was determined as described by Cowan & Steel (1965) while the methods of Lányi (1987), except that, in all cases, artificial seawater containing (1 M): 23.6 g NaCl, 0.64 g KCl, 4.53 g MgCl\textsubscript{2}, 6H\textsubscript{2}O, 5.94 g MgSO\textsubscript{4}, 7H\textsubscript{2}O and 1.3 g CaCl\textsubscript{2}, 2H\textsubscript{2}O; Bruns et al., 2001] was used instead of distilled water. DNase activity was determined on DNase test agar (Difco) made with artificial seawater. Hydrolysis of hypoxanthine, tyrosine, and xanthine was determined on MA using the sub-.

...
calculate pairwise 16S rRNA gene sequence similarities. The results of the comparison of 16S rRNA gene sequences indicated that strain UDC329<sup>T</sup> was closely related to several established species in the genus *Shewanella*, particularly *S. fodinae* JC15<sup>T</sup> (97.9 % 16S rRNA gene sequence similarity), *S. indica* KJW27<sup>T</sup> (95.0 %), *S. alga* ATCC 51192<sup>T</sup> (94.8 %), *S. haliotis* DW01<sup>T</sup> (94.5 %) and *S. chilikensis* JC5<sup>T</sup> (93.9 %). The levels of 16S rRNA gene sequence similarity between strain UDC329<sup>T</sup> and the other type strains of the genus *Shewanella* were all between 91.2 % and 93.8 %. A phylogenetic tree based on 16S rRNA gene sequences was then constructed by using the neighbour-joining algorithm (Saitou & Nei, 1987). The method described by Jukes & Cantor (1969) was used to generate evolutionary distance matrices. The tree topology was evaluated by bootstrap analyses (Felsenstein, 1985) based on 1000 replications. In the tree, strain UDC329<sup>T</sup> was clustered with *S. alga* ATCC 51192<sup>T</sup>, *S. chilikensis* JC5<sup>T</sup>, *S. fodinae* JC15<sup>T</sup>, *S. haliotis* 

**Fig. 1.** Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences, showing the relationship between strain UDC329<sup>T</sup> and members of the genus *Shewanella*. Bootstrap values (%) based on 1000 replications are shown at branch points. *Vibrio cholerae* ATCC 14035<sup>T</sup> was used as an outgroup. Bar, 0.01 substitutions per nucleotide position.
DW01\(^\top\) and S. indica KJW27\(^\top\) (Fig. 1). In the present study, since similarity of 16S rRNA gene sequences is not a sufficient criterion to guarantee species identification among bacteria belonging to the genera Bacillus, Vibrio, Pseudomonas, Aeromonas and Shewanella (Yamamoto & Harayama, 1995), the more rapidly evolving gyrB gene was also investigated. In the analyses based on the sequences of this gene, strain UDC329\(^\top\) showed sequence similarities of only 77–78.4 % with type strains of all established species of the genus Shewanella. For gyrB gene sequence similarities, Venkateswaran et al. (1999) considered the cut-off point for the delineation of genomic species to be 90 %. In a neighbour-joining tree based on gyrB gene sequences, strain UDC329\(^\top\) was clustered with S. algae ATCC 51192\(^\top\) (HM016090), S. haliotis DW01\(^\top\) (HM016099), S. indica KJW27\(^\top\) (HM016092), S. chilikensis JC5\(^\top\) (HM016091) and S. amazonensis SB2B\(^\top\) (AF005257). The detailed morphological, physiological and biochemical characteristics of strain UDC329\(^\top\) are provided in the species description and, with comparative data on S. algae ATCC 51192\(^\top\), S. chilikensis JC5\(^\top\), S. fiodiae JC15\(^\top\), S. haliotis DW01\(^\top\) and S. indica KJW27\(^\top\), in Table 1. The isoprenoid quinones detected in strain UDC329\(^\top\) included both menaquinones and ubiquinones – a characteristic observed in established Shewanella species (Nogi et al., 1998; Venkateswaran et al., 1999; Bozal et al., 2002). The predominant menaquinone was MK-7 (about 88 %) and the predominant ubiquinones were Q-8 (about 67 %) and Q-7 (about 29 %). Several phenotypic characteristics listed in Table 1 can be used to differentiate strain UDC329\(^\top\) from its closest relatives in the genus Shewanella.

The cellular fatty acid profiles of strain UDC329\(^\top\) and the type strains of closely related species of the genus Shewanella are shown in Table 2. The major fatty acids of the novel strain were iso-C\(_{15}:0\) and C\(_{17}:0\) 3OHc and its DNA G + C content, 50.2 mol% (Table 1), lies within the
range described for the genus *Shewanella* (42–55 mol%; Venkateswaran *et al.*, 1999). Based on phylogenetic, genomic, chemotaxonomic and phenotypic data, strain UDC329\(^\text{T}\) represents a novel species within the genus *Shewanella*, for which the name *Shewanella dokdonensis* sp. nov. is proposed.

**Description of *Shewanella dokdonensis* sp. nov.**

*Shewanella dokdonensis* (dok.do.nen'sis. N.L. fem. adj. dokonensis of Dokdo, the island from where the type strain was isolated).

Cells are Gram-staining-negative and non-spore-forming rods (0.4–0.6 × 1.4–2.0 \(\mu\)m) that are motile by means of a single polar flagellum. Cells grow well on MA and TSA plates. Colonies on MA are circular, convex with entire margins, smooth, glistening, dark orange–yellow in colour and 2.0–2.5 mm in diameter after 5 days incubation at 30 °C. Growth occurs at 10–40 °C (optimum between 25 and 30 °C), with 0–7 % (w/v) NaCl (optimum 1 %) and at pH 5.0–8.5 (optimum pH 7). Growth occurs under anaerobic conditions on MA. When using lactate as the electron donor, DMSO, TMAO, sodium thiosulfate and sodium nitrate are reduced, but manganese dioxide, sodium nitrite and sodium sulfite are not. Cells are catalase-positive and weakly oxidase-positive. Casein, DNA, and Tweens 20, 40, 60, and 80 are hydrolysed but aesculin, starch, xanthine, hypoxanthine, tyrosine, gelatin and urea are not. Reduces nitrate to nitrite. \(\text{H}_2\text{S}\), acetoin and indole are not produced. Acids are not produced from carbohydrates. Negative for

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3*</th>
<th>4</th>
<th>5</th>
<th>6*</th>
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<tbody>
<tr>
<td>Habitat</td>
<td>Seawater</td>
<td>Salt marsh</td>
<td>Marine lagoon sediment</td>
<td>Coal mine</td>
<td>Abalone</td>
<td>Marine sediment</td>
</tr>
<tr>
<td>Colony colour</td>
<td>Dark orange–yellow</td>
<td>Pink</td>
<td>Pale brown</td>
<td>Red</td>
<td>Pink–orange</td>
<td>Pale brown</td>
</tr>
<tr>
<td>Optimal temperature for growth (°C)</td>
<td>25–30</td>
<td>37*</td>
<td>28–30</td>
<td>30–37*</td>
<td>37*</td>
<td>37</td>
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<tr>
<td>Growth at 42 °C</td>
<td>–</td>
<td>+*</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>+*</td>
</tr>
<tr>
<td>Optimal pH for growth</td>
<td>7</td>
<td>7–8*</td>
<td>8</td>
<td>6–7*</td>
<td>7*</td>
<td>7.5</td>
</tr>
<tr>
<td>Tolerance to 10 % (w/v) NaCl</td>
<td>–</td>
<td>+*</td>
<td>–</td>
<td>–</td>
<td>–*</td>
<td>+*</td>
</tr>
<tr>
<td>Nitrate reduction</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

Production of:

- Oxidase: W + + + + + +
- Acid phosphatase: + W W W ND + ND
- \(\alpha\)-Chymotrypsin: + W ND + + ND
- \(\beta\)-Galactosidase: + – + – – –
- \(\text{H}_2\text{S}\): – + + – + +
- Ornithine decarboxylase: – + + – + –
- Urease: – – + – – +
- Gelatinase: – + + – + +
- Tyrosinase: – + ND ND + ND
- Haemolysis (type): + (\(\alpha\)) + (\(\alpha\)) + (\(\beta\)) + (\(\beta\)) + (\(\beta\))

Utilization of:

- Glucose: + + – + + +
- \(\alpha\)-Galactose: + – ND + + ND
- Lactose: + – ND – ND
- N-Acetyl-\(\alpha\)-glucosamine: – + + – + +

Resistance to:

- Ampicillin (10 \(\mu\)g): + + – + + –
- Nalidixic acid (30 \(\mu\)g): – + ND + + ND
- Penicillin (10 IU): + + – + + –
- Polymyxin B (300 IU): $ + ND – + ND
- Tetracycline (30 \(\mu\)g): + + + – + +
- Vancomycin (30 \(\mu\)g): + + – + + +

DNA G + C content (mol%): 50.2 52–54* 54.6 53.6* 53.7* 51.2

Table 2. Cellular fatty acid compositions (%) of strain UDC329<sup>T</sup> and the type strains of closely related species of the genus Shewanella.

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
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<tbody>
<tr>
<td>Straight-chain saturated</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>C&lt;sub&gt;12:0&lt;/sub&gt;</td>
<td>1.11</td>
<td>3.03</td>
<td>2.87</td>
<td>1.01</td>
<td>2.54</td>
<td>2.30</td>
</tr>
<tr>
<td>C&lt;sub&gt;13:0&lt;/sub&gt;</td>
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<td>tr</td>
<td>1.45</td>
<td>tr</td>
<td>tr</td>
<td>1.01</td>
</tr>
<tr>
<td>C&lt;sub&gt;14:0&lt;/sub&gt;</td>
<td>2.62</td>
<td>3.51</td>
<td>4.80</td>
<td>7.37</td>
<td>3.63</td>
<td>4.63</td>
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<tr>
<td>C&lt;sub&gt;15:0&lt;/sub&gt;</td>
<td>3.66</td>
<td>16.14</td>
<td>14.26</td>
<td>4.78</td>
<td>12.71</td>
<td>12.65</td>
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<tr>
<td>C&lt;sub&gt;17:0&lt;/sub&gt;</td>
<td>tr</td>
<td>2.40</td>
<td>4.50</td>
<td>3.77</td>
<td>3.79</td>
<td>4.29</td>
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<tr>
<td>iso-C&lt;sub&gt;13:0&lt;/sub&gt;</td>
<td>3.87</td>
<td>2.41</td>
<td>1.96</td>
<td>1.33</td>
<td>2.48</td>
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<td>2.06</td>
<td>2.01</td>
<td>1.89</td>
<td>1.88</td>
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<td>Branched saturated</td>
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<tr>
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<td>5.75</td>
<td>3.85</td>
<td>3.49</td>
<td>3.75</td>
<td>4.87</td>
<td>3.54</td>
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<tr>
<td>iso-C&lt;sub&gt;15:0&lt;/sub&gt;</td>
<td>33.52</td>
<td>18.62</td>
<td>16.17</td>
<td>24.71</td>
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<td>2.52</td>
<td>2.88</td>
<td>3.35</td>
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<td>7.43</td>
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<td>2.19</td>
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<td>Mono-unsaturated</td>
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<tr>
<td>C&lt;sub&gt;17:1&lt;/sub&gt;&lt;sup&gt;9c&lt;/sup&gt;</td>
<td>11.73</td>
<td>7.85</td>
<td>11.93</td>
<td>16.50</td>
<td>10.62</td>
<td>13.55</td>
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<td>C&lt;sub&gt;16:1&lt;/sub&gt;&lt;sup&gt;7c&lt;/sup&gt;</td>
<td>2.28</td>
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<td>3.33</td>
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<tr>
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<td>2.82</td>
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<td>Summed feature*</td>
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<tr>
<td>1</td>
<td>2.33</td>
<td>1.68</td>
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<td>2.75</td>
<td>1.83</td>
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<tr>
<td>2</td>
<td>2.56</td>
<td>2.02</td>
<td>1.96</td>
<td>1.89</td>
<td>1.82</td>
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<tr>
<td>3</td>
<td>6.54</td>
<td>13.34</td>
<td>11.85</td>
<td>4.29</td>
<td>12.82</td>
<td>11.87</td>
</tr>
</tbody>
</table>

*Summed features represent groups of two or more fatty acids that could not be separated by GLC using the MIDI system. Summed feature 1 contained C<sub>13:0</sub> 3-OH and/or iso-C<sub>15:0</sub> 1/H. Summed feature 2 contained C<sub>14:0</sub> 3-OH and/or iso-C<sub>16:0</sub> 1. Summed feature 3 contained iso-C<sub>15:0</sub> 2-OH and/or C<sub>16:1</sub><sup>9c</sup>.

citrate utilization. Causes α-haemolysis on blood agar. When assayed with the API ZYM system, positive result for alkaline phosphatase, leucine arylamidase, α-chymotrypsin, acid phosphatase, β-galactosidase and (weakly) esterase lipase (C8) activities but negative for esterase (C4), lipase (C14), valine arylamidase, cystine arylamidase, trypsin, naphthol-AS-BL-phosphohydrolase, α-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, N-acetyl-β-glucosaminidase, α-mannosidase and α-fructosidase activities. Glucose, galactose, acetate, pyruvate, capric acid and malic acid are utilized as sole carbon and energy sources. Sensitive to erythromycin (15 μg), kanamycin (30 μg), streptomycin (10 μg), chloramphenicol (30 μg), gentamicin (10 μg), amikacin (30 μg), nalidixic acid (30 μg) and polymyxin B (300 IU) but resistant to penicillin (10 IU), ampicillin (10 μg), vancomycin (30 μg) and tetracycline (30 μg). Other physiological and biochemical characteristics are provided in Table 1. Both menaquinones and ubiquinones are present. The predominant menaquinone is MK-7 and the predominant ubiquinones are Q-7 and Q-8. Major fatty acids are iso-C<sub>15:0</sub> and C<sub>17:1</sub><sup>ω8c</sup>. Table 2 shows the complete fatty acid composition.

The type strain, UDC329<sup>T</sup> (=KCTC 22898<sup>T</sup>=DSM 23626<sup>T</sup>), was isolated from seawater collected off the coast at Dongdo, on Dokdo Island, in the East Sea of the Republic of Korea. The genomic DNA G+C content of the type strain is 50.2 mol%.

Acknowledgements

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References


1943–1949.


