Hafnia psychrotolerans sp. nov., isolated from lake water

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A psychrotolerant, Gram-stain-negative, motile, aerobic, peritrichous bacterium, strain DJC1-1T, was isolated from Lake Dajiaco, Tibetan Plateau, China. The strain was negative for citrate utilization, lipase activity and α-glucosidase, but positive for the Voges–Proskauer reaction and \( N \)-acetyl-β-glucosaminidase. 16S rRNA gene sequence analysis indicated that *Hafnia paralvei* ATCC 29927T, *Hafnia alvei* ATCC 13337T, *Serratia grimesii* DSM 30063T and *Serratia plymuthica* DSM 4540T were the closest relatives of strain DJC1-1T, with similarities of 97.76, 96.80, 97.71 and 97.58 %, respectively. The DNA G+C content of strain DJC1-1T was 53.9 mol%. The predominant fatty acids were C16 : 0 and C17 : 0 cyclo. Based on these characteristics, strain DJC1-1T can be assigned to the genus *Hafnia*. In DNA–DNA hybridization tests, strain DJC1-1T shared 50.6, 35.1, 36.5 and 18.1 % DNA–DNA relatedness with the type strains of *H. paralvei*, *H. alvei*, *S. grimesii* and *S. plymuthica*, respectively. The growth temperature ranged from 0 to 40 °C, with optimum growth at 15 °C. Physiological and biochemical tests differentiated strain DJC1-1T from the type strains of recognized species of the genus *Hafnia*. Therefore, strain DJC1-1T is identified as representing a novel species of the genus *Hafnia*, for which the name *Hafnia psychrotolerans* sp. nov. is proposed. The type strain is DJC1-1T (=JCM 30077T =CGMCC 1.12806T).

The genus *Hafnia* was first recognized by Møller et al. (1954). Subsequently, DNA–DNA relatedness investigations demonstrated that these bacteria represented a unique taxon (Brenner, 1978). Huys et al. (2010) reclassified strains of *Hafnia alvei* hybridization group 2 as representing a novel species. The genus thus comprises, at the time of writing, two recognized species. Strains of the genus are characterized by Gram-negative, non-spore-forming, facultatively anaerobic, peritrichously flagellated rods, and are typically methyl-red-positive, and positive for lysine decarboxylase, ornithine decarboxylase and the Voges–Proskauer reaction. They are negative for arginine dihydrolase, citrate utilization, gelatin hydrolysis and lipase activity (Huys et al., 2010; Møller, 1954; McBee & Schauer, 2006). DNA G+C contents vary within the genus from 47 to 57 mol% (Greipsson & Priest, 1983). In this study, a psychrotolerant bacterium isolated from lake water is proposed as a representative of a novel species of the genus *Hafnia*.

Strain DJC1-1T was isolated from the water of Lake Dajiaco, Tibetan Plateau, China (29° 53.369’ N 85° 44.773’ E, 5170 m above sea level), where mean ambient annual temperature is 2 °C, the pH of the water is 9.4 and salinity is 3.33 g l\(^{-1}\). To prevent contamination, the lake water samples were coated directly on R2A agar (containing 0.03 % sodium pyruvate, 0.03 % K\(_2\)HPO\(_4\), 0.005 % MgSO\(_4\), 0.05 % yeast extract, 0.05 % peptone, 0.05 % glucose, 0.05 % Casamino acids, 0.05 % starch, 1.5 % agar; pH 7.2) (Reasoner & Geldreich, 1985) immediately after sampling and all equipment used in this process was sterilized. Plates were then transported to the laboratory at room temperature.

Genomic DNA was extracted according to the methods of Marmur (1961), and purity was checked using a NanoDrop spectrophotometer (2000c; Thermo) (Marmur, 1961). The 16S rRNA gene was amplified with primers
27F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1492R (5′-CGGTTACCTTGTTACGACTT-3′) (Embley, 1991). The 16S rRNA gene sequence of strain DJC1-1T was compared with those in GenBank using the BLAST program (NCBI) and the EzBioCloud (http://www.ezbiocloud.net/eztaxon) (Kim et al., 2012) to determine its approximate phylogenetic affiliation. 16S rRNA gene sequences were then analysed with the software package MEGA 5.05 (Tamura et al., 2011). A phylogenetic tree was reconstructed using the neighbor-joining and maximum-likelihood methods with bootstrap values based on 1000 replications (Fig. 1). 16S rRNA gene sequence analysis indicated that strain DJC1-1T shared 97.76, 96.80, 97.71 and 97.58 % similarity, respectively, with Hafnia paralvei ATCC 29927T, Hafnia alvei ATCC 13337T, Serratia grimesii DSM 30063T and Serratia plymuthica DSM 4540T. 16S rRNA gene sequence similarity can be used to distinguish between two species when it is below 98.65 % (Kim et al., 2014). Therefore, we considered that strain DJC1-1T may represent a novel species of the genus Hafnia.

A series of phenotypic and genotypic approaches were employed to further determine the taxonomic position of the novel isolate. Morphology was observed by transmission electron microscopy (JEM-1400; JEDL) (Fig. S1, available in the online Supplementary Material). Gram staining and catalase activity tests were conducted according to the standard protocol of the Microbial Identification System (MIDI, version 6.0). The main fatty acids of strain DJC1-1T were C16:0 (29.43 %) and C17:0 cyclo (29.35 %); no significant differences in the fatty acid profile were found between the novel isolate and those of closely related bacteria, although small quantitative differences were observed (Table 2). Strain DJC1-1T, H. paralvei ATCC 29927T and H. alvei ATCC 13337T do not have C12:0 2-OH, in contrast to recognized species of the genus Serratia.

DNA G+C content analysis was based on the midpoint value ($T_m$) determined from the thermal denaturation profile (De Ley et al., 1970). The DNA G+C content of strain DJC1-1T was 53.9 mol%.

**Fig. 1.** Neighbour-joining phylogenetic tree showing the position of strain DJC1-1T, based on 16S rRNA gene sequence analysis. Numbers at nodes indicate bootstrap percentages (based on 1000 replications). Bar, 0.01 accumulated changes per nucleotide position.
The type strains of *H. paralvei*, *H. alvei*, *S. grimesii* and *S. plymuthica* and strain DJC1-1T were examined by rep-PCR fingerprinting using the (GTG)5 primer (Gevers et al., 2006b; Hussey et al., 2005a, b). DNA was extracted by using a TIANamp bacterial DNA kit (Tiangen Biotech) following the manufacturer’s instructions. PCR amplifications were performed with a TIANamp bacterial DNA kit (Tiangen Biotech) as described by Masco et al. (2003) and the annealing temperature was set at 40 °C. The PCR products were electrophoresed in a 1.5 % agarose gel for 4 h at a constant voltage of 60 V cm−1 in 1× TAE (40 mM Tris/acetate, 1 mM EDTA, pH 8.0) at room temperature. The analysis was carried out using the BioNumerics 7.1 software package (Applied Maths). The five strains produced banding patterns containing 15, 18, 12, 15 and 15 bands from the top down (Fig. 2). The results showed that strain DJC1-1T differed markedly from the other strains.

We further compared the five strains at the genomic level by DNA–DNA hybridization experiments, which were carried out applying the optical renaturation method (Huss et al., 1983; De Ley et al., 1970). Temperature used in the optimal renaturation method was 74.49 °C. Hybridizations were repeated three times and means of the resulting values were determined. The level of DNA–DNA relatedness between strain DJC1-1T and *H. paralvei* ATCC 22927T was 50.6 %. DNA–DNA relatedness between strain DJC1-1T and the type strains of *H. alvei*, *S. grimesii* and *S. plymuthica* was 35.1, 36.5 and 18.1 %, respectively. Based on the genotypic and phenotypic data presented in this study, strain DJC1-1T represents a novel species of the genus *Hafnia*, for which the name *Hafnia psychrotolerans* sp. nov. is proposed.

**Description of Hafnia psychrotolerans sp. nov.**

*Hafnia psychrotolerans* (psy.chro.to.l'e.rans. Gr. adj. psychr. cold; L. part. adj. tolerans tolerating; N.L. part. adj. psychrotolerans tolerating cold).

Cells are aerobic, non-pigmented, Gram-stain-negative, motile, peritrichously flagellated rods (0.8–1.2 μm wide, 1.8–2.4 μm long). White, round, smooth, convex and opaque colonies are produced on R2A agar after incubation at 20 °C for 2–3 days. Grows at 0–40 °C (optimally at 15 °C) on R2A agar, at pH 4–11 (optimally at pH 7) and with 0–8 % NaCl (optimally in the absence of NaCl). In API tests, negative for lysine decarboxylase, tryptophan decarboxylase and

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**Table 1. Biochemical characteristics of strain DJC1-1T and the type strains of species of the genera *Serratia* and *Hafnia***

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Optimum growth temperature (°C)</td>
<td>15</td>
<td>37</td>
<td>37</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Nitrate reduction</td>
<td>++</td>
<td>ND</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Arginine dihydrolase</td>
<td>−−</td>
<td>−−</td>
<td>−−</td>
<td>−−</td>
<td>−−</td>
</tr>
<tr>
<td>Lysine decarboxylase</td>
<td>−−</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Ornithine decarboxylase</td>
<td>−−</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Tryptophan decarboxylase</td>
<td>−</td>
<td>−</td>
<td>ND</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Citrate utilization</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>Aesculin hydrolysis</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Gelatin hydrolysis</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
</tbody>
</table>

**Acid production from:**

- **D-Glucose**: + + ND + +
- **Inositol**: − − − − + +
- **D-Sorbitol**: − − − − + +
- **L-Rhamnose**: − − − − + +
- **Sucrose**: + + − − + +
- **Melibiose**: − − − − − +
- **Amygdalin**: − − ND + +
- **L-Arabinose**: − − + + + +

**Enzyme activities:**

- **Leucine arylamidase**: + + + + −
- **α-Glucosidase**: − − − − + +
- **N-Acetyl-β-D-glucosaminidase**: + + − − −
- **DNA G+C content (mol%)**: 53.9 49.8 51.5 ND ND

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
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<tr>
<td>C12:0</td>
<td>5.91</td>
<td>4.47</td>
<td>5.93</td>
<td>3.15</td>
<td>3.98</td>
</tr>
<tr>
<td>C12:0 2-OH</td>
<td>TR</td>
<td>TR</td>
<td>TR</td>
<td>1.20</td>
<td>TR</td>
</tr>
<tr>
<td>C14:0</td>
<td>2.50</td>
<td>6.78</td>
<td>9.23</td>
<td>6.68</td>
<td>6.36</td>
</tr>
<tr>
<td>C16:0</td>
<td>29.43</td>
<td>41.42</td>
<td>29.03</td>
<td>31.59</td>
<td>31.06</td>
</tr>
<tr>
<td>C17:0 cyclo</td>
<td>29.35</td>
<td>20.62</td>
<td>31.48</td>
<td>26.65</td>
<td>19.34</td>
</tr>
<tr>
<td>C17:0</td>
<td>3.22</td>
<td>TR</td>
<td>TR</td>
<td>3.49</td>
<td>3.78</td>
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<tr>
<td>C18:0</td>
<td>TR</td>
<td>TR</td>
<td>1.08</td>
<td>1.07</td>
<td></td>
</tr>
<tr>
<td>C19:0 cyclo 2-OH</td>
<td>2.73</td>
<td>9.17</td>
<td>TR</td>
<td>3.19</td>
<td>1.64</td>
</tr>
</tbody>
</table>

Summed features:

- **1**: TR TR TR 1.11 TR
- **2**: 8.53 8.58 12.14 9.01 9.14
- **3**: 8.65 3.65 6.84 5.36 10.10
- **4**: 5.68 3.66 2.52 5.55 8.00

*Summed features represent two or more fatty acids that cannot be separated by GLC with the MIDI system. Summed feature 1 comprises iso-C15:0 3-OH; summed feature 2 comprises iso-C16:1 7c and/or C16:1 6c; summed feature 3 comprises C16:1 7c and/or C16:1 6c and/or C17:0 cyclo; summed feature 4 comprises C18:1 9c and/or C18:1 10c.*
ornithine decarboxylase. Negative for production of indole, H₂S, arginine dihydrolase and urease, degradation of gelatin and citrate utilization. Positive for the Voges–Proskauer reaction and aesculin hydrolysis. Positive for β-galactosidase, alkaline phosphatase, leucine arylamidase and acid phosphatase. Produces acid from D-glucose, sucrose and D-mannitol, but not from inositol, L-arabinose, D-sorbitol, L-rhamnose, amygdalin or melibiose. The major fatty acids are C₁₆:0 and C₁₇:0 cyclo.

The type strain is DJC1-1ᵀ (=JCM 30077ᵀ=CGMCC1.12806ᵀ), which was isolated from water of Lake Dajiaco, Tibetan Plateau, China. The genomic DNA G+C content of the type strain is 53.9 mol% (Tₘ).

Acknowledgements

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References


