Idiomarina baltica sp. nov., a marine bacterium with a high optimum growth temperature isolated from surface water of the central Baltic Sea

Ingrid Brettar, Richard Christen and Manfred G. Höfle

1GBF-German Research Centre for Biotechnology, Dept Environmental Microbiology, Mascheroder Weg 1, D-38124 Braunschweig, Germany
2UMR 6078 CNRS and Université Nice Sophia Antipolis, Batiment Jean Maetz, F-06230 Villefranche sur Mer, France

Two bacterial strains isolated from the Baltic Sea, OS145T and OS146, were characterized on the basis of their physiological and biochemical features, their fatty acid profiles and their phylogenetic position based on 16S rDNA sequence analyses. The strains were isolated from the upper oxic water column of the central Baltic Sea. Phylogenetic analyses of the 16S rDNA gene sequences revealed a clear affiliation of the novel strains with members of the genus Idiomarina, of the Gammaproteobacteria. Closest sequence similarity was seen with Idiomarina abyssalis and Idiomarina zobellii (95–96%). The mean G + C content of the DNA of strains OS145 and OS146 was 49.7 mol%. Both strains were non-pigmented, Gram-negative, polarly flagellated organisms that were strictly aerobic. Growth of the strains was observed at salinities ranging from 0–8 to 10% NaCl. Temperature range for growth was rather broad and high for marine bacteria: both strains grew between 8 and 46 °C, showed good growth between 20 and 44 °C, and had an optimum between 30 and 40 °C. The fatty acids of the two strains were dominated by iso-branched fatty acids (54–80%), with a high abundance of C15:0 iso (36%), C16:1ω7c, C17:0 iso and C17:1ω9c. Growth temperature (8–40 °C) influenced the fatty acid composition of the strains in a way that the content of iso-branching fatty acids increased with increasing temperatures, while the mono-unsaturated fatty acids increased with decreasing temperatures. Salinity (1–7–10% NaCl) had only a minor effect on the fatty acid composition. According to their morphology, physiology, fatty acid composition and 16S rDNA sequences, strains OS145T and OS146 fitted well into the genus Idiomarina, but could be easily distinguished from the recognized species of the genus. Because of their unique nature, it is proposed that the strains isolated from the Baltic Sea represent a novel species, for which the name Idiomarina baltica (type strain OS145T = DSM 15154T = LMG 21691T) is proposed.

INTRODUCTION

At the time of writing, the genus Idiomarina contained two species, Idiomarina abyssalis and Idiomarina zobellii, of strictly aerobic Gammaproteobacteria, which were isolated from deep-sea samples from the northwest Pacific Ocean (Ivanova et al., 2000). Comparison of the 16S rRNA gene sequences of these two species with those of submitted sequences of clones and isolates showed that related sequences were derived from deep-sea sediments, with sequences from a hydrothermal mound of the Mid-Atlantic Ridge showing the highest similarity, while those of Pacific deep-sea organisms were more distantly related.

An eminent feature of the genus Idiomarina is the unique composition of its fatty acids, with a high percentage of iso-branched fatty acids. Strains OS145T and OS146, isolated from the central Baltic Sea, have an unsaturated iso-branched fatty acid (C17:1ω9c, 8.2%) in addition to the two iso-branched fatty acids observed for I. abyssalis and I. zobellii (C15:0 iso and C17:0 iso) (Ivanova et al., 2000). A high proportion of iso-branching fatty acids is atypical for the Proteobacteria, and has so far only been seen in the Xanthomonas-branch Proteobacteria (Finkmann et al., 2000). Since strains OS145T and OS146 showed broad temperature and salinity spectra for growth, and fatty acids...
can be crucial for adaptation to temperature (Klein et al., 1999) and salinity (Lopez et al., 1998), the responses of the fatty acid composition of these two strains to temperature and salinity were analysed, and are described here.

In this study, we describe two strictly aerobic strains (OS145T and OS146) of the genus *Idiomarina* that were isolated from the oxic water column of the Gotland Deep, a basin with anoxic deep water in the central Baltic Sea. The strains were first recognized by their unique low-molecular-weight RNA fingerprints (Höflé & Brettar, 1996). Phylogenetic analyses of the 16S rRNA gene sequences of strains OS145T and OS146 showed them to be included in the robust cluster formed by the genus *Idiomarina* and suggest, together with their physiological features, fatty acid compositions and low-molecular-weight RNA fingerprints, that the strains represent a novel species of the genus *Idiomarina*, for which the name *Idiomarina baltica* is proposed. This novel species is the only representative of the genus *Idiomarina* not derived from deep-sea samples, but is instead from the surface water of an estuarine environment, the Baltic Sea.

**METHODS**

**Bacterial strains, isolation and growth conditions.** Strains OS145T and OS146 were isolated during a cruise of the RV *Poseidon* in August 1986 from the oxic part of the water column (30 m, 6 °C, 8 % NaCl) of a basin in the Central Baltic Sea (Gotland Deep; BY 15, 57-1920 N, 20-0302 E). All details on environmental conditions, sampling and isolation procedures have been described previously (Brettar & Rheinheimer, 1991, 1992; Brettar & Höflé, 1993; Höflé & Brettar, 1995, 1996). Medium for isolation of the strains was ZoBell agar (Oppenheimer & Zobell, 1952). Strains grew well on ZoBell agar, in marine broth or on marine agar (Difco).

**Physiological and biochemical tests and morphology.** Strains OS145T and OS146 were tested for a number of key characteristics using standard procedures (Gerhardt et al., 1994), such as Gram reaction (Gram-staining, Gram string test), cell size and morphology (phase-contrast microscopy, electron microscopy after negative staining with 1 % uranyl acetate), cytochrome oxidase and catalase (phase-contrast microscopy, electron microscopy after negative staining), haemolysis (bovine blood agar), acid production from glucose, ribose and arabinose, and hydrolysis of starch, gelatin, Tween 80 and lecithin were tested. Chitinase activity was tested as described by Cottrell et al. (2000). Strains were additionally characterized by using the whole test spectrum of the identification systems API 50CH, API 20NE and API ZYM (bioMérieux) at 20 °C and BIOLOG GN2 at 28 °C. Growth at different temperatures was assessed at 4, 8, 10, 20, 25, 30, 36, 40, 44, 49 and 60 °C. Growth at different salinities was tested at 0, 0-8, 1, 3, 6 and 10 % NaCl. For these tests we used half-strength marine broth (Difco; catalogue no. 2216), except for the salinity test where half-strength Caso medium (DSMZ; catalogue no. 220) was supplemented with the respective amount of NaCl.

For testing anaerobic respiration, the strains were inoculated into marine broth (Difco) containing the electron acceptors at a final concentration of 10 mmol l⁻¹. Incubation was done anaerobically in the dark for up to 18 days at 20 °C. No growth was observed in marine broth in an anoxic environment without electron acceptor addition. Growth under anaerobic conditions in the presence of the added electron acceptors was considered an indicator of electron-acceptor utilization. Additionally, growth on triple-sugar/iron agar (Difco) was tested. Denitrification was studied additionally in nutrient broth (Difco) (plus 20 mmol nitrate l⁻¹) as described in more detail by Brettar & Höflé (1993). As a positive control, *Sheewanella baltica* OS155, a strain isolated from the Baltic Sea and able to use the provided electron acceptors (Ziemke et al., 1998), was used for comparisons.

**Phylogenetic analyses based on 16S rRNA gene sequence comparisons.** Genomic DNA was prepared from individual colonies as described by Moore et al. (1996). 16S rRNA genes were amplified by PCR (Mulhis & Faloona, 1987), and the PCR products were sequenced directly as described previously (Moore et al., 1999).

The 16S rDNA sequences of strains OS145T and OS146 were automatically and then manually aligned by reference to a database of 37 000 already aligned bacterial 16S rDNA sequences. The same sequences were then compared (using BLAST) against the current content of the EMBL database (Bacteria division) to check for the presence of newly submitted related sequences. Phylogenetic trees were constructed according to three different methods (BIONJ, maximum-likelihood and maximum-parsimony). For the neighbour-joining analysis, a matrix distance was calculated according to the Kimura 2-parameter correction. Bootstrapping was done using 500 replications, BIONJ and the Kimura 2-parameter correction. BIONJ was according to Gascuel (1997); maximum-likelihood and maximum-parsimony programs were from PHYLIP (Felsenstein, 1995). The phylogenetic trees were drawn using NIPILOT (Perrière & Gouy, 1996) and CLARISDRAW software for Apple Macintosh. The nucleotide sequences of strains OS145T and OS146 were deposited in the EMBL DNA database under accession numbers AJ440214 and AJ440215, respectively. Domains used to construct phylogenetic trees were regions of the small-subunit rRNA sequences available for all sequences, and excluded positions likely to show homoplaspy.

For the dendrogram shown in Fig. 2, retaining only sequences of related genera, mostly from type strains (26 sequences), allowed us to include in the analysis the almost-complete 16S rDNA sequences, corresponding to nucleotide positions 75–183 and 200–1429, of strains OS145T and OS146. The topology shown is that of the bootstrap analysis, as it has been demonstrated that this topology is often better than that of a simple neighbour-joining analysis (Berry & Gascuel, 1996). As a result, there is no distance bar in this tree; note also that one should consider the distance bar with caution in a simple tree, as the distance bar represents the distances calculated after corrections (Kimura 2-parameter correction; Jukes & Cantor, 1969), and that the lengths of the branches simply do not represent the real number of differences between the sequences themselves.

**DNA isolation and spectroscopic analysis of DNA–DNA hybridization.** DNA was isolated by chromatography on hydroxyapatite (Cashion et al., 1977). DNA–DNA hybridization was carried out as described by De Ley et al. (1970) with the modifications described by Huss et al. (1983) and Escara & Hutton (1980) using a Gilford System model 2600 spectrometer. Renaturation rates were calculated with the TRANSFERBAS program (Jahnke, 1992).

**Determination of the DNA G+C content.** For strains OS145T and OS146, this was done using HPLC analysis of hydrolysed DNA according to Tamaoka & Komagata (1984) and Mesbah et al. (1989).

**Cellular fatty acids profiles.** Strains OS145T and OS146 were grown on marine agar (Difco) for 24 h at 28 °C. The fatty acid methyl esters (FAMEs) were obtained from washed cells by saponification, methylation and extraction. Analysis by gas chromatography was controlled by MÉS software (Microbial ID), and peaks were automatically integrated and identified by the MICROBIAL IDENTIFICATION software package (Sasser, 1990). In addition to the standard procedures, i.e. growth at 28 °C on marine agar (3-4 % NaCl), the influence of temperature and salinity on the fatty acid pattern were tested. To assess the influence of temperature, strains were grown on
marine agar (3.4 % NaCl) at 8 °C (15 days), 10 °C (6 days) and 40 °C (1 day). To assess the influence of salinity, the strains were grown in the presence of 1-7 and 10 % NaCl at 28 °C, and in the presence of 10 % NaCl at 40 °C.

RESULTS AND DISCUSSION

Physiological, biochemical and morphological characteristics

Strains OS145T and OS146 were Gram-negative, curved rods of 0.4–0.7 µm in width and 0.7–1.6 µm in length, with a polar flagellum (Fig. 1). Colonies were circular, smooth, opaque and slightly yellowish on marine agar. In terms of their physiological features [Table 1, and supplementary data available in IJSEM Online (http://ijis.sgmjournals.org)], strains OS145T and OS146 showed almost identical responses. The strains were catalase-, cytochrome oxidase- and aminopeptidase-positive. Growth was observed between 8 and 46 °C for strain OS145T, and between 10 and 46 °C for strain OS146. Both strains showed good growth between 20 and 44 °C, and had an optimum between 30 and 40 °C. Growth of the two strains was observed in the presence of 0.8–10 % NaCl, with the optimum being between 3 and 6 % NaCl. Both strains were able to grow in marine broth under oxic conditions. Growth did not occur under anoxic conditions, nor did it occur in the presence of sulphite, nitrate, nitrite, trimethylaminoxide, thiosulfate or ferric iron as electron acceptors. Strains OS145T and OS146 were able to grow in marine broth at pH 5.7. They were also able to produce H2S from cysteine (Dye, 1968). There was no haemolysis of blood. Both strains hydrolysed gelatin and Tween 80, but not starch or lecithin. Acid production by the strains was observed from D-glucose, but not from D-ribose or D-arabinose. The strains were unable to use any of the substrates provided by the API 50CH system or to assimilate the substrates provided by the API NE20 system. Both strains showed a broad spectrum of enzymic activities and were positive for β-glucosidase, protease (gelatinase) (API 20NE), alkaline phosphatase, acid phosphatase, esterase (C4), lipase (C8), leucine arylamidase, valine arylamidase, cysteine arylamidase, chymotrypsin and naphthol-phosphohydralase activities (API ZYM).

In the BIOLOG GN2 test system, strains OS145T and OS146 used Tween 40, Tween 80, L-arabinose, acetic acid, α-ketobutyric acid and α-ketovaleric acid.

A comparison of strains OS145T and OS146 with the two validly described species of *Idiomarina* showed pronounced differences in the temperature ranges for growth and the substrates utilized in the BIOLOG assay (Table 1).

Phylogenetic position of strains OS145T and OS146 based on 16S rDNA sequence comparisons

General phylogenetic analyses based on the 16S rDNA sequences of the novel strains (Fig. 2) revealed that they were members of the *Gammaproteobacteria*. More-detailed analyses showed that the strains isolated from the Baltic Sea formed a robust cluster with *I. abyssalis* and *I. zobellii*, of the genus *Idiomarina*. As the Baltic Sea strains were clearly an outgroup to the genus *Idiomarina*, and according to phylogenetic principles, they could be described as a novel species of the genus *Idiomarina* or they could be assigned to a novel genus. Phenotypic traits, especially their fatty acids profiles, suggested that strains OS145T and OS146 could be included in the genus *Idiomarina*. Sequences of strains originating from the Atlantic (i.e. TAG C7 and DIII1c) clustered most closely with the novel strains from the Baltic Sea (Fig. 2).

16S rDNA sequence similarity of strain OS145T with strain OS146 was 99.2 %. Sequence similarity of the two strains with related sequences was highest, in terms of validly described species, with *I. zobellii* and *I. abyssalis* (96.8–95.9 %), both of which were isolated from the northwest Pacific Ocean (at depths of 4000–5000 m) (Ivanova et al., 2000). The highest similarity (98.5 % with OS145T) for a submitted sequence was obtained for strain TAG C7, a halophilic bacterium isolated from the TAG hydrothermal mound of the Mid-Atlantic Ridge (at a depth of 3700 m). Another strain that showed high 16S rDNA sequence similarity with OS145T (98.3 %) was strain DIII1c, isolated from the New England Shelf sediment (at a depth of 1500 m) (Teske et al., 2000). Isolates and sequences derived from the deep sea of the Pacific Ocean showed a lower level of 16S rDNA similarity with the two Baltic Sea strains, which ranged from 97.1 to 96.5 %, e.g. ‘*Idiomarina lothiensis*’, a strain derived from the hydrothermal fluids of a submarine volcano at Hawaii, and
sequences from northwest Pacific Ocean deep-sea sediments (BD1-9, BD6-1 and BD4-11; from depths of 1160–6300 m) (Li et al., 1999) and the Mariana Trench (Mariana eubacterium no. 1; from a depth of 11 000 m) (Kato et al., 1997). Most of the Pacific Ocean strains and clones clustered more closely with each other than with the ‘Atlantic branch’ represented by strains OS145T and OS146 (Fig. 2).

DNA–DNA hybridization between strains OS145T and OS146 showed 81.6 % DNA similarity (at 66 °C, 2x SSC), indicating that both strains were members of the same species (Wayne et al., 1987). This finding is consistent with 16S rDNA sequence analyses. Therefore, the two strains can be perceived as being members of the same species.

The DNA G+C content values determined for strains OS145T and OS146 were 49.9 and 49.4 mol%, respectively (Table 1). The DNA G+C content values determined for the related species I. abyssalis and I. zobellii were 50 and 48 mol%, respectively, which were within the range of the two novel strains described here.

**Cellular fatty acid profiles and changes with temperature and salinity**

An important biochemical feature of members of the genus *Idiomarina* is their high percentage of iso-branched fatty acids, which differentiate them from related Gammaproteobacteria (Table 2). The dominant fatty acids for cells of strains OS145T and OS146 grown at 28 °C were C15:0 iso (31.3–36.9%), C16:1ω7c (8.4–11.0%), C17:0 iso (9.4–11.2%), C17:1ω7c (5.6–10.0%), C18:1ω9c (6.0–9.7%) and C16:0 (4.8–9.7%). These profiles compare well with those of *I. abyssalis* and *I. zobellii*, with the only major difference being that C17:1ω7c was not observed in *I. abyssalis* and *I. zobellii*. The cellular fatty acids of all *Idiomarina* species were dominated by iso-branched fatty acids (OS145T, 73.1%; OS146, 57.6%). When iso-branched

---

**Table 1.** Features that can be used to differentiate strains OS145T and OS145 from *I. abyssalis* and *I. zobellii*

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell shape</td>
<td>Curved rod</td>
<td>Curved rod</td>
<td>Rod</td>
<td>Rod</td>
</tr>
<tr>
<td>DNA G+C content (mol%)</td>
<td>49.9</td>
<td>49.4</td>
<td>50</td>
<td>48</td>
</tr>
<tr>
<td>Growth temp. range (°C)</td>
<td>8–46</td>
<td>10–46</td>
<td>4–30</td>
<td>4–30</td>
</tr>
<tr>
<td>Optimum growth temp. (°C)</td>
<td>30–40</td>
<td>30–40</td>
<td>20–22</td>
<td>20–22</td>
</tr>
<tr>
<td>Utilization of substrates*</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

* Determined using the BIOLOG GN2 system.
Table 2. Cellular fatty acid composition (%) of strains OS145T and OS146 grown at different temperatures and salinities in comparison to other *Idiomarina* species

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Temperature (°C)</th>
<th>Salinity (% NaCl)</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10</td>
<td>28</td>
<td>40</td>
<td>40</td>
<td>10</td>
<td>28</td>
</tr>
<tr>
<td>Straight-chain fatty acids</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C10:0 3-0H</td>
<td>2 06</td>
<td>1 16</td>
<td>0 95</td>
<td>0 66</td>
<td>2 77</td>
<td>1 91</td>
</tr>
<tr>
<td>C16:0</td>
<td>5 06</td>
<td>4 81</td>
<td>4 87</td>
<td>3 73</td>
<td>9 29</td>
<td>9 70</td>
</tr>
<tr>
<td>C17:0 cyclo</td>
<td>0 61</td>
<td>1 19</td>
<td>2 72</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C18:0</td>
<td>0 73</td>
<td>0 94</td>
<td>0 82</td>
<td>1 18</td>
<td>0 65</td>
<td>1 83</td>
</tr>
<tr>
<td>Terminal-branched fatty acids</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C11:0 iso</td>
<td>3 09</td>
<td>2 48</td>
<td>3 03</td>
<td>2 97</td>
<td>2 95</td>
<td>2 22</td>
</tr>
<tr>
<td>C11:0 iso 3-0H</td>
<td>3 50</td>
<td>3 65</td>
<td>4 61</td>
<td>4 56</td>
<td>2 3</td>
<td>2 74</td>
</tr>
<tr>
<td>C13:0 iso</td>
<td>0 83</td>
<td>0 81</td>
<td>0 77</td>
<td>1 19</td>
<td>0 82</td>
<td>0 60</td>
</tr>
<tr>
<td>C15:0 iso 3-0H</td>
<td>4 22</td>
<td>3 22</td>
<td>3 64</td>
<td>3 36</td>
<td>3 35</td>
<td>3 22</td>
</tr>
<tr>
<td>C15:0 iso</td>
<td>27 80</td>
<td>36 88</td>
<td>41 98</td>
<td>39 48</td>
<td>30 52</td>
<td>31 31</td>
</tr>
<tr>
<td>C15:1 iso F</td>
<td>4 31</td>
<td>1 53</td>
<td>1 69</td>
<td>2 53</td>
<td>4 11</td>
<td>0 92</td>
</tr>
<tr>
<td>C17:0 iso</td>
<td>5 29</td>
<td>11 21</td>
<td>12 97</td>
<td>14 74</td>
<td>2 78</td>
<td>9 44</td>
</tr>
<tr>
<td>C17:1 iso o9c</td>
<td>12 65</td>
<td>9 99</td>
<td>8 96</td>
<td>9 47</td>
<td>7 39</td>
<td>5 62</td>
</tr>
<tr>
<td>Mono-unsaturated fatty acids</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C15:1 o9c</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1 30</td>
</tr>
<tr>
<td>C16:1 o7c</td>
<td>17 91</td>
<td>8 37</td>
<td>5 83</td>
<td>4 00</td>
<td>21 19</td>
<td>11 01</td>
</tr>
<tr>
<td>C17:1 o9c</td>
<td>0 39</td>
<td>0 71</td>
<td>0 74</td>
<td>0 61</td>
<td>1 06</td>
<td>1 04</td>
</tr>
<tr>
<td>C17:1 o9c</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1 50</td>
</tr>
<tr>
<td>C18:1 iso o9c</td>
<td>12 65</td>
<td>9 99</td>
<td>8 96</td>
<td>9 47</td>
<td>7 39</td>
<td>5 62</td>
</tr>
<tr>
<td>C18:1 iso o7c</td>
<td>3 91</td>
<td>4 98</td>
<td>2 95</td>
<td>1 46</td>
<td>1 96</td>
<td>9 17</td>
</tr>
<tr>
<td>C19:1 iso o9c</td>
<td>0 39</td>
<td>0 54</td>
<td>0 55</td>
<td>0 91</td>
<td>0 87</td>
<td>0 52</td>
</tr>
<tr>
<td>mono-Branched (% total fatty acids)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>62 9</td>
<td>73 1</td>
<td>80 4</td>
<td>80 0</td>
<td>54 4</td>
<td>57 6</td>
<td>64 8</td>
</tr>
<tr>
<td>Mono-unsaturated (% total fatty acids)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>40 9</td>
<td>28 7</td>
<td>20 2</td>
<td>17 28</td>
<td>37 2</td>
<td>31 2</td>
<td>26 3</td>
</tr>
</tbody>
</table>

and mono-unsaturated fatty acids are summarized, they contribute to 83–91 % of the total fatty acid composition of strains OS145T and OS146. A high contribution of iso-branched fatty acids has previously only been seen for the *Xanthomonas*-branch *Proteobacteria* (Finkmann et al., 2000), and has not been observed for genera closely related to the genus *Idiomarina*. Therefore, due to their characteristic fatty acid patterns, a closer relationship between the recognized *Idiomarina* species and strains OS145T and OS146 can be assumed.

Since adaptation to temperature and salinity is often connected to a change in the fatty acid composition of bacteria (Klein et al., 1999), the composition of the fatty acids of strains OS145T and OS146 was analysed after cultivation at lower (10 °C) and higher (40 °C) temperatures in comparison to the standard cultivation at 28 °C (Table 2). At 10 °C, in both strains the proportion of iso-branched fatty acids decreased while the proportion of mono-unsaturated fatty acids increased, with C16:1 o7c showing a doubling of its contribution. At 40 °C, the proportion of iso-branched fatty acids increased and the proportion of mono-unsaturated fatty acids decreased. The response of the fatty acid composition to temperature was more pronounced for strain OS145T than for strain OS146, especially for the iso-branched fatty acids. With an increase in the growth temperature from 10 to 40 °C, the proportion of iso-branched fatty acids increased from 63 to 80 % for strain OS145T and from 54 to 65 % for strain OS146, respectively. For the same temperature range, the mono-unsaturated fatty acids decreased from 41 to 20 % for strain OS145T and from 37 to 26 % for strain OS146. By contrast, different salinities (1-7, 3-4 and 10 % NaCl) did not have a major effect on the composition of the fatty acids [Table 2, and supplementary data available in IJSEM Online (http://ijis.sgmjournals.org)].

In terms of the response of their mono-unsaturated fatty acids, OS146 can be assumed.
acids to temperature, strains OS145T and OS146 are comparable to other marine *Gammaproteobacteria* such as *Shewanella*, i.e., the fraction of mono-unsaturated fatty acids increases with decreasing temperatures (Bozal *et al.*, 2002). Temperature effects on the fatty acid compositions of the *Xanthomonas*-branch *Proteobacteria* have been reported for *Stenotrophomonas maltophilia* and a narrow temperature range of 30–37 °C (Rahmati-Bahram *et al.*, 1995). The latter study showed a temperature response of the iso-branched and mono-unsaturated fatty acids comparable to our findings. The bacilli can be compared to our strains as they are a well-studied group with a comparable temperature range and a high fraction of iso-branched fatty acids. For bacilli, an increase in the proportion of anteiso-branched fatty acids is considered a major factor allowing adaptation to lower temperatures; this phenomenon has been also been observed for other low-GC Gram-positive organisms (Announ *et al.*, 1997, Klein *et al.* 1999). By contrast, in strains OS145T and OS146, anteiso-branched fatty acids occurred only at very low concentrations (<1%) at all culture conditions. As a response to high salinities, bacilli decrease the fraction of iso-branched fatty acids (Lopez *et al.*, 1998), which contrasts with our findings for strains OS145T and OS146. Thus, strains OS145T and OS146 have a fatty acid spectrum that is different from other *Gammaproteobacteria*, but the temperature response of their fatty acids is most comparable to *S. maltophilia*.

**Conclusion**

Phylogenetic analyses based on 16S rDNA sequences, DNA–DNA homology, physiological features and fatty acid profiles suggest that strains OS145T and OS146 belong to the genus *Idiomarina* and represent a novel species of this genus. The name *Idiomarina baltica* is proposed for the novel species (type strain OS145T). Physiological features that differentiate I. *baltica* from recognized *Idiomarina* species are the different temperature range for growth and the spectrum of utilizable substrates. All *Idiomarina* species have a comparable pattern of fatty acids dominated by iso-branched fatty acids, with *I. baltica* also having C17:1 iso (ω9c-2).

**Description of *Idiomarina baltica* sp. nov.**

*Idiomarina baltica* (bal.ti’ca. N.L. fem. adj. *baltica* from the Baltic sea, referring to the source of the type strain).

Cells are Gram-negative and polarly flagellated, with one flagellum. Slightly curved rods, with dimensions 0.4–0.7 × 0.7–1.6 μm. Oxidase- and catalase-positive. Colonies are circular, smooth and non-pigmented to slightly yellowish on marine agar. Aerobic and chemoheterotrophic. Grows between 8 and 46 °C, with optimum growth between 30 and 40 °C. NaCl is needed for growth; tolerant towards high salinities (>6% NaCl) and shows optimal growth in the presence of between 3 and 6% NaCl. Dominant fatty acids (>5%) are C15:0 iso, C16:1 ω7c, C17:0 iso, C17:1 iso ω9c, and C18:1 ω7c. Utilizes Tween 40, Tween 80, L-arabinose and acetic acid (BIOLOG GN2). Positive for β-glucosidase, protease (API 20NE), alkaline phosphatase, acid phosphatase, esterase (C4 and C8), leucine arylamidase, valine arylamidase, cysteine arylamidase, chymotrypsin, naphtholphosphohydrolase (API ZYM) and aminopeptidase activities. Hydrolyses Tween 80 and gelatin, but not starch or lecithin. Acid is produced from D-glucose, but not from D-ribose or D-arabinose. Produces H2S from protein. Does not hydrolyse bovine blood. Shows no chitinase activity. Does not utilize fumarate or succinate. Does not grow under anoxic conditions, nor in the presence of nitrate, nitrite and sulfite, trimethylaminoxide, ferric iron or thiosulfate as electron acceptors.

Of marine or estuarine origin. The mean DNA G+C content is 49.7 mol%. The type strain of *Idiomarina baltica* is OS145T (=DSM 15154T =LMG 21691T).

**ACKNOWLEDGEMENTS**

J. Bötel is acknowledged for excellent technical assistance. The support of the scientific and technical crew of RV Poseidon in August 1986 is greatly acknowledged. Special thanks to G. Rheinheimer, J. Wesnigk, R. Schmaljohann and H. Sell. Thanks to H. Lünsdorf and E. Barth for electronic microscopy of the strains. The analytical services of the DSMZ (Deutsche Sammlung von Mikroorganismen und Zellkulturen, Braunschweig, Germany) are greatly acknowledged. Many thanks to S. Verbarg, R. M. Kroppenstedt and P. Schumann and their staff. Many thanks for the excellent support of A. Fröhling. D. Kirchman is acknowledged for providing data on chitinase activities. This work was part of the EU project ‘Marine Bacterial Genes and Isolates as Sources for Novel Biotechnological Products’ (MARGENES). The project was funded by the Marine Science and Technology (MAST III) programme of the European Commission (contract no. MAS3-CT97-0125).

**REFERENCES**


