**Marinobacter aquaeolei sp. nov., a halophilic bacterium isolated from a Vietnamese oil-producing well**

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Several strains of moderately halophilic and mesophilic bacteria were isolated at the head of an oil-producing well on an offshore platform in southern Vietnam. Cells were Gram-negative, non-spore-forming, rod-shaped and motile by means of a polar flagellum. Growth occurred at NaCl concentrations between 0 and 20%; the optimum was 5% NaCl. One strain, which was designated VT8T, could degrade n-hexadecane, pristane and some crude oil components. It grew anaerobically in the presence of nitrate on succinate, citrate or acetate, but not on glucose. Several organic acids and amino acids were utilized as sole carbon and energy sources. The major components of its cellular fatty acids were C12:0 3-OH, C16:1 09c, C16:0 and C18:1 09c. The DNA G+C content was 55.7 mol%. 16S rDNA sequence analysis indicated that strain VT8T was closely related to Marinobacter sp. strain CAB (99.8% similarity) and Marinobacter hydrocarbonoclasticus (99.4% similarity). Its antibiotic resistance, isoprenoid quinones and fatty acids were similar to those of Marinobacter hydrocarbonoclasticus and Pseudomonas nautica. However, the whole-cell protein pattern of VT8T differed from that of other halophilic marine isolates, including P. nautica. DNA-DNA hybridization indicated that the level of relatedness to Marinobacter hydrocarbonoclasticus was 65% and that to P. nautica was 75%. Further differences were apparent in Fourier-transformed IR spectra of cells and lipopolysaccharide composition. It is proposed that VT8T should be the type strain of a new species and should be named *Marinobacter aquaeolei*. *P. nautica* may have been misclassified, as suggested previously, and may also belong to the genus *Marinobacter*.

**Keywords**: *Marinobacter aquaeolei*, 16S rRNA gene analysis, Fourier-transformed IR spectroscopy, lipopolysaccharide, *Pseudomonas nautica*

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**INTRODUCTION**

The oil/gas fields are new and special ecosystems because of their physico-chemical and geochemical conditions. Environmental parameters such as temperature, pressure, pH, salinity, heavy metal concentration and petroleum composition vary widely from reservoir to reservoir (Bernard et al., 1992; Bhupathiraju et al., 1993). Recently, several reports have described halophilic bacteria obtained from such environments (Adkins et al., 1993; Dang et al., 1996; Tardy-Jacquenod et al., 1996a, b). In our microbial investigation of offshore oil/gas fields near the coastal town of Vung Tau in southern Vietnam, several novel micro-organisms have been isolated, including halophilic sulfate-reducing bacteria (Dang et al., 1996). In this study, properties of a new moderately halophilic aerobic isolate (strain VT8T) are reported which, on the basis of genotypic and phenotypic analyses, should be placed in the recently created genus *Marinobacter* (Gauthier et al., 1992). Strain VT8T shared several phenotypic properties with *Marinobacter hydrocarbonoclasticus* and *Pseudomonas nautica*, but
differences in whole-cell protein pattern, Fourier-transformed IR (FT-IR) spectra and lipopolysaccharide (LPS) composition justified its designation as a distinct species. It has been pointed out by several authors that *P. nautica* could be clearly separated from other *Pseudomonas* species, i.e. that it was probably misclassified (De Ley, 1992; De Vos et al., 1989; Zumft, 1992). Our data suggest that *P. nautica* may also belong to the genus *Marinobacter*.

**METHODS**

**Isolation, cultivation and maintenance of bacterial strains.** Samples were collected at the head of an oil-producing well on the offshore oil/gas platform near the coastal town of Vung Tau in southern Vietnam. For enrichments and isolation, a culture medium similar to that described in American Petroleum Institute Research Publication 38 (1975) was used. It contained (l−1 distilled water): peptone, 10 g; meat extract, 1 g; glucose, 5 g; NaCl, 100 g; MgCl₂.6H₂O, 3 g; and phenol red, 0.018 g. The pH was adjusted to 6.9−7.3 with NaOH before autoclaving. When necessary, agar was added at 2% (w/v). Samples of 2 ml each were inoculated into 12 ml vials containing 7 ml liquid medium. Following growth of the community by incubation at ambient temperature (30−37 °C), samples were transferred to agar plates by spreading. Single colonies were picked and purified further by streaking repeatedly on agar plates. Pure cultures were maintained by suspending cells in a small volume of 25% glycerol and 5% NaCl in screw-capped plastic vials and keeping them frozen at −70 °C.

**Reference strains.** The following strains were used for biochemical and morphological comparisons and obtained from the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ): *Halomonas elongata* DSM 2581\(^T\), *Marinobacter hydrocarbonoclasticus* DSM 8798\(^T\), *Marinomonas communis* DSM 5604, *Marinomonas vagina* DSM 5605\(^T\), *Pseudomonas nautica* DSM 50418\(^T\), *Salinivibrio (former* *ly Vibriod* *costicola* DSM 11403\(^T\). Sequence data from the following additional strains were used for phylogenetic comparisons (see also Fig. 4): *Marinobacter* sp. strain CAB, *Marinobacterium georgiense* ATCC 700074\(^T\), *Oceanospirillum japonicum* ATCC 19191, *Oceanospirillum commune* ATCC 27118, *Oceanospirillum jannaschii* ATCC 27135\(^T\), Str. clone SAR92 (Sargasso Sea bacterioplankton), Str. clone agg. 53 (marine snow-associated), *Chromohalobacter marismortui* DSM 6770\(^T\) and *Piscirickettsia salmonis* ATCC VR-1361.

**Nutritional and growth characteristics.** Marine broth 2216 (ZoBell, 1941; Difco), *Halomonas* complex (HMC) or defined (HMD) medium (Vreeland & Martin, 1980; Vreeland et al., 1980) with slight modifications were used. HMC medium contained (l−1 distilled water): Casamino acids (Difco), 7−5 g; peptone (Roth), 5 g; yeast extract (Difco), 1 g; NaCl, 50 g; MgSO₄.7H₂O, 20 g; sodium chloride, 3 g; K₂HPO₄, 0−5 g; and FeSO₄·(NH₄)₂SO₄·6H₂O, 0.05 g. The pH was adjusted to 7.3 with KOH or HCl before autoclaving. HMD medium contained (l−1 distilled water): NaCl, 50 g; MgCl₂.6H₂O, 5.3 g; KCl, 0.75 g; FeSO₄·(NH₄)₂SO₄·6H₂O, 0.04 g; K₂HPO₄, 0.87 g; (NH₄)₂SO₄, 4 g. When required, agar was added at 2% (w/v) concentration. Cultures in liquid media were grown in 100 ml side-arm flasks containing 20 ml medium. Growth was monitored by measuring OD₆oo in a Novaspec II instrument (Pharmacia). The temperature range for growth was between 4 and 55 °C in HMC medium. The requirement for NaCl was determined in the same medium, which was supplemented with 0−32% NaCl (w/v). The pH range for growth was determined in Marine broth 2216, which was supplemented with 50 g NaCl and whose pH was adjusted to 4.0−10.0 with HCl or KOH. The utilization of carbohydrates as sole carbon and energy sources was tested in HMD medium (Vreeland & Martin, 1980), which was supplemented with 5% NaCl and whose pH was adjusted to 7.3 with KOH. Carbohydrates were added at 10 mM final concentration. The utilization of pristane (2,6,10,14-tetramethylpentadecane) and n-hexadecane (both from Sigma) was tested by inoculating strain VT8\(^T\) into HMD medium, which was supplemented with 1% (v/v) of the hydrocarbon compounds. Growth was scored by measuring OD₆oo. Degradation of crude oil fractions was tested by inoculating strain VT8\(^T\) into mineral medium, which contained (1-1 tap water): KNO₃, 3 g; NaCl, 50 g; MgSO₄.7H₂O, 0.4 g; KH₂PO₄, 0.3 g; Na₂HPO₄, 0.7 g. Crude oil (5%, v/v) from White tiger oil-field (Vietnam) was added and incubation proceeded for 7 d at 30 °C. The residual oil was analysed by GC. Control experiments were performed under identical conditions, but omitting the bacterial inoculum.

**Ion specificity determination.** KCl, LiCl and NH₄Cl were substituted for NaCl at concentrations of 0.85 M in HMC medium to test for a specific ion requirement.

**Phenotypic analysis.** Standard tests (Gram staining, cytochrome oxidase, catalase, gelatinase) were performed as described previously (Smibert & Krieg, 1994). The Analytical Profile Index system (API 20NE and API ZYM; bioMérieux) was used for analysis of additional enzyme activities (Humble et al., 1977) and for assimilation tests, respectively. Strips were inoculated with a bacterial suspension in minimal medium and incubated for up to 2 weeks. Antibiogram susceptibility was tested by spreading bacterial suspensions on agar plates and applying filter-paper disks (Schleicher and Schüll; 6 mm diameter) on which the following antibiotics were dispensed (µg per disk shown in parentheses): ampicillin (20), anisomycin (15), bacitracin (20), chloramphenicol (30), erythromycin (15), gentamicin (30), kanamycin (30), novobiocin (30), oleandomycin (25), streptomycin (20) and tetracycline (20). Zones of inhibition were measured after 2 d incubation at 30 °C.

**Analysis of isoprenoid quinones.** Lipoquinones were extracted from lyophilized cells with methanol:hexane (2:1, v/v) as described by Tindall (1990). The hexane phase contained the lipoquinones, which were separated by TLC on silica gel plates (Kieselgel F₂₅₄; Merck), using hexane:diethylether (85:15, v/v) as the developing solvent, and compared with lipoquinones of type strains based on Rₚ values (Collins, 1994).

**Electron microscopy.** Cells were harvested following 1 d incubation in HMC medium and prepared for scanning (Denner et al., 1994) or transmission electron microscopy (Dang et al., 1996) as described previously.

**Cellular fatty acid analysis.** Fatty acid methyl esters were prepared and analysed by GC as described by Kuykendall et al. (1988). This work was performed by R. Kroppenstedt, DSMZ, Braunschweig, Germany.

**Whole-cell protein pattern analysis.** SDS-PAGE of whole-cell proteins was performed as described previously (Stan-Lotter et al., 1989). Briefly, approximately 50 mg cells (wet
weight) ml⁻¹ were lysed by boiling in SDS sample buffer (Laemmli, 1970) for 10 min, and then centrifuged at 10000 g for 15 min, to remove any precipitates. The gel system of Laemmli (1970) was used. Proteins were visualized by staining with Coomassie blue. Marker proteins of 25–200 kDa were from Novex. Protein gels were run at least in triplicate.

Preparation of cell envelopes and analysis of LPS. Cell envelopes were prepared by passing cells through a French press and subsequent centrifugation at 20000 g as described previously (Stan-Lotter & Sanderson, 1981), except that a buffer consisting of 50 mM Tris/HCl, 10 mM MgCl₂, pH 7.4, was used in all operations. Cell envelopes were suspended in SDS sample buffer (Laemmli, 1970) and 0·25% proteinase K (Sigma) was added (Selma et al., 1994). Following digestion at 37 °C for 1 h, samples were separated on the same gel system as that used for whole-cell proteins and stained with silver (Bio-Rad kit) according to the manufacturer’s instructions.

FT-IR spectrometry. Bacterial strains were streaked on agar plates containing HMC medium and incubated overnight. Bacterial films were prepared by suspending one loopful (1 mm diameter) in 80 µl distilled water and applying an aliquot of 35 µl to a preformed sample area on a zinc selenite optical plate. Following drying of samples under reduced pressure, spectra were recorded between wave numbers 4000 and 500 cm⁻¹ in an FT-IR spectrometer type IFS 28/B (Bruker) as described by Helm et al. (1991a, b). Data analysis was carried out using the OPUS 3·0 software for bacterial identification from the same manufacturer. At least six independent preparations of each investigated strain were measured and a mean spectrum was calculated.

DNA base composition. Cells were harvested in the late exponential phase of growth and G+C contents were determined by the Identification Service of the DSMZ. DNA was isolated by the procedure of Visuvanathan et al. (1989) and was analysed by HPLC according to Mesbah et al. (1984) and Tamaoka & Komagata (1984).

DNA–DNA hybridization. DNA was isolated as described by Cashion et al. (1977). Levels of DNA–DNA hybridization between isolate VT8 and two type strains were determined spectrophotometrically by the renaturation method of De Ley et al. (1970), with modifications by Hubs et al. (1983) and Escara & Hutton (1980). Renaturation rates were computed by the program TRANSFER.BAS (Jahnke, 1992). These experiments were carried out by the Identification Service of the DSMZ.

16S rRNA sequence analysis. Sequencing of the 16S rRNA gene was carried out by C. Spröer at the DSMZ (see under DNA base composition). Extraction of the genomic DNA, amplification of the 16S rDNA by PCR and purification of the products were performed as described by Rainey et al. (1996). The purified 16S rDNA was sequenced using the ABI PRISM Dye Terminator Cycle Sequencing Ready Reaction kit (Applied Biosystems) as indicated by the manufacturer. The DNA fragments of the sequencing reactions were separated and analysed using an Applied Biosystems 373 DNA Sequencer. The resulting sequences were read into the Alignment Editor ae2 (Maidak et al., 1996), aligned manually and compared with 16S rRNA gene sequences of representative organisms of the gamma subclass of the Proteobacteria. Sequences which were used for comparisons were obtained from the EMBL or the RDP (Maidak et al., 1996) databases.

RESULTS

Cellular and colonial morphology

Several rod-shaped isolates were obtained from the head of an oil-producing well on the offshore oil/gas platform at Vung Tau; all were Gram-negative, formed yellowish colonies on HMC medium and exhibited a wide range of NaCl tolerances. Strain VT8 was characterized further; it was a short rod-shaped bacterium of 0·4–0·5 µm in diameter and 1·4–1·6 µm in length (Fig. 1). It was characteristically found growing singly or in pairs. Cells were actively motile; transmission electron microscopy (not shown) revealed a single, frequently polar flagellum, which is also visible in some areas of Fig. 1. Spore formation was not observed under any of the growth conditions described in this work. Colonies on HMC medium containing 5% NaCl were yellow, circular, convex, smooth with entire margins and 1·5–2·0 mm in diameter after 2 d cultivation at 30 °C.

Cultural and biochemical characteristics

Strain VT8 grew at 0·0–3·4 M NaCl and growth was optimal at 0·85 M NaCl. K⁺, Li⁺ or NH₄⁺ ions were not able to replace Na⁺ ions. The optimum growth temperature was 30 °C. The temperature range for growth was 13–50 °C; no growth occurred at 55 or 4 °C. The pH range for growth was 5–10 with an optimum pH of 7·3. Growth was aerobic; catalase and oxidase activities were present. VT8 could grow anaerobically with nitrate on succinate, citrate or acetate, but not on glucose. DL-Lactate, acetate, succinate, fumarate, citrate, l-glutamate and butyrate could be used as sole carbon and energy sources, but not D(-)-arabinose, D(+)-fructose, D(+)-galactose, D(+)-glucose, D(+)-mannose, cellobiose, lactose, melibiose, sucrose, maltose, raffinose, glycerol, mannitol, sorbitol, benzoate, glycerate, oxalate, salicin, N-acetylglucosamine or amino acids (except l-Pro, l-Leu, l-Ile, l-Gln, l-Glu and l-Ala). On HMC medium,

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**Fig. 1.** Scanning electron micrograph of strain VT8 grown on Halomonas complex medium containing 5% NaCl. Bar, 1 µm.
Table 1. Fatty acid compositions of strain VT8\textsuperscript{T}, Marinobacter hydrocarbonoclasticus and P. nautica

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Total fatty acid (%) in:</th>
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<tbody>
<tr>
<td></td>
<td>Strain VT8\textsuperscript{T}</td>
</tr>
<tr>
<td>10:0</td>
<td>0.57</td>
</tr>
<tr>
<td>12:0</td>
<td>7.89</td>
</tr>
<tr>
<td>11:0 3-OH</td>
<td>0.62</td>
</tr>
<tr>
<td>13:0</td>
<td>0.62</td>
</tr>
<tr>
<td>12:0 3-OH</td>
<td>9.94</td>
</tr>
<tr>
<td>14:0</td>
<td>2.60</td>
</tr>
<tr>
<td>15:0</td>
<td>2.24</td>
</tr>
<tr>
<td>16:0 iso</td>
<td>0.38</td>
</tr>
<tr>
<td>16:1 \omega 9c</td>
<td>11.62</td>
</tr>
<tr>
<td>16:1 \omega 5c</td>
<td>0.46</td>
</tr>
<tr>
<td>16:0</td>
<td>22.63</td>
</tr>
<tr>
<td>16:0 10-methyl</td>
<td>2.84</td>
</tr>
<tr>
<td>17:1 \omega 8c</td>
<td>4.54</td>
</tr>
<tr>
<td>17:0</td>
<td>3.78</td>
</tr>
<tr>
<td>17:0 10-methyl</td>
<td>0.46</td>
</tr>
<tr>
<td>18:1 \omega 9c</td>
<td>19.82</td>
</tr>
<tr>
<td>18:0</td>
<td>1.44</td>
</tr>
<tr>
<td>Summed feature 4*</td>
<td>6.78</td>
</tr>
<tr>
<td>Summed feature 7*</td>
<td>0.77</td>
</tr>
</tbody>
</table>

* Summed features are groups of fatty acids which were not resolved chromatographically; they consisted of fatty acids \(16:1 \omega 7t\) and \(15:0 \text{iso} 2-\text{OH}\) (feature 4) and isomers \(18:1 \omega 7c\), \(18:1 \omega 9t\) and \(18:1 \omega 12t\) (feature 7), respectively.

Strain VT8\textsuperscript{T} was susceptible to the following antibiotics (diameter of inhibition zone is given in mm): bacitracin (12), chloramphenicol (22), erythromycin (18), gentamicin (13), kanamycin (10) and streptomycin (9); it was resistant to ampicillin, anisomycin, novobiocin, oleandomycin and tetracycline. Antibiotic susceptibility results for Marinobacter hydrocarbonoclasticus and P. nautica were similar to those of strain VT8\textsuperscript{T}. The following enzymes were present in strain VT8\textsuperscript{T} when assayed with the API ZYM system: alkaline phosphatase, esterase (C4), lipase (C8), leucine arylamidase, valine arylamidase, acid phosphatase, naphthol AS-BI phosphohydrolase and \(N\)-acetyl-\(\beta\)-glucosaminidase. These enzymes were also found in P. nautica. The following enzymes were not present in either strain VT8\textsuperscript{T} or P. nautica when assayed for with the API ZYM system: cystine arylamidase, trypsin, chymotrypsin, \(\alpha\)-galactosidase, \(\beta\)-galactosidase, \(\beta\)-glucuronidase, \(\alpha\)-glucosidase, \(\alpha\)-mannosidase or \(\alpha\)-fucosidase. The following tests were positive when assayed with the API 20NE system: nitrate reduction to nitrite, urease activity and gelatin liquefaction. Except for urease activity, these tests were also positive for P. nautica. The following tests were negative for both VT8\textsuperscript{T} and P. nautica when assayed for with the API 20NE system: nitrite reduction to \(N_2\), indole production, glucose oxidation, glucose fermentation, arginine dihydrolase and aesculin hydrolysis.

Utilization of hydrocarbons and crude oil

Strain VT8\textsuperscript{T} was able to utilize pristane, \(n\)-hexadecane and crude oil components as sole carbon sources. Preliminary results showed that saturated hydrocarbons were reduced by 41\%, aromatic hydrocarbons by 52\% and asphaltene by 20\% over a course of 7 d shaking at 30 °C in minimal medium (Dang T. C. H. & Nguyen B. H., unpublished data).

Isoprenoid quinones

The lipoquinones of strain VT8\textsuperscript{T} were compared with lipoquinones of P. nautica and Marinobacter hydrocarbonoclasticus, following separation by TLC on silica gel. All three strains displayed similar levels of fluorescence quenching bands with \(R_f\) values of about 0.3 (data not shown); this indicated the presence of ubiquinones, which are typical for many Gram-negative bacteria and are clearly distinct from menaquinones, which occur predominantly in Gram-positive bacteria and archaea, and whose \(R_f\) values are about 0.7–0.8 (Collins, 1994).

Fatty acid composition

The fatty acid compositions of VT8\textsuperscript{T} isolate and the reference strains are shown in Table 1. The major fatty acids of strain VT8\textsuperscript{T} were \(C_{12:0} 3\text{-OH}\), \(C_{16:1} \omega 9c\), \(C_{16:0} \).
Marinobacter aquaeolei sp. nov.

The results of cellular fatty acid analysis confirmed that strain VTST, *Marinobacter hydrocarbonoclasticus* and *P. nautica* had similar profiles in general, but strain VTST differed from both strains by lower levels of the fatty acids C\(_{16:1}\) \(\omega 9c\) and C\(_{18:1}\) \(\omega 9c\).

**Gel electrophoresis of whole-cell proteins**

SDS-PAGE of whole-cell proteins is a rapid method for distinguishing bacterial species and has a similar level of discrimination to DNA–DNA hybridization (Jackman, 1987). Bacterial cells which are grown under identical conditions produce constant protein patterns which greatly facilitate the identification of strains under investigation (Vauterin et al., 1993). This method has been widely used in the systematics of numerous bacterial strains (Kersters & De Ley, 1980); Gram-negative bacteria with very similar protein patterns in particular exhibit unusually high DNA–DNA reassociation values (Vandamme et al., 1997, 1998, and references therein). Cells of strain VTST were compared with several type strains of the gamma Proteobacteria with similar morphological and biochemical characteristics (Fig. 2). Strain VTST showed a unique protein profile which did not resemble the profiles of *H. elongata*, *C. marismortui*, *Marinobacter hydrocarbonoclasticus*, *Marinomonas vaga*, *Marinomonas communis*, *S. costicola* or *P. nautica*.

**FT-IR spectrometry**

FT-IR spectra of bacteria are recorded from whole cells, without any preparation other than drying, and provide fingerprint-like, highly reproducible patterns which can distinguish strains at the subspecies level (Helm et al., 1991a; Naumann et al., 1991). The spectra of strain VTST and *P. nautica* showed differences in terms of prominent additional peaks or shoulders between wave numbers 1200 and 900 (data not shown); this window represents the polysaccharide...
region (Helm et al., 1991a). Some additional differences between the two isolates were apparent between wave numbers 900 and 700 cm⁻¹; according to Helm et al. (1991a), this region is the true fingerprint region, where remarkable spectral patterns often become apparent, although the chemical groups responsible for the spectra in this region have not yet been assigned.

LPS content
Since FT-IR spectra indicated possible differences in the polysaccharide composition of strains VT8ᵀ and P. nautica, the LPS content of these strains was examined by SDS-PAGE (Fig. 3). The LPS patterns from strain VT8ᵀ, P. nautica and Marinobacter hydrocarbonoclasticus appeared as the typical series of regularly spaced bands, which represent LPS molecules with varying lengths of O-linked side-chains and where different distances between the bands are due to different repeating sugar units in the polysaccharide chains (Palva & Mäkelä, 1980). While the LPS from Marinobacter hydrocarbonoclasticus and P. nautica (Fig. 3, lanes 2 and 3) showed a wide range of chain lengths, albeit with different spacing, that from strain VT8ᵀ was apparently more homogeneous (Fig. 3, arrow); in addition, the distances between bands in the LPS of strain VT8ᵀ were shorter than those of the LPS from the other two strains, suggesting less sugar molecules per repeating unit. While not strictly proven, these results would be consistent with the observed differences in the polysaccharide region of the FT-IR spectra of P. nautica and strain VT8ᵀ.

G + C content
The DNA G+C content of VT8ᵀ was 55.7 ± 0.1 mol%. This value was higher than that of Marinobacter hydrocarbonoclasticus (52.7 mol%; Gauthier et al., 1992) and lower than that of P. nautica (59.2%; De Vos et al., 1989).

DNA–DNA hybridization
The measurement of DNA–DNA hybridization showed the following homology values: 65% between strain VT8ᵀ and Marinobacter hydrocarbonoclasticus; 75.4% between strain VT8ᵀ and P. nautica; 85.1% between Marinobacter hydrocarbonoclasticus and P. nautica. A DNA homology value of 65% is well below the usual criterion of approximately 70% or higher, which justifies a different species (Wayne et al., 1987). The homology values of approximately 75 and 85% between VT8ᵀ and P. nautica, and P. nautica and Marinobacter hydrocarbonoclasticus, respectively, could indicate a closer relationship on the DNA level. However, the variation in several methods used for DNA–DNA hybridization is considered to range from ±4–5% or higher (Balkwill et al., 1997; DSMZ Identification Service).

Phylogeny
A matrix of sequence similarity of strain VT8ᵀ and selected reference organisms from marine habitats belonging to the gamma Proteobacteria was constructed. Pairwise evolutionary distances were computed using the correction of Jukes & Cantor (1969). From these numbers, a phylogenetic dendrogram (Fig. 4) was drawn using the neighbour-joining method (Saitou & Nei, 1987) and the PHYLIP program (Felsenstein, 1993). Results of 16S rRNA sequence analysis suggested that strain VT8ᵀ was more closely related to Marinobacter sp. strain CAB (99.8% similarity) and Marinobacter hydrocarbonoclasticus (99.4% similarity) than to Marinobacterium georgiense (90.1% similarity), Marinomonas vaga (89.2% similarity) and strains in the genus Oceanospirillum (similarity levels of 88.8–91.7%). It also appeared to be closely related to P. nautica (99.8% similarity; C. Spröer, unpublished data).

DISCUSSION
Strain VT8ᵀ grew optimally at 0.85 M (5%) NaCl and could thus be classified as a moderately halophilic bacterium according to Kushner & Kamekura (1988). It utilized a range of sugars, organic and amino acids, and other carbon sources that might be available in marine waters. The ability to grow over a range of salinities and moderate temperatures as well as to utilize a variety of substrates appear characteristic of
numerous marine isolates. Strain VT8\textsuperscript{T} was also able to
degradate hydrocarbons and some crude oil
components; this was consistent with its origin from
an oil-field brine. Analysis of its 16S rRNA sequence
placed strain VT8\textsuperscript{T} in the gamma subclass of the
Proteobacteria, which includes, among others, the
genera Oceanospirillum, Marinobacter, Marinomonas,
Halomonas and Pseudomonas. The genus Marinobacter
was created by Gauthier et al. (1992), and
recently Rontani et al. (1997) discovered a new
Marinobacter sp., strain CAB. Growth characteristics,
nutritional properties and 16S rRNA sequence data
suggested that VT8\textsuperscript{T} should be placed in this genus.
Since the results of phylogenetic analysis indicated that
strain VT8\textsuperscript{T} was related to Marinobacter hydrocarbono-
clasticus and also to P. nautica (C. Spröer, unpublished
data), several experiments were performed to compare
strain VT8\textsuperscript{T} with these two species. The overall
antibiotic susceptibility of strain VT8\textsuperscript{T} was similar to
those of Marinobacter hydrocarbonoclasticus and P. nautica. The fatty acid composition of the three strains
(Table 1) indicated a somewhat lower amount of both
C\textsubscript{16:1}, \textit{69c} and C\textsubscript{18:1} \textit{9}c in strain VT8\textsuperscript{T}, as compared to
Marinobacter hydrocarbonoclasticus and P. nautica.
The \textit{G + C} content of \textit{P. nautica}, strain VT8\textsuperscript{T} and
Marinobacter hydrocarbonoclasticus were different
(59.2, 55.7 and 52.7\%, respectively). The DNA–DNA
hybridization value of 65\% between strain VT8\textsuperscript{T} and
Marinobacter hydrocarbonoclasticus was below the
value of approximately 70\%, which has been suggested for
delineating a species (Wayne et al., 1987). The DNA
homology values were higher between VT8\textsuperscript{T} and
\textit{P. nautica} (75\%) and between \textit{P. nautica} and Marinobacter
hydrocarbonoclasticus (85\%), respectively; this could
partially be due to the known inherent experimental variation. Even so, high DNA–DNA
hybridization values, approaching the range for species
identity, should be reflected in very similar whole-cell protein patterns (Vandamme et al., 1998). The comparison of whole-cell protein patterns following separation by SDS-PAGE (Fig. 2) showed, however, that
strain VT8\textsuperscript{T} differed markedly from other selected
strains of the gamma Proteobacteria, including Marinobacter hydrocarbonoclasticus and \textit{P. nautica};
protein patterns of the latter two were also very
different. Other differences in cell constituents were
apparent from the LPS patterns of the three strains.
The different spacing of bands suggested not only
different repeating sugar units, but also a pronounced
protein patterns (Vandamme identity, should be reflected in very similar whole-cell
The different spacing of bands suggested not only
experimental variation. Even so, high DNA–DNA
comparison of whole-cell protein patterns following sep-
paration of strain VT8\textsuperscript{T} to the recently described isolate Marinobacter sp. strain CAB (Rontani et al., 1997). This strain is not yet available for direct comparisons. However, several properties of strain CAB suggested that it differed from strain VT8\textsuperscript{T};
optimal growth was at 0.6 M NaCl (VT8\textsuperscript{T} was 0.85 M);
it could not grow beyond 2.5 M NaCl (VT8\textsuperscript{T} could
grow up to 3.4 M); it could not liquefy gelatin; of the
20 amino acids tested, only Glu, Leu and Pro were
utilized as carbon and energy sources (VT8\textsuperscript{T} could
utilize L-Ile, L-Ala, L-Gln, in addition to L-Glu, L-Leu and L-Pro).

Marine bacteria belonging to the species \textit{P. nautica} are
Gram-negative heterotrophs; their taxonomic position
remains to be established (Baumann et al., 1972; De Vos et al., 1989; Kita-Tsukamoto et al., 1991; Zumft, 1992). Kita-Tsukamoto et al. (1993) suggested that
halophilic, marine pseudomonads should be separated from the genus \textit{Pseudomonas} and should be placed in
newly created groups. Results from the comparison of
nutritional requirements, antibiotic susceptibility, iso-
prenoid quinones, fatty acid composition, FT-IR
spectra and 16S rRNA sequence data, which were
obtained during our studies with strain VT8\textsuperscript{T}, would
be consistent with a placement of \textit{P. nautica} into the
genus Marinobacter.

Description of Marinobacter aquaeolei sp. nov.

\textit{Marinobacter aquaeolei} (a.\textit{qua.e.0`le.i. L. n.}\textit{ aqua}
water; \textit{L. n. oleum} oil; \textit{M.L. gen. n.}\textit{ aquaeolei} from
water of oil, isolated from an oil-field brine).

Cells are Gram-negative, rod-shaped, non-spore-
forming and motile by means of a polar flagellum.
Cells are 0.4–0.5 \textmu m wide and 1.4–1.6 \textmu m long. Col-
onies on \textit{Halomonas} complex medium are circular,
yellow, convex, with entire margins; diameter is
1.5–2.0 mm after 2 d incubation at 30 °C. Growth
occurs at 13–50 °C and pH 5–10; optimal growth
occurs at 30 °C and pH 7.3. The range of NaCl for
growth is 0–20\%; optimal concentration is 5\%.
Anaerobic growth occurs in the presence of nitrate on
sucinate, citrate or acetate, but not on glucose.
Dl-Lactate, acetate, succinate, fumarate, citrate, L-
glutamate, butyrate and the amino acids L-Pro, L-Leu,
L-Ile, L-Gln, L-Glu and L-Ala are utilized as sole
carbon and energy sources. \textit{n}-Hexadecane, pristane
and some crude oil components are degraded. Sus-
sceptible to chloramphenicol, erythromycin, strepto-
mycin, kanamycin, gentamicin and bacitracin; not
susceptible to tetracycline, novobiocin, oleandomycin,
anisomycin or ampicillin. The DNA G + C content is
55.7 ± 0.1 mol\% (as determined by HPLC). The type
strain is VT8\textsuperscript{T}, which was isolated from an oil-
producing well on an offshore platform in southern
Vietnam. It has been deposited in the Deutsche
Sammlung von Mikroorganismen und Zellkulturen as DSM 11845T and in the American Type Culture Collection as ATCC 700491T.

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