Yangia pacifica gen. nov., sp. nov., a novel member of the Roseobacter clade from coastal sediment of the East China Sea

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An aerobic, Gram-negative bacterial isolate, strain DX5-10T, was isolated from coastal sediment of the East China Sea. The taxonomy of strain DX5-10T was studied by phenotypic and phylogenetic methods. Strain DX5-10T was motile, formed faint-yellowish colonies and was positive for catalase reaction and weakly positive for oxidase reaction. The nearly complete 16S rRNA gene of strain DX5-10T was obtained and sequence analysis indicated that strain DX5-10T represented an independent lineage within the Roseobacter clade of Alphaproteobacteria. Strain DX5-10T was phylogenetically related to members of the genera Roseobacter, Loktanella, Roseisalinus, Silicibacter, Antarctobacter, Sulfitobacter, Salipiger, Ruegeria and Roseivivax, and the sequence identities among them were less than 95·0%. The predominant respiratory ubiquinone of strain DX5-10T was Q-10 and the DNA G+C content of strain DX5-10T was 63·3 mol%. Therefore, strain DX5-10T represents a novel species of a novel genus, for which the name Yangia pacifica gen. nov., sp. nov. is proposed, with the type strain DX5-10T (=CGMCC 1.3455T =JCM 12573T).

Members of the Roseobacter clade are aerobic, Gram-negative bacteria and have been widely detected in marine or saline lacustrine samples by ribosomal probing (González & Moran, 1997; Zubkov et al., 2001) and 16S rRNA gene cloning (Eilers et al., 2000; Rappé et al., 2000). Bacterial species that belong to the Roseobacter clade have been frequently isolated from such environments. The Roseobacter clade within the order Rhodobacterales contains 33 genera (Garrity et al., 2004), and recently several novel genera, including Oceanicola (Cho & Giovannoni, 2004), Loktanella (Van Trappen et al., 2004), Salipiger (Martínez-Cánovas et al., 2004), Roseisalinus (Labrenz et al., 2005) and Thalassobacter (Macián et al., 2005), have been established. The physiological characterization of described species within these genera indicates that they are metabolically diverse and are potentially important players in the degradation of lignin and aromatic compounds (Buchan et al., 2001) and in biogeochemistry of organic (González et al., 2003) or inorganic sulfur-containing compounds (Sorokin, 1995; Pukall et al., 1999).

During an ecological survey of microbial diversity of coastal sediments, an aerobic, Gram-negative bacterium, strain DX5-10T, was obtained that is phylogenetically related to members of the genera Roseobacter, Loktanella, Roseisalinus, Roseivivax, Salipiger, Silicibacter and Sulfitobacter. This bacterial strain, together with some uncharacterized marine isolates (Teske et al., 2000; Buchan et al., 2001), formed a distinct lineage within the Roseobacter clade. In this note, we describe the characterization and classification of strain DX5-10T.

Strain DX5-10T was isolated from coastal sediment of the East China Sea located in Fujian Province. The sample (from 4–6 cm beneath the surface) was diluted with 9 ml sterile saline solution and 0·1 ml 10−3 and 10−4 dilutions were plated onto artificial sea water basal medium with 1% peptone and 0·5% yeast extract (Eguchi et al., 1996). Routine cultivation of strain DX5-10T was done at 30°C in marine broth 2216 (MB; Difco). Methods for observation of morphology, physiological and biochemical tests, including catalase and oxidase reactions, nitrate reduction, requirement for NaCl, ranges of temperature and pH for growth, decomposition of gelatin and casein and hydrolysis of starch...
were described and cited in a previous report (Dai et al., 2005).

Biomass for chemotaxonomic analysis was harvested from MB cultures on a rotary shaker (100 r.p.m., 30 °C). Quinones were extracted and purified according to Collins (1985) and were analysed by HPLC equipped with a Hewlett Packard 1050 system and a Zorbax ODS column (Agilent Technologies) operating at 40 °C, with Ruegeria gelatinovorans DSM 5887T as reference strain. The mobile phase was a mixture of acetonitrile/2-propanol (2:1·2) with a flow rate of 1 ml min⁻¹. The UV detection wavelength was set at 270 nm. The fatty-acid profile of whole cells was analysed by gas chromatography by using a model HP6890GC equipped with a hydrogen-ionization detector. Peaks were identified with pre-installed software, HPCHEM-STATION (version A5.01).

Genomic DNA of strain DX5-10T was extracted according to Marmur (1961) and G+C content was determined by thermal denaturation. The 16S rRNA gene of strain DX5-10T was amplified and sequenced as described previously (Zhang et al., 2003). Alignments of 16S rRNA gene sequences of strain DX5-10T and species of the Roseobacter clade (Fig. 1) were carried out with CLUSTAL X program (version 1·8; Thompson et al., 1997). Phylogenetic trees were constructed using the neighbour-joining method (Saitou & Nei, 1987) with the Kimura two-parameter model (Kimura, 1980) by using the programs of TREECON (Van de Peer & De Wachter, 1994).

Bacteriochlorophyll a (Bchl a) was detected spectrophotometrically in vivo and in vitro. For in vivo measurements, 5 ml of strain DX5-10T culture in MB incubated under a natural daylight rhythm was collected, washed and resuspended in 5 ml PBS (137 mmol NaCl l⁻¹, 2·7 mmol KC1 l⁻¹, 4·3 mmol Na₂HPO₄.7H₂O l⁻¹, 1·4 mmol KH₂PO₄ l⁻¹, pH 7·8). For in vitro measurements, 5 ml liquid culture was centrifuged at 5000 g for 10 min. The cells were lysed in liquid nitrogen and Bchl a was extracted using 5 ml iced-cold acetone/methanol solution (7:2, v/v) in the dark at −20 °C for 12 h. Cell fragments were then removed by centrifugation. Spectrophotometric measurements were performed with an HP8453 UV/Vis spectrometer by scanning the wavelength range 400–900 nm. Pigments were detected using HPLC Angelent (ODS column: 5 µm, 4 × 250 mm; UV2000 detector λ = 362 nm) as described by Kobližek et al. (2003). Roseobacter litoralis DSM 6996T was used as a control.

Strain DX5-10T formed faint-yellowish colonies on MB agar. Cells of strain DX5-10T were motile, Gram-negative rods (0·8 × 1·0–1·5 µm in size). The catalase reaction was positive and oxidase reaction was weakly positive. Growth occurred at temperatures of 22–40 °C and at pH 5·0–10·0, with optima at 37 °C and pH 7·5. NaCl was required but

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<td>62·8†</td>
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*Fatty acid 16:1ω7c.
†Fatty acid ω7c.

Table 1. Comparison of fatty-acid compositions of strain DX5-10T with some type species of the family Rhodobacteraceae.

Values are percentages of total fatty acids and only percentages higher than 1% are shown; –, negative or percentages lower than 1%; ND, no data available. Species strains: 1, Yangia pacifica sp. nov. DX5-10T; 2, Salipiger mucosus A3T (data from Martinez-Cainovas et al., 2004); 3, Roseisalinus halodurans OCh 239T (Suzuki et al., 1999; Martinez-Cainovas et al., 2004); 4, Loktania salsula (10 strains) (Van Trappen et al., 2004); 5, Oceaniculture granulosum HTCC 2516T (Cho & Giovannoni, 2004); 6, Roseisalinus antarcticus EL-88T (Labrenz et al., 2005); 7, Ketogulonicigenium vulgare DSM 4025T (Urbance et al., 2001); 8, Antarcctobacter heliothermus EL-219T (Labrenz et al., 1998).
A list of properties that differentiate strain DX5-10 T from *cyclobacter heliothermus* in vivo detected either...

**Genera:** 1. **Table 2.** Characteristics that differentiate DX5-10 T was related to species of various genera: in the GenBank database and phylogenetic analysis based on the nearly complete 16S rRNA gene sequence of strain DX5-10 T amplified and partially sequenced (1351 bp). A BLASTN search with the 16S rRNA gene sequence of strain DX5-10 T in Tables 1 and 2. In brief, strain DX5-10 T was different from members of the *Roseobacter* clade and strain DX5-10 T from some related members of the *Roseobacter* clade is provided in Tables 1 and 2. In brief, strain DX5-10 T was different from members of the genera *Roseivivax* and *Salipiger* in that Bchl *a* was not detected either in *vivo* or *in vitro* (Table 2).

The nearly complete 16S RNA gene of strain DX5-10 T was amplified and partially sequenced (1351 bp). A BLASTN search with the 16S rRNA gene sequence of strain DX5-10 T in the GenBank database and phylogenetic analysis based on 16S rRNA gene sequence identities showed that strain DX5-10 T was related to species of various genera: *Roseobacter galileaezensis* BS107 T (95.0 %), *Loktanella hongkongensis* JCM 12479 T (94.7 %), *Roesalisinus antarcticus* EL-88 T (94.5 %), *Silicibacter pomeroyi* DSS-3 T (94.5 %), *Antartococcus heliothermus* EL-219 T (94.2 %), *Sulfitobacter dubius* KMM 3554 T (94.2 %), *Salipiger mucosus* A3 T (94.1 %), *Ruengeria atlantica* IAM 14463 T (94.0 %) and *Roseivivax halodurans* OCh1 210 T (93.9 %). Following these discoveries, a detailed phylogenetic analysis on the species with validly published names of the *Roseobacter* clade and strain DX5-10 T was performed, and the phylogenetic tree based on the

**Table 2.** Characteristics that differentiate *Yangia pacifica* gen. nov., sp. nov. from related genera

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**Description of *Yangia* gen. nov.**

*Yangia* (Yan’gi.a. N.L. fem. n. *Yangia* after the Chinese microbiologist H.-F. Yang, who founded the research of environmental microbiology in the early 1960s in China).

**Description of *Yangia pacifica* sp. nov.**

*Yangia pacifica* (pa.ci’fi.ca N.L. fem. adj. pacifica pertaining to the Pacific Ocean, the origin of the type strain).

In addition to the properties described above for the genus, the following properties are observed. Cells are 0.8 × 1.0–1.5 μm in size and tend to aggregate after cell division.
Strictly aerobic; does not grow under anaerobic conditions. Cells grow at 22–40 °C and at pH 5–10, with optima at 37 °C and pH 7–9. NaCl is required and the species grows in the NaCl concentration range of 1–10% (optimal growth occurs at 5% NaCl). Bchl a is not detected either in vivo or in vitro. Nitrate reduction, hydrolysis of starch, gelatin liquefaction and indole formation are negative. Accumulates poly-(3-hydroxybutyric) acids. Urease formation is positive. Citric acid is not assimilated. Tests for methyl red and Voges–Proskauer are negative. Maltose, lactate, malate, arginine and glutamate support growth as sole carbon sources. Glucose, lactose, mannitol, sorbitol, inositol, arabinose, fructose, sucrose, L-lysine, L-leucine and L-phenylalanine do not support growth. The cellular fatty-acid profile is outlined in Table 1. DNA G+C content is 63.3 mol%. The type strain, DX5-10T (=CGMCC 1.3455T = JCM 12573T), was isolated from a sample of coastal sediment from the East China Sea of the Pacific Ocean.

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