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 DX5-10^T, was isolated from coastal sediment of the East China Sea. The taxonomy of strain DX5-10^T was studied by phenotypic and phylogenetic methods. Strain DX5-10^T 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 DX5-10^T was obtained and sequence analysis indicated that strain DX5-10^T represented an independent lineage within the *Roseobacter* clade of *Alphaproteobacteria*. Strain DX5-10^T 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 DX5-10^T was Q-10 and the DNA G+C content of strain DX5-10^T was 63.3 mol%. Therefore, strain DX5-10^T represents a novel species of a novel genus, for which the name *Yangia pacifica* gen. nov., sp. nov. is proposed, with the type strain DX5-10^T (=CGMCC 1.3455^T=JCM 12573^T).

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 biogeochemical cycling 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 DX5-10^T, 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 DX5-10^T.

Strain DX5-10^T 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 DX5-10^T 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

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Abbreviation: Bchl *a*, bacteriochlorophyll *a*.

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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 5887^T 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 DX5-10^T was extracted according to Marmur (1961) and G + C content was determined by thermal denaturation. The 16S rRNA gene of strain DX5-10^T was amplified and sequenced as described previously (Zhang *et al.*, 2003). Alignments of 16S rRNA gene sequences of strain DX5-10^T 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 DX5-10^T 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 KCl 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 ice-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 Angilent (ODS column: 5 µm, 4 × 250 mm; UV2000 detector λ = 362 nm) as described by Koblížek *et al.* (2003). *Roseobacter litoralis* DSM 6996^T was used as a control.

Strain DX5-10^T formed faint-yellowish colonies on MB agar. Cells of strain DX5-10^T 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

Table 1. Comparison of fatty-acid compositions of strain DX5-10^T 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. DX5-10^T; 2, *Salipiger mucosus* A3^T (data from Martínez-Cánovas *et al.*, 2004); 3, *Roseivivax halodurans* OCh 239^T (Suzuki *et al.*, 1999; Martínez-Cánovas *et al.*, 2004); 4, *Loktanella salsilacus* (10 strains) (Van Trappen *et al.*, 2004); 5, *Oceanicola granulosus* HTCC 2516^T (Cho & Giovannoni, 2004); 6, *Roseisalinus antarcticus* EL-88^T (Labrenz *et al.*, 2005); 7, *Ketogulonicigenium vulgare* DSM 4025^T (Urbance *et al.*, 2001); 8, *Antarctobacter heliothermus* EL-219^T (Labrenz *et al.*, 1998).

Fatty acid	1	2	3	4	5	6	7	8
10:0 3-OH	—	—	—	2·4	1·5	1·9	3·6	—
12:0 3-OH	3·20	—	—	—	1·6	—	2·9	—
12:1 3-OH	—	2·3	—	ND	—	—	—	3·1
14:0	—	—	—	—	—	—	2·4	—
16:0	8·95	12·4	—	2·9	11·9	15·1	33·5	2·5
15:0-iso 2-OH/16:1ω7c	3·93*	1·3*	—	2·8	1·2	2·1*	2·7	—
17:0	1·44	—	—	—	—	—	—	—
17:1ω8c	3·93	—	—	—	—	—	—	—
18:0	—	2·0	—	1·4	—	2·3	2·9	1·0
18:1ω7c/ω9c/ω12t	81·67	78·0†	Not quantified	87·7†	62·8†	62·0†	52·0	83·2
Methyl 18:1	—	1·9	—	—	8·1	—	—	—
cyclo 19:0	—	2·3	—	ND	10·8	—	—	2·4

*Fatty acid 16:1ω7c.

†Fatty acid ω7c.

concentrations greater than 13 % were inhibitory to growth. Maximal growth was observed at 5 % NaCl. Bchl *a* was not detected either *in vivo* or *in vitro*. Additional phenotypic properties are given in the following genus and species description. The cells of strain DX5-10^T contained ubiquinone 10 (Q-10). The fatty-acid profile of strain DX5-10^T grown in MB is detailed in the species description and in Table 1. The genomic DNA G + C content was 63.3 mol%. A list of properties that differentiate 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). Furthermore, strain DX5-10^T contained 17:1 ω 8c (3.93 %) and 17:0 (1.44 %), which were not detected in any other genera as listed in Table 1, and the fatty acid methyl ester 11-methyl 18:1 ω 7c and cyclo-substituted fatty acid 19:0 cyclo ω 8c that were present in *Salipiger mucosus* A3^T were not detected in strain DX5-10^T.

The nearly complete 16S rRNA 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 gallaeciensis* BS107^T (95.0 %), *Loktanella hongkongensis* JCM 12479^T (94.7 %), *Roseisalinus antarcticus* EL-88^T (94.5 %), *Silicibacter pomeroyi* DSS-3^T (94.5 %), *Antarctobacter heliothermus* EL-219^T (94.2 %), *Sulfitobacter dubius* KMM 3554^T (94.2 %), *Salipiger mucosus* A3^T (94.1 %), *Ruegeria atlantica* IAM 14463^T (94.0 %) and *Roseivivax halodurans* OCh 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

nearly complete 16S RNA gene sequence (1324 bp) similarity indicates that strain DX5-10^T, together with previously isolated but not taxonomically described strains NF18, AG33 and SE45 (Teske *et al.*, 2000; Buchan *et al.*, 2001), formed an independent lineage representing a novel genus within the *Roseobacter* clade (Fig. 1). This lineage represented by strain DX5-10^T is the neighbour to the clade of the species of *Roseivivax* and *Salipiger mucosus* A3^T (Fig. 1), and the 16S rRNA gene identities of strain DX5-10^T between them ranged from 93.7 to 94.1 %.

Combining the phenotypic and phylogenetic studies, we propose that strain DX5-10^T represents a novel species of a novel genus, *Yangia pacifica* gen. nov., sp. nov.

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).

Gram-negative rods, motile. Aerobic heterotroph. When grown on marine agar 2216 plates, small, wettish, shiny, faint-yellowish colonies develop within 3–5 days. Cells do not form spores. Growth requires NaCl. Catalase reaction is positive and oxidase reaction is weakly positive. The predominant respiratory quinone is Q-10. Major fatty acids are 18:1 and 16:0. Cells also contain 3-hydroxylated 12:0. The type species is *Yangia pacifica*.

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 $\times$ 1.0–1.5 μ m in size and tend to aggregate after cell division.

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

Genera: 1, *Salipiger* (data from Martínez-Cánovas *et al.*, 2004); 2, *Roseivivax* (Suzuki *et al.*, 1999); 3, *Loktanella* (Van Trappen *et al.*, 2004); 4, *Oceanicola* (Cho & Giovannoni, 2004); 5, *Roseisalinus* (Labrenz *et al.*, 2005); 6, *Ketogulonicigenium* (Urbance *et al.*, 2001); 7, *Antarctobacter* (Labrenz *et al.*, 1998). +, Positive; –, negative; W, weakly positive; ND, no data available.

Characteristic	DX5-10 ^T	1	2	3	4	5	6	7
Morphology	Rods	Rods	Rods	Rods	Rods	Rods	Ovoid to rods	Rods
Colony colour	Faint yellow	–	Pink	Pink–beige	Faint yellow	Red	Tan	Brown–yellow
Motile	+	–	+	–	–	–	+	+
Oxidase	W	+	+	+	+	+	+	+
Bchl <i>a</i>	–	–	+	ND	–	+	–	–
Na ⁺ requirement	+	+	–	+ / –	+	+	–	+
Optimum NaCl concentration (%)	5	9–10	ND	ND	ND	5–9	0.5–2.5	2–6
Optimum temperature (°C)	37	20–40	27–30	25	28–30	16–26	27–31	16–26
Nitrate to nitrite	–	–	+ / –	–	–	+	ND	+
Acids from glucose	–	–	+ / W / –	ND	–	–	ND	–
Quinone type	Q-10	Q-10	Q-10	ND	ND	Q-10	ND	Q-10
DNA G + C content (mol%)	63.3	64.5	59.7–64.4	59.4–66.4	67.3–71.5	67	52.1–54.0	62.3–62.8

Fig. 1. Neighbour-joining tree based on 16S rRNA gene sequence analysis indicates that strain DX5-10^T, hydrothermal vent strains NF18 and AG33, and marine isolate SE45 (Buchan *et al.*, 2001; Teske *et al.*, 2000) form a coherent cluster within the *Roseobacter* clade, representing the novel genus *Yangia*. *Rubrimonas cliftonensis* OCh 317^T (D85834) was used as the outgroup. Bootstrap confidence values obtained from 1000 bootstrap replications are given at nodes. Bar, 0.05 substitutions per nucleotide position.

Strictly aerobic; does not grow under anaerobic conditions. Cells grow at 22–40 °C and at pH 5.0–10.0, with optima at 37 °C and pH 7.5. 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, DX5-10^T (=CGMCC 1.3455^T=JCM 12573^T), was isolated from a sample of coastal sediment from the East China Sea of the Pacific Ocean.

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