Wenxinia marina gen. nov., sp. nov., a novel member of the *Roseobacter* clade isolated from oilfield sediments of the South China Sea

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An aerobic and heterotrophic, Gram-negative bacterial isolate, strain HY34^T, was isolated from sediment of an oilfield in the South China Sea, China. The taxonomy of strain HY34^T was studied by phenotypic and phylogenetic methods. Strain HY34^T formed faint-pink colonies on marine agar 2216. Cells of strain HY34^T were non-motile, ovoid or short rods. Strain HY34^T was positive for catalase and oxidase, and nitrate was reduced to nitrite. The nearly complete 16S rRNA gene sequence of strain HY34^T was obtained and sequence analysis showed that it, together with the genus *Rubellimicrobium*, formed a distinct clade close to some members of the *Roseobacter* clade in the family *Rhodobacteraceae*, and it showed highest sequence similarities to *Oceanicola granulosus* HTCC2516^T (93.8%), *Silicibacter lacuscaerulensis* ITI-1157^T (93.3%), *Dinoroseobacter shibae* DFL 12^T (93.3%) and *Rubellimicrobium thermophilum* C-lvk-R2A-2^T (92.2%). Bacteriochlorophyll *a* was not detected. The ubiquinone system was Q-10. The major polar lipids were phosphatidylglycerol, phosphatidylcholine and an unidentified glycolipid. The major fatty acids (>10%) were C_{18:1}ω7c and C_{16:0}. The DNA G+C content of this strain was 69.4 mol%. A polyphasic analysis supported the conclusion that this strain represents a novel genus and species, which we designated *Wenxinia marina* gen. nov., sp. nov. The type strain of *Wenxinia marina* is HY34^T (=CGMCC 1.6105^T =JCM 14017^T).

The *Roseobacter* clade (also called the ‘*Roseobacter–Sulfitobacter–Silicibacter*’ group; Wagner-Döbler *et al.*, 2003) of the *Alphaproteobacteria*, which was named after the genus *Roseobacter* by Giovannoni & Rappé (2000), is the second most abundant group of organisms detected by ribosomal probing (González & Moran, 1997; Zubkov *et al.*, 2001) and 16S rRNA gene cloning (Eilers *et al.*, 2000; Rappé *et al.*, 2000) in marine environments. This clade contains 33 genera (Garrity *et al.*, 2004), and many strains from this group have recently been isolated from diverse marine environments and identified as members of novel genera such as *Oceanicola* (Cho & Giovannoni, 2004), *Thalassobacter* (Macián *et al.*, 2005a), *Dinoroseobacter* (Biebl *et al.*, 2005) and *Yangia* (Dai *et al.*, 2006). The abundance of this group in marine environments and the diversity of their physiological properties suggest that they

play important roles in marine ecosystems such as the degradation of aromatic compounds (Buchan *et al.*, 2001, 2005), in the biogeochemical cycles of elements such as carbon and sulfur (Buchan *et al.*, 2005; Pukall *et al.*, 1999; Wagner-Döbler & Biebl, 2006) and in the oxidation of the greenhouse gas carbon monoxide (Wagner-Döbler & Biebl, 2006).

In our study of the microbial diversity of marine sediment in the South China Sea, an aerobic and heterotrophic, Gram-negative bacterium, strain HY34^T, was obtained that is phylogenetically related to members of the genera *Rubellimicrobium* (Denner *et al.*, 2006), *Oceanicola*, *Silicibacter* and *Dinoroseobacter*. In this communication, we describe the isolation and polyphasic taxonomic study of strain HY34^T.

Strain HY34^T was isolated from sediment of the Xijiang oilfield in the South China Sea near Fujian province, China, from a depth of about 100 m. For isolation, serially diluted sediment samples were spread onto low-organic marine agar 2216 (LOM) plates [containing 0.5 g peptone and 0.1 g yeast extract l⁻¹; the composition and concentrations of salts are the same as marine broth 2216 (MB)]

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain HY34^T is DQ640643.

A transmission electron micrograph of cells of strain HY34^T, results of 2D TLC of the polar lipids of strain HY34^T and a comparison of the fatty acid profiles of HY34^T and related strains are available as supplementary material with the online version of this paper.

and cultured at 30 °C for 10 days. Individual colonies were picked up and cultured on marine agar 2216 (MA; Difco). Cell morphology was examined by transmission electron microscopy (H600; Hitachi) and scanning electron microscopy (FEI QUANTA 200). The Gram reaction was determined on cells grown on MA at 30 °C for 24 h according to the method described by Gerhardt *et al.* (1994). Endospore formation was determined after malachite green staining (Dong & Cai, 2001) of cells grown on MA. Cells of strain HY34^T are Gram-negative, non-motile and non-spore-forming ovoid or short rods (Supplementary Fig. S1 in IJSEM Online).

The 16S rRNA gene of strain HY34^T was amplified using two bacterial universal primers, 27F and 1492R (Lane, 1991). Database searches by using BLAST on NCBI (Altschul *et al.*, 1990) and similarities of the 16S rRNA gene sequence showed that the most closely related strains were from the genera *Oceanicola* (92.7–93.8%) and *Silicibacter* (92.3–93.3%). Other close relatives include the type strains of *Dinoroseobacter shibae* (93.3% 16S rRNA gene sequence similarity), *Paracoccus solventivorans* (92.7%), *Ruegeria atlantica* (92.6%), *Roseicyclus mahoneyensis* (92.4%), *Roseivivax halodurans* (92.6%) and *Citricella thiooxidans* (92.6%). A phylogenetic tree of 16S rRNA gene sequences was constructed by the neighbour-joining method with Kimura's two-parameter calculation model in MEGA version 3.1 (Kumar *et al.*, 2004). The phylogenetic tree (Fig. 1) showed that strain HY34^T, together with genus *Rubellimicrobium*, formed a distinct clade within the *Roseobacter* group, although the similarity of its 16S rRNA gene

sequence to that of *Rubellimicrobium thermophilum* C-lvk-R2A-2^T was 92.2%, a bit lower than that to *Oceanicola granulosus* HTCC2516^T (93.8%).

Physiological tests such as growth temperature and pH ranges, tolerance of NaCl and carbon sources for growth were determined as described by Cho & Giovannoni (2003). Enzyme activities were detected by API ZYM (bioMérieux) according to the manufacturer's instructions. Susceptibility to antibiotics was determined using filter-paper discs (Beijing Pharmaceutical Company) containing various antibiotics as specified in the species description. Other phenotypic characteristics were determined according to Dong & Cai (2001) with artificial seawater. Presence of bacteriochlorophyll was checked as described by Dai *et al.* (2006). The phenotypic properties are given in Table 1 and in the genus and species descriptions.

Fatty acids were determined from cells cultured at 30 °C on MA for 3 days. Cellular fatty acids were extracted, methylated and analysed by the Sherlock Microbial Identification System following the manufacturer's instructions. For the analysis of polar lipids and quinines, strain HY34^T was grown in MB for 2 days; cells were then harvested and lyophilized. Polar lipids were examined by two-dimensional TLC and characterized with spray reagents specific for α -glycols (periodate-Schiff), sugars (α -naphthol/sulfuric acid, anisaldehyde/sulfuric acid) and phosphate (Zinzadze reagent) (Ventosa *et al.*, 1993). Quinones were determined according to Collins (1985) and Wu *et al.* (1989). Fatty acids of strain HY34^T (>1%)

Fig. 1. Neighbour-joining phylogenetic tree based on the 16S rRNA gene sequences of strain HY34^T and related members of the family *Rhodobacteraceae*. Bootstrap values (expressed as percentages of 1000 replications) $\geq 50\%$ are shown at branching points. *Terracoccus luteus* DSM 44267^T was used as an outgroup. Filled circles indicate that the corresponding nodes were also recovered in the tree generated with the maximum-parsimony algorithm. Bar, 0.1 substitutions per nucleotide position.

Table 1. Differential properties between strain HY34^T and related genera

Genera: 1, *Wenxinia* gen. nov. (strain HY34^T); 2, *Oceanicola* (Cho & Giovannoni, 2004); 3, *Silicibacter* (Petursdottir & Kristjansson, 1997; González *et al.*, 2003); 4, *Ruegeria* (Rüger & Höfle, 1992; Uchino *et al.*, 1998; Martens *et al.*, 2006); 5, *Dinoroseobacter* (Biebl *et al.*, 2005); 6, *Roseicyclus* (Rathgeber *et al.*, 2005); 7, *Rubellimicrobium* (Denner *et al.*, 2006); 8, *Jannaschia* (Wagner-Döbler *et al.*, 2003; Adachi *et al.*, 2004; Macián *et al.*, 2005b; Choi *et al.*, 2006); 9, *Loktanella* (Van Trappen *et al.*, 2004; Lau *et al.*, 2004; Ivanova *et al.*, 2005; Weon *et al.*, 2006); 10, *Ketogulonicigenium* (Urbance *et al.*, 2001); 11, *Marinosulfonomonas* (Holmes *et al.*, 1997); 12, *Roseisalinus* (Labrenz *et al.*, 2005); 13, *Roseibacterium* (Suzuki *et al.*, 2006); 14, *Roseivivax* (Suzuki *et al.*, 1999); 15, *Marinovum* (Lafay *et al.*, 1995; Uchino *et al.*, 1998; Martens *et al.*, 2006). +, Positive; w, weakly positive; –, negative; v, variable; ND, no data available.

Characteristic	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Motility	–	–	–	–	+	–	+	v	–	v	–	+	–	+	+
Colony colour	Faint pink	Faint yellow	Tan	Beige	Red, beige	Pink to purple	Red	Brown to red*	Pink–beige	Tan	Whitish	Red	Pink	Pink	Beige/pink
Bacteriochlorophyll <i>a</i>	–	–	–	–	+	+	–	–	–	–	ND	+	+	+	–
Catalase	+	v	+	+	ND	+	+	+	+	+	+	+	+	+	+
Growth at 37 °C	+	+	+	–	+	+	+	–	v	v	+	–	–	–	+
Nitrate reduction	+	–	v	+	+	ND	–	v	–	ND	ND	+	–	v	–
Hydrolysis of:															
Gelatin	w	–	ND	–	+	ND	–	v	–	ND	ND	–	+	v	+
Aesculin	+	+	ND	ND	ND	+	–	v	+	ND	ND	+	ND	ND	+
Utilization of:															
Glucose	+	v	v	+	+	+	+	+	v	+	+	+	–	+	+
Mannose	+	–	ND	+	ND	ND	+	v	v	+	ND	–	ND	–	–
Citrate	–	+	v	+	+	+	–	v	v	–	+	–	–	+	+
Xylose	+	–	–	+	ND	ND	+	v	v	–	ND	+	ND	–	–
Fructose	+	–	–	ND	+	+	+	v	v	+	+	+	ND	+	+
Succinic acid	+	–	v	+	+	+	ND	–	–	+	+	+	–	+	–
Lysine	–	–	v	+	ND	ND	ND	–	–	–	ND	–	ND	ND	ND
Ribose	+	v	ND	–	ND	ND	+	v	v	–	ND	+	ND	–	–
Arabinose	+	v	ND	–	ND	ND	+	–	–	+	ND	+	ND	v	+
Acetate	+	ND	–	+	+	+	+	v	–	ND	+	+	–	+	–
Formation of acid from:															
Glucose	+	–	–	+	ND	ND	–	–	v	ND	ND	–	–	+	ND
Lactose	+	ND	ND	–	ND	ND	–	–	–	ND	ND	ND	ND	v	ND
Mannitol	+	ND	ND	–	ND	ND	–	–	v	ND	ND	ND	ND	–	ND
Major fatty acids (>10 %)	C _{18:1} ω7c, C _{16:0}	C _{18:1} ω7c, C _{16:0} , cyc-C _{19:0}	C _{18:1} ω7c	C _{18:1} ω7c, 2-OH C _{16:0}	C _{18:1} ω7c	ND	cyc-C _{19:0} , C _{16:0} , C _{18:0}	C _{18:1} ω7c, cyc-C _{19:0} †	C _{18:1} ω7c	C _{16:0} , C _{18:1}	ND	C _{18:1} ω7c, C _{16:0}	C _{18:1}	C _{18:1}	C _{18:1} ω7c
DNA G + C content (mol%)	69.4	67.3–71.5	66.1–68.0	55	64.8	66.2	69.4–70.0	59.1–64.6	59–66	52.1–54.0	56.8–57	67	68.1	59.7–64.4	60

*No pigmentation in *J. helgolandensis* (Wagner-Döbler *et al.*, 2003).

†Cyclo C_{19:0} not detected in *J. cystaogens* (Adachi *et al.*, 2004).

are C_{18:1}ω7c (57.1 %), C_{16:0} (16.5 %), 11-methyl C_{18:1}ω7c (5.4 %), C_{18:0} (3.9 %), C_{14:0} (3.7 %), iso-C_{15:1} G plus iso-C_{15:1} I (3.4 %), summed feature 3 (C_{16:1}ω7c and/or iso-C_{15:0} 2-OH) (1.9 %), C_{12:0} (1.6 %) and C_{13:0} 2-OH (1.2 %). Strain HY34^T contained Q-10 as the major ubiquinone. Strain HY34^T contained phosphatidylglycerol and phosphatidylcholine as the major identified polar lipids (Supplementary Fig. S2). The unidentified glycolipid L1 was also a major polar lipid in strain HY34^T, and it was not detected in *Ruegeria atlantica* DSM 5823^T, *Marinovum algicola* FF3^T or other members of *Roseobacter* clade (Martens *et al.*, 2006). The unidentified phospholipid PL1 was probably identical to the unidentified phospholipid PL2 of *Ruegeria atlantica* DSM 5823^T (Martens *et al.*, 2006). Strain HY34^T showed fewer polar lipids when compared with *Ruegeria atlantica* DSM 5823^T and *Marinovum algicola* FF3^T, the two closest relatives. These differences clearly distinguish HY34^T from members of the genera *Ruegeria* and *Marinovum* and other members of the *Roseobacter* clade.

The G + C content of the DNA was determined by thermal denaturation (Marmur & Doty, 1962; Seidler & Mandel, 1971) using DNA from *Escherichia coli* K-12 as a control. The G + C content of strain HY34^T was 69.4 mol%, and this value is close to those reported for strains of *Oceanicola* and *Silicibacter* (Table 1).

A comparison of the properties of HY34^T and members of related genera showed that strain HY34^T was similar to members of *Oceanicola* and *Silicibacter*. Differential characteristics of HY34^T and related genera are listed in Table 1. Furthermore, strain HY34^T was distinct in the presence of C_{13:0} 2-OH, iso-C_{15:1} G plus iso-C_{15:1} I and C_{18:1}ω9c and the absence of C_{10:0} 3-OH; strain HY34^T also differed from strains of *Oceanicola* in the presence of 11-methyl C_{18:1}ω7c and the absence of cyc-C_{19:0}, and from strains of *Silicibacter* and *Ruegeria atlantica* in the absence of C_{12:0} 3-OH (Supplementary Table S1). There are also significant phenotypic differences between these taxa, as listed in Table 1.

Based on the phylogenetic analysis of the 16S rRNA gene sequence, chemotaxonomic and phenotypic characteristics, we conclude that strain HY34 represents a novel genus and species, for which we propose the name *Wenxinia marina* gen. nov., sp. nov.

Description of *Wenxinia* gen. nov.

Wenxinia (Wen.xi'ni.a. N.L. fem. n. *Wenxinia* named after Professor Wen-Xin Chen, one of the academicians of the Chinese Academy of Sciences and a pioneer of soil microbiology in China).

Cells are Gram-negative, non-motile and non-spore-forming ovoid or short rods. Strictly aerobic and heterotrophic. NaCl is required for growth. Colonies are faint pink when cultured on MA. Bacteriochlorophyll *a* is absent. Oxidase- and catalase-positive. Nitrate is reduced to nitrite.

The ubiquinone system is Q-10. The major polar lipids are phosphatidylglycerol (PG), phosphatidylcholine (PC) and an unidentified glycolipid (L1). The major fatty acids (>10 %) are C_{18:1}ω7c and C_{16:0}. The type species is *Wenxinia marina*.

Description of *Wenxinia marina* sp. nov.

Wenxinia marina (ma.ri'na. L. fem. adj. *marina* of or belonging to the sea).

Exhibits the following properties in addition to those given in the genus description and in Table 1. Cells are 0.7–0.8 µm wide and 1.3 µm long. Grows at 15–42 °C (optimum 34–38 °C) but not at 10 or 45 °C, at 0.5–9 % NaCl (optimum 1–4 %) but not at 0.4 or 9.2 % and at pH 6.5–8.5 (optimum pH 7.5–8.0) but not at pH 6.0 or 9.0. Indole and H₂S are not produced. Hydrolyses urea and Tween 20 and hydrolyses Tweens 40 and 80 weakly; does not hydrolyse agar, casein, starch, DNA or CM-cellulose. Tests for arginine dihydrolase and lecithinase are negative. Accumulates polyhydroxyalkanoates in its cells. Contains phosphatidylethanolamine and an unidentified phospholipid as minor polar lipids. Fatty acids of the type strain (>1 %) are C_{18:1}ω7c (57.1 %), C_{16:0} (16.5 %), 11-methyl C_{18:1}ω7c (5.4 %), C_{18:0} (3.9 %), C_{14:0} (3.7 %), iso-C_{15:1} G plus iso-C_{15:1} I (3.4 %), summed feature 3 (C_{16:1}ω7c and/or iso-C_{15:0} 2-OH; 1.9 %), C_{12:0} (1.6 %) and C_{13:0} 2-OH (1.2 %). Utilizes sucrose, lactose, galactose, maltose, melezitose, L-rhamnose, L-fucose, trehalose, cellobiose, gluconate, lactic acid, malate and L-glutamic acid and utilizes D-melibiose, inulin, methyl α-D-glucoside, glycerol, sorbitol, butanol, pyruvate, formic acid, L-alanine and L-proline weakly; does not utilize D-raffinose, mannitol, L-sorbose, dulcitol, adonitol, *myo*-inositol, methanol, ethanol, citrate, malonate, butyric acid or caprate. Forms acid from D-xylose, cellobiose, lactose, L-rhamnose, L-arabinose, D-raffinose and forms acid weakly from sucrose, maltose, mannose, trehalose and ribose; does not form acid from other carbon sources tested. Results of API ZYM analysis show strong activities for esterase (C8) and α- and β-glucosidases and weak activities for alkaline phosphatase, leucine arylamidase, valine arylamidase and naphthol-AS-BI-phosphohydrolase; no activity is found for acid phosphatase, N-acetyl-β-cysteine arylamidase, glucosaminidase, α- and β-galactosidase, α-mannosidase, α-chymotrypsin, β-glucuronidase, α-fucosidase and lipase (C₁₄). Resistant to norfloxacin (10 µg), tetracycline (30 µg) and gentamicin (10 µg; weakly resistant). Sensitive to neomycin (30 µg), polymyxin B (300 µg), streptomycin (10 µg), ampicillin (10 µg), carbenicillin (100 µg), vancomycin (30 µg), ciprofloxacin (5 µg), rifampicin (5 µg), chloramphenicol (30 µg), benzylpenicillin (10 µg), kanamycin (30 µg) and erythromycin (15 µg). The G + C content of the DNA of the type strain is 69.4 mol%.

The type strain, HY34^T (=CGMCC 1.6105^T =JCM 14017^T), was isolated from sediment of the Xijiang oilfield in the South China Sea, China.

Acknowledgements

This work was supported by grants from the National Natural Science Foundation of China (30230010) and the Chinese Academy of Sciences (KSCX2-SW-113).

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