**Oceanihabitans sediminis** gen. nov., sp. nov., a member of the family *Flavobacteriaceae* isolated from the Yellow Sea

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A Gram-stain-negative, rod-shaped bacterial strain, designated S9-10T, was isolated from a sediment sample from the Yellow Sea near China. Phylogenetic analysis of 16S rRNA gene sequences revealed that strain S9-10T was a member of the family *Flavobacteriaceae* and formed a distinct lineage. The genomic DNA G+C content of strain S9-10T was 34.2 mol%. The major respiratory quinone was MK-6. The predominant cellular fatty acids were iso-C₁₅:₀ (21.1%), iso-C₁₇:₀ 3-OH (12.0%). The polar lipid profile contained phosphatidylethanolamine, aminophospholipid, aminoglycolipid, two unidentified aminolipids and five unidentified polar lipids. On the basis of phenotypic, genotypic, chemotaxonomic and phylogenetic analyses, strain S9-10T represents a novel species of a novel genus of the family *Flavobacteriaceae*, for which the name *Oceanihabitans sediminis* gen. nov., sp. nov. is proposed. The type strain of *Oceanihabitans sediminis* is S9-10T (=DSM 28133T =LMG 28074T).

The bacterial family *Flavobacteriaceae* (Bernardet et al., 2002) is one of the major branches of the phylum *Bacteroidetes*. The family *Flavobacteriaceae* accommodates bacteria that are chemo-organotrophs, Gram-negative, rod-shaped and are characterized chemotaxonomically by the presence of menaquinone 6, which can be either the only respiratory quinone or the major respiratory quinone, and the absence of sphingophospholipids (Bernardet et al., 2002). The cells can be nonpigmented, or pigmented by carotenoid or flexirubin pigments or both; they can move by gliding or are non-motile (Bernardet et al., 2002). Members of the family occur in marine environments (Bernardet & Nakagawa, 2006) and form a distinct clade in the phylogenetic tree (Bowman, 2006; Bowman & Nichols, 2005). Most novel genera within this family described recently were isolated from marine environments (Yoon et al., 2014; Hameed et al., 2014; Hsu et al., 2014; Zhang et al., 2014; Liu et al., 2014; Kwon et al., 2014; Hameed et al., 2014; Song et al., 2015; Liu et al., 2016). In this study, we report the characterization of a novel species in a novel genus within the family *Flavobacteriaceae* isolated from sediment sample of the Yellow Sea in China.

Strain S9-10T was isolated from a marine sediment sample collected using a 0.1 m² modified Gray–O’Hara box corer from the Northern Yellow Sea in China (38° 25′ N, 121° 57′ E) during November, 2012 (Zhang & Margesin, 2015). A sample of subsurface sediment (collected at 5 cm depth) was stored at −20 °C until it could be processed. Briefly, sediment was shaken with 0.1 % (w/v) sodium pyrophosphate solution and appropriate dilutions were then plated on Marine agar 2216 (MA, Difco) at 25 °C.

DNA was extracted and purified as described by Sambrook et al. (1989). The 16S rRNA gene was amplified, cloned and sequenced according to previously published protocols (Zhang et al., 2006, 2011). On the basis of pairwise comparisons of the 16S rRNA gene sequences using the recent version of EzTaxon-e program (Kim et al., 2012), the closest relatives of the novel isolate were members of recognized species of the genera *Bizonia*, *Olleya*, *Lacinutrix*, *Algibacter*,...
Winogradskyella and Gaetbulibacter with sequence similarities to the type strains of the type species of the genera listed of 95.0%, 94.7%, 94.1%, 93.6%, 93.2% and 93.6%, respectively. All other species belonging to the family Flavobacteriaceae showed 16S rRNA gene sequence similarities of <94.4%. Multiple sequence alignments were performed using the clustal w program integrated in mega version 6 (Tamura et al., 2013). Phylogenetic trees were reconstructed by using the neighbour-joining (NJ) and maximum-likelihood (ML) algorithms using mega version 6.0. For the NJ and ML algorithms genetic distances were calculated by the Kimura two-parameter model (Kimura, 1980) and the pairwise deletion option was used. The resultant tree topologies generated by two methods were evaluated by bootstrap analysis based on 1000 replicates. The reconstructed phylogenetic tree based on the neighbour-joining algorithm (Saitou & Nei, 1987) (Fig. 1) revealed that S9-10T formed a distinct lineage within the family Flavobacteriaceae. This phylogenetic position was confirmed in the tree generated using the ML algorithm (Fig. S1, available in the online Supplementary Material).

The morphology of cells grown on MA at 25°C was examined. The morphology of cells grown on MA at 25°C was examined by phase-contrast microscopy (×1000; Leitz Diaplan) and by transmission electron microscopy (Zeiss Libra 120 EFTEM). Motility was examined by microscopy (×1000) and on semi-solid MA soft agar. Gram-staining was determined by bubble production in 3% (v/v) Lacinutrix copepodicola DJ3T (AY694001)
Lacinutrix himadiensis E4-9aT (FN377744)
Lacinutrix mariniflava AKS432T (DQ167239)
Lacinutrix algicola AKS293T (DQ167238)
Olleya marilimosa CAM030T (EF660466)
Olleya aquimaris L-4T (FJ886713)
Olleya namhaensis MY15T (JQ327134)

*Oceanihabitans sediminis* S9-10T (KU517448)
Pontirhabdus pectinivorans JC2675T (HM475134)
Algibacter lectus KMM 3902T (AY187689)
Flavivirga amylovorans JC2681T (HM475138)
Flavivirga jejuensis JC2682T (HM475139)
Psychroserpens mesophilus KOPRIT (DQ001321)
Psychroserpens burtonensis ACAM188T (U62913)
Psychroserpens damuponensis F051-1T (H336890)
Mesoflavibacter zeaxanthinifaciens DSM 18436T (AB265181)
Gaetbulibacter saemankumensis SMK-12T (AY893937)
Gaetbulibacter marinus IMCC1914T (EF108219)
Bizionia argentinensis JUB59T (EU021217)
Bizionia echini KMM 61777T (FJ716799)
Bizionia algoritergicola APA-1T (AY694003)
Bizionia hallyeonensis T-y7T (JN885199)
Winogradskyella pacifica KMM6019T (GQ181061)
Winogradskyella eximia KMM 3944T (AY521225)
Winogradskyella epiphytica KMM 3906T (AY521224)
Gillisia myxillae JCM 13564T (DQ202393)
Gillisia illustrilutea JCM 13564T (DQ202393)
Bacteroides fragilis ATCC 25285T (X83935)

**Fig. 1.** Neighbor-joining tree, based on 16S rRNA gene sequence data, showing the phylogenetic position of strain S9-10T, and representatives of other related taxa within the family Flavobacteriaceae. Numbers at nodes represented percentage levels of bootstrap support based on a neighbor-joining analysis of 1000 re-sampled datasets. *Bacteroides fragilis* ATCC 25285T (GenBank accession number X83935) was used as an outgroup. GenBank accession numbers of 16S rRNA sequences are given in parentheses. Bar, 2% sequence divergence.
H₂O₂ and cytochrome c oxidase activity was determined using 1 % (w/v) N,N,N′,N′-tetramethyl-p-phenylenediamine. Oxidative/fermentative metabolism of glucose was determined as described by Süßmuth et al. (1987) on Hugh and Leifson’s OF basal medium (1 % glucose, 0.2 % peptone, 0.1 % yeast extract, 0.5 % NaCl, 0.02 % K₂HPO₄, 0.008 % bromthymol blue, 3 % agar) supplemented with sea salts (S9883, Sigma). API 20 NE (supplemented with sea salts), API 20 E and API ZYM systems (bioMérieux) were used for testing assimilation and enzyme activities and incubated at 25 °C. Degradation of casein (using casein Hammarsten grade as substrate), starch, pectin, polygalacturonic acid, carboxymethyl cellulose, alginic acid and agar were tested on MA plates supplemented with appropriate substrates (0.5 % w/v) as described previously (Margesin et al., 2003). Hydrolysis of DNA was tested on DNAase agar supplemented with 2 % (w/v) NaCl. Growth under anaerobic conditions was examined after 5 days of incubation at 25 °C in an anaerobic jar (containing Anaerocult A (Merck) to produce anaerobic conditions) on MA supplemented with 10 mm KNO₃. Growth under microaerophilic conditions was investigated at 25 °C on MA after incubation in the microaerophilic atmosphere containing 8–10 % (v/v) carbon dioxide and 5–7 % (v/v) oxygen; this atmosphere was generated in sealed jars containing Anaerocult C (Merck). Growth at 1, 5, 10, 15, 20, 25, 30, 35 and 40 °C was generated in sealed jars containing Anaerocult C (Merck). Growth at pH 5, 6, 7, 8, 9 and 10 was assessed in marine broth buffered with acetic acid/sodium acetate (pH 5, 7 % (v/v) nitrogen and 40 % (w/v) NaCl was tested in salt-free medium (0.5 % peptone, 0.1 % yeast extract). All tests were carried out simultaneously with strain S9-10T and the reference strains Winogradskyella thalassocola KCTC 12221T, Bizonia paragorgiae KACC 15409T, Algibacter lectus JCM 21761T and Olleya marilimosa CIP 108537T. The morphological, physiological and biochemical characteristics of the strains are given in the species description and the features that differentiate the investigated strain from the reference strains are given in Table 1.

The presence or absence of flexirubin type pigments was determined by flooding bacterial cells on a glass slide with 20 % (w/v) KOH (Bernardet & Bowman, 2006). The immediate color change of the cell biomass indicated the presence of flexirubin-type pigments in cells of S9-10T. In contrast, the absence of a color change of the yellow cell biomass indicated the absence of flexirubin-type pigments in cells of W. thalassocola KCTC 12221T, B. paragorgiae KACC 15409T, A. lectus JCM 21761T and O. marilimosa CIP 108537T. Carotenoids were extracted from whole cells grown at 25 °C with acetone/methanol (7:2, v/v) as described by Alarico et al. (2002) and Margesin & Zhang (2013). Absorption spectra were recorded spectrophotometrically. The presence of carotenoids was verified by absorption peaks at 452 and 479 nm in the spectra of the acetone–methanol extracts of W. thalassocola KCTC 12221T, B. paragorgiae KACC 15409T, A. lectus JCM 21761T and O. marilimosa CIP 108537T. Carotenoids were absent in cells of S9-10T.

For fatty acid methyl ester analysis, S9-10T and the reference strains W. thalassocola KCTC 12221T, B. paragorgiae KACC 15409T, A. lectus JCM 21761T and O. marilimosa CIP 108537T were grown on MA at 25 °C for 2 days. All five strains shared similar growth parameters and a sufficient amount of cells of similar physiological age could be harvested from the third streak quadrant of the MA plates after cultivation under the applied conditions. The fatty acid methyl esters were extracted and prepared according to the standard protocol of the Sherlock Microbial Identification System (MIDI, version 6.1) (Sasser, 1990), using the data bank TSBA40 for calculation. Fatty acid analyses were carried out by the Identification Service of the DSMZ, Braunschweig, Germany. The predominant cellular fatty acids (>10 % of total fatty acids) of strain S9-10T were iso-C₁₅:₀ (21.1 %), iso-C₁₅:₁ (6.7 %), iso-C₁₆:₁ (3.9 %), 3-OH (12.0 %), and anteiso-C₁₅:₀ (7.6 %). Thus, the fatty acid profile of S9-10T resembled those of other genera within the family Flavobacteriaceae (Nedashkovskaya et al., 2004, 2005a, b; Mancuso Nichols et al., 2005). Details of the fatty acid profiles of strain S9-10T and the reference strains are available as Table S1.

Respiratory quinones were extracted according to the protocol of Altenburger et al. (1996) and were analyzed by HPLC (Stolz et al., 2007). The quinone system of strain S9-10T consisted predominantly of menaquinone-6 (MK-6) (99.7 %), and also traces of MK-7 (0.3 %). This is in agreement with the described species of the family Flavobacteriaceae.

Analysis of polar lipids was carried out by the Identification Service, DSMZ, Braunschweig (Germany) according to the methods of Bligh & Dyer (1959); Tindall (1990 a, b) and Tindall et al. (2007). The polar lipid profile of strain S9-10T consisted predominantly of phosphatidylethanolamine, aminophospholipid, aminoglycolipid, two unidentified aminolipids and five unidentified polar lipids (Fig. S2). The members of its closely related genera Bizonia, Algibacter, Winogradskyella, Lacinutrix and Gaetbulibacter in the family Flavobacteriaceae also contain phosphatidylethanolamine as the major polar lipid (Nedashkovskaya et al., 2004; Jeong et al., 2013; Li et al., 2015; Park et al., 2015). However, common major polar lipids of the members of the genus Olleya in the family Flavobacteriaceae do not include phosphatidylethanolamine and include an unidentified aminolipid and an unidentified lipid (Lee et al., 2010).

The DNA G+C content was determined using the thermal denaturation method (Marmur & Doty, 1962) using Escherichia coli K12 as calibration standard. This experiment was carried out at 260 nm with a model Lambda 35 UV/VIS spectrometer equipped with a Peltier System (PTP 1+1) (Perkin–Elmer). The genomic DNA G+C content of strain S9-10T was 34.2 mol%, which is inside the range reported for the other genera in the family
The data presented in this study demonstrate that strain S9-T represents a member of a novel genus that is distinguished from W. thalassocola KCTC 12221T, B. paragorgiae KACC 15409T, A. lectus JCM 21761T and O. marilimosa CIP 108537T by the presence of flexirubin-type pigments, the absence of carotenoid pigments, the ability to grow at 35°C and the inability to grow under microaerophilic conditions.

### Phenotypic characteristics that differentiate strain S9-10T from Winogradskyella thalassocola KCTC 12221T, Bizonia paragorgiae KACC 15409T, Allobacter lectus JCM 21761T and Olleya marilimosa CIP 108537T.

Table 1. Phenotypic characteristics that differentiate strain S9-10T from Winogradskyella thalassocola KCTC 12221T, Bizonia paragorgiae KACC 15409T, Allobacter lectus JCM 21761T and Olleya marilimosa CIP 108537T.

<table>
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<tr>
<td>Flexirubin-type pigments</td>
<td>+</td>
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<tr>
<td>Carotenoid pigments</td>
<td>–</td>
<td>+</td>
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<td>Growth on MA at 35°C</td>
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<td>Microaerophilic growth</td>
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<td>Degradation of casein (Hammarsten grade)</td>
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<td>Degradation of starch</td>
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<td>Enzyme activities (API ZYM)</td>
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<td>Chymotrypsin</td>
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<td>W</td>
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<td>β-Glucosidase</td>
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<td>+</td>
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<td>N-Acetyl-glucosamine</td>
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<tr>
<td>DNA G+C content (mol%)</td>
<td>34.2</td>
<td>34.6+</td>
<td>37.6</td>
<td>31.0–33.0‡</td>
<td>49§</td>
</tr>
</tbody>
</table>

*Data from Nedashkovskaya et al. (2005a).
†Data from Nedashkovskaya et al. (2005b).
‡Data from Nedashkovskaya et al. (2004).
§Data from Mancuso Nichols et al. (2005).

Flavobacteriaceae (Bernardet et al., 2002). This value was higher than that of A. lectus JCM 21761T (31.0–33.0%) and lower than those of W. thalassocola KCTC 12221T (34.6%) and B. paragorgiae KACC 15409T (37.6%) and O. marilimosa CIP 108537T (49%) (Nedashkovskaya et al., 2004; Mancuso Nichols et al., 2005, 2005a, b).

The data presented in this study demonstrate that strain S9-10T represents a member of a novel genus that is distinguished from W. thalassocola KCTC 12221T, B. paragorgiae KACC 15409T, A. lectus JCM 21761T and O. marilimosa CIP 108537T by the presence of flexirubin-type pigments, the absence of carotenoid pigments, the ability to grow at 35°C and the inability to grow under microaerophilic conditions.

### Description of Oceanihabitans gen. nov.

Oceanihabitans (O.ce.a.ni.ha.bitans. L. masc. n. oceanus the ocean; L. pres. part. habitans, inhabiting; N.L. part. adj. oce-anihabitans inhabitant of the ocean).

Cells are Gram-stain-negative rods, often in short chains. Gliding motility. Absence of flagella (Fig. S3), Absence of carotenoid pigments, presence of flexirubin-type pigments. Positive for cytochrome c oxidase and catalase. The predominant menaquinone is MK-6. The predominant cellular fatty acids are iso-C₁₅:₀, iso-C₁₅:₁G and iso-C₁₇:₀ 3-OH. The polar lipid profile contains phosphatidylethanolamine, aminophospholipid, aminoglycolipid, two unidentified aminolipids and five unidentified polar lipids. The genus is a member of the family Flavobacteriaceae, phylum Bacteroidetes. The type species is Oceanihabitans sediminis.
Description of Oceanihabitans sediminis sp. nov.

Oceanihabitans sediminis (se.di.mi.nis. L. gen. n. sediminis of sediment).

Displays the following characteristics in addition to those given in the genus description.

Colonies on MA are creamy white, shiny, circular and with entire margins. Grows under aerobic conditions, unable to grow under microaerophilic and anaerobic conditions. Grows at 1–35 °C on MA and in marine broth. Grows at 25 °C in buffered marine broth at pH 6–9 (optimal growth at pH 7–8). Cannot grow in sea-salts-free medium supplemented with NaCl only. Grows in the presence of 2–8 % (w/v) NaCl in marine broth. Positive for alkaline phosphatase, acid phosphatase, esterase lipase (C8), cysteine arylamidase, valine arylamidase, naphthol-AS-Bi-phosphohydrolase and chymotrypsin, on agar plates for degradation of DNA, alginic acid and casein (Hammarsden grade). Negative for lipase (C14), α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, α-mannosidase, α-fucosidase, lysine dihydrolyase, ornithine dihydrolyase, urease, indole production, H₂S production, citrate utilization and nitrate reduction, on agar plates negative for degradation of starch, carboxymethyl cellulose and agar. Positive in API20NE (supplemented with sea salts) for assimilation of D-glucose, D-mannose, D-mannitol, D-maltose, L-arabinose, N-acetyl-glucosamine, potassium gluconate, malic acid, adipic acid and phenylacetic acid; negative for assimilation of capric acid and trisodium citrate. Negative for D-glucose fermentation.

The genomic DNA G+C content is 34.2 mol%. The type strain is S9-10¹ (=DSM 28133¹=LMG 28074¹) and was isolated from marine sediment collected in the Northern Yellow Sea in China.

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References


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