Achromobacter sediminum sp. nov., isolated from deep subseafloor sediment of South Pacific Gyre

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A Gram-stain-negative, facultatively anaerobic, rod-shaped, motile bacterium with a subpolar or lateral flagellum, designated strain XH089T, was isolated from deep-sea sediment sample collected from the South Pacific Gyre (41°51' S 153°06' W) during the Integrated Ocean Drilling Program (IODP) Expedition 329. Phylogenetic analysis based on 16S rRNA gene sequences indicated that strain XH089T belonged to the genus Achromobacter and showed the highest 16S rRNA gene sequence similarities with Achromobacter ruhlandii ATCC 15749T (96.95%), Achromobacter denitrificans DSM 30026T (96.70%) and Achromobacter marplatensis B2T (96.66%). The DNA G+C content of strain XH089T was 66.5 mol%. The major fatty acids were C₁₆ : 0 and C₁₇ : 0 cyclo. The major polar lipids were phosphatidylethanolamine, phosphatidylmonomethylethanolamine, diphosphatidylglycerol, three unknown phospholipids and four unknown polar lipids. On the basis of data from the polyphasic analysis, strain XH089T is considered to represent a novel species of the genus Achromobacter, for which the name Achromobacter sediminum sp. nov. is proposed. The type strain is XH089T (=DSM 27279T=JCM 19223T).

The genus Achromobacter, of the family Alcaligenaceae in the class Betaproteobacteria, was first proposed by Bergey et al. (1923) with the type species Achromobacter liquefaciens. Because of the lack of an identifiable culture of Achromobacter liquefaciens, the generic name Achromobacter was excluded from the Approved Lists of Bacterial Names (Skerman et al., 1980). Yabuuchi & Yano (1981) proposed the name Achromobacter with a different type species Achromobacter xylosoxidans. Then the genus Achromobacter was emended by Yabuuchi et al. (1998) and Alcaligenes ruhlandii (Packer & Vishniac, 1955), Alcaligenes piechaudii (Kiredjian et al., 1986) and Alcaligenes denitrificans (Rüger & Tan, 1983) were transferred from the genus Alcaligenes to the genus Achromobacter. At the time of writing, 11 species have been recognized and have validly published names: Achromobacter xylosoxidans (Yabuuchi & Yano, 1981), Achromobacter ruhlandii and Achromobacter piechaudii (Yabuuchi et al., 1998), Achromobacter denitrificans (Coenye et al., 2003a), Achromobacter insolitus and Achromobacter spanius (Coenye et al., 2003b), Achromobacter marplatensis (Gomila et al., 2011), Achromobacter animicus, Achromobacter mucilolens, Achromobacter pulmonis and Achromobacter spiritus (Vandamme et al., 2013). These bacteria have been isolated from many sources including human clinical samples, soil and water. A novel rod-shaped bacterium, designated XH089T, was isolated from sediment at a depth of 18.1–18.2 m below the sea floor in the South Pacific Gyre at station U1370 (41°51’ S 153°06’ W) during the Integrated Ocean Drilling Program (IODP) Expedition 329. The aim of the present study was to determine the exact taxonomic position of strain XH089T using a polyphasic taxonomic approach.

Sediment samples were spread on marine agar 2216 (MA; Difco) plates after being mixed with sterile 0.85 % (w/v) saline and incubated at 4 °C for 3 months or 28 °C for up to 2 weeks. Strain XH089T, which formed circular (1.0–1.5 mm diameter), convex, slightly transparent colonies with entire margins on MA after culturing at 28 °C for 7 days, was picked and purified by streaking three times on MA. The culture was maintained on MA plates at 28 °C, and stocks were preserved in sterile 0.85 % (w/v) saline supplemented with 15 % (v/v) glycerol at −80 °C. Achromobacter ruhlandii DSM 653T and Pigmentiphaga kullae DSM 13608T obtained from the DSMZ (German Collection of Microorganisms and Cell Cultures) were used as reference strains, which were cultivated on nutrient agar (NA; Difco) at 28 °C as was XH089T since P. kullae DSM 13608T grew better on NA.

Gram-staining and flagellum staining were investigated using standard methods (Beveridge et al., 2007). Cell morphology was determined by transmission electron microscopy (JEM-1200EX; JEOL) after cells had been negatively stained with 1 % (w/v) phosphotungstic acid. Motility was determined by

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The GenBank accession number for the 16S rRNA gene sequence of Achromobacter sediminum XH089T is KC986352.

Two supplementary figures are available with the online version of this paper.
the hanging-drop method (Mackie & McCartney, 1989). To test anaerobic growth, bacterial strains were cultured on NA with resazurin as an indicator of anaerobic condition in an anaerobic jar filled with nitrogen and a packet of Aneropack-Anaero (Mitsubishi Gas Chemical) at 28 °C for one month. The temperature range for growth was determined on NA by incubating cultures at 4–46 °C (4, 8, 12, 16, 20, 24, 28, 32, 37, 42 and 46 °C) for 5 days and at 0 °C on NA for 30 days. The salinity and pH range supporting growth were investigated in 96-well microplates by measuring the OD595 at 0, 12, 24, 48, 72, 96 and 120 h in nutrient broth (NB; Difco). The NaCl concentration was adjusted to 0–15.0 % (w/v, at intervals of 1.0 %) in NB. Growth in NB was evaluated at pH 2.0–10.0 at intervals of 1 pH unit using the following buffer systems: Na2HPO4/citric acid (pH 2.0–7.0), Tris/HCl (pH 8.0–9.0) and Na2CO3/NaHCO3 (pH 9.0–10.0). Various phenotypic characterizations of strain XH089T and two reference strains were tested according to standard approaches (Tindall et al., 2007), including catalase, oxidase and hydrolysis of agar, starch, casein, gelatin and Tween 20, 40 and 80 (method 2). DNase activity was examined by using DNase agar (Qingdao Hope Bio-technology) according to the manufacturer’s instructions. Activities of constitutive enzymes and other physiological properties were determined after growth on NA at 28 °C for 3 days by using API 20E, API 50CH and API ZYM strips (BioMérieux) and GN2 MicroPlates (Biolog) according to the manufacturers’ instructions.

Cells of XH089T were Gram-stain-negative, facultatively anaerobic, motile rods (0.8–1.2 μm in length, 0.2–0.3 μm in width) with a single subpolar or lateral flagellum which occurred as single units or in pairs (Fig. 1). Colonies were cream, circular (1.0–1.5 mm in diameter), convex and opaque with entire margins after growth on NA for 3 days at 28 °C. Growth occurred at 4–42 °C (optimum 28 °C). The salinity range for growth of XH089T was 0–5 % (w/v) NaCl (optimum 0–2 %). The pH range for growth was 5.0–8.0 (optimum 7.0). Oxidase and catalase were positive. Tween 20 and Tween 40 could be degraded. Gelatin, DNA, starch, casein, agar and Tween 80 were not hydrolysed. Other morphological, physiological and biochemical characteristics of strain XH089T are given in Fig. 1, Table 1 and the species description.

For cellular fatty acid analysis, strain XH089T and the reference strains were grown on NA at 28 °C for 2–3 days when the bacterial communities reached the late-exponential stage of growth according to the four quadrants streak method (Sasser, 1990). Fatty acid methyl esters were prepared and analysed according to the standard protocol of MIDI (Sherlock Microbial Identification System, version 6.0) and identified by the TSBA6.0 database of the Microbial Identification System (Sasser, 1990). Cell biomass for polar lipids was obtained by centrifugation after shaking at 28 °C in NB for 3 days. Polar lipids were extracted according to the procedures described by Minnikin et al. (1984) and separated by two-dimensional TLC on silica gel 60 F254 plates (Merck) using chloroform : methanol : acetic acid : water (80 : 12 : 15 : 4, v/v) for the first dimension and chloroform : methanol : acetic acid : water (65 : 25 : 15 : 4, v/v) for the second dimension (Collins & Shah, 1984). The identification of individual lipid was performed by spraying with the appropriate detection reagents (Komagata & Suzuki, 1987). DNA of strain XH089T was extracted according to the procedure of Moore et al. (1999) and the G+C content was

**Fig. 1.** Transmission electron micrographs of negatively stained cells of *Achromobacter sediminum* XH089T cultured on NA at 28 °C for 2 days. Bars,500 nm. a: a single cell; b: cells in a pair.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>1</th>
<th>2</th>
<th>3</th>
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<tbody>
<tr>
<td>Colony colour (on NA)</td>
<td>Cream</td>
<td>Yellow</td>
<td>Cream</td>
</tr>
<tr>
<td>Growth at 42 °C</td>
<td>+</td>
<td>+</td>
<td>w</td>
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<tr>
<td>Flagellum</td>
<td>Subpolar or lateral</td>
<td>Peritrichous</td>
<td>–</td>
</tr>
<tr>
<td>Motility</td>
<td>+</td>
<td>–</td>
<td>–</td>
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<tr>
<td>Hydrolysis</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>DNA</td>
<td>+</td>
<td>+</td>
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<td>Tween 20</td>
<td>+</td>
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<tr>
<td>Tween 40</td>
<td>+</td>
<td>–</td>
<td>–</td>
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<tr>
<td>API 20E</td>
<td>–</td>
<td>–</td>
<td>–</td>
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<tr>
<td>Urease</td>
<td>+</td>
<td>–</td>
<td>–</td>
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<tr>
<td>Acetoin production</td>
<td>+</td>
<td>–</td>
<td>–</td>
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<tr>
<td>Nitrate reduction</td>
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<td>+</td>
<td>+</td>
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<tr>
<td>Assimilation of d-glucose</td>
<td>+</td>
<td>+</td>
<td>–</td>
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<tr>
<td>API ZYM</td>
<td>–</td>
<td>–</td>
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<tr>
<td>Esterase lipase (C8)</td>
<td>w</td>
<td>–</td>
<td>–</td>
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<tr>
<td>Valine arylamidase</td>
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<td>+</td>
<td>w</td>
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<tr>
<td>α-chymotrypsin</td>
<td>w</td>
<td>+</td>
<td>+</td>
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<tr>
<td>DNA G+C content (mol%)</td>
<td>68.5</td>
<td>68.0*</td>
<td>68.5†</td>
</tr>
</tbody>
</table>

*Data from Vandamme et al. (2013).†Data from Blümel et al. (2001).
determined by reversed-phase HPLC according to Mesbah & Whitman (1989).

The cellular fatty acid profiles of strain XH089\textsuperscript{T}, Achromobacter ruhlandii DSM 653\textsuperscript{T} and P. kulae DSM 13608\textsuperscript{T} are listed in Table 2. The dominant fatty acids of strain XH089\textsuperscript{T} were C\textsubscript{16:0} (36.1 \%) and C\textsubscript{17:0} cyclo (26.2 \%), which were similar to those of Achromobacter ruhlandii though some quantitative differences were observed. XH089\textsuperscript{T} contained more C\textsubscript{18:0} and less C\textsubscript{17:0} cyclo than Achromobacter ruhlandii. P. kulae differed from XH089\textsuperscript{T} and Achromobacter ruhlandii in possessing a large quantity of C\textsubscript{16:0}. The polar lipids profile of strain XH089\textsuperscript{T} comprised phosphatidylethanolamine (PE), phosphatidyldimonoethylenelethanolamine (PME), diphosphatidylglycerol (DPG), three unknown phospholipids (PL1–PL3) and four unknown polar lipids (L1–L4). The major polar lipids of Achromobacter ruhlandii were PE, phosphatidylglycerol (PG), DPG, three unknown phospholipids (PL1, PL4–5) and one unknown polar lipid (L3), and the major polar lipids of P. kulae were PE, PG, DPG, one unknown phospholipid (PL6) and one unknown polar lipid (L5) (Fig. S1, available in the online Supplementary Material). With regard to the polar lipids profile, XH089\textsuperscript{T} differed from the two reference strains in containing PME and more unknown phospholipids and unknown polar lipids. The DNA G+C content of strain XH089\textsuperscript{T} was 66.5 mol\%, which was similar to that found in other species of the genus Achromobacter (65–68 mol\%). For 16S rRNA gene sequencing, the genomic DNA of XH089\textsuperscript{T} was extracted and purified using standard methods (Ausubel et al., 1995). The 16S rRNA gene was amplified by PCR with two universal primers (27F: 5'-AGAGTTTGATCCTGCTCAG-3' and 1492R: 5'-GTTACCTTGGTACGACTT-3'). For the cloning and sequencing of the 16S rRNA gene, the PCR product was purified using a TIANgel Midi Purification kit (TIANGEN Biotech), ligated into the pUCm-T vector (TaKaRa), cloned into Escherichia coli JM109 and sequenced at BGI (Qingdao, China). Pairwise similarity values between strain XH089\textsuperscript{T} and closely related type strains were calculated using the EzTaxon-e server (http://eztaxon-e.ezbiocloud.net/; Kim et al., 2012). The 16S rRNA gene sequences of related strains were downloaded from the NCBI database (http://www.ncbi.nlm.nih.gov) and aligned using the CLUSTAL_X program (Thompson et al., 1997). Phylogenetic trees were reconstructed using neighbour-joining and maximum-likelihood methods with Kimura two-parameter model analyses (Kimura, 1980) implemented in the program MEGA version 5 (Tamura et al., 2011). In each case, bootstrap values were calculated based on 1000 replicates.

Pairwise alignment according to the nearly complete 16S rRNA gene sequence (1454 nt) of strain XH089\textsuperscript{T} showed 96.95 \% sequence similarity to Achromobacter ruhlandii ATCC 15749\textsuperscript{T}, 96.70 \% to Achromobacter denitrificans DSM 30026\textsuperscript{T} and 96.66 \% to Achromobacter marplatensis B2\textsuperscript{T}. The phylogenetic trees using both neighbour-joining and maximum-likelihood methods showed that XH089\textsuperscript{T} formed a tight phylogenetic cluster with the type strains of species of the genus Achromobacter (Fig. 2, Fig. S2). However, the low levels of sequence similarity to the type strains of recognized species of the genus Achromobacter implied that it represented a novel species (Stackebrandt & Goebel, 1994). In addition, the phylogenetic position of strain XH089\textsuperscript{T} was strongly supported by its phenotypic characteristics. Strain XH089\textsuperscript{T} was clearly distinguishable from other species of the genus Achromobacter with validly published names by the differences in the morphology of flagella, nitrate reduction, activities of hydrolytic enzymes and assimilation of different sugars (Table 1). Moreover, the fatty acid composition of strain XH089\textsuperscript{T} differed by containing more C\textsubscript{18:0} and less C\textsubscript{17:0} cyclo than Achromobacter ruhlandii (Table 2).

On the basis of phenotypic characteristics and phylogenetic inferences, strain XH089\textsuperscript{T} is assigned to the genus Achromobacter as representing a novel species, for which the name Achromobacter sediminum sp. nov. is proposed.

**Description of Achromobacter sediminum sp. nov.**

Cells are Gram-stain-negative, facultatively anaerobic, motile rods (0.8–1.2 μm in length, 0.2–0.3 μm in width) with single subpolar or lateral flagellum which occur as single units or in pairs. Colonies are cream, circular (1.0–1.5 mm in diameter), convex, opaque with entire margins after growth on NA for 3 days at 28 °C. Growth occurs at 4–42 °C (optimum 28 °C). The salinity and pH ranges for growth are 0–5 % (w/v) NaCl (optimum 0–2 %) and pH 5.0–8.0 (optimum pH 7.0) respectively. Oxidase and catalase are positive. Tween 20 and Tween 40 can be degraded. Gelatin, DNA, starch, casein, agar and Tween 80 catalase are positive. Tween 20 and Tween 40 can be degraded. Gelatin, DNA, starch, casein, agar and Tween 80 are not hydrolysed. With API 20E strips, there are positive reactions in the Biolog GN2 MicroPlate system for Tween 40, Tween 80, pyruvic acid methyl ester, succinic acid monomethyl ester, acetic acid, cis-aconitic acid, citric acid, formic acid, α-hydroxybutyric acid, β-hydroxybutyric acid, γ-hydroxybutyric acid, p-hydroxyphenylactic acid, α-keto butyric acid, α-ketoglutaric acid, α-ketovaleric acid, DL-lactic acid, propionic acid, sebacic acid, succinic acid, succinamic acid, L-alaninamide, L-alanine, L-aspartic acid, L-glutamic acid, L-leucine, L-phenylalanine, L-proline, L-pyroglutamic acid and L-threonine can be oxidized and negative reactions for α-cyclodextrin, dextrin, glycogen, N-acetyl-D-galactosamine, N-acetyl-D-glucosamine, adonitol, L-arabinose, D-arabitol, D-mannitol, L-rhamnose, sucrose, melibiose, amygdalin and L-arabinose.

In the API ZYM strip, alkaline phosphatase, esterase (C4), leucine arylamidase, acid phosphatase and naphthol-AS-BI-phosphohydrolase activities are present; esterase lipase (C8), valine arylamidase and α-chymotrypsin activities are weak; and lipase (C14), cystine arylamidase, trypsin, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, N-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase activities are absent. Acid is produced from glycerol, erythritol, L-arabinose, D-ribose, D-adonitol, D-glucose, D-fructose, D-mannose, inositol, D-sorbitol, melibiose, sucrose, xylitol, gentiobiose, D-fucose, D-arabitol and L-arabitol, but not from D-arabinose, D-xyllose, L-xyllose, methyl β-D-xlyopyranoside, D-galactose, L-sorbose, L-rhamnose, dulcitol, D-mannitol, methyl α-D-mannopyranoside, methyl α-D-glucopyranoside, N-acetyl-glucosamine, amygdalin, arbutin, aesculin, salicin, cellobiose, maltose, lactose, trehalose, inulin, melezitose, raffinose, starch, glyogen, turanose, D-lyxose, D-tagatose, L-fucose, potassium gluconate, potassium 2-ketogluconate and potassium 5-ketogluconate in the API 50CH system. There are positive reactions in the Biolog GN2 MicroPlate system for Tween 40, Tween 80, pyruvic acid methyl ester, succinic acid monomethyl ester, acetic acid, cis-aconitic acid, citric acid, formic acid, α-hydroxybutyric acid, β-hydroxybutyric acid, γ-hydroxybutyric acid, p-hydroxyphenylactic acid, α-keto butyric acid, α-ketoglutaric acid, α-ketovaleric acid, DL-lactic acid, propionic acid, sebacic acid, succinic acid, succinamic acid, L-alaninamide, L-alanine, L-aspartic acid, L-glutamic acid, L-leucine, L-phenylalanine, L-proline, L-pyroglutamic acid and L-threonine can be oxidized and negative reactions for α-cyclodextrin, dextrin, glycogen, N-acetyl-D-galactosamine, N-acetyl-D-glucosamine, adonitol, L-arabinose, D-arabitol, cellobiose, L-erythritol, D-fructose, L-fucose, D-galactose, gentiobiose, myo-inositol, α-lactose, lactulose, maltose, D-mannitol, D-mannose, melibiose, methyl β-D-glucoside, D-psicose, raffinose, L-rhamnose, D-sorbitol, sucrose, trehalose, turanose, xyitol, D-galactonic acid lactone, D-galacturonic acid, D-glucokinase acid, D-glucosaminic acid, D-glucuronic acid, itaconic acid, malonic acid, quinic acid, D-saccharic acid, bromosuccinic acid, glucuronamidase, L-alanine, L-aryl glycine, L-aspar-
agine, glycyl L-aspartic acid, glycyl L-glutamic acid, L-histidine, hydroxy-L-proline, L-ornithine, D-serine, L-serine, DL-carnitine, γ-aminobutyric acid, urocanic acid, inosine, uridine, thymidine, phenylalanine, putrescine, 2-aminooctanol, 2,3-butandiol, glycerol, α-DL-glycerol phosphate, glucose 1-phosphate and glucose 6-phosphate. The dominant fatty acids are C16:0 and C17:0 cyclo. The major polar lipids are phosphatidylethanolamine, phosphatidylmonomethylethanolamine, diphosphatidylglycerol, three unknown phospholipids and four unknown polar lipids.

The type strain, XH089T (=DSM 27279T = JCM 19223T), was isolated from deep-sea sediment of the South Pacific Gyre (41°51’S 153°06’W). The DNA G+C content of the type strain is 66.5 mol%.

Acknowledgements

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Reference


