Oceanirhabdus sediminicola gen. nov., sp. nov., an anaerobic bacterium isolated from sea sediment

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A novel anaerobic bacterium, designated NH-JN4^T was isolated from a sediment sample collected in the South China Sea. Cells were Gram-stain-positive, spore-forming, peritrichous and rod-shaped (0.5–1.2×2.2–7 μm). The temperature and pH ranges for growth were 22–42 °C and pH 6.0–8.5. Optimal growth occurred at 34–38 °C and pH 6.5–7.0. The NaCl concentration range for growth was 0.5–6 % (w/v) with an optimum of 2.5 %. Catalase and oxidase were not produced. Substrates which could be utilized were peptone, tryptone, yeast extract, beef extract and glycine. Main fermentation products from PYG medium were formate, acetate, butyrate and ethanol. Strain NH-JN4^T could utilize sodium sulfite as an electron acceptor. No respiratory quinone was detected. The predominant fatty acids were anteiso-$C_{15:0}$, $C_{16:0}$, iso-$C_{15:0}$, anteiso-$C_{17:0}$ and $C_{16:0}$ DMA. The major polar lipids were diphosphatidylglycerol, phosphatidylglycerol and glycolipids. The DNA G+C content was 35.8 mol%. Phylogenetic analysis based on the 16S rRNA gene sequence revealed that strain NH-JN4^T was a member of family *Clostridiaceae*, and was most closely related to *Clostridium limosum* ATCC 25620^T, *Clostridium proteolyticum* DSM 3090^T, *Clostridium histolyticum* ATCC 19401^T and *Clostridium tepidiprofundi* SG 508^T, showing 94.0, 93.0, 92.9 and 92.3 % sequence similarity, respectively. On the basis of phenotypic, genotypic and chemotaxonomic properties, strain NH-JN4^T represents a novel species of a new genus in the family *Clostridiaceae*, for which the name *Oceanirhabdus sediminicola* gen. nov., sp. nov. is proposed. The type strain of the type species is NH-JN4^T (=JCM 18501^T=CCTCC AB 2013103^T=KCTC 15322^T).

Members of the family *Clostridiaceae* are Gram-positive, rod-shaped and obligatory anaerobic strains (Wiegel, 2009). There are 11 genera in the family *Clostridiaceae* according to the third edition of *Bergey's Manual of Systematic Bacteriology* (Wiegel, 2009), but recently, several genera have been proposed as new members of the family *Clostridiaceae* such as *Brassicibacter* (Fang *et al.*, 2012) and *Sporosalibacterium* (Rezgui *et al.*, 2011), increasing the number of genera to 18. *Clostridium* is the type genus of the family *Clostridiaceae* (Wiegel, 2009). Members of this genus are usually endospore-forming, chemo-organotrophic and produce mixtures of organic acids and alcohols from carbohydrates or peptones (Wiegel, 2009). At the time of writing there are more than 190 species in this genus (Jung *et al.*, 2010) and the species were divided into 19 cluster groups according to their 16S rRNA gene sequences by Collins *et al.* (1994). Some species of this genus were isolated from sediments (Jung *et al.*, 2010; Kim *et al.*, 2006, 2007; Lee *et al.*, 2007). Here, we describe an obligatory anaerobic, spore-forming and Gram-stain-positive strain, which represents a novel species of a new genus belonging to the family *Clostridiaceae*.

Strain NH-JN4^T was isolated with CJW medium from a sediment sample of the South China Sea (118° 67′ E 21° 95′ N), collected by the Second Institute of Oceanography, State Oceanic Administration of China in 2010. CJW medium contained (l^{-1} distilled water) 1.0 g Na_2SO_4, 30.0 g NaCl, 5.0 g $MgCl_2.6H_2O$, 0.7 g KCl, 1.0 g $CaCl_2.2H_2O$, 0.5 g NH_4Cl, 0.4 g K_2HPO_4, 0.4 g KH_2PO_4, 2.0 g $NaHCO_3$, 2.0 mg $Fe(NH_4)_2(SO_4)_2$, 3.0 g yeast extract (Difco), 4.0 g tryptone (Difco), 0.6 g L-cysteine, 0.001 g resazurin, pH 6.8. To make PYG medium 10.0 g glucose, 7.0 g yeast extract, 1.0 g tryptone and 5.0 g peptone were added to CJW medium. To make solid medium, 1.5 % agar was added. Sediment was dispersed and suspended in sterile CJW medium. The clear liquid of the upper layer was inoculated onto plates anaerobically and cultivated at 37 °C in the anaerobic chamber (Bugbox; Ruskinn) until colonies appeared. The Hungate roll-tube technique (Hungate, 1969; Bryant, 1972) was used to

Abbreviation: FAME, fatty acid methyl ester.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain NH-JN4^T is JQ771468.

Three supplementary figures are available with the online version of this paper.

purify strains at least twice before preservation at −80 °C with 20 % (v/v) glycerol and in dry-freeze ampoules. One of the strains, designated NH-JN4^T, was further analysed. *Clostridium tepidiprofundi* DSM 19306^T and *Clostridium proteolyticum* DSM 3090^T were used as reference strains.

Colonies of strain NH-JN4^T were white and semi-transparent with a smooth surface. They were circular with a diameter of 0.5–1 mm on CJW slopes after 24 h incubation at 37 °C. Cell morphology was examined using optical microscopy (BX40; Olympus) and transmission electron microscopy (JEM-1230; JEOL) during stationary growth phase (Tan *et al.*, 2012). Cells were rods (0.5–1.2 × 2.2–7 μm) and peritrichous (Fig. S1, available in IJSEM Online). In the stationary phase of growth, the rods formed terminal endospores. Specific sporulation genes *ssp* and *spo0A* were found in strain NH-JN4^T by the PCR method (Brill & Wiegel, 1997). *Escherichia coli* and *Bacillus subtilis* were used as negative and positive controls, respectively. Gram staining was performed, and the strain was Gram-stain-positive. Ultra-thin section electron micrographs also revealed a typical Gram-positive cell wall structure (Fig. S1).

Genomic DNA was extracted and the 16S rRNA gene was amplified by PCR using the universal bacterial 16S rRNA gene amplification primers 27F (5′-AGAGTTTGATCC-TGGCTCAG-3′) and 1492R (5′-GGTTACCTTGTTAC-GACTT-3′); the annealing temperature for PCR was 55 °C. PCR products were ligated into T-vector and the 16S rRNA gene was sequenced. The sequence was compared with closely related sequences of reference organisms in the database of the Eztaxon-e using Eztaxon-e's identifying function (Kim *et al.*, 2012). Sequence data of the most closely related species and strain NH-JN4^T were aligned using CLUSTAL W (Thompson *et al.*, 1994). Neighbour-joining (Saitou & Nei, 1987), maximum-parsimony (Fitch, 1971) and maximum-likelihood (Felsenstein, 1981) methods in the MEGA 5 software package (Tamura *et al.*, 2011) were used to reconstruct the phylogenetic trees. Evolutionary distances were calculated with a bootstrap value of 1000 for the neighbour-joining method. The algorithm of the Jukes–Cantor model was used according to the function of model selection in MEGA 5. An almost-complete 16S rRNA gene sequence (1473 nt) was obtained from strain NH-JN4^T. Phylogenetic analysis based on this 16S rRNA gene sequence suggested that strain NH-JN4^T was a member of family *Clostridiaceae* and was closely related to the genus *Clostridium* (Fig. 1). This result was also supported by the maximum-parsimony and maximum-likelihood trees both with bootstrap values of 1000 (Fig. S3). The results revealed that strain NH-JN4^T was most closely related to *C. tepidiprofundi* SG 508^T and *C. proteolyticum* DSM 3090^T, which belong to Cluster I and Cluster II of the genus *Clostridium*, respectively (Collins *et al.*, 1994; Slobodkina *et al.*, 2008). The DNA G+C content of strain NH-JN4^T determined by the HPLC method (Mesbah & Whitman, 1989) was 35.8 mol% using salmon sperm DNA as the calibration standard.

To test the growth range and optimal growth conditions, the strain was cultured in CJW medium at 4, 15, 19, 22, 28, 30, 32, 34, 36, 38, 40, 42, 46 and 50 °C as well as at pH 5.5, 6, 6.5, 7, 7.5, 8, 8.5 and 8.8. MES (for pH 5.5–6.5), PIPES (pH 6.1–7.5) or Tricine (pH 7.5–8.8) were added at a concentration of 25 mM to maintain a stable pH. To test the NaCl concentration growth range and optimum, sodium and chloride ions were removed from CJW medium. NaCl concentrations (w/v) of the medium were adjusted to 0, 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 6, 7 and 7.5 %. All tests were performed in triplicate. Optimal growth of strain NH-JN4^T was at 34–38 °C (range 22–42 °C), pH 6.5–7 (range 6.0–8.5) and with 2.5 % NaCl (range 0.5–6 %). No growth was observed when the strain was cultured below 22 °C or above 42 °C after incubation for 10 days. No growth was observed below pH 6.0 or above pH 8.5, or when the NaCl concentration (w/v) was higher than 6 % or in the absence of NaCl. Strain NH-JN4^T did not grow in aerobic medium, which indicated that it was an obligatory anaerobic strain. Catalase and oxidase tests were also performed (Zhu *et al.*, 2011). Catalase activity was detected with 3 % hydrogen peroxide. Oxidase activity was detected with tetramethyl-*p*-phenylenediamine. Catalase and oxidase activities were negative.

To test utilization of carbon sources, basal medium based on CJW medium with yeast extract (Difco) and tryptone (Difco) removed was used. Complex proteinaceous substrates (10 g l^{-1}) such as Casamino acids and peptone and glycerol were added into basal medium which was then autoclaved for 20 min at 121 °C. Sugars and amino acids powder were sterilized by UV light overnight before added into the medium as described by Tan *et al.* (2012). Growth was observed in the presence of peptone (10 g l^{-1}), tryptone (10 g l^{-1}), yeast extract (10 g l^{-1}), beef extract (10 g l^{-1}) and glycine (20 mM). Growth was not observed in the presence of starch (10 g l^{-1}), pyruvate (25 mM), L-valine (25 mM), DL-alanine (20 mM), L-proline (10 mM), DL-alanine (20 mM) + L-proline (10 mM), L-arginine (25 mM), glucose (25 mM), maltose (25 mM), arabinose (25 mM), fructose (25 mM), xylose (25 mM), cellobiose (25 mM), sucrose (25 mM), formate (20 mM), acetate (20 mM), butyrate (20 mM), fumarate (20 mM), olive oil (10 g l^{-1}), carboxymethyl cellulose (10 g l^{-1}), filter paper (10 g l^{-1}), chitin (10 g l^{-1}), Casamino acids (10 g l^{-1}) or glycerol (20 mM). The major fermentation products detected by the HPLC method (Ehrlich *et al.*, 1981) in PYG medium after incubation for 48 h were formate, acetate, butyrate and ethanol. The results of these phenotypic tests are given in Table 1 and in the species description.

Chemotaxonomic analysis was performed on strain NH-JN4^T, *C. tepidiprofundi* DSM 19306^T and *C. proteolyticum* DSM 3090^T. Cells were cultivated in PYG medium at 37 °C until the culture reached late exponential phase. Fatty acids methyl esters (FAMEs) were obtained as described by Kuykendall *et al.* (1988). Identification and qualification of the FAMEs were automatically performed by the Sherlock

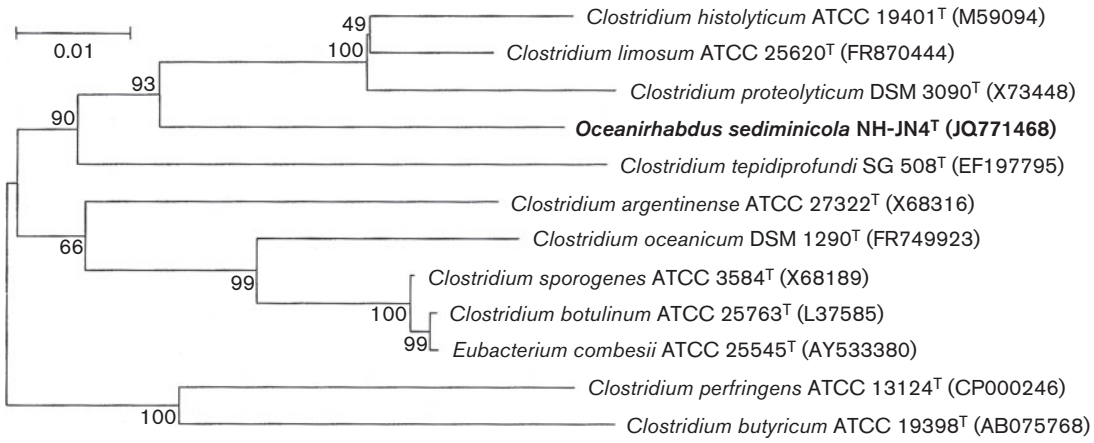

Fig. 1. Neighbour-joining phylogenetic tree based on a comparison of the 16S rRNA gene sequences of strain NH-JN4^T and its closest relatives. GenBank accession numbers are given in parentheses. Percentages of bootstrap support are shown at branch nodes. Bootstrap value is 1000. Bar, 0.01 substitutions per nucleotide position.

Microbial Identification System with the standard MIS Library Generation Software (Microbial ID Inc.) and the results were matched with the ANAEROBE MOORE 3.90 library. The main fatty acids of strain NH-JN4^T were anteiso-$C_{15:0}$ (18.1 %), $C_{16:0}$ (13.6 %), iso-$C_{15:0}$ (12.0 %), anteiso-$C_{17:0}$ (8.7 %) and $C_{16:0}$ DMA (8.2 %). Significant differences existed in the proportion and major compositions of fatty acids of strain NH-JN4, *C. tepidiprofundi* 19306^T and *C. proteolyticum* DSM 3090^T (Table 2).

The polar lipids of strain NH-JN4^T, *C. tepidiprofundi* 19306^T and *C. proteolyticum* DSM 3090^T were extracted, separated on silica gel plates (10 × 10 cm, Merck 5554) and further analysed (Minnikin *et al.*, 1984). Concentrated sulfuric acid and 5 % ethanolic molybdatophosphoric acid were used to reveal total lipids, ninhydrin for aminolipids, α-naphthol for glycolipids and Zinzadze's reagent for phospholipids (Fang *et al.*, 2012). The results suggested that the major polar lipids of strain NH-JN4^T were diphosphatidylglycerol, phosphatidylglycerol and a diversity of glycolipids (Fig. S2, panel 1).

Isoprenoid quinones were extracted using the method described by Minnikin *et al.* (1984), and were analysed by HPLC as described by Tindall (1990). No quinone was detected in strain NH-JN4^T.

To analyse the reduction of electron acceptors, sodium thiosulfate (20 mM), sodium sulfite (5 mM), sodium sulfate (20 mM), sodium nitrite (5 mM) and sodium nitrate (20 mM) were added from filter-sterilized solutions to the basal medium (CJW medium lacking L-cysteine and resazurin). Amorphous iron (III) oxyhydroxide (90 mM) and elemental sulfur (1 %) were added into basal medium before autoclaving. Iron (III) citrate (20 mM) was sterilized by UV light overnight. Reduction of elemental sulfur, sodium thiosulfate, sodium sulfite and sodium sulfate were tested using the method of Ramamoorthy *et al.* (2006). Additional 0.5 % ammonium iron (II) sulfate was added into the cultural tubes after the cells reached stationary phase and tubes were scored positive if black iron (II) sulfide precipitation formed. The reduction of nitrate to nitrite was determined by using Griess reagent; tubes were scored positive if the Griess reagent turned red. The reduction of nitrite to nitrogen gas was determined with Durham tubes as described by Ogg & Patel (2009); tubes were scored positive if gas levels in Durham tubes were larger than that of control group. The reduction of amorphous iron (III) oxyhydroxide was inferred when a transformation of the reddish-brown colour of the iron (III) oxide to a dark precipitate [iron (II)] and a clearing of the media was observed (Ogg & Patel, 2009). The reduction of iron (III) citrate was inferred when the amount of solid iron (III) citrate decreased and the medium turned green. Strain NH-JN4^T could utilize sodium sulfite as the electron acceptor when peptone (10 g l^{-1}) was used as the electron donor. It could not utilize elemental sulfur, sodium thiosulfate, sodium sulfate, sodium nitrate, sodium nitrite, amorphous iron (III) oxyhydroxide and iron (III) citrate as electron acceptors.

16S rRNA gene sequence analysis indicated that strain NH-JN4^T was related to the family *Clostridiaceae* and was most closely related to six uncultured bacterial clones which were retrieved from Caribbean coral (GenBank accession numbers FJ202830, FJ202578, FJ425601, GU118553 and GU118536; similarity value 99 %) and Cedar Key, Florida (EU488325; similarity value 99 %). The novel strain NH-JN4^T formed a separate branch on the phylogenetic tree and showed significant phylogenetic divergence from *C. limosum* ATCC 25620^T, *C. proteolyticum* DSM 3090^T, *C. histolyticum* ATCC 19401^T and *C. tepidiprofundi* SG 508^T (94.0, 93.0, 92.9 and 92.3 % 16S rRNA gene sequence similarity, respectively). The DNA G+C content of strain NH-JN4^T was significantly higher (35.8 mol%) than those

Table 1. Differential phenotypic, physiological and genotypic characteristics of strain NH-JN4^T and its closest phylogenetic relatives

Taxa: 1, strain NH-JN4^T; 2, *C. tepidiprofundi* DSM 19306^T (Slobodkina *et al.*, 2008); 3, *C. proteolyticum* DSM 3090^T (Wiegel, 2009); 4, *C. histolyticum* (Wiegel, 2009); 5, *C. limosum* (Wiegel, 2009; Cato *et al.*, 1970); 6, *C. butyricum* (Wiegel, 2009). +, Positive; −, negative; ±, 61–89% of strains positive; ND, not determined.

Characteristic	1	2	3	4	5	6
Isolation source	Sea sediment	Deep-sea hydrothermal vent	Chicken manure	Gas gangrene in humans	Mud	Soil
Cell width (μm)	0.5–1.2	0.4–0.6	0.5	0.5–0.9	0.6–1.6	0.5–1.7
Cell length (μm)	2.2–7.0	2.0–3.0	2.2	1.3–9.2	1.7–16	2.4–7.6
Motility	+	+	−	±	±	±
Temperature (°C)						
Optimum	34–38	50	30–37	37	37	30–37
Range	22–42	22–60	22–45*	ND	ND	ND
pH						
Optimum	6.5–7.0	6.0–6.8	6.0–8.0	ND	ND	ND
Range	6.0–8.5	4.0–8.5	5.5–9.0*	ND	ND	ND
NaCl concentration (%)						
Optimum	2.5	2.5	0*	ND	ND	ND
Range	0.5–6	1–6	0–3.5*	ND	ND	ND
DNA G+C content (mol%)	35.8	30.9	29.5	ND	24	28–29
Carbohydrate fermentation:						
Glucose	−	+	−	ND	±	ND
Maltose	−	+	−	−	−	+
Amino acid fermentation						
Glycine	+	−	−	+	ND	ND
Reduction of electron acceptors						
Elemental sulfur	−	+	−*	ND	ND	ND
Sodium sulfite	+	−	−*	ND	ND	ND
Sodium nitrate	−	−	+*	ND	ND	ND
16S rRNA gene sequence similarity to strain NH-JN4^T (%)	100	92.3	93.0	92.9	94.0	90.0
Fermentation products in PYG medium†	F, A, B, E	B,E*	A,M*	A	A, F, S, L	B, A, F, L, S

*Data from this study.

†Fermentation products: F, formate; A, acetate; B, butyrate; E, ethanol; M, methanol; S, succinate; L, lactate.

Table 2. Fatty acid contents of strain NH-JN4^T and its closest phylogenetic relatives

Taxa: 1, strain NH-JN4^T; 2, *C. tepidiprofundi* DSM 19306^T; 3, *C. proteolyticum* DSM 3090^T. Data are from this study. Values are percentages of total fatty acids. Fatty acids representing >5 % of the total fatty acids are in bold. −, Not detected.

Fatty acid	1	2	3
$C_{9:0}$	0.2	−	−
$C_{10:0}$	0.2	−	−
$C_{13:0}$ iso	1.3	1.1	−
$C_{13:0}$ anteiso	−	0.2	−
$C_{13:0}$ iso 3OH	−	0.5	−
$C_{13:1}$ *cis* 12	−	0.5	−
$C_{14:0}$	5.1	3.6	**19.7**
$C_{14:0}$ DMA	1.5	2.3	1.3
$C_{14:0}$ iso	−	0.6	−
$C_{15:0}$	−	−	0.9
$C_{15:0}$ iso	**12.0**	**38.4**	−
$C_{15:0}$ iso ALDE	2.1	2.4	−
$C_{15:0}$ anteiso	**18.1**	3.3	1.0
$C_{15:0}$ iso DMA	4.1	**11.4**	−
$C_{15:0}$ anteiso DMA	−	2.4	−
$C_{15:2}$	1.6	−	0.7
$C_{16:0}$ ALDE	2.4	0.3	−
$C_{16:0}$	**13.6**	**14.5**	**31.6**
$C_{16:0}$ DMA	**8.2**	1.9	2.30
$C_{16:0}$ iso	−	2.6	−
$C_{16:1}$ *cis* 7 DMA	1.5	0.2	−
$C_{16:1}$ *cis* 7	−	0.4	−
$C_{16:1}$ *cis* 9	1.0	0.8	1.7
$C_{16:1}$ *cis* 9 DMA	1.6	0.6	4.0
$C_{16:1}$ *cis* 11	−	−	0.7
Unknown 16.107 C_{16} DMA	−	0.6	−
$C_{17:2}$	−	−	3.7
$C_{17:0}$ iso	2.0	**9.0**	−
$C_{17:0}$ anteiso	**8.7**	1.1	−
$C_{17:0}$ cyc	1.5	0.6	−
Unknown 17.103 $C_{17:0}$ DMA	4.1	0.6	−
$C_{18:0}$	4.8	0.3	1.4
$C_{18:0}$ DMA	−	−	0.5
$C_{18:1}$ *cis* 9	−	0.1	−
$C_{18:1}$ c11/t9/t6	−	−	**9.2**
$C_{18:1}$ *cis* 11 DMA	−	−	**20.5**
$C_{18:1}$ cis 13	−	−	0.8
$C_{19:0}$ anteiso	1.2	−	−
$C_{20:0}$	3.6	−	−

of *C. limosum* ATCC 25620^T (24 mol%), *C. proteolyticum* DSM 3090^T (29.5 mol%) and *C. tepidiprofundi* SG 508^T (30.9 mol%).

Moreover, *C. tepidiprofundi* DSM 19306^T is a moderately thermophilic bacterium with an optimal growth temperature of 50 °C and endospore formed only below pH 5.5 (Slobodkina *et al.*, 2008), whereas the upper growth temperature of strain NH-JN4^T was 42 °C and endospore formed in the optimal environment. Both strain NH-JN4^T and *C. tepidiprofundi* DSM 19306^T were tolerant to high NaCl concentrations (6 %) while *C. proteolyticum* DSM 3090^T could not grow when the NaCl concentration was above 4 %. Strain NH-JN4^T utilized glycine as its sole carbon source while *C. tepidiprofundi* DSM 19306^T was not observed to utilize any amino acid (Slobodkina *et al.*, 2008). However, *C. tepidiprofundi* DSM 19306^T could use some kinds of common mono- and disaccharides (Slobodkina *et al.*, 2008) while strain NH-JN4^T could not. As for *C. proteolyticum* DSM 3090^T, it can use neither carbohydrates nor amino acids (Wiegel, 2009). The fermentation products of strain NH-JN4^T in PYG medium were mainly formate, acetate, butyrate and ethanol. In contrast, end products of fermentation in PYG medium were acetate and methanol for *C. proteolyticum* DSM 3090^T, and butyrate and ethanol for *C. tepidiprofundi* DSM 19306^T. Strain NH-JN4^T could only utilize sodium sulfite as an electron acceptor, while *C. tepidiprofundi* DSM 19306^T used elemental sulfur (Slobodkina *et al.*, 2008) and *C. proteolyticum* DSM 3090^T used sodium nitrate. Genomic and phenotypic traits suggest that strain NH-JN4^T is distinct from its most related genus *Clostridium*.

Finally, the evidence from chemotaxonomy also showed distant relatedness among strain NH-JN4^T, *C. proteolyticum* DSM 3090^T and *C. tepidiprofundi* DSM 19306^T. The most abundant FAME of strain NH-JN4^T was anteiso-$C_{15:0}$ (18.1 %) while those of *C. proteolyticum* DSM 3090^T and *C. tepidiprofundi* DSM 19306^T were $C_{16:0}$ (31.6 %) and iso-$C_{15:0}$ (38.4 %), respectively. Strain NH-JN4^T possessed anteiso-$C_{19:0}$ and $C_{20:0}$ while other two strains lacked these fatty acids. Additionally, *cis*-$C_{18:1}$ 11 DMA (20.5 %) and $C_{18:1}$ c11/t9/t6 were present in cell extracts of *C. proteolyticum* DSM 3090^T but were not detected in cell extracts of strain NH-JN4^T or *C. tepidiprofundi* DSM 19306^T. Significant differences among strain NH-JN4^T, *C. tepidiprofundi* DSM 19306^T and *C. proteolyticum* DSM 3090^T also existed in the composition of polar lipids. Strain NH-JN4^T had a variety of glycolipids whereas *C. tepidiprofundi* DSM 19306^T as well as *C. proteolyticum* DSM 3090^T had some aminolipids and a diversity of phospholipids (Fig. S2).

On the basis of the genotypic and phenotypic differences, and the large phylogenetic distance separating strain NH-JN4^T from the most closely related genus *Clostridium* of the family *Clostridiaceae*, it is proposed that the strain represents a novel species of a new genus, for which the name *Oceanirhabdus sediminicola* gen. nov., sp. nov. is proposed.

Description of *Oceanirhabdus* gen. nov.

Oceanirhabdus (O.ce.a.ni.rhab′dus. L. n. *oceanus* ocean; Gr. fem. n. *rhabdos* rod; N.L. fem. n. *Oceanirhabdus* a rod of the ocean).

Cells are obligatory anaerobic, spore-forming rods and stain Gram-positive. Catalase and oxidase were not produced. Species of this genus can utilize complex

proteinaceous substrates as carbon and energy source. The major cellular fatty acids are anteiso-$C_{15:0}$, $C_{16:0}$, iso-$C_{15:0}$, anteiso-$C_{17:0}$ and $C_{16:0}$ DMA. No isoprenoid quinone is detected. The major polar lipids are diphosphatidylglycerol, phosphatidylglycerol and glycolipids. The DNA G+C content of the only known strain of the type, and only known, species is 35.8 mol%. 16S rRNA gene sequence analysis indicates that the genus is a member of the family *Clostridiaceae* and is most closely related to the genus *Clostridium*. The type species is *Oceanirhabdus sediminicola*.

Description of *Oceanirhabdus sediminicola* sp. nov.

Oceanirhabdus sediminicola [se.di.mi.ni′co.la. L. n. *sedimen -inis* sediment; L. suff. *-cola* (from L. n. *incola*) inhabitant, dweller; N.L. n. *sediminicola* sediment dweller].

Cells are 0.5–1.2 × 2.2–7.0 μm and peritrichous. Growth is observed at 22–42 °C (optimum 34–38 °C), at pH 6.0–8.5 (optimum 6.5–7.0) and with 0.5–6 % (w/v) NaCl (optimum 2.5 %). The substrates used are peptone, tryptone, yeast extract, beef extract and glycine. Growth can be observed in the presence of peptone, tryptone, yeast extract, beef extract and glycine. Growth is not observed in the presence of starch, pyruvate, L-valine, DL-alanine, L-proline, DL-alanine + L-proline, L-arginine, glucose, maltose, arabinose, fructose, xylose, cellobiose, sucrose, formate, acetate, butyrate, fumarate, olive oil, CM-cellulose, filter paper, chitin, Casamino acids or glycerol. The major fermentation products from PYG medium are formate, acetate, butyrate and ethanol. Sodium sulfite can be utilized as an electron acceptor but not elemental sulfur, sodium thiosulfate, sodium sulfate, sodium nitrate, sodium nitrite, amorphous iron (III) oxyhydroxide or iron (III) citrate.

The type strain, NH-JN4^T (=JCM 18501^T=CCTCC AB 2013103^T=KCTC 15322^T) was isolated from a sediment sample of the South China Sea. The DNA G+C content of the type strain is 35.8 mol%.

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