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Aceticlastic and NaCl-Requiring Methanogen "*Methanosaeta pelagica*" sp. nov., Isolated from Marine Tidal Flat Sediment

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Among methanogens, only 2 genera, *Methanosaeta* and *Methanosarcina*, are known to contribute to methanogenesis from acetate, and *Methanosaeta* is a specialist that uses acetate specifically. However, *Methanosaeta* strains so far have mainly been isolated from anaerobic digesters, despite the fact that it is widespread, not only in anaerobic methanogenic reactors and freshwater environments, but also in marine environments, based upon extensive 16S rRNA gene-cloning analyses. In this study, we isolated an aceticlastic methanogen, designated strain 03d30q^T, from a tidal flat sediment. Phylogenetic analyses based on 16S rRNA and *mcrA* genes revealed that the isolate belongs to the genus *Methanosaeta*. Unlike the other known *Methanosaeta* species, this isolate grows at Na^+ concentrations of 0.20 to 0.80 M, with an optimum concentration of 0.28 M. Quantitative estimation using real-time PCR detected the 16S rRNA gene of the genus *Methanosaeta* in the marine sediment, and relative abundance ranged from 3.9% to 11.8% of the total archaeal 16S rRNA genes. In addition, the number of *Methanosaeta* organisms increased with increasing depth and was much higher than that of *Methanosarcina* organisms, suggesting that aceticlastic methanogens contribute to acetate metabolism to a greater extent than previously thought in marine environments, where sulfate-reducing acetate oxidation prevails. This is the first report on marine *Methanosaeta* species, and based on phylogenetic and characteristic studies, the name "*Methanosaeta pelagica*" sp. nov. is proposed for this novel species, with type strain 03d30q.

The global budget of atmospheric CH_4 is approximately 500 to 600 Tg per year (28), and approximately 74% of the emitted CH_4 is derived from biological methanogenesis (6, 20, 64). Acetate is a key compound for methanogenic degradation of organic matter, and the methanogenesis accounts for two-thirds of the total CH_4 generated (22). However, only 2 genera, *Methanosarcina* and *Methanosaeta*, are capable of aceticlastic methanogenesis. *Methanosarcina* is a versatile methanogen that prefers methanol, methylamine, and hydrogen to acetate (2), whereas *Methanosaeta* is a specialist that uses acetate only (4). The 2 genera use different enzymes to activate acetate (20). Complete genome sequencing of *Methanosarcina acetivorans* C2A^T, *Methanosarcina mazei* Go1, *Methanosaeta concilii* GP6, and *Methanosaeta thermophila* P_T^T revealed that the 2 genera use different enzymes to catalyze the first step of aceticlastic methanogenesis but that the core steps of methanogenesis are similar (1, 10, 17, 54). A major difference between the genera is their affinity for acetate; the minimum threshold for acetate use is much lower in *Methanosaeta* (7 to 70 μM) than in *Methanosarcina* (0.2 to 1.2 mM) (20). Accordingly, *Methanosaeta* adapts to low-acetate environments and predominates over *Methanosarcina* in environments such as rice fields (18), landfill sites (25, 35), and anaerobic digesters (27, 52). *Methanosaeta* may be widely distributed in nature and may be the predominant CH_4 producer on earth.

Methanogens are abundant in habitats where electron acceptors, such as oxygen, nitrate, and sulfate, are strictly limited. In anaerobic marine habitats, because of the abundance of sulfate in seawater, sulfate-reducing bacteria usually degrade organic matter, including acetate. Meanwhile, many studies on marine sediment communities have demonstrated that methanogenesis also occurs in the high-sulfate-concentration and anaerobic methane oxidation layers, as well as in the deep sulfate-depleted zone (7, 12, 44). Methylotrophic methanogens, including *Methanosarcina*, are regarded as major contributors to CH_4 production in these areas, since methylated compounds cannot be used by sulfate-reducing bacteria and thus remain available for methanogens as noncompetitive substrates (41). In marine sediments, particularly in the deep sulfate-depleted zone, CH_4 is thought to originate from H_2-CO_2 rather than from acetate (63). Also, various methanogens have been retrieved from brackish environments, such as estuarine and tidal flat sediments, by molecular approaches (13, 47, 48). In these environments, methanogens probably do not compete with sulfate-reducing bacteria, because organic substrates are not limiting.

As aceticlastic methanogens, several strains of *Methanosarcina*, but not *Methanosaeta*, have been isolated from marine sediments (14, 24, 56). However, as stated previously, *Methanosarcina* is a versatile methanogen that uses a wide range of substrates, and it may function as a methylotrophic or hydrogenotrophic methanogen. Acetate concentrations in the pore water of marine sediments are usually less than 20 μM (23, 43); thus, conditions appear to be suitable for *Methanosaeta* rather than for *Methanosarcina*. Many clones related to *Methanosaeta* have been detected in marine sediments (11, 24, 32, 42, 46), but their identities remain unknown. In the present study, we report on an aceticlastic methanogen belonging to the genus *Methanosaeta* isolated from a tidal flat

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TABLE 1 PCR primer sets for this study

Gene	Primers (forward/reverse)	Target group	Amplicon length (bp)	Reference
16S rRNA	530f/1392r	Universal	900	26
	A109f/ARC915	*Archaea*	800	18, 58
	Ar0023mLF/Ar1530R	*Archaea*	1,500	36
	27f/1492r	*Bacteria*	1,500	26
	Bac349F/Bac806R	*Bacteria*	460	59
	A344F/ARC915	*Archaea*	570	5, 58
	MX825cm/ARC915	*Methanosaeta*	90	This study, 58
	MS821c/ARC915	*Methanosarcina*	90	49, 58
mcrA	MR1mod/ME2mod	*Archaea*	1,100	This study

sediment. In addition, based on the results of phylogenetic and ecological investigations, we discuss the contribution to CH_4 production from acetate in marine ecosystems.

MATERIALS AND METHODS

Microorganisms and growth conditions. Strain 03d30q^T (= NBRC 105920^T = DSM 24271^T) was isolated as described below. *Escherichia coli* NBRC 3301 (= K-12), *Methanosarcina barkeri* NBRC 100474^T, *M. thermophila* NBRC 101360^T, *M. concilii* NBRC 103675^T, and *Methanosaeta harundinacea* NBRC 104789^T were used for analyses. The cultivation media were NBRC media no. 802, 837, 897, 994, and 925 (39), respectively, and all cultures were cultivated at a temperature of 37°C, except *M. thermophila* (55°C).

Study area, sample collection, and measurements. The Futtsu tidal flat is a foreshore sand flat located from the Koito River estuary to the sea in Tokyo Bay, Chiba Prefecture, Japan. Sediment samples were collected on 24 June 2005 using a peat sampler (model DIK-105A; Daiki Rika Kogyo). The collected samples were immediately (approximately 2 h) transported to the laboratory in a sealed nylon bag with an O_2-absorbing and CO_2-generating agent (Anaero-Pack; Mitsubishi Gas Chemical) and inoculated into medium as soon as possible. Samples for molecular analysis were stored in 2-ml tubes at −80°C until extraction of DNA. Sediment temperature was measured on site using a thermometer. Pore water salinity and pH were measured on site using a refractometer (ATC-S/Mill-E; Atago) and pH meter (D-13; Horiba), respectively.

Enrichment, isolation, and purity test for aceticlastic methanogens. NBRC medium no. 1108 was used to enrich and isolate the aceticlastic methanogens. The medium was composed of the following salts and solutions (liter^{-1}): 1.19 g KH_2PO_4, 0.21 g K_2HPO_4, 3.05 g $MgCl_2 \cdot 6H_2O$, 0.15 g $CaCl_2 \cdot 2H_2O$, 0.54 g NH_4Cl, 20 g NaCl, 6.56 g sodium acetate, 1.5 g Bacto Yeast Extract (Difco), 0.4 g Bacto Tryptone (Difco), 0.14 g coenzyme M (2-mercaptoethanesulfonic acid sodium salt), 2.5 g $NaHCO_3$, 1 mg resazurin, 2 ml trace element solution (37), 10 ml vitamin solution (37), and 0.36 g $Na_2S \cdot 9H_2O$. The medium was prepared in culture vessels with butyl-rubber stoppers and aluminum seals under N_2-CO_2 (80/20 [vol/vol]). Enrichments and routine cultivations were conducted in 50-ml culture vessels containing 20 ml of the liquid medium.

The purity of the isolate was verified by microscopic observation, inoculation into the various media, and determination of the 16S rRNA gene sequences amplified using the following primer sets: universal primer set 530f and 1392r, bacterial primer set 27f and 1492r (26), and archaeal primer set A109f (18) and ARC915 (58) (Table 1).

Phenotypic and chemotaxonomic features. Optimum temperatures, initial pH values, and Na^+ concentrations for growth were determined by examining the time course of CH_4 production. The pH of the medium was adjusted by adding Na_2CO_3 or HCl at room temperature. The range of Na^+ concentrations for the growth of each *Methanosaeta* species was examined using each medium and adding various concentrations of NaCl. The gas phase of the cultures was analyzed by gas chromatography using a thermal-conductivity detector and a Molecular Sieve 60/80 column (both from Shimadzu) (34).

The G+C content of extracted genomic DNA was analyzed using high-performance liquid chromatography (HPLC) with a reverse-phase column (31). The Gram reaction and susceptibility and motility tests were performed as described by Boone and Whitman (3). To determine DNA-DNA relatedness, the fluorometric microplate hybridization method developed by Ezaki et al. (15, 16) was applied.

Microscopy. An Olympus AX70 microscope was used for routine observation. For the observation of ultrathin sections of cells, cells were prepared by using rapid freezing and the freeze-substitution method (65). After the procedures, the cells were embedded, stained with uranyl acetate and lead citrate after prestaining with 0.2% oolong tea extract in potassium phosphate buffer (51, 65), and observed using a transmission electron microscope (H-7650; Hitachi) operating at 100 kV.

DNA extraction, PCR, sequencing, and quantitative PCR. Extraction and purification of the genomic DNA of the microorganisms were performed as described previously (38). The PCR primers used in this study are summarized in Table 1. The 16S rRNA gene of the microorganisms was amplified and sequenced as described previously (36). The partial *mcrA* (alpha subunit of methyl-coenzyme M reductase) gene was amplified using primers MR1mod (5′-GAC CTS CAC TWC GTV AAC AAC-3′) and ME2mod (5′-TCA TBG CRT AGT TNG GRT AGT-3′), which were slightly modified MR1 (53) and ME2 (19) primers based on the genome sequence of *M. thermophila* (CP000477). PCR amplification and sequencing of the products were performed as described previously (33). The genomic DNA of the microbial cells in the sediments was extracted using a Power Soil DNA Isolation Kit (MoBio). Quantitative PCR was performed as described previously (36), except for the primer sets and standards. The following 16S rRNA gene-targeted PCR primer sets were used: Bac349F and Bac806R (59) for the domain *Bacteria*, A344F (5) and ARC915 (58) for the domain *Archaea*, MX825cm (modified MX825c; 5′-GCT AGG TGT CRG YCA CGG TGC GA-3′) (8) and ARC915 for the genus *Methanosaeta*, and MS821c (5′-GCT CGC TAG GTG TCA GGC ATG GCG-3′) (49) and ARC915 for the genus *Methanosarcina*. The template standards for each primer set were constructed using the dilution series of the 16S rRNA gene PCR products of *E. coli* (for *Bacteria*), *M. thermophila* (for *Archaea* and *Methanosaeta*), and *M. barkeri* (for *Methanosarcina*). These PCR products were used in each real-time PCR analysis to calculate the copy number of the 16S rRNA genes in the sediment samples. The efficiencies of quantitative PCR analyses for *Bacteria*, *Archaea*, *Methanosaeta*, and *Methanosarcina* were 1.01, 0.77, 0.77, and 0.78, respectively. Copy numbers were calculated from duplicate data in 2 independent analyses using the same DNA extract. Two tests were performed to confirm the specificity of the real-time PCR assay: a melting-curve analysis performed after the amplification and a size analysis of the PCR products confirmed by gel electrophoresis. In addition, to confirm specificity, real-time PCR products for the assays of the genera *Methanosaeta* and *Methanosarcina* were cloned using a TOPO TA Cloning Kit (Invitrogen), and several representatives were sequenced using the M13 primer set.

Phylogenetic analysis. Phylogenetic analyses were carried out using the 16S rRNA gene sequence and deduced amino acid sequence of the *mcrA* gene. The 16S rRNA gene sequences were aligned with an ARB data set using ARB software (29), and the resulting alignment was recorrected manually considering primary and secondary structures. The data set of the *mcrA* gene was aligned using Clustal X software (60). Phylogenetic trees were inferred using the neighbor-joining (NJ) method in the Clustal X packages, and 1,000 replicate data sets were used for the bootstrap analysis (50, 60).

Nucleotide sequence accession numbers. The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA and *mcrA* gene sequences are AB679167 to AB679171.

RESULTS

Site description. Core samples of sandy sediments were collected from 2 points (sample no. 031 and 032) at low tide. As some sea grass grew near sample no. 031 and the sediments were predominantly blackish, the conditions were inferred to be completely anaerobic. For sample no. 032, the sediments changed gradually from gray to black with increasing depth, and the deeper sediments probably maintained anaerobic conditions. Sediment samples were collected at 3 depths (10, 35, and 60 cm) for molecular analysis, and the sediments from sample no. 032 at depths of 35 cm and 60 cm were used for the enrichment of aceticlastic methanogens. The average temperature, pH, and salinity were 21°C, 7.3, and 32‰, respectively, in the sediments and 25°C, 7.8, and 31‰, respectively, in the surrounding seawater. The measurement values of the sediments were not much different at different depths.

Enrichment and isolation. Primary enrichment cultures of aceticlastic methanogens were obtained by inoculating the medium with 1 g of each sediment sample and incubating it at 20°C and 30°C. After 1 month of cultivation, microbial growth and CH_4 formation were observed in all cultures, and the primary enrichment cultures were transferred to fresh medium. Microscopic observation of enrichment after several passages indicated that *Methanosaeta*-like cells were enriched in the culture of sample no. 032 (60-cm depth) at 30°C. Therefore, the acquisition of pure culture was focused on the enrichment. Since colony formation in medium solidified with 1.5% agar was not successful, serial dilution was used for the isolation. It became clear that the *Methanosaeta*-like cells were dominant and that the transferred culture at higher dilution ($>10^7$) grew continuously in the medium even after removing yeast extract and tryptone, although more than 3 months was required to confirm growth. After repeating the dilution culture several times, an aceticlastic methanogen, designated strain 03d30q^T, was obtained (Fig. 1A). The purity of the isolate was verified by determining the 16S rRNA gene sequence amplified from the extracted DNA using various primer sets and inoculation into test media. No growth was observed in medium under H_2-CO_2 (80/20 [vol/vol]; 150 kPa) as a substitute for N_2-CO_2; medium supplemented with 10 mM sodium lactate, 10 mM sodium sulfate, and 10 mM sodium thiosulfate; and NBRC medium no. 325 (39) for aerobic heterotrophs. These results confirmed that the culture was free of hydrogenotrophic methanogens and heterotrophs.

Phylogenetic analysis. We determined nearly full-length 16S rRNA gene sequences of strain 03d30q^T (1,383 bp; AB679167) and *M. concilii* NBRC 104675^T (1,407 bp; AB679168). Phylogenetic analysis based on the 16S rRNA gene sequence (Fig. 2) indicated that strain 03d30q^T should be placed in the genus *Methanosaeta* and that the sequence similarities between the isolate and the valid species *M. concilii*, *M. thermophila*, and *M. harundinacea* were 92.8%, 92.5%, and 97.0%, respectively. In the genus *Methanosaeta*, strain 03d30q^T formed a distinct cluster with environmental clone sequences retrieved from saline environments, such as hydrothermal sediments, cold-seep sediments, oil reservoirs, marine methane hydrates, and ancient seawater (11, 24, 32, 40, 42, 46), though it is distantly related to the sequences from terrestrial environments, anaerobic methanogenic reactors, and *M. harundinacea* isolated from an upflow anaerobic sludge blanket (UASB) reactor treating brewery wastewater (30). The cluster was clearly separated from the anaerobic methane-oxidizing archaeal (ANME) clusters that have often been retrieved from marine sediments. In addition to the 16S rRNA gene sequence, the *mcrA* gene sequences of strain 03d30q^T (1,150 bp; AB679169), *M. concilii* NBRC 103675^T (1,150 bp; AB679170), and *M. harundinacea* NBRC 104789^T (1,150 bp; AB679171) were also determined. The NJ tree for McrA (Fig. 3) demonstrated that strain 03d30q^T was also associated with the genus *Methanosaeta*, and the similarities between the isolate and *M. concilii*, *M. thermophila*, and *M. harundinacea* were 82.2%, 85.6%, and 90.9%, respectively. DNA-DNA hybridization studies between strain 03d30q^T and *M. harundinacea* NBRC 104789^T showed relatedness values of less than 1%.

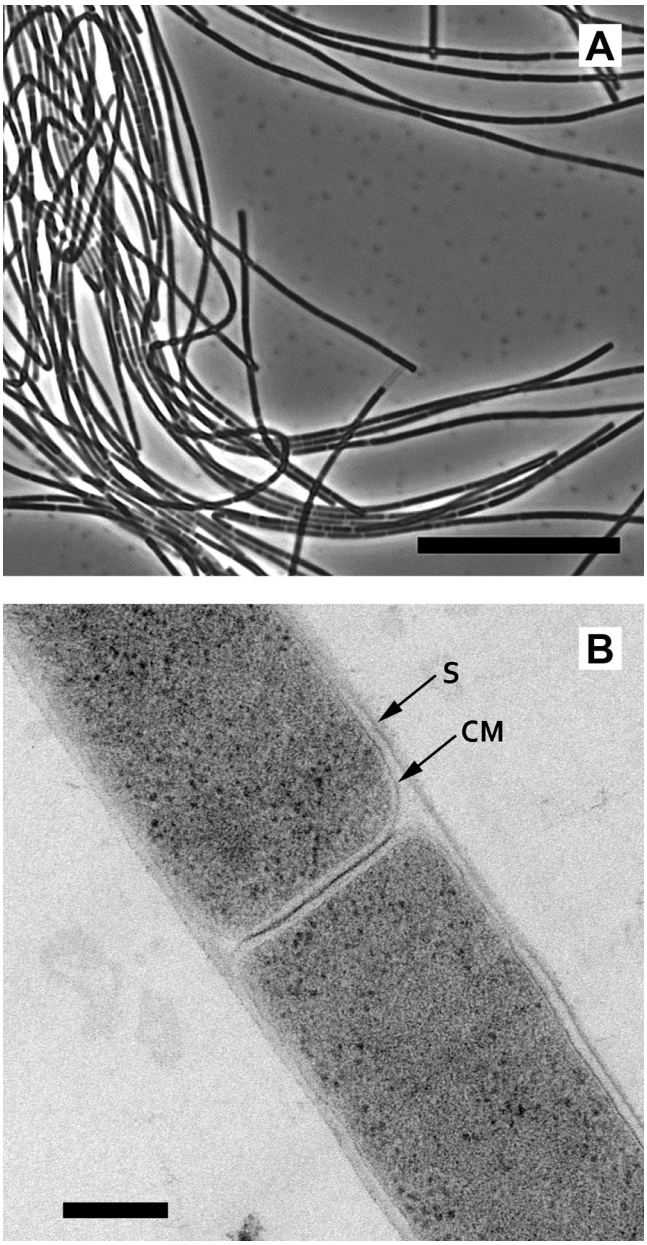

FIG 1 (A) Phase-contrast micrograph of strain 03d30q^T (bar, 10 μm). (B) Ultrathin section of strain 03d30q^T observed with a transmission electron microscope (bar, 0.2 μm). CM, cytoplasmic membrane; S, sheath.

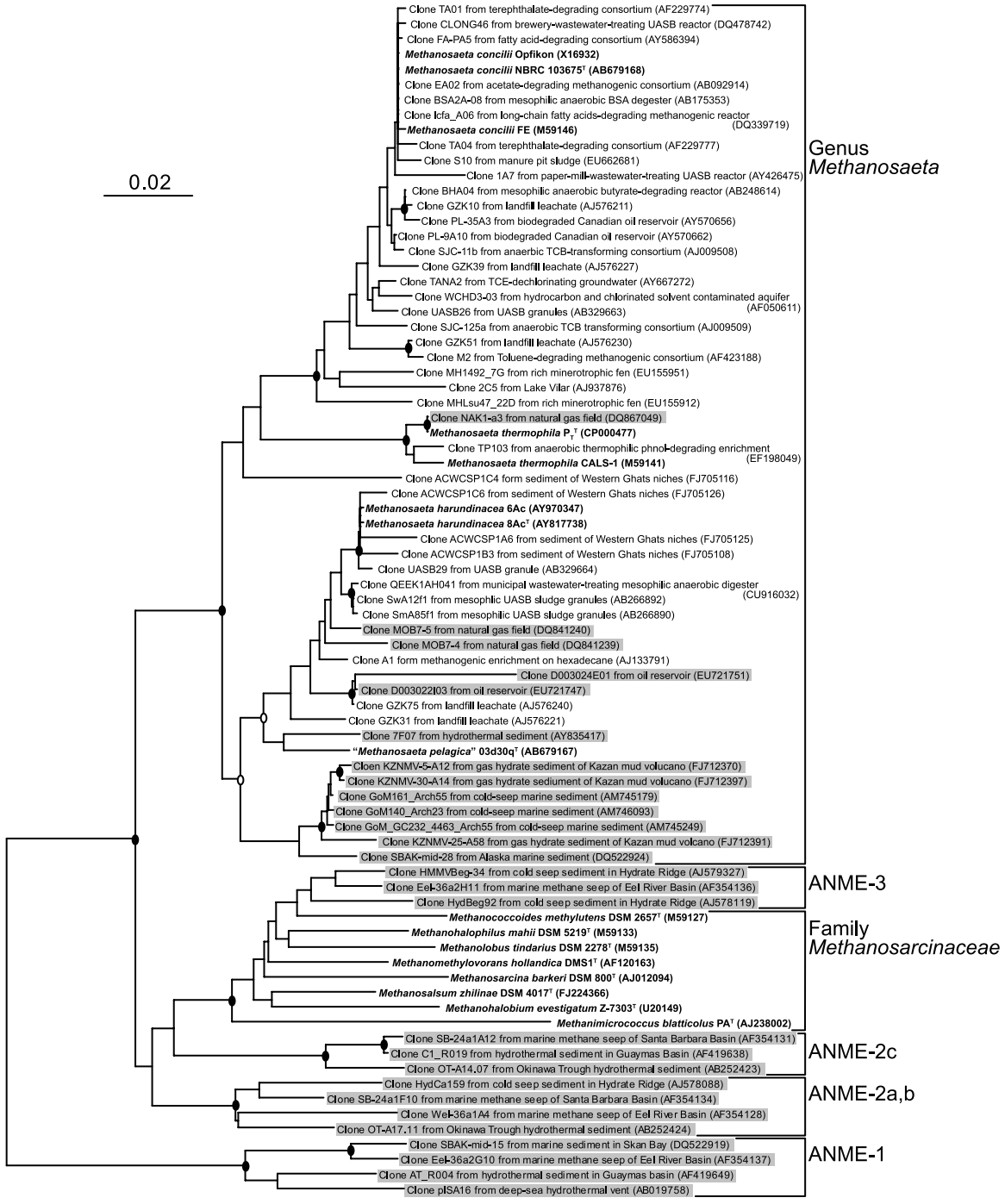

FIG 2 Neighbor-joining tree based on 16S rRNA gene sequences of strain 03d30q^T and relatives. The probability scores obtained at branch points are indicated by solid circles (above 95%) and open circles (above 90%). The sequences obtained from isolates are in boldface. Environmental clonal sequences retrieved from saline environments are shaded. GenBank/EMBL/DDBJ accession numbers are shown in parentheses. Bar, 0.02 substitutions per compared nucleotide site.

Physiology of the isolate and Na^+ requirement. Morphologically, the cells of strain 03d30q^T were weakly autofluorescent nonmotile rods, and many multicellular filaments were observed microscopically (Fig. 1A). A tubular sheath enclosed the cells, and the individual cells within the sheath had a cytoplasmic membrane (Fig. 1B). Gram staining of the cells was negative, and susceptibility to lysis by detergent (SDS; 0.1 g liter^{-1}) or hypotonic conditions was not observed. The G+C content of the genomic DNA of strain 03d30q^T was 45.4 mol%. Strain 03d30q^T used acetate as the sole energy and carbon source, and yeast extract stimulated its growth. Growth and methane formation were not observed on H_2-CO_2 (80/20; 125 kPa), formate (40 mM), methanol (20 mM), ethanol (20 mM), methylamine (20 mM), dimethylamine (20 mM), trimethylamine (20 mM), or dimethyl sulfide (4

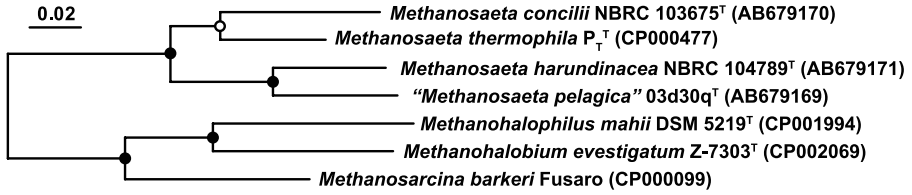

FIG 3 Neighbor-joining tree showing the relationships between strain 03d30q^T and relatives based on deduced McrA sequences. The probability scores obtained at branch points are indicated by solid circles (above 95%) and an open circle (above 90%). GenBank/EMBL/DDBJ accession numbers are shown in parentheses. Bar, 0.02 substitutions per compared position.

mM). Strain 03d30q^T was able to grow at temperatures between 20°C and 35°C and initial pH values between 6.0 and 7.8, with optimum growth at 30°C and initial pH 7.5 (Fig. 4). The effect of the Na^+ concentration in the medium on growth, determined by CH_4 production of strain 03d30q^T and other *Methanosaeta* species, is shown in Fig. 5. The Na^+ concentrations for growth of strain 03d30q^T ranged from 0.20 to 0.80 M, with optimum growth at 0.28 M. The other *Methanosaeta* species grew optimally in each medium containing the lowest Na^+ concentration. The upper Na^+ concentrations for growth of *M. concilii*, *M. thermophila*, and *M. harundinacea* were 0.40 M, 0.56 M, and 0.34 M, respectively. Strain 03d30q^T had a doubling time of 12.4 days under optimum growth conditions (30°C, pH 7.5, and 0.28 M Na^+).

Environmental quantification. Field estimation of aceticlastic methanogens in tidal flat sediments was performed with quantitative real-time PCR assays using the 16S rRNA gene copy number (Fig. 6). The relative abundances of *Methanosaeta* and *Methanosarcina* were calculated as the percentages of the total archaeal 16S rRNA gene copy number in the DNA extracts (Fig. 6, in parentheses). The number of *Bacteria* tended to decrease and the number of *Archaea* tended to increase with increasing depth. The number of *Methanosaeta* organisms increased slightly with increasing depth, and the proportion of total *Archaea* ranged from 3.9 to 11.8%. The number of *Methanosarcina* organisms was less than 1/10 the number of *Methanosaeta* organisms, and the maximum proportion was 0.9% of total *Archaea*.

DISCUSSION

Ecology and physiology of a marine *Methanosaeta* strain. Previous studies predicted the occurrence of *Methanosaeta*-type organisms in marine environments based on molecular analyses. They indicated that *Methanosaeta* species inhabit saline environments, such as marine sediments, gas hydrate sediments, hydrothermal sediments, oil reservoirs, and natural gas fields (11, 24, 32, 40, 42, 46) (Fig. 2), but the identities of the organisms remained unknown. Sowers reported that an acetate-utilizing rod-shaped methanogen was obtained from marine sediments (55), but it is

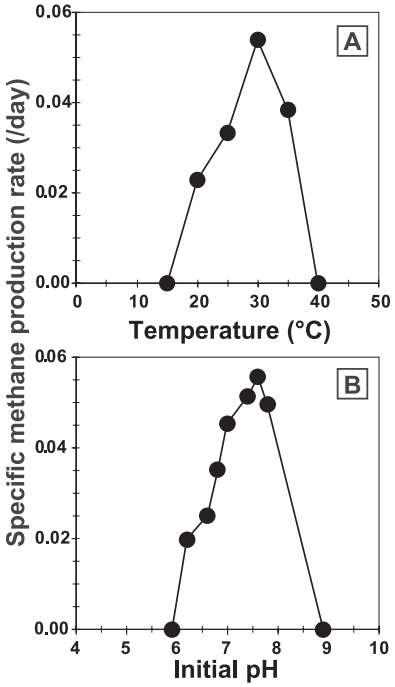

FIG 4 Effect of temperature (A) and initial pH (B) on CH_4 production of strain 03d30q^T.

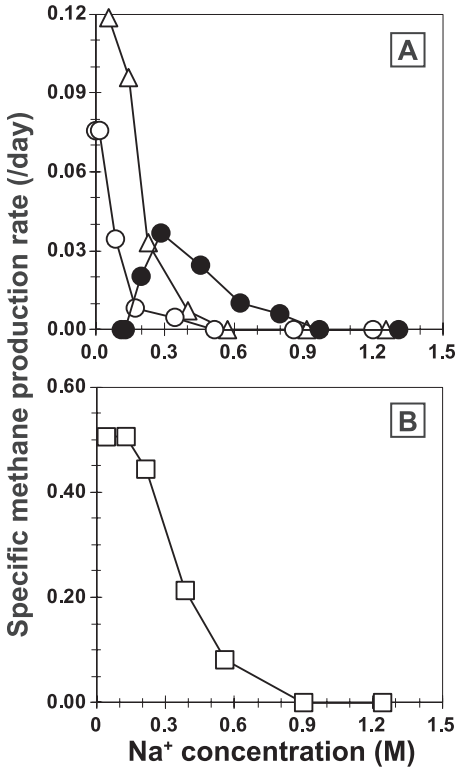

FIG 5 Effect of Na^+ concentration on CH_4 production of strain 03d30q^T (solid circles), *M. concilii* (triangles), and *M. harundinacea* (open circles) (A) and *M. thermophila* (B). The Na^+ concentration was adjusted by the addition of NaCl to each growth medium.

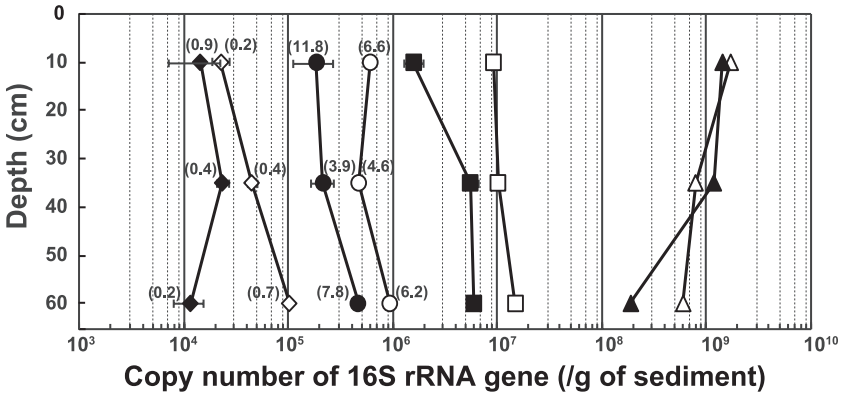

FIG 6 Copy numbers of 16S rRNA genes of *Bacteria* (triangles), *Archaea* (squares), *Methanosaeta* (circles), and *Methanosarcina* (diamonds) detected by real-time PCR analyses. The open and solid symbols represent samples 031 and 032, respectively. The percentages of *Methanosaeta* and *Methanosarcina* among the total *Archaea* are shown in parentheses.

impossible to confirm it as a marine *Methanosaeta* species. In the present study, we successfully isolated an aceticlastic methanogen belonging to the genus *Methanosaeta* from a marine environment. Unlike the other *Methanosaeta* species isolated so far, our isolate is obviously "moderately halophilic" and adapts to saline environments (Fig. 5). The results directly show that *Methanosaeta* thrives and contributes to anaerobic acetate degradation, not only in terrestrial environments, but also in saline environments. This information indicates that *Methanosaeta*-type organisms in marine environments may have evolved specifically by adapting to a saline environment, in which all previously known *Methanosaeta* species would not survive.

In marine ecosystems, hydrogen and acetate, the major substrates for methanogens, are consumed mainly by sulfate reducers in the sulfate-rich surface layer of sediments. However, sulfate is generally depleted at a depth of tens of centimeters to several meters, and methanogenesis potentially becomes the dominant terminal oxidation process in the sulfate-depleted zone. In addition, methane production has been detected and methanogens have been retrieved even from sediments with high sulfate concentrations (7, 12, 23, 24, 44). In the present study, the 16S rRNA genes of *Methanosaeta* were detected in tidal flat sediments by real-time PCR analysis, and the amount (number of copies per gram sample) increased slightly with increasing depth (Fig. 6). Interestingly, the number of *Methanosaeta* genes was 10 times higher than that of *Methanosarcina* genes. The results may indicate that the methane produced from acetate originates from *Methanosaeta* rather than from *Methanosarcina* and that *Methanosaeta* contributes significantly to the anaerobic degradation of organic matter in tidal flat sediments. Acetate concentrations in the pore water of marine sediments are usually less than 20 μM (24, 43). Therefore, in marine sediments, *Methanosaeta* has an advantage over *Methanosarcina* in acetate use based on affinity (20).

Although several strains of aceticlastic methanogens belonging to the genus *Methanosarcina* have been isolated from marine sediments (14, 24, 56, 61), isotopic evidence suggests that acetate is a minor precursor for methanogenesis and that the rate of acetate oxidation to CO_2 is higher than the rate of CH_4 production in the surface layer (43, 63). On the other hand, sometimes *Methanosaeta* growth was not observed although the organisms were detected by molecular analysis (25). Certainly, *Methanosaeta* is one of the most recalcitrant methanogens and is difficult to enrich and isolate, primarily because of slow growth. In addition, Wellsbury et al. reported difficulty distinguishing between abiological thermogenic and biological aceticlastic methanogenesis in marine sediments (9, 62). These facts suggest that aceticlastic methane generation in marine sediments has been underestimated. Parkes et al. showed that traces of *Methanosaeta* were detected and acetate concentrations in marine sediments remained low, although the rate of CH_4 production from acetate was equivalent to one-quarter of the rate from H_2-CO_2 (43), indicating the presence of sufficient acetate-using populations to maintain the final steps of *in situ* degradation of organic matter. Aceticlastic methanogens may be widespread and perhaps dominant in contributing to acetate degradation in marine sediments, in particular the tidal flat sediments, which have an abundant supply of organic matter.

In conclusion, we have isolated marine *Methanosaeta* species from tidal flat sediments and suggested a possible contribution to acetate metabolism in saline environments. Further analyses for acetate degradation in tidal flat sediments, such as competition between methanogens and sulfate-reducing bacteria, are now under way.

Taxonomy. The characteristics of strain 03d30q^T and *Methanosaeta* species are summarized in Table 2. The phylogenetic tree based on the 16S rRNA gene sequence (Fig. 2) revealed that strain 03d30q^T belongs to the genus *Methanosaeta* and that *M. harundinacea* is a related cultured relative (97.0% sequence similarity). Phylogenetic analysis based on McrA showed a similar trend (Fig. 3). The results from a DNA-DNA hybridization study indicated that the degree of DNA relatedness between strain 03d30q^T and *M. harundinacea* was $<$1%. These results suggest that a new species should be created for the isolate (57). In addition, the G+C content of genomic DNA, sensitivity to the Na^+ concentration, and temperature for growth differentiated strain 03d30q^T from the other *Methanosaeta* species. In particular, the difference in Na^+ sensitivity was a criterion for discriminating strain 03d30q^T from the other *Methanosaeta* species, because strain 03d30q^T was the only moderately halophilic species. For these reasons, strain 03d30q^T represents a novel species within the genus *Methanosaeta*, for which the name "*Methanosaeta pelagica*" sp. nov. is proposed.

Description of "*Methanosaeta pelagica*" sp. nov. "*Methano-*

TABLE 2 Comparison of properties of *Methanosaeta* species

Property	Value			
	"*M. pelagica*"	*M. concilii*[a]	*M. thermophila*[a]	*M. harundinacea*[a]
Type strain	03d30q	GP6	P_T	8Ac
Habitat	Tidal flat sediment	Anaerobic digester	Anaerobic digester	Anaerobic digester
Optimum growth conditions				
Temp (°C)	30	35–40	55–60	34—37
pH	7.5	7.1–7.5	6.5–6.7	7.2–7.6
Na^+ concn (M)	0.28	<0.06	<0.13	<0.02
Effect on yeast extract for growth	Stimulate	Inhibit	Inhibit[b]	Required
G+C content (mol%)	45.4 (HPLC)	50.3 (T_m)[c]	52.7 (HPLC)	55.7 (T_m)

[a] Reference data are from Patel (45) and Ma et al. (30).
[b] Yeast extract inhibits the growth of some strains of *M. thermophila*.
[c] T_m, melting temperature.

saeta pelagica" (pe.la′gi.ca. L. fem. adj. *pelagica*, pertaining to the sea). Single cells are 0.5 μm wide and 2.5 to 11.0 μm long, and long sheathed chains of cells form. It shows weakly blue-green autofluorescence by epifluorescence microscopy. Nonmotile. Gram negative. Susceptibility to lysis by SDS or hypotonic conditions is not observed. Strictly anaerobic. Acetate is the only substrate for growth and CH_4 production: H_2-CO_2, formate, methanol, ethanol, methylamine, dimethylamine, trimethylamine, or dimethyl sulfide does not support growth. Yeast extract stimulates growth. The optimum growth conditions are 30°C, initial pH 7.5, and 0.28 M Na^+ concentration. The DNA G+C content of the type strain is 45.4 mol%.

The type strain, 03d30q (= NBRC 105920 = DSM 24271) was isolated from a tidal flat sediment in Tokyo Bay, Chiba Prefecture, Japan.

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