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Vibrio halioticoli* sp. nov., a non-motile alginolytic marine bacterium isolated from the gut of the abalone *Haliotis discus hannai

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Six alginolytic, facultatively anaerobic, non-motile marine bacteria were isolated from the gut of abalone *Haliotis discus hannai*. DNA–DNA hybridization data showed that the six strains constituted a single genospecies. Phylogenetic analyses of 16S rDNA sequences indicated that the isolates should be assigned to the genus *Vibrio*. The phenotypic features of the isolates were closely related to *Vibrio fischeri* and *Vibrio pelagius* biovar I, but 13 traits (motility, luminescence, alginase production, lipase production, lysine decarboxylase, indole production, growth in 1 and 6% NaCl and assimilation of five carbon compounds) distinguished these strains from *V. fischeri*, and 17 traits (motility, growth at 37 °C, lipase production, indole production, growth in 1 and 6% NaCl, acid from sucrose and D-sorbitol, and assimilation of nine carbon compounds) distinguished these strains from *V. pelagius*. The G+C content of the isolates was 41.6–43.1 mol%. According to DNA–DNA hybridization data and 16S rDNA phylogenetic analyses, it was concluded that the six isolates constitute a new species different from any other *Vibrio* species. The name *Vibrio halioticoli* sp. nov. (type strain IAM 14596^T) is proposed. A set of phenotypic features which enables differentiation of the new species from other species of the *Vibrionaceae* family is described.

Keywords: *Vibrio halioticoli* sp. nov., marine bacterium, alginolytic, gut microflora, abalone

INTRODUCTION

An alginolytic, non-motile fermentative marine bacterium was found to form the main microflora of the gut of the abalone *Haliotis discus hannai* (16). Most of the isolates degraded polyguluronate rather than polymannuronate (16). Abalone *Haliotis* spp. secreted, in their gastrointestinal tract, polymannuronate-degrading enzymes. We therefore speculated that the bacterium colonized the gut of the abalone, where it

further degraded and utilized a residual part of ingested algae (16). The bacterium anaerobically produced acid from glucose without gas, showed Na⁺ requirement and was oxidase-positive, but non-motile. We presumed that this bacterium may belong to the family *Vibrionaceae* (16). DNA–DNA hybridization experiments, phenotypic characterizations and phylogenetic analyses demonstrated that this bacterium is a new species of *Vibrio*, for which we propose the name *Vibrio halioticoli*.

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Abbreviations: SEM, scanning electron microscopy; TEM, transmission electron microscopy.

The DDBJ/EMBL/GenBank accession numbers for the nucleotide sequences of *V. halioticoli* are AB000390 (IAM 14596^T = A431), AB000391 (IAM 14597 = A611), AB000392 (IAM 14598 = A1013) and AB000393 (IAM 14599 = A1112).

METHODS

Bacterial strains, and morphological and phenotypic characterization. Six isolates of *V. halioticoli* IAM 14596^T (strain A431), IAM 14597 (strain A611), IAM 14598 (strain A1013), IAM 14599 (strain A1112), strain A42 and strain A11122 isolated from the gut of abalones (16) were used in this study. Strains used in DNA–DNA hybridization experiment are listed in Table 2. All strains were cultured on ZoBell 2216E

agar (12) and their main characteristics were determined as described previously (1, 6, 7, 9, 13, 19). Alginate hydrolysis activity was determined using previously described methods (16).

V. haliotocoli IAM 14956^T was cultured in ZoBell 2216E broth and a broth containing 0.5% sodium alginate. Morphological characteristics of *V. haliotocoli* IAM 14956^T under both culture conditions were examined by transmission (TEM) and scanning electron microscopy (SEM). For TEM observation, fresh cultured cells were transferred to a collodion-coated grid, stained with 2% uranyl acetate, and then observed with a H-300 transmission electron microscope (Hitachi). For SEM, the cells of IAM 14956^T were fixed for 3 h with glutaraldehyde/natural seawater (2.5/50%, v/v). After washing with slightly modified Millonig's phosphate buffer, pH 7.4, cells were stained with 2.0% tannic acid solution for 30 min. Post-fixation with 2.0% OsO₄ solution was for 6–8 h, the material was dehydrated by a series of graded ethanol solutions and finally exchanged to *tert*-butanol. The specimen was freeze-dried, and the preparation was coated with gold in a JFC-1100 ion sputter (Nihon Denshi) and examined with a S-2300 scanning electron microscope (Hitachi).

Determination of G+C content and DNA–DNA hybridizations. DNAs of bacterial strains were prepared by the procedures of Marmur (10), with minor modification. G+C contents of DNA from *V. haliotocoli* IAM 14956^T, 14597, 14598, 14599, A42 and A1112 were determined by HPLC (18). DNA–DNA hybridization experiments were performed in microdilution wells using a fluorometric direct binding method (4). DNAs of *V. haliotocoli* IAM 14956^T and *Vibrio pelagius* biotype I ATCC 25916^T were labelled with photobiotin (Vector Laboratories). Four micrograms of unlabelled single-stranded DNA were immobilized in microdilution wells (Immuron 200, FIA/LIA plate, black type; Greiner labortechnik), then hybridization mixture containing 20 ng labelled DNA was added to each microdilution well and the hybridization was performed under optimal conditions following pre-hybridization. Formamide concentration in the hybridization mixtures was determined according to the methods of Meinkoth & Wahl (11). The hybridization of the biotinylated DNA to immobilized DNAs was performed under optimal conditions following pre-hybridization and detected by fluorometry after binding streptavidin- β -galactosidase to labelled DNA. 4-Methylumbelliferyl- β -D-galactopyranoside (0.6 mM; Wako) was added to each well as fluorogenic substrate for β -galactosidase and then incubated at 30 °C. The fluorescence intensity of each well was then measured using a Corona Electric MicroFluoro reader (MTP-22) at a wavelength of 360 nm for excitation and 450 nm for emission. DNA–DNA homology was calculated according to the method of Ezaki *et al.* (5).

DNA amplification and sequencing. Bacterial DNAs of *V. haliotocoli* IAM 14956^T, IAM 14597, IAM 14598 and IAM 14599 for PCR were prepared according to Enright *et al.* (3). Fresh overnight cultures in marine broth were harvested and resuspended in 300 μ l 10 mM Tris (pH 8.0), 2 mM EDTA to which 100 μ l lysozyme (50 mg ml⁻¹), 10 μ l RNase A (1 mg ml⁻¹), 10 μ l proteinase K (5 mg ml⁻¹), 300 μ l of a lysis solution containing 10 mM EDTA, 2% Triton X-100, and 50 mM Tris (pH 8.0) were added. After mixing, samples

were incubated for 30 min at 37 °C. Samples were extracted once with phenol and once with phenol/chloroform/isoamyl alcohol (25:24:1, by vol.). DNA was precipitated with ethanol at –20 °C in the presence of ammonium acetate and collected by centrifugation (14000 r.p.m.). After removal of ethanol, the DNA pellet was resuspended to a final concentration of 50 ng μ l⁻¹ in 10 mM Tris (pH 8.0)/1 mM EDTA. The 100 ng of DNA template was used to amplify the small-subunit rRNAs genes by PCR. The initial denaturation step consisted of heating the reaction mixture at 94 °C for 180 s, and this was followed by an annealing step (55 °C for 60 s) and an extension step (72 °C for 90 s). The thermal profile then consisted of 30 cycles of annealing at 55 °C for 60 s, extension at 72 °C for 90 s, and denaturation at 94 °C for 60 s. The PCR products were analysed on a 1.5% agarose gel with a molecular mass standard for quantification of the PCR yield. The amplification primers used in this study gave a 1.5 kbp PCR product and corresponded to positions 25–1521 in the *Escherichia coli* sequence. The PCR products which produced a single band on agarose gels were purified for sequencing by PEG 6000 precipitation. Approximately 100 ng template was directly sequenced by using a *Taq* FS Dye Terminator Sequencing kit (ABI) according to the protocol recommended by the manufacturer. DNA sequencing was done with an Applied Biosystems model 373A automated sequencer.

Primer position. Nine DNA primers were used in the sequencing reactions. These primers corresponded to the following position in the *E. coli* sequence: primer 24F, positions 8–24; 390F, 379–397; 800F, 800–819; 1100F, 1092–1112; 389R, 399–381; 520R, 528–509; 920R, 928–909; 1190R, 1176–1195; 1540R, 1541–1522. 'F' indicates a primer for sequencing the sense direction of the rDNA, and 'R' indicates a primer for sequencing the anti-sense direction of the primer of the rDNA.

Phylogenetic analysis. The sequences were aligned and studied using a set of programs developed by R. Christen. In all phylogenetic analysis, we used the sequences determined in this study and small-subunit rDNA sequences obtained from the EMBL database. For Fig. 2, the following sequences were used: *Vibrio campbellii* ATCC 25920^T (X74692), *Vibrio hollisae* ATCC 33564^T (X74707), *Photobacterium leiognathi* ATCC 25521^T (X74686), *Vibrio cholerae* CECT 514^T (X76337), *Photobacterium phosphoreum* (Z19107), *Photobacterium angustum* ATCC 25915^T (X74685), *Vibrio fischeri* ATCC 7744^T (X74702), *Vibrio logei* ATCC 15832 (X74708), *Vibrio salmonicida* NCIMB 2262 (X70643), *Vibrio navarrensis* CIP 103381^T (X74715), *Vibrio vulnificus* ATCC 27562^T (X76333), *Vibrio diazotrophicus* ATCC 33466^T (X74701), *Vibrio ordalii* ATCC 33509^T (X74718), *Vibrio anguillarum* ATCC 12964^T (X16895), *Vibrio gazogenes* ATCC 29988^T (X74705), *Vibrio metschnikovii* CIP 69.14^T (X74711), *Vibrio cincinnatiensis* ATCC 35912^T (X74698), *Vibrio splendidus* biovar I SCB 8 (Z31657), *Vibrio aestuarianus* ATCC 35048^T (X74689), *Vibrio furnissii* ATCC 35016^T (X74704), *Vibrio fluvialis* NCTC 11327^T (X76335), *Vibrio orientalis* ATCC 33934^T (X74719), *Vibrio mediterranei* CIP 103203^T (X74710), *Vibrio tubiashii* ATCC 19109^T (X74725), *Vibrio nereis* ATCC 25917^T (X74716), *Vibrio proteolyticus* ATCC 15338^T (X74723), *Vibrio nigrapulchritudo* ATCC 27043^T (X74717), *Vibrio carchariae* ATCC 35084^T (X74693), *Vibrio harveyi* ATCC 14126^T (X74706), *Vibrio alginolyticus* ATCC 17749^T (X74690), *Vibrio pelagius* biovar I ATCC 25916^T (X74722),

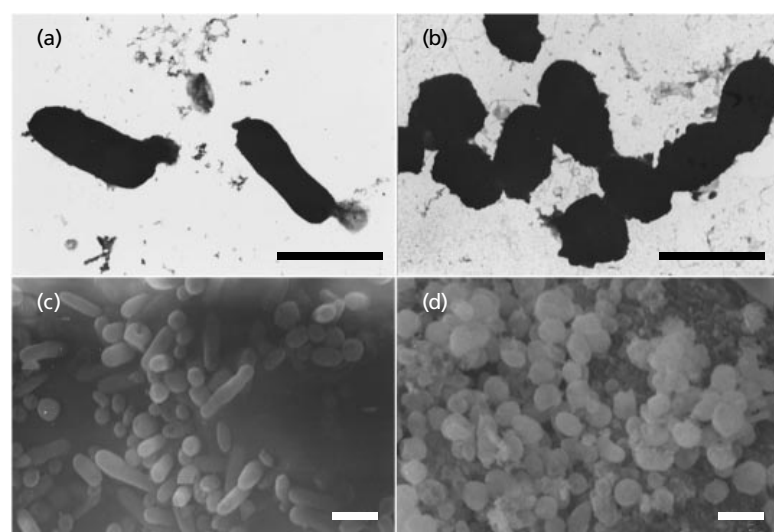


Fig. 1. Negatively stained (a, b) and scanning electron (c, d) micrographs of *Vibrio halioticoli* IAM 14596^T. Cells cultured in ZoBell 2216E broth without (a, c) or with (b, d) 0.5% sodium alginate. Bars, 2 μm.

Vibrio natriegens ATCC 14048^T (X74714), *Vibrio mimicus* ATCC 33653^T (X74713), *Vibrio parahaemolyticus* ATCC 17802^T (X74720), *Vibrio tapetis* CECT 4600 (Y08430), *Vibrio scophthalmi* CECT 4638^T (U46579), *Vibrio* sp. (X97988), *Vibrio* sp. (X97990), *Vibrio* sp. (X97989), *Vibrio mytili* (X99761), *Vibrio diabolus* HE800 (X99762), *Vibrio* sp. (X97987), *Vibrio* sp. (U57919) and *Vibrio aspartigenicus* GSP-1 (M98446).

Domains used to construct the dendrogram shown in Fig. 2 were regions of the small-subunit rDNA sequences available for all sequences and excluding positions likely to show homoplasy: positions 101–178, 186–1019, 1024–1353 (*E. coli* small-subunit rDNA sequence J01695 numbering). Phylogenetic analyses were performed by using three different methods, neighbour-joining (15), maximum-likelihood (options QFYG, fastDNAm1 program of G. J. Olsen, University of Illinois, Urbana, USA) and maximum-parsimony [PAUP 3.0s for the Macintosh, heuristic search, (17)]. The robustness of each topology was checked by using the neighbour-joining method and 100 bootstrap replications. Trees were drawn by using the njplot program for the Macintosh (M. Gouy, CNRS URA 243, Université Claude Bernard, Lyon, France).

RESULTS AND DISCUSSION

We isolated more than 100 strains of *V. halioticoli* including IAM 14596^T, IAM 14597, IAM 14598, IAM 14599, A42 and A11I22. All of them appeared to be non-motile, Gram-negative, fermentative (16); no flagellated cells were observed by TEM or SEM (Fig. 1a, c). Bacterial cells cultured in ZoBell 2216E broth with 0.5% sodium alginate showed the unique property of binding to a gel formed of alginate in seawater (data not shown); attached cells were Gram-negative, coccoid in irregular clusters or often in chains (Fig. 1b, d). The binding of the bacterium to insoluble alginate was similar to that reported for some cellulolytic ruminal bacteria (8). These ruminal bacteria are organized as cellulase multisubunit complexes called

'cellulosomes', responsible both for efficient cellulolysis and for the adhesion of the bacterium to ingested plant materials containing cellulose in ruminal environments (2, 8). This bacterium required salt for its growth, did not accumulate poly-β-hydroxybutyrate and was oxidase-positive (Table 1). No peritrichous cells were observed when the bacterium was cultivated on solid media. Mean G+C content was 42.5 mol% (Table 2), suggesting that this bacterium should be assigned either to the genera *Vibrio* or *Photobacterium* in the family *Vibrionaceae* (1, 7).

Specific biochemical and physiological features of *V. halioticoli* are shown in Table 1. The new bacterium displayed the highest phenotypic similarity with *V. fischeri* ATCC 7744^T from which it differed by 13 out of 78 traits. Motility, luminescence, alginate production, lipase production, lysine decarboxylase, indole production, growth in 1 and 6% NaCl and assimilation of five carbon compounds were different from *V. fischeri* ATCC 7744^T (Table 1). The bacterium was also closely related to *V. pelagius* biovar I ATCC 25916^T from which it differed by 17 traits (motility, growth at 37 °C, lipase production, indole production, growth in 1 and 6% NaCl, acid from glucose and D-sorbitol, and assimilation of nine carbon compounds).

The 16S rDNA sequences of strains IAM 14596^T, IAM 14597, IAM 14598 and IAM 14599 were aligned by comparison to a database containing about 5000 already aligned eubacterial small-subunit rDNA sequences. The results of broad phylogenetic analyses clearly showed that the strains which we studied belonged to the gamma-3 sub-class of the *Proteobacteria* of the domain *Bacteria* (data not shown). More detailed analyses showed that they were included in the genus *Vibrio* and more precisely in a sub-group that includes *V. cholerae* and *V. alginolyticus* (14). Final analyses were conducted to include all known

Table 1. Phenotypic characteristics for distinguishing *Vibrio haliotocoli* from previously described alginate-producing *Vibrio* species

Species/strain: 1, *V. haliotocoli* IAM 14596^T; 2, *V. haliotocoli* IAM 14597-9, A42, A11122;
3, *V. pelagius* biovar I ATCC 25916^T; 4, *V. fischeri* ATCC 7744^T; 5, *V. alginolyticus* V447;
6, *V. harveyi* NCIMB 1280^T; 7, *V. splendidus* biovar I HUPF 9117^T.

Characteristic	Species/strain						
	1	2	3	4	5	6	7
Pigmentation	—	—	—	—	—	—	—
Na ⁺ requirement	+	+	+	+	+	+	+
OF test	F	F	F	F	F	F	F
Motility	—	—	+	+	+	+	+
Swarming	—	—	—	—	+	—	—
PHB accumulation	—	—	—	—	—	—	—
Luminescence	—	—	—	+	—	+	—
Growth at (°C):							
4	—	—	—	—	—	—	—
15	+	+	+	+	+	+	+
30	+	+	+	+	+	+	+
37	—	—	+	—	+	+	+
40	—	—	—	—	+	—	—
Oxidase	+	+	+	+	+	+	+
Catalase	+	+	+	+	+	+	+
Production of:							
Amylase	—	—	—	—	+	+	+
Gelatinase	—	—	—	—	+	+	+
Chitinase	—	—	—	—	+	+	+
Alginase	+	+	+	—	—	+	—
Agarase	—	—	—	—	—	—	—
Lipase	—	—	+	+	+	+	+
Nitrate reduction	+	+	+	+	+	+	+
O/129 sensitivity	+	+	+	+	+	+	+
Gas from D-glucose	—	—	—	—	—	—	—
Acetoin production	—	—	—	—	+	—	—
Methyl red test	+	+	+	+	+	+	+
Lysine decarboxylase	—	—	—	+	+	+	—
Arginine dehydrolase	—	—	—	—	—	—	—
Ornithine decarboxylase	—	—	—	—	+	+	—
Indole production	+	+	—	—	+	+	+
ONPG	+	D	+	—	—	—	+
Growth on TCBS	+	+	+	+	+	+	+
Growth in:							
1 % NaCl broth	—	—	+	+	+	+	+
3 % NaCl broth	+	+	+	+	+	+	+
6 % NaCl broth	—	—	+	+	+	+	+
Acid from:							
D-Glucose/D-mannitol/maltose	+	+	+	+	+	+	+
L-Arabinose/inositol/L-rhamnose	—	—	—	—	—	—	—
Sucrose	—	—	+	—	+	+	+
D-Sorbitol	—	—	+	—	—	—	+
Requirement for organic growth factors	—	—	—	—	—	—	—

16S rDNA sequences from this clade and a few outgroup sequences (Fig. 2). Resulting phylogenetic analyses demonstrated that all four strains of *V. haliotocoli* formed a robust clade that could be associated with no other *Vibrio* species (Fig. 2). The

sequences of 16S rDNAs among the different strains of *V. haliotocoli* showed high levels of similarity, ranging from 99.6 to 99.9 % (Table 3), whereas relatively low levels of sequence similarity were found to other closely related species.

Table 1 (cont.)

Characteristic	Species/strain						
	1	2	3	4	5	6	7
Utilization of:							
D-Mannose	—	D	+	+	+	+	+
Sucrose	—	—	+	—	+	+	+
D-Gluconate	—	—	+	—	+	+	+
D-Sorbitol	—	—	—	—	—	—	+
Glycerol	+	+	+	+	+	—	+
α -Ketoglutarate	—	—	+	+	+	+	+
D-Galactose	—	D	+	+	+	+	+
Cellobiose	—	D	—	+	—	+	+
Melibiose	—	—	—	—	—	—	+
Lactose	—	—	—	—	—	—	+
D-Glucronate	—	—	—	—	—	+	+
Trehalose	—	—	+	—	+	+	+
Putrescine	—	—	+	—	—	—	+
γ -Aminobutyrate	—	—	+	—	—	—	—
Acetate	—	—	+	+	+	—	+
Pyruvate	—	D	+	+	+	+	+
L-Tyrosine	—	—	—	+	—	—	—
Propionate	—	—	+	—	+	+	+
L-Glutamate	—	—	+	+	—	+	+
Alginate	+	+	+	—	+	+	—
D-Fructose/D-glucose/maltose/ D-mannitol/D-glucosamine/ N-acetylglucosamine/fumarate/succinate	+	+	+	+	+	+	+
meso-Erythritol/D-xylose/L-arabinose/ citrate/DL-malate/ δ -aminovariate/aconitate	—	—	—	—	—	—	—

F, Fermentative. D, Different reactions.

Table 2. DNA relatedness among *Vibrio* spp. and *Photobacterium* spp.

Species	Strain	G + C content (mol %)	Reassociation (%) with biotinylated DNA from:	
			<i>V. halioticoli</i> IAM 14596 ^T	<i>V. pelagius</i> ATCC 25916 ^T
<i>V. halioticoli</i>	IAM 14596 ^T	43.1	100.0	19.3
<i>V. halioticoli</i>	A42	42.4	107.7	19.3
<i>V. halioticoli</i>	IAM 14597	42.8	96.4	20.9
<i>V. halioticoli</i>	IAM 14598	42.9	79.7	26.0
<i>V. halioticoli</i>	IAM 14599	41.6	105.6	15.1
<i>V. halioticoli</i>	A11122	42.4	95.5	13.1
<i>V. alginolyticus</i>	V447	45–47*	5.5	27.9
<i>V. pelagius</i> biovar I	ATCC 25916 ^T	46.0	6.3	100.0
<i>V. campbellii</i>	ATCC 25920 ^T	46–48*	9.4	13.4
<i>V. fischeri</i>	ATCC 7744 ^T	39–41*	8.3	12.6
<i>V. splendidus</i> biovar I	HUPF 9117 ^T (= ATCC 33125 ^T)	45–46*	7.5	16.2
<i>V. parahaemolyticus</i>	HO5	46–47*	7.8	22.6
<i>V. harveyi</i>	NCIMB 1280 ^T	46–48*	14.2	20.1
<i>P. leiognathi</i>	NCIMB 391 ^T	42–44*	8.5	7.2
<i>P. phosphoreum</i>	IAM 12085 ^T	41–42*	7.8	8.1

* Data from reference (1).

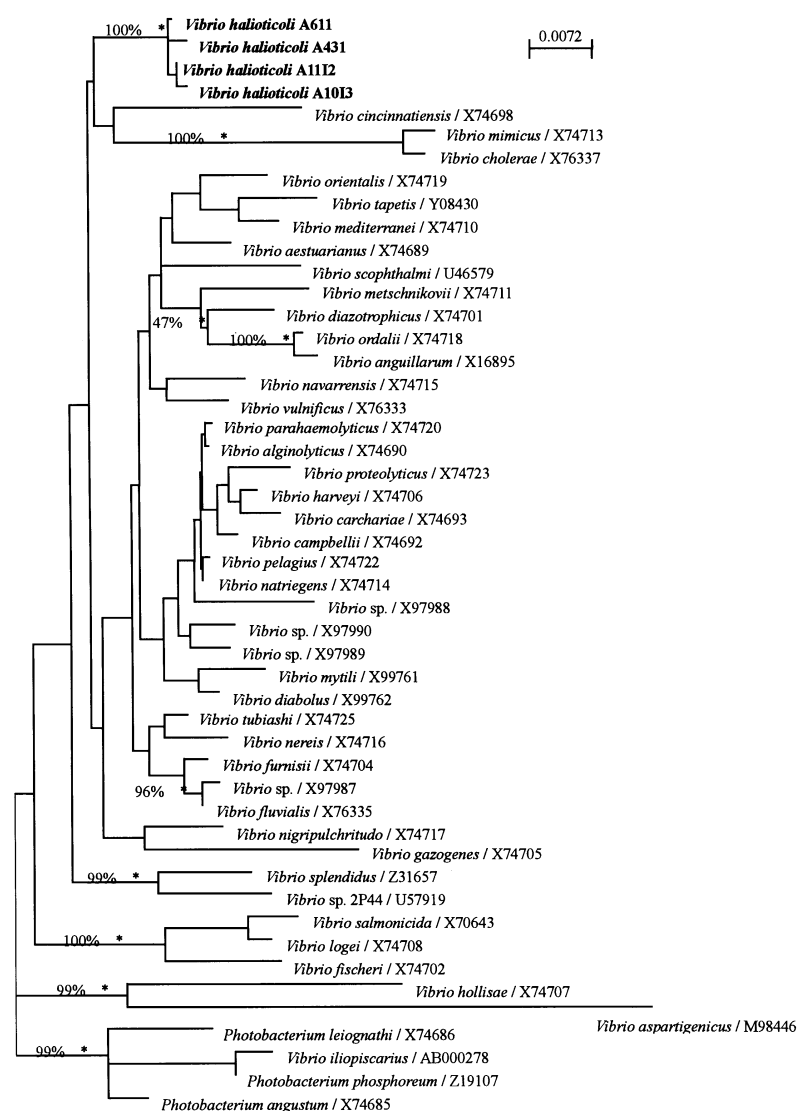


Fig. 2. Unrooted phylogenetic tree on the basis of 16S rDNA sequences. Scale bar, 0.0072 accumulated changes per nucleotide. This figure combines the results of three analyses, i.e. neighbour-joining, maximum-parsimony and maximum-likelihood. The topology shown was obtained by neighbour-joining, and the percentage values are the results of a bootstrap analysis using 100 replications. Branches also obtained in the maximum-likelihood analysis ($P < 0.01$) and in the most parsimonious tree are indicated by *. All four strains studied were from a robust clade within the genus *Vibrio*, but were distinct from any other species.

DNA–DNA hybridization results (Table 2) showed that six strains of *V. halioticoli* IAM 14596^T, IAM 14597, IAM 14598, IAM 14599, A42 and A11122 were conspecific. Strain IAM 14596^T was not genotypically related to any of the phenotypically closely related species (Table 2). In conclusion, DNA–DNA hybridization experiments (Table 2) and phylogenetic analyses (Fig. 2) showed that strains IAM 14596^T, IAM 14597, IAM 14598 and IAM 14599 should be recognized as a new species. The name *Vibrio halioticoli* is proposed for this bacterium, which is of marine ecological interest because of its interactions in the microbiota of the gut in marine herbivores.

Description of *Vibrio halioticoli* sp. nov.

Vibrio halioticoli (hal.i.o.ti'co.li. M.L. n. *Haliotis* systematic name of an abalone genus. Gr. n. *colon* gut. M.L. gen. n. *halioticoli* the *Haliotis* gut, from which the organism was isolated).

Gram-negative, facultatively anaerobic, non-motile, non-flagellated rod. Cells are rod-shaped, with rounded ends, and are 0.6–0.8 µm in diameter and 1.7–2.0 µm long when the organism is grown in ZoBell 2216E broth, but cells are spherical shaped 0.7–1.0 µm in diameter and 1.0–1.6 µm long when the organism is grown in ZoBell 2216E broth containing alginate; the cells occur singly in the broth, or in irregular clusters, or chained in the broth containing alginate. No endospores or capsules are formed. Flagellation is not observed when the organism is cultivated on solidified media and/or in liquid media. Colonies on ZoBell 2216E agar are beige, circular, smooth and convex with entire edge. Sodium ion is essential for growth. Mesophilic and neutrophilic chemo-organotroph which grows at 15–30 °C. No growth occurs at 40 °C. Produces acid from glucose; reduces nitrate; hydrolyses alginate; indole-, oxidase- and catalase-positive; assimilates D-fructose, maltose, D-glucosamine, N-acetylglucosamine, D-mannitol, fumarate,

Table 3. Levels of homology for nucleotides of 16S rDNAs from *V. halioticoli* and closely related species

	A1013	1	2	3	4	5	6	7	8	9	10
1. <i>V. halioticoli</i> A1112	99.9										
2. <i>V. halioticoli</i> A431 ^T	99.8	99.6									
3. <i>V. halioticoli</i> A611	99.9	99.8	99.7								
4. <i>V. cholerae</i>	93.4	93.1	93.8	93.3							
5. <i>V. mediterranei</i>	96.5	96.7	96.7	96.7	92.4						
6. <i>V. alginolyticus</i>	97.7	97.8	97.5	97.8	92.0	97.0					
7. <i>V. nigrapulchritudo</i>	97.0	97.2	97.2	97.1	92.1	96.3	97.3				
8. <i>V. splendidus</i>	95.4	95.2	95.5	95.2	92.0	95.9	94.6	94.2			
9. <i>V. fischeri</i>	96.2	96.1	95.9	95.9	91.6	94.4	95.2	94.6	94.6		
10. <i>V. hollisae</i>	92.3	92.5	92.6	92.4	90.7	92.2	92.4	93.2	92.0	91.1	
11. <i>P. leiognathi</i>	95.7	95.7	95.6	95.9	91.6	94.2	95.2	95.5	93.1	94.9	93.3

succinate, glycerol, D-glucose and alginate. Non-motile; does not produce gas from glucose; does not produce acetoin; negative for lysine decarboxylase, arginine dehydrolase and ornithine decarboxylase; no luminescence or pigmentation; does not require organic growth factors; does not hydrolyse starch, gelatin, chitin, Tween 80 or agar; does not accumulate poly- β -hydroxybutyrate; does not assimilate D-mannose, sucrose, D-gluconate, D-sorbitol, citrate, meso-erythritol, DL-malate, α -ketoglutarate, D-galactose, cellobiose, melibiose, lactose, D-glucuronate, D-xylose, trehalose, putrescine, δ -aminovariate, γ -aminobutyrate, acetate, pyruvate, L-tyrosine, propionate, aconitate, L-glutamate or L-arabinose. The DNA G+C content is 41.6–43.1 mol%. Isolated from the gut of the abalone *Haliotis discus hannai*. The type strain is IAM 14596^T.

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REFERENCES

- Baumann, P. & Schubert, R. H. W. (1984). Family II. *Vibrionaceae* Veron 1965, 5245^{AL}. In *Bergey's Manual of Systematic Bacteriology*, vol. 1, pp. 516–550. Edited by N. R. Krieg & J. G. Holt. Baltimore: Williams & Wilkins.
- Bayer, E. A., Kenig, R. & Lamed, R. (1983). Adherence of *Clostridium thermocellum* to cellulose. *J Bacteriol* **156**, 818–827.
- Enright, M. C., Carter, P. E., MacLean, I. A. & McKenzie, H. (1994). Phylogenetic relationships between some members of the genera *Neisseria*, *Acinetobacter*, *Moraxella*, and *Kingella* based on partial 16S ribosomal DNA sequence analysis. *Int J Syst Bacteriol* **44**, 387–391.
- Ezaki, T., Hashimoto, Y., Takeuchi, N., Yamamoto, H., Liu, S.-L., Miura, H., Matsui, K. & Yabuuchi, E. (1988). Simple genetic method to identify viridans group *Streptococci* by colorimetric dot hybridization and fluorometric hybridization in microdilution wells. *J Clin Microbiol* **26**, 1708–1713.
- Ezaki, T., Hashimoto, Y. & Yabuuchi, E. (1989). Fluorometric deoxyribonucleic acid-deoxyribonucleic acid hybridization in microdilution wells as an alternative to membrane filter hybridization in which radioisotopes are used to determine genetic relatedness among bacterial strains. *Int J Syst Bacteriol* **39**, 224–229.
- Hidaka, T. & Sakai, M. (1968). Comparative observation of inorganic salt requirement of the marine and terrestrial bacteria. In *Proceedings of US and Japan Seminar on Marine Microbiology*, pp. 125–149. Edited by H. Kadota & N. Taga. Bulletin of Misaki Marine Biology Institute of Kyoto University, no. 12.
- Holt, J. G., Krieg, N. R., Sneath, P. H. A., Staley, J. T. & Williams, S. T. (1994). Facultatively anaerobic gram-negative rods. Subgroup 2: family *Vibrionaceae*. In *Bergey's Manual of Determinative Bacteriology*, 9th edn, pp. 190–253. Baltimore: Williams & Wilkins.
- Lamed, R., Naimark, J., Morgenstern, E. & Bayer, E. A. (1987). Specialized cell surface structures in cellulolytic bacteria. *J Bacteriol* **168**, 3792–3800.
- Leifson, E. (1963). Determination of carbohydrate metabolism of marine bacteria. *J Bacteriol* **82**, 33–36.
- Marmur, J. (1961). A procedure for the isolation of deoxyribonucleic acid from microorganisms. *J Mol Biol* **3**, 208–218.
- Meinkoth, J. & Wahl, G. (1984). Hybridization of nucleic acids immobilized on solid supports. *Anal Biochem* **138**, 267–284.
- Oppenheimer, C. H. & ZoBell, C. E. (1952). The growth and viability of sixty-three species of marine bacteria as influenced by hydrostatic pressure. *J Mar Res* **11**, 10–18.
- Ostle, A. G. & Holt, J. G. (1982). Nile blue A as fluorescent stain for poly- β -hydroxybutyrate. *Appl Environ Microbiol* **44**, 238–241.
- Ruimy, R., Breittmayer, V., Elbaze, P., Lafay, B., Boussemart, O., Gauthier, M. & Christen, R. (1994). Phylogenetic analysis and assessment of the genera *Vibrio*, *Photobacterium*, *Aeromonas*, and *Pleisiomonas* deduced from small-subunit rRNA sequences. *Int J Syst Bacteriol* **44**, 416–426.
- Saitou, N. & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406–425.
- Sawabe, T., Oda, Y., Shiomi, Y. & Ezura, Y. (1995). Alginate

- degradation by bacteria isolated from the gut of sea urchins and abalones. *Microb Ecol* **30**, 193–202.
17. **Swofford, D. (1992).** PAUP: phylogenetic analysis using parsimony, version 3.0s. Illinois Natural History Survey, Champaign, IL, USA.
18. **Tamaoka, J. & Komagata, K. (1984).** Determination of DNA base composition by reversed-phase high-performance liquid chromatography. *FEMS Microbiol Lett* **25**, 125–128.
19. **West, M., Burdash, N. M. & Freimuth, F. (1977).** Simplified silver plating stain for flagella. *J Clin Microbiol* **6**, 414–419.