J. Gen. Appl. Microbiol., **56**, 509–518 (2010)

Short Communication

Aliivibrio sifiae sp. nov., luminous marine bacteria isolated from seawater

Susumu Yoshizawa,[1,*] Hajime Karatani,[2] Minoru Wada,[3] Akira Yokota,[4] and Kazuhiro Kogure[1]

[1] *Atmosphere and Ocean Research Institute, The University of Tokyo, Kashiwa, Chiba 277–8564, Japan*
[2] *Department of Biomolecular Engineering, Graduate School of Science and Technology, Kyoto Institute of Technology, Sakyo-ku, Kyoto 606–8585, Japan*
[3] *Graduate School of Science and Technology, Nagasaki University, Nagasaki 852–8521, Japan*
[4] *Institute of Molecular and Cellular Biosciences, The University of Tokyo, Bunkyo-ku, Tokyo 113–0032, Japan*

(Received May 28, 2010; Accepted August 18, 2010)

Key Words——*Aliivibrio sifiae* sp. nov.; luminous bacteria; marine bacteria; *Vibrionaceae*

Species of the Fischeri clade (Sawabe et al., 2007) in the genus *Vibrio* were reclassified as a new genus *Aliivibrio* (Gammaproteobacteria: *Vibrionaceae*) based on phylogenetic and phenotypic differences (Urbanczyk et al., 2007). At present, the genus comprises 5 species: *Aliivibrio fischeri* (Beijerinck, 1889), *Aliivibrio logei* (Harwood et al., 1980), *Aliivibrio salmonicida* (Egidius et al., 1986), *Aliivibrio wodanis* (Lunder et al., 2000) and *Aliivibrio finisterrensis* (Beaz-Hidalgo et al., 2010). The strains within genus *Aliivibrio* occur in certain kinds of marine animals such as fish or squid (Dunlap and Kita-Tsukamoto, 2006; Dunlap et al., 2007). Except for *A. finisterrensis*, it was reported that the *Aliivibrio* species contain luminous strains (Ast et al., 2009; Dunlap and Kita-Tsukamoto, 2006). Additionally, some strains of *A. fischeri* have been studied intensively as a model organism of luminous bacteria because of their quorum regulation of luminescence and bioluminescent symbiosis (Lupp and Ruby, 2005; Nyholm and Mcfall-Ngai, 2004).

The aim of this study was to determine the taxonomic position of strains H1-1^T and H1-2 by a polyphasic taxonomic approach that included multilocus sequence analysis (MLSA), chemotaxonomic characterization, DNA–DNA hybridization and physiological properties.

Strain H1-1^T (NBRC 105001^T) and H1-2 (NBRC 105002) were isolated from surface seawater at Harumi Pier in Tokyo Bay, Japan in 2007. The samples were filtered through a polycarbonate filter (pore size of 0.2 μm) (Whatman International, Ltd., Maidstone, UK), which was then placed on half strength marine agar 2216E (Difco) and kept at 20°C. Luminous colonies grown on the agar plate were isolated with sterile toothpicks utilizing a CCD camera (ATTO Bioscience & Technology, Tokyo, Japan) and transferred to new marine agar 2216E plates for isolation. The isolates were repeatedly purified and stored at −80°C in marine broth containing 20% (v/v) glycerol.

Cell morphology and flagella were observed using atomic force microscopy (model SPM-9500 J2; Shimadzu) as was described before (Nishino et al., 2004). The temperature range for growth was determined by incubating the isolates on the marine agar 2216E (Difco). The growth on different NaCl concentrations

* Address reprint requests to: Dr. Susumu Yoshizawa, Atmosphere and Ocean Research Institute, The University of Tokyo, 5–1–5 Kashiwanoha, Kashiwa, Chiba 277–8564, Japan.

Tel and Fax: +81–4–7136–6417

E-mail: yoshizawa@aori.u-tokyo.ac.jp

The GenBank/EMBL/DDBJ accession numbers for the 16S rDNA, *gyrB, pyrH, gapA, ftsZ, topA, mreB, recA* and *rpoA* gene sequences used in this study are shown in Table 6.

[0.5−10% (w/v)] was determined on tryptone soya agar (Difco). The growth pH range was determined using the marine agar 2216E after adjusting the pH to 4, 5, 6, 9, 10, 10.5 and 11 using HCl or NaOH. The temperature range, pH range and NaCl range for growth were determined after 2 days' incubation. Catalase activity was determined by bubble formation in a 3% (v/v) H_2O_2 solution. Oxidase activity was determined by cytochrome oxidase paper (Nissui Pharmaceutical). API 20E and API ZYM strips (bioMérieux) were used to determine physiological and biochemical characteristics. API 20E and API ZYM were read after 48 h incubation at 20°C and 6 h incubation at 20°C, respectively. All suspension media for API test strips were supplemented with 2% (w/v) NaCl solution (final concentration). Hydrolysis reaction (Starch, Tween 20, Tween 40, Tween 60 and Tween 80) and nitrate reduction were analyzed by conventional methods. Cellular fatty acid composition (MIDI system) was determined as described previously (Xie and Yokota, 2003). The cells were grown for 48 h at 20°C on plates of marine agar 2216E. A few fatty acid compositions could not be clearly identified by using the MIDI system. Therefore, summed features 2 and 3 were further analyzed as follows: the fatty acid samples, together with non-polar fatty acid and hydroxyl fatty acid standards used for comparison, were developed on TLC plates (silica-gel F254; Merck) with hexane/ethyl ether (1 : 1), sprayed with a 0.02% dichlorofluorescein solution in ethanol, dried and detected under UV light. The separated spots of the non-polar fatty acids and hydroxyl fatty acids were scraped from the plates, transferred to tubes and extracted with ethyl ether. The extracts were then concentrated under a stream of nitrogen gas and dissolved in hexane/methyl *tert*-butyl ether (1 : 1). The separated and purified non-polar fatty acids and hydroxyl fatty acids were detected by using the MIDI system again.

DNA was prepared according to the method of Marmur (1961) from cells grown on marine agar 2216E and the DNA base composition was determined by using an HPLC method (Mesbah et al., 1989). DNA−DNA hybridizations were carried out with photobiotin-labeled probes in microplate wells as described by Ezaki et al. (1989). The hybridization temperature was set at 40.7°C. A fragment (approx. 1,500 bp) of the 16S rRNA gene was amplified from the extracted DNA by using bacterial universal primers specific to the 16S rRNA gene (27F and 1492R) (Lane, 1991). The genes encoding urydilate kinase (*pyrH*), DNA gyrase β subunit (*gyrB*), glyceraldehyde 3-phosphate dehydrogenase (*gapA*), RNA polymerase α subunit (*rpoA*) and DNA recombination protein (*recA*) were used for multilocus sequence analysis (MLSA) (Sawabe et al., 2007). PCR primers for the six genetic loci and reaction conditions were used according to Sawabe et al. (2007) and Thompson et al. (2005). To test the evolutionary relationships of the genus *Aliiviibrio*, phylogenetic analysis was performed with the program MEGA4 (Tamura et al., 2007). Multiple alignments of the sequences were performed using CLUSTAL_W (version 1.6) (Thompson et al., 1994). Distances were calculated using the Kimura two-parameter model (Kimura, 1980). Clustering based on the neighbor-joining method (Saitou and Nei, 1987) and the maximum-parsimony method were determined using bootstrap values based on 1,000 replication (Felsenstein, 1985). Sequence data for other *Vibrio* species from online electronic taxonomic scheme for vibrios (http://www.taxvibrio.lncc.br) and GenBank were used in this study.

The 16S rRNA gene sequences analysis indicated that H1-1^T and H1-2 belong to the genus *Aliivibrio* using the neighbor-joining method and maximum-parsimony method (Figs. 1 and 2). The 16S rRNA gene sequences similarities between H1-1^T and species of *Aliivibrio* were 99.3% to *A. wodanis*, 99.0% to *A. logei*, 98.4% to *A. salmonicida*, 98.5% to *A. finisterrensis* and 97.2% to *A. fischeri*. A phylogenetic tree based on MLSA (16S rRNA, *pyrH*, *recA*, *rpoA*, *gapA* and *gyrB*; 4,195 bp) using the neighbor-joining method confirmed their distinction from other species of genus *Aliivibrio*; sequence similarities between H1-1^T and species of *Aliivibrio* were 96.4% to *A. wodanis*, 95.3% to *A. logei*, 95.1% to *A. salmonicida*, 95.1% to *A. finisterrensis* and 91.8% to *A. fischeri* (Fig. 3 and Table 1). Phylogenetic trees using each gene (*pyrH*, *recA*, *rpoA* and *gyrB*) also showed that H1-1^T and H1-2 were differentiated from known *Aliivibrio* species (Figs. 4−7), whereas phylogenetic analysis using *gapA* could not distinguish these strains from other *Aliivibrio* species (Fig. 8). Thompson et al. (2007) also reported that *gapA* was not high-resolution among *Vibrio harveyi* species group.

Additionally, we conducted DNA−DNA hybridization of the close relatives. DNA−DNA hybridization values between H1-1^T and other *Aliivibrio* species were 40.0% (*A. wodanis* ATCC BAA-104^T), 38.9% (*A. logei* ATCC

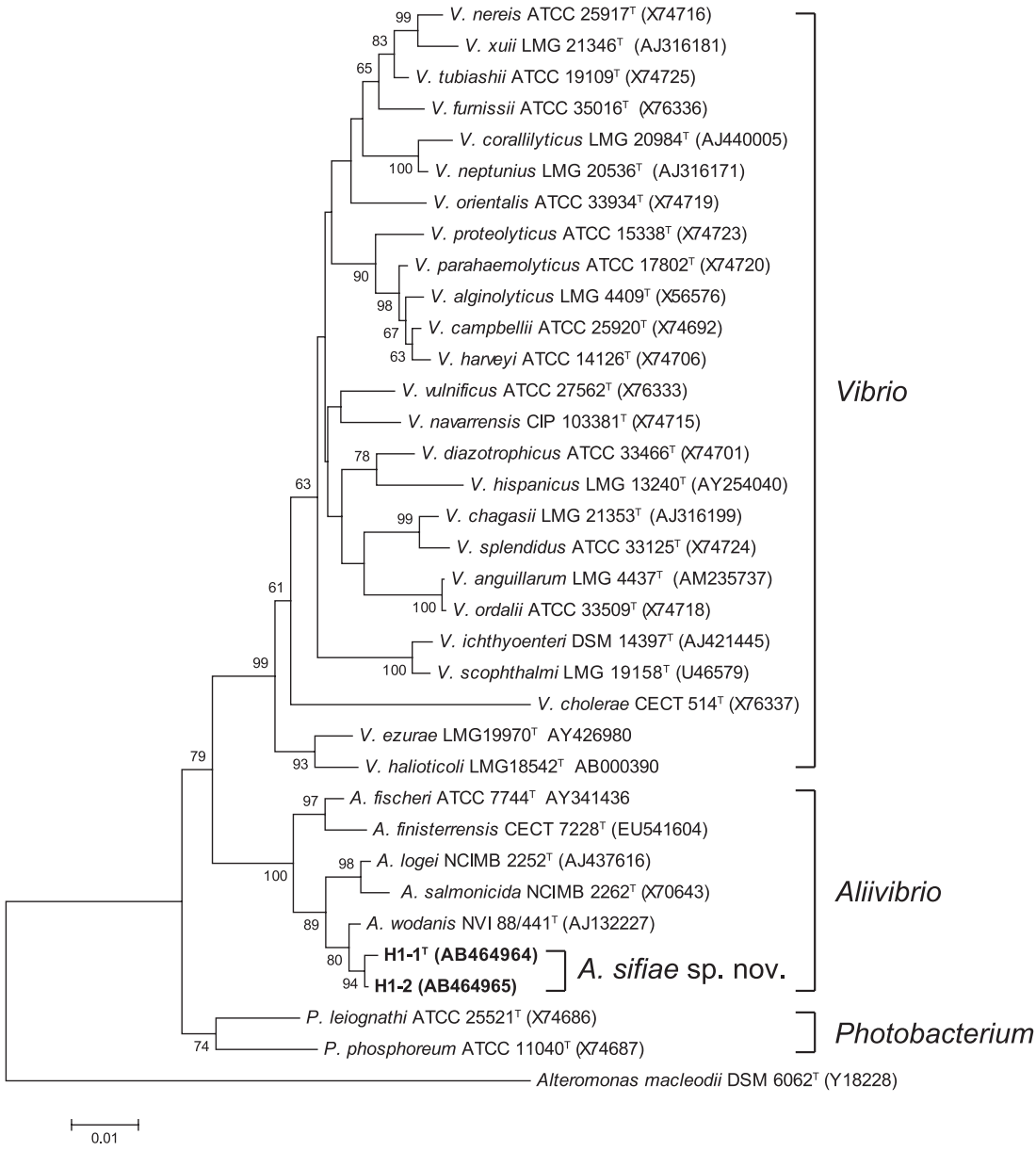

Fig. 1. Phylogenetic tree based on the partial 16S rRNA gene sequences.

This tree was constructed based on neighbor-joining. Numbers at nodes denote the level of bootstrap based on 1,000 replications; only values greater than 50% are shown. *Alteromonas macleodii* was used as an outgroup. Scale bar, 1% estimated sequence divergence.

29985^T) and 19.7% (*A. salmonicida* NCIMB 1281^T). The DNA–DNA hybridization value between H1-1^T and H1-2 strains was 71.3% (Table 2). These genetic results indicated that these strains were included in same genospecies and distinguished from any known *Aliivibrio* species. The DNA G+C contents of H1-1^T and H1-2 were 40.2 and 40.2 mol%, respectively (Table 2).

Strain H1-1^T and H1-2 grow on TCBS agar as yellow and green colonies, respectively. These strains grow on marine agar as luminescent and unpigmented translucent colonies. On the basis of the API 20E and API ZYM tests, strain H1-1^T and H1-2 can be discriminated from other *Aliivibrio* species. In contrast to most of their phylogenetic neighbors, these strains can produce β-galactosidase, lysine decarboxylase, leucine-arylamidase and *N*-acetyl-β-glucosaminidase and can utilize glucose and mannitol (Table 3 and description).

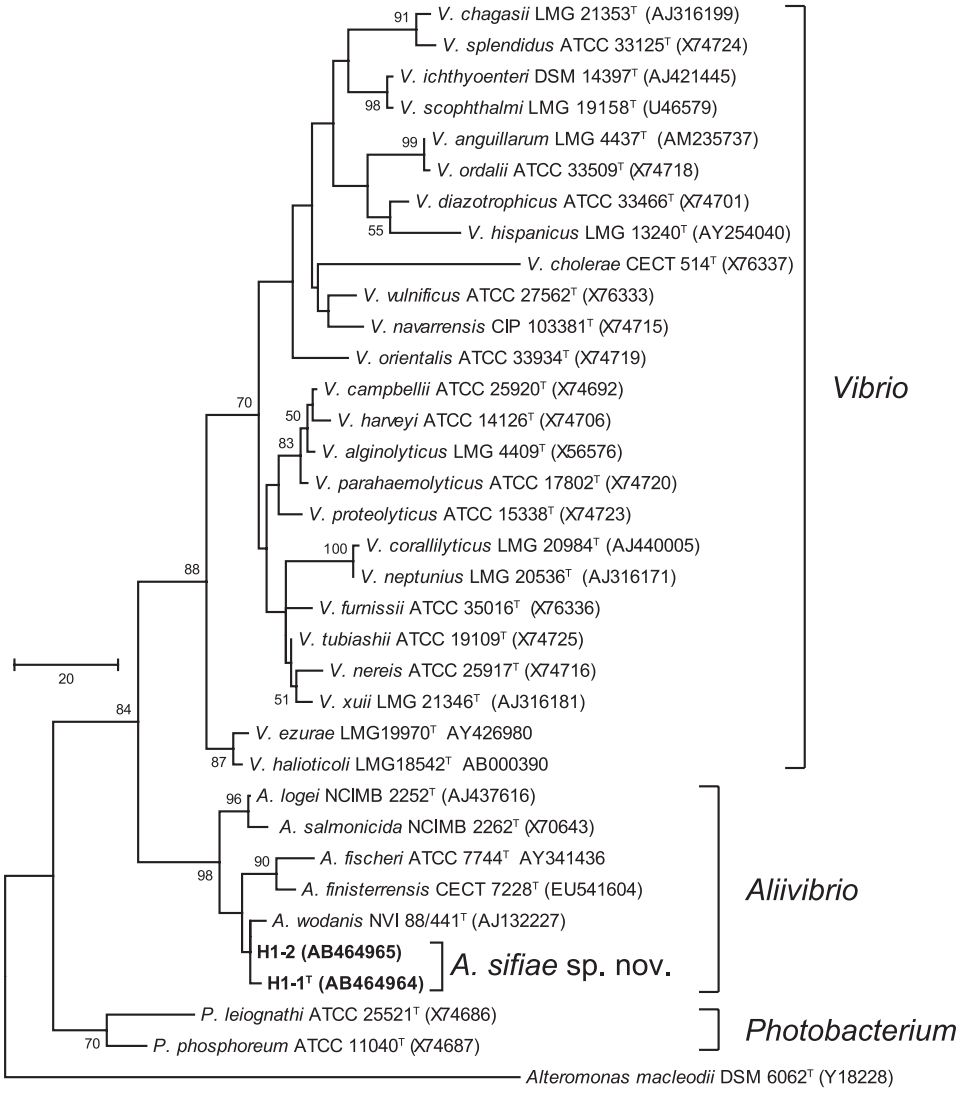

Fig. 2. Phylogenetic tree based on the maximum-parsimony method, using partial 16S rRNA sequences.

Bootstrap values are expressed as percentages of 1,000 replications; only values greater than 50% are shown. *Alteromonas macleodii* was used as an outgroup. Scale bar, 20 substitutions. on 1,000 replicates.

The predominant cellular fatty acids of strain H1-1^T and H1-2 are $C_{16:1}$ ω7c (44.2 and 46.4%), $C_{16:0}$ (17.6 and 20.1%), $C_{18:1}$ ω7c (8.9 and 6.4%), $C_{14:0}$ (6.8 and 6.9%) and $C_{12:0}$ (5.6 and 5.5%). The profiles of the seven tested strains are similar and could not clearly discriminate these three strains from other *Aliivibrio* species (Table 4).

Morphological, cultural, physiological and biochemical characteristics of H1-1^T and H1-2 are given in the species description or are shown in Table 3. These results showed that strain H1-1^T and H1-2 belong to genus *Aliivibrio* and are differentiated from other *Aliivibrio* species based on the phylogenetic analysis and DNA−DNA relatedness, as well as several phenotypic traits. Therefore, strain H1-1^T (=NBRC 105001^T) and H1-2 (=NBRC 105002) should be identified as a novel species in the genus *Aliivibrio*, for which the name *Aliivibrio sifiae* sp. nov. is proposed.

Description of *Aliivibrio sifiae* sp. nov.

Aliivibrio sifiae (*si.fi'a.e.* N.L. fem. gen. n. *sifiae* of *Sif,* the name of Norse goddess. Sif's hair was made of gold, and the name was chosen to reflect the yellow color of the luminescence).

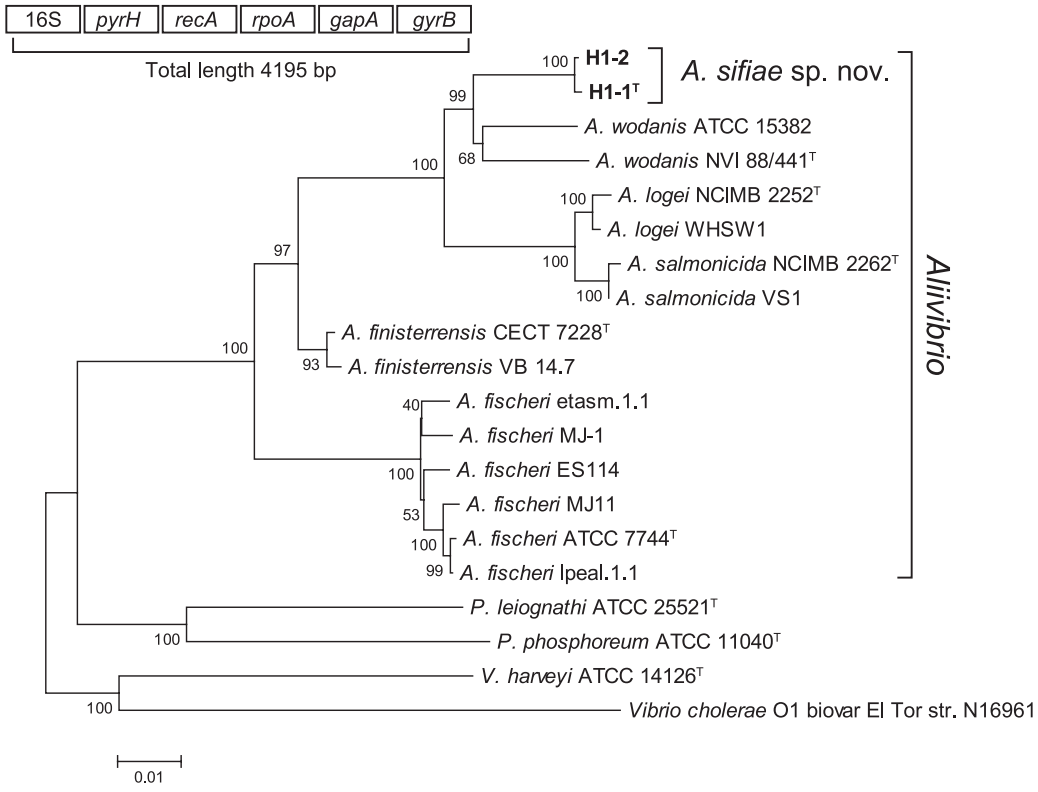

Fig. 3. Phylogenetic tree based on the concatenated gene sequences of the six loci (4,195 bp). This tree was constructed based on neighbor-joining. Numbers at nodes denote the level of bootstrap based on 1,000 replicates. *Vibrio harveyi* and *Vibrio cholerae* were used as an outgroup. Bar, 1% sequence divergence.

Table 1. Pairwise comparison of concatenated six genetic loci similarities between representative strains of the genus *Aliivibrio*.

		1	2	3	4	5	6	7
1	H1-1^T	100.0						
2	H1-2	99.8	100.0					
3	*A. wodanis* NVI 88/441^T	96.4	96.4	100.0				
4	*A. logei* NCIMB 2252^T	95.3	95.4	95.0	100.0			
5	*A. salmonicida* NCMB 2262^T	95.1	95.1	94.7	98.7	100.0		
6	*A. finisterrensis* CECT 7228^T	95.1	95.2	94.9	94.6	94.3	100.0	
7	*A. fischeri* ATCC 7744^T	91.8	91.8	91.6	91.2	91.1	95.7	100.0

Values are percentage similarity between concatenated six loci (16S rRNA, *pyrH, recA, rpoA, gapA* and *gyrB*) gene sequences. All results are based on the pairwise analysis of 7 sequences. Analyses were conducted using the Kimura 2-parameter method in MEGA4. All positions containing alignment gaps and missing data were eliminated only in pairwise sequence comparisons (Pairwise deletion option). There were a total of 4,195 positions in the final dataset.

Gram-negative, oxidase-positive, catalase-positive and motile by means of more than one polar flagellum. Cells are approximately 1.0 μm wide and 1.7–2.5 μm long, with coccoid–rods. Circular with entire margin, non-pigmented, translucent, luminescent colonies on marine agar with weak swarming. Round, 5–7 mm green or yellow colonies in TCBS agar. H1-1^T grows in the presence of 0.5–5% (optimum 3–4%), but not 8 or 10% NaCl (w/v); grows at 4–30°C (optimum 10–25°C), but not at 37°C; grows at pH 5.0–10.5 (optimum pH 7.0–8.0); grows under anaerobic conditions. Nitrate is reduced to nitrite but not further to N_2O or N_2.

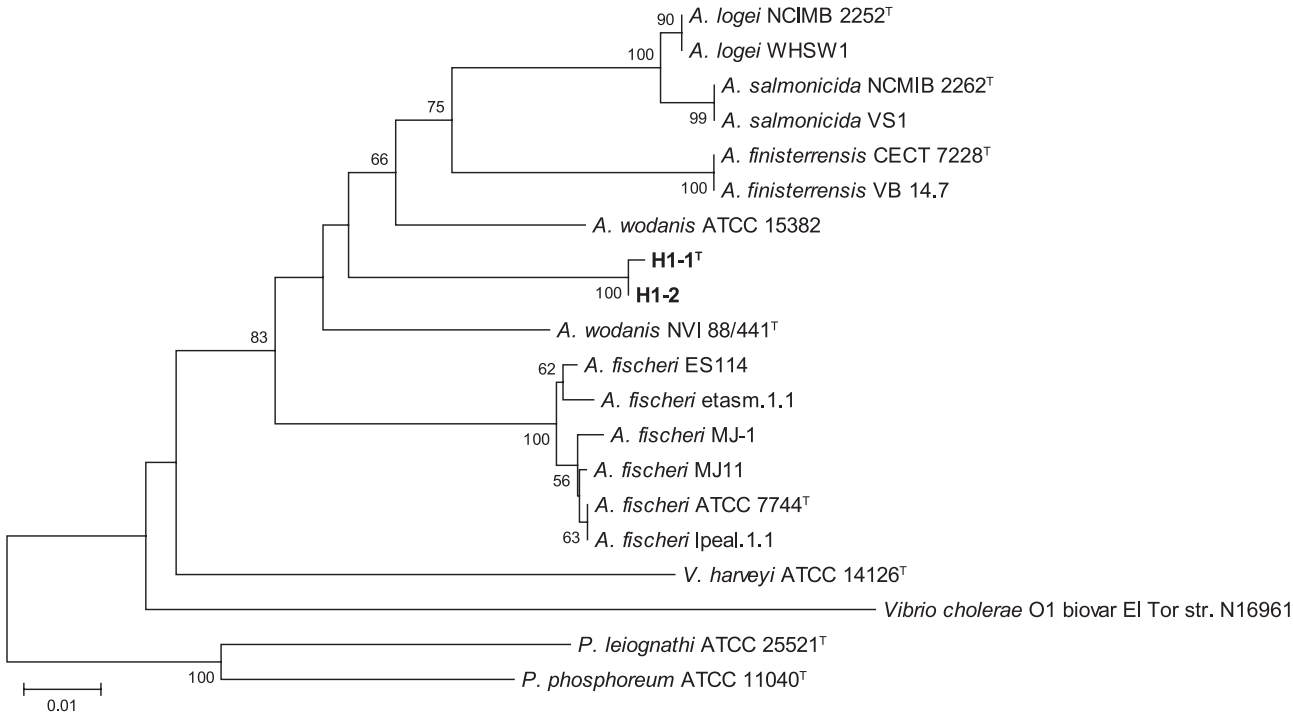

Fig. 4. Phylogenetic tree based on the neighbor-joining method, using partial *pyrH* sequences (348 bp).

Bootstrap values are expressed as percentages of 1,000 replications; only values greater than 50% are shown. Scale bar, 1% estimated sequence divergence.

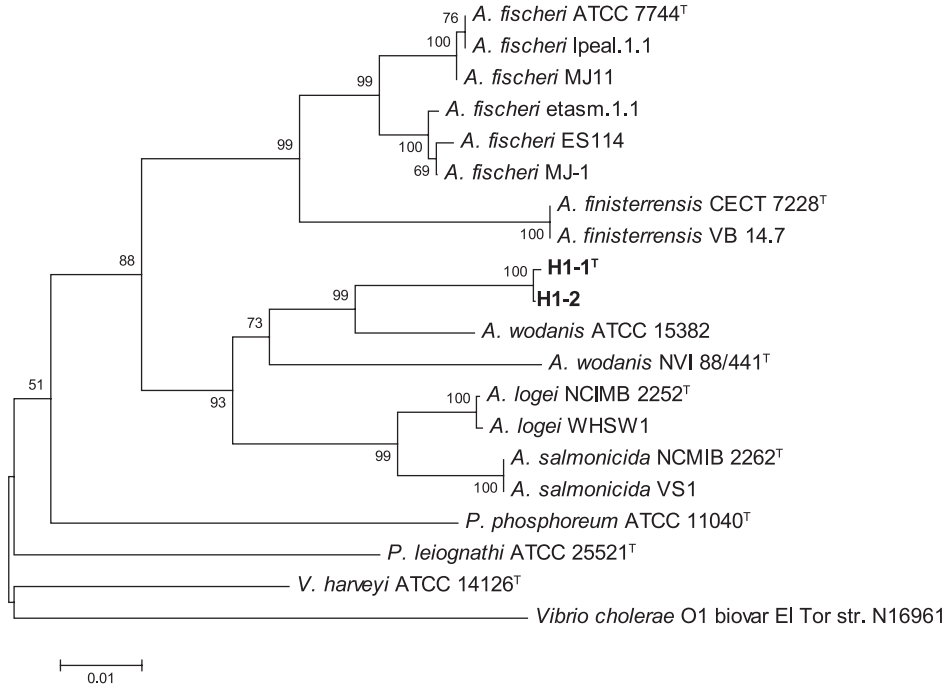

Fig. 5. Phylogenetic tree based on the neighbor-joining method, using partial *recA* sequences (497 bp).

Bootstrap values are expressed as percentages of 1,000 replications; only values greater than 50% are shown. Scale bar, 1% estimated sequence divergence.

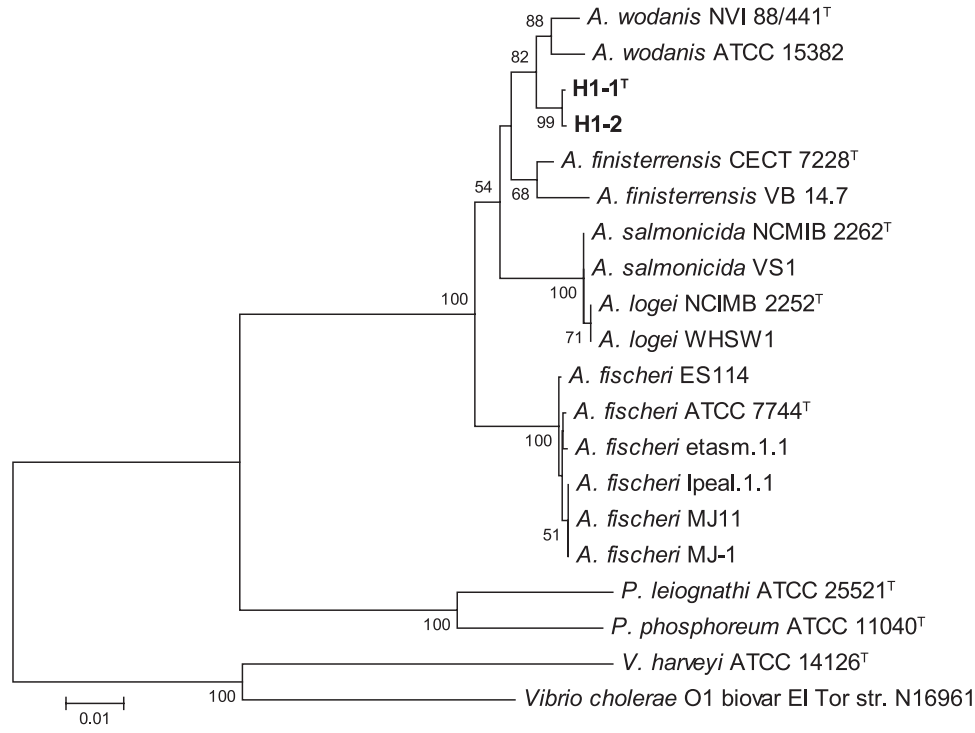

Fig. 6. Phylogenetic tree based on the neighbor-joining method, using partial *rpoA* sequences (864 bp).

Bootstrap values are expressed as percentages of 1,000 replications; only values greater than 50% are shown. Scale bar, 1% estimated sequence divergence.

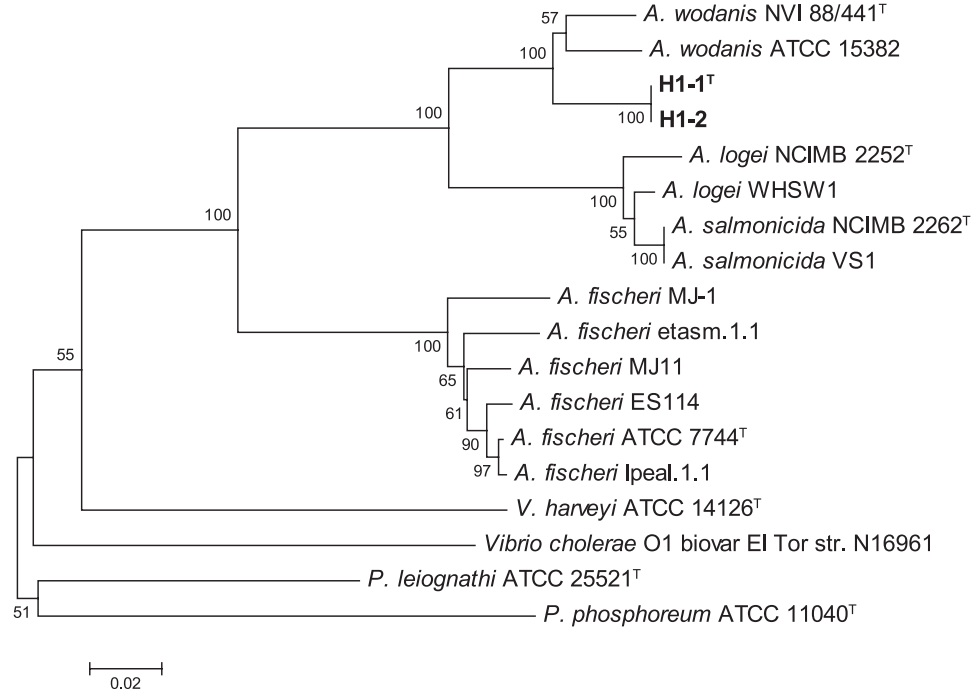

Fig. 7. Phylogenetic tree based on the neighbor-joining method, using partial *gyrB* sequences (586 bp).

Bootstrap values are expressed as percentages of 1,000 replications; only values greater than 50% are shown. Scale bar, 2% estimated sequence divergence.

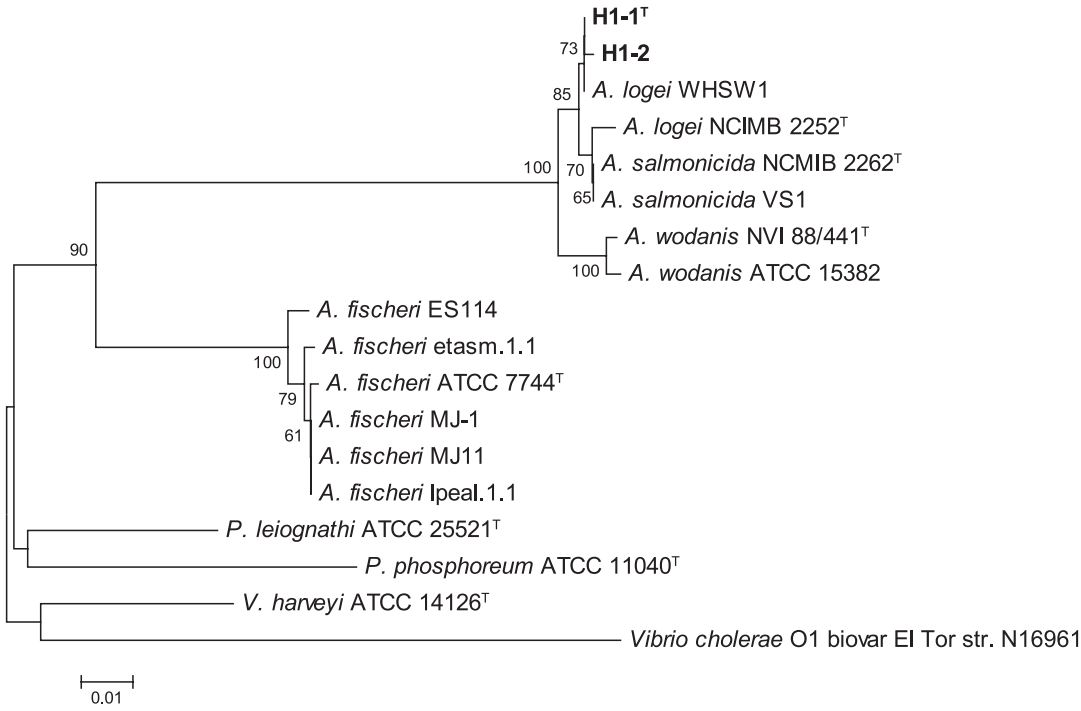

Fig. 8. Phylogenetic tree based on the neighbor-joining method, using partial *gapA* sequences (634 bp).

Bootstrap values are expressed as percentages of 1000 replications; only values greater than 50% are shown. Scale bar, 1% estimated sequence divergence.

Table 2. DNA relatedness among *Aliivibrio* related species.

Species	Strain	G+C content (mol%)	DNA–DNA hybridization values (%) H1-1^T
A. sifiae	H1-1^T	40.2	100
	H1-2	40.2	71.3
A. wodanis	ATCC BAA-104^T	40[a]	40
A. logei	ATCC 29985^T	40–42[a]	38.9
A. salmonicida	NCIMB 2262^T	39–40[a]	19.7
A. fischeri	NCIMB 1281^T	42[a]	41.4

[a]Data were described by Farmer III et al. (2005).

Negative for H_2S production from sodium thiosulfate. Tween 20, Tween 40, Tween 60 and Tween 80 are hydrolyzed. Starch and gelatin are not hydrolyzed. Positive for utilization of glucose, mannitol and amygdalin. Positive enzyme activities are seen for alkaline phosphatase, leucine-arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase, *N*-acetyl-β-glucosaminidase, β-galactosidase and lysine decarboxylase. The major fatty acids of strain H1-1^T and H1-2 are $C_{16:1}$ ω7c (44.2 and 46.4%), $C_{16:0}$ (17.6 and 20.1%), $C_{18:1}$ ω7c (8.9 and 6.4%), $C_{14:0}$ (6.8 and 6.9%), $C_{12:0}$ (5.6 and 5.5%), $C_{18:0}$ (3.9 and 3.3%), $C_{14:0}$ 3-OH (3.4 and 3.1%) and $C_{12:0}$ 3-OH (3.0 and 2.9%). G+C content of both strain H1-1^T and H1-2 is 40.2 mol%. The type strain is H1-1^T (=NBRC 105001^T = KCTC 22535^T). The reference strain is H1-2 (=NBRC 105002 = KCTC 22536).

Table 3. Differential phenotypic characteristics of *Aliivibrio sifiae* sp. nov. and the related species of genus *Aliivibrio.*

	1	2	3	4	5	6
Luminescence	+	+	−	+	+	−
Production of						
β-Galactosidase	+	+	−	−	−	+
Arginine dihydrolase	−	−	−	−	−	+
Lysine decarboxylase	+	+	−	w	+	−
Urease	−	−	−	−	+	−
Esterase C4	−	−	−	−	−	w
Esterase-lipase C8	−	−	w	w	−	w
Leucine-arylamidase	+	+	−	w	w	+
Valine-arylamidase	w	−	−	−	−	w
Acid phosphatase	+	+	−	+	+	+
α-Glucosidase	−	−	−	−	−	+
N-Acetyl-β-glucosaminidase	w	w	−	−	−	+
Indole production	+	−	−	−	−	+
Fermentation of						
Glucose	w	w	−	+	−	+
Mannitol	+	+	−	+	+	+
Sucrose	+	−	−	−	−	+
Amygdalin	+	+	−	w	+	+
Arabinose	−	−	w	−	−	+
Growth at:						
4°C	+	+	+	+	−	−
30°C	+	+	−	−	+	+
37°C	−	−	−	−	−	+
Growth in NaCl (%)						
0.5	+	+	−	w	+	+
6	−	w	−	−	−	+
8	−	−	−	−	−	+
TCBS agar	Y	G	−	Y	G	Y

Taxa: 1, H1-1^T; 2, H1-2; 3, *A. wodanis* ATCC BAA-104^T; 4, *A. logei* ATCC 29985^T; 5, *A. fischeri* NCIMB 1281^T; 6, *A. salmonicida* NCIMB 2262^T. +, Positive; w, weakly positive; −, negative; G, green colony; Y, yellow colony.

Table 4. Cellular fatty acid compositions (as percentages of the total) of *Aliivibrio sifiae* sp. nov. and the related taxa of the genus *Aliivibrio.*

Fatty acid	1	2	3	4	5	6
$C_{12:0}$	5.6	5.5	3.8	5.4	5.0	5.0
$C_{14:0}$ ISO E	—	1.6	1.9	1.0	—	—
$C_{12:0}$ 3-OH	3.0	2.9	3.7	2.7	2.9	2.8
$C_{14:0}$	6.8	6.9	6.2	4.0	5.4	5.2
$C_{15:0}$	—	—	—	1.4	—	1.3
$C_{16:0}$ ISO	—	—	—	—	3.9	—
$C_{16:0}$	17.6	20.1	22.4	23.5	18.8	18.2
$C_{18:1}$ ω7c	8.9	6.4	5.7	4.5	19.6	9.2
$C_{18:0}$	3.9	3.3	3.3	2.1	2.0	2.0
Summed feature 2	3.4	3.1	4.1	3.5	3.6	3.1
Summed feature 3	44.2	46.4	43.5	47.5	31.6	47.8

Taxa: 1, H1-1^T; 2, H1-2; 3, *A. wodanis* ATCC BAA-104^T; 4, *A. logei* ATCC 29985^T; 5, *A. salmonicida* NCIMB 2262^T; 6, *A. fischeri* NCIMB 1281^T. Summed feature 2 comprises $C_{16:1}$ ISO I and/or $C_{14:0}$ 3-OH. Summed feature 3 comprises $C_{15:0}$ ISO 2-OH and/or $C_{16:1}$ ω7c. Data are expressed as percentages of total fatty acids. Fatty acids representing less than 1% are not shown.

Table 5. Pairwise comparison of 16S rRNA gene sequences similarities between representative strains of the genus *Aliivibrio*.

		1	2	3	4	5	6	7
1	H1-1^T	100.0						
2	H1-2	99.8	100.0					
3	*A. wodanis* NVI 88/441^T	99.3	99.5	100.0				
4	*A. logei* NCIMB 2252^T	99.0	99.0	98.5	100.0			
5	*A. salmonicida* NCMB 2262^T	98.4	98.3	98.1	99.1	100.0		
6	*A. finisterrensis* CECT 7228^T	98.5	98.6	98.0	97.6	97.1	100.0	
7	*A. fischeri* ATCC 7744^T	97.2	97.4	97.1	96.7	96.2	97.9	100.0

Values are percentage similarity between 16S rRNA gene sequences. All results are based on the pairwise analysis of 8 sequences. Analyses were conducted using the Kimura 2-parameter method in MEGA4. All positions containing alignment gaps and missing data were eliminated only in pairwise sequence comparisons (Pairwise deletion option). There were a total of 1,555 positions in the final dataset.

Table 6. GenBank accession numbers of all housekeeping genes in this study.

	16S rRNA	*gyrB*	*pyrH*	*gapA*	*ftsZ*	*topA*	*mreB*	*recA*	*rpoA*
H1-1^T	**AB464964**	**AB464968**	**AB464972**	**AB464977**	**AB464982**	**AB464987**	**AB464992**	**AB464996**	**AB465000**
H1-2	**AB464965**	**AB464969**	**AB464973**	**AB464978**	**AB464983**	**AB464988**	**AB464993**	**AB464997**	**AB465001**
A. logei ATCC 29985^T	AJ437616	EF380255	EF380234	EU185847	**AB464981**	**AB464986**	**AB464991**	AJ842457	AJ842643
A. salmonicida ATCC 43839^T	X70643	EF380256	EU118245	DQ907308	DQ907375	DQ907517	DQ907447	EF380243	EF380249
A. wodanis BAA-104^T	AJ132227	EF380257	EU118246	DQ907314	DQ907383	DQ907523	DQ907455	EF380244	EF380250
A. finisterrensis CECT 7228^T	EU541604	—	EU889126	—	—	—	—	EU541586	EU541561
A. fischeri ATCC 7744^T	AY341436	AY455874	EF415528	EF415488	DQ907344	DQ907482	DQ907415	AJ842417	AJ842604
P. leiognathi ATCC 25521^T	X74686	AY455879	EF380238	EF415492	DQ907324	DQ907463	DQ907391	AJ842364	AJ842550

Bold types indicate the sequences determined in this study.

Acknowledgments

We are grateful to Dr. Jaewoo Yoon, Eiko Ikemoto and TechnoSuruga Co., Ltd. for technical support and Kumiko Kita-Tsukamoto for suggestions in the early stages of this work. This work was supported by the Sasakawa Scientific Research Grant from The Japan Science Society and a research grant from Institute for Fermentation, Osaka, Japan.

References

Ast, J. C., Urbanczyk, H., and Dunlap, P. V. (2009) Multi-gene analysis reveals previously unrecognized phylogenetic diversity in *Aliivibrio*. *Syst. Appl. Microbiol.,* **32**, 379–386.

Beaz-Hidalgo, R., Doce, A., Balboa, S., Barja, J. L., and Romalde, J. L. (2010) *Aliivibrio finisterrensis* sp. nov., isolated from Manila clam, *Ruditapes philippinarum* and emended description of the genus *Aliivibrio*. *Int. J. Syst. Evol. Microbiol.,* **60**, 223–228.

Beijerinck, M. W. (1889) Le *Photobacterium luminosum*, bacterie lumineuse de la Mer du Nord. *Arch. Neerl. Sci. Exactes Nat.*, **23**, 401–427.

Dunlap, P. V. and Kita-Tsukamoto, K. (2006) Luminous bacteria. The Prokaryotes, 3rd ed., Vol. 2, **2**, pp. 863–892.

Dunlap, P. V., Ast, J. C., Kimura, S., Fukui, A., Yoshino, T., and Endo, H. (2007) Phylogenetic analysis of host-symbiont specificity and codivergence in bioluminescent symbioses. *Cladistics*, **23**, 507–532.

Egidius, E., Wiik, R., Andersen, K., Hoff, K. A., and Hjeltnes, B. (1986) *Vibrio salmonicida* sp. nov., a new fish pathogen. *Int. J. Syst. Bacteriol.*, **36**, 518–520.

Ezaki, T., Hashimoto, Y., and 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.

Farmer, J. J., III, Janda, J. M., Brenner, F. W., Cameron, D. N., and Birkhead, K. M. (2005) Genus *Vibrio*. *In* Bergey's Manual of Systematic Bacteriology, 2nd ed., Vol. 2, Part B, The Proteobacteria, ed. by Brenner, D. J., Krieg, N. R., and Staley, J. T., Springer, New York, pp. 494–546.

Felsenstein, J. (1985) Confidence-limits on phylogenies—An approach using the bootstrap. *Evolution,* **39**, 783–791.

Harwood, C. S., Bang, S. S., Baumann, P., and Nealson, K. H. (1980) *Photobacterium logei* sp. nov., nom. rev.; *Beneckea nereida* sp. nov., nom. rev.; and *Beneckea gazogenes* sp. nov., nom. rev. *Int. J. Syst. Bacteriol.,* **30**, 655–655.

Kimura, M. (1980) A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide-sequences. *J. Mol. Evol.,* **16**, 111–120.

Lane, D. J. (1991) 16S/23S rRNA sequencing. *In* Nucleic Acid Techniques in Bacterial Systematics, ed. by Stackebrandt, E. and Goodfellow, M., John Wiley and Sons, New York, pp. 115–147.

Lunder, T., Sorum, H., Holstad, G., Steigerwalt, A. G., Mowinckel, P., and Brenner, D. J. (2000) Phenotypic and genotypic characterization of *Vibrio viscosus* sp. nov. and *Vibrio wodanis* sp. nov. isolated from Atlantic salmon (*Salmo salar*) with 'winter ulcer'. *Int. J. Syst. Evol. Microbiol.,* **50**, 427–450.

Lupp, C. and Ruby, E. G. (2005) *Vibrio fischeri* uses two quorum-sensing systems for the regulation of early and late colonization factors. *J. Bacteriol.*, **187**, 3620–3629.

Marmur, J. (1961) Procedure for isolation of deoxyribonucleic acid from micro-organisms. *J. Mol. Biol.,* **3**, 208–218.

Mesbah, M., Premachandran, U., and Whitman, W. B. (1989) Precise measurement of the G+C content of deoxyribonucleic-acid by high-performance liquid-chromatography. *Int. J. Syst. Bacteriol.*, **39**, 159–167.

Nishino, T., Ikemoto, E., and Kogure, K. (2004) Application of atomic force microscopy to observation of marine bacteria. *J. Oceanogr.*, **60**, 219–225.

Nyholm, S. V. and Mcfall-Ngai, M. J. (2004) The winnowing: Establishing the squid-Vibrio symbiosis. *Nat. Rev. Microbiol.*, **2**, 632–642.

Saitou, N. and Nei, M. (1987) The neighbor-joining method: A new method for reconstructing phylogenetic trees. *Mol. Biol. Evol.*, **4**, 406–425.

Sawabe, T., Kita-Tsukamoto, K., and Thompson, F. L. (2007) Inferring the evolutionary history of vibrios by means of multilocus sequence analysis. *J. Bacteriol.,* **189**, 7932–7936.

Tamura, K., Dudley, J., Nei, M., and Kumar, S. (2007) MEGA4: Molecular evolutionary genetics analysis (MEGA) software version 4.0. *Mol. Biol. Evol.*, **24**, 1596–1599.

Thompson, F. L., Gevers, D., Thompson, C. C., Dawyndt, P., Naser, S., Hoste, B., Munn, C. B., and Swings, J. (2005) Phylogeny and molecular identification of Vibrios on the basis of multilocus sequence analysis. *Appl. Environ. Microbiol.*, **71**, 5107–5115.

Thompson, F. L., Gomez-Gil, B., Vasconcelos, A. T. R., and Sawabe, T. (2007) Multilocus sequence analysis reveals that *Vibrio harveyi* and *V. campbellii* are distinct species. *Appl. Environ. Microbiol.*, **73**, 4279–4285.

Thompson, J. D., Higgins, D. G., and Gibson, T. J. (1994) Clustal W: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res.*, **22**, 4673–4680.

Urbanczyk, H., Ast, J. C., Higgins, M. J., Carson, J., and Dunlap, P. V. (2007) Reclassification of *Vibrio fischeri*, *Vibrio logei*, *Vibrio salmonicida* and *Vibrio wodanis* as *Aliivibrio fischeri* gen. nov., comb. nov., *Aliivibrio logei* comb. nov., *Aliivibrio salmonicida* comb. nov. and *Aliivibrio wodanis* comb. nov. *Int. J. Syst. Evol. Microbiol.,* **57**, 2823–2829.

Xie, C. H. and Yokota, A. (2003) Phylogenetic analyses of *Lampropedia hyalina* based on the 16S rRNA gene sequence. *J. Gen. Appl. Microbiol.*, **49**, 345–349.