Photobacterium histaminum sp. nov., a Histamine-Producing Marine Bacterium

MASAYO OKUZUMI,^{1†} AKIRA HIRAISHI,² TAKESHI KOBAYASHI,¹ AND TATEO FUJII^{1*}

Department of Food Science and Technology, Tokyo University of Fisheries, Konan, Minato-ku, Tokyo 108,¹ and
Laboratory of Environmental Biotechnology, Konishi Co., Yokokawa, Sumida-ku, Tokyo 130,² Japan

A total of 23 strains of halophilic histamine-producing bacteria previously isolated from marine fish and seawater were studied phenotypically, genotypically, and phylogenetically. These organisms are facultatively anaerobic, gram-negative, short rods that are motile by means of one to three unsheathed polar flagella. They are mesophilic and nonluminescent, produce oxidase, utilize D-glucose but not D-mannitol as a carbon and energy source, and accumulate poly- β -hydroxybutyrate. The guanine-plus-cytosine contents of the DNAs are ca. 41 mol%. Genomic DNA hybridization experiments revealed that some of the isolates exhibited low levels of reassociation (less than 40%) with previously described *Photobacterium* species. 16S rRNA gene sequence information confirmed the phylogenetic position of one of the isolates, strain C-8^T (T = type strain), as a member of the genus *Photobacterium*. Therefore, we concluded that these organisms are members of a new species of the genus *Photobacterium* and propose the name *Photobacterium histaminum* sp. nov. for them. The type strain is strain C-8 (= JCM 8968).

Most of the histamine-producing bacteria that have been isolated previously as the causative agents of scombroid fish poisoning are members of the family *Enterobacteriaceae*; these bacteria include *Citrobacter freundii* (27), *Enterobacter cloacae* (31), *Escherichia coli* (7, 8), *Hafnia alvei* (8), *Klebsiella pneumoniae* (26), and *Proteus morganii* (*Morganella morganii*) (15, 16) strains. Some gram-positive bacteria, such as *Clostridium perfringens*, have also been reported to be histamine producers (34). On the other hand, less information about histamine-producing bacteria of marine origin has been available. During surveys of histamine-producing bacteria in marine fish and seawater, we isolated several strains of facultatively anaerobic, halophilic bacteria that produced appreciable amounts of histamine (22, 32, 33). On the basis of growth temperature characteristics, these isolates were divided into two groups; the members of one group were psychrophilic strains that grew at 4°C but not at 40°C, and the members of the other group were mesophiles which did not grow at 4°C. We paid special attention to the mesophilic strains, because their ability to produce histamine was as great as that of *M. morganii* and because they appeared to belong to the genus *Photobacterium* on the basis of their phenotypic traits but could not be assigned to any previously described member of this genus.

In this study, we further characterized the new histamine-producing isolates phenotypically, genotypically, and phylogenetically. Phylogenetic relationships among members of marine genera, including the genus *Photobacterium*, have been inferred from partial 16S rRNA sequences consisting of about 600 bases (18). In this study, the phylogenetic position of a representative isolate was determined on the basis of almost complete 16S ribosomal DNA (rDNA) sequences. On the basis of our data, we concluded that the isolates which we studied are members of a new species of the genus *Photobacterium*, for which we propose the name *Photobacterium histaminum*; strain C-8 is the type strain of this species.

MATERIALS AND METHODS

Bacterial strains and growth conditions. A total of 23 isolates previously obtained from different sources (31–33) were studied; 15 of these strains (C-4-1, C-4-2, C-5, C-6, C-7, C-12, C-13, C-15, C-18, C-19, C-20, C-21, C-22, C-23, and C-24) were isolated from coastal seawater obtained from Tokyo Bay, Japan (32), 5 strains (C-1, C-2, C-3, C-10, and C-11) were isolated from seawater obtained from Sagami Bay, Japan (32), and 3 strains (C-8^T [T = type strain], C-9, and C-41) were isolated from skins of a labracoglossid fish (*Labracoglossa argentiventris*), a Japanese bluefish (*Scombrops boops*), and a sardine (*Sardinops melanostictus*) purchased from a fish market in Kanagawa, Japan, between June and August 1987 (33). The type strains of three *Photobacterium* species, *Photobacterium phosphoreum* ATCC 11040, *Photobacterium angustum* ATCC 25915, and *Photobacterium leiognathi* ATCC 25521, were obtained from the American Type Culture Collection, Rockville, Md., and were used as reference organisms. These strains were stabbed into 50% artificial seawater (ASW)–peptone–yeast extract agar (0.5% Polypeptone, 0.3% yeast extract, and 1.5% agar in 50% ASW [pH 7.0]) (22) and were maintained at 15°C. Unless indicated otherwise, the inocula used for phenotypic tests were prepared from 18- to 24-h-old cultures in 50% ASW–PMYG broth (0.5% Polypeptone 0.25% meat extract, 0.25% yeast extract, and 0.1% glucose in 50% ASW [pH 7.0]) (22) and incubated at 30°C.

Morphology. Cell morphology, Gram staining characteristics, and motility were studied as described previously (31). Flagella were observed by using negatively stained cells and electron microscopy (1). Accumulation of poly- β -hydroxybutyrate (PHB) was determined by the Sudan Black staining method (5).

Cultural, physiological, and biochemical characteristics. Hydrogen sulfide production was determined with SIM medium (Difco) after 6 days of incubation. Production of 2,3-butanediol was determined by the method of Bullock (4) after 3 days of growth in medium supplemented with 2% NaCl. Bioluminescence was determined by examining colonies grown on plates containing Hidaka agar (9) (10 g of Polypeptone, 2 g of yeast extract, 3 g of glycerol, 15 g of agar, 750 ml of ASW, 250 ml of distilled water; [pH 7.2]). Cytochrome oxidase was

* Corresponding author. Tokyo University of Fisheries, Konan, Minato-ku, Tokyo 108, Japan.

† Present address: Natori Co. Ltd., Toshima, Kita-ku, Tokyo 114, Japan.

detected by using commercially available test papers (Nissui Co., Tokyo, Japan). Starch hydrolysis was detected by covering the bacterial surface growth on a 2% NaCl-peptone-yeast extract-glucose (2% NaCl-PYG) agar plate containing 0.1% soluble starch with Gram's iodine solution. Chitin decomposition was determined with a 2% NaCl-PYG agar plate containing 10% chitin. Alginate hydrolysis was determined with 75% ASW-yeast extract medium supplemented with 2% sodium alginate (24). Gelatin hydrolysis was detected by flooding the growth on a 2% NaCl-PYG agar plate containing 0.4% gelatin with acidic mercuric chloride (24). The NaCl requirement for optimal growth was determined by the method of Lee et al. (19). Utilization of organic compounds as carbon and energy sources was determined by the method of Baumann and Baumann (2). Most of the organic compounds used were added at a concentration of 1% (wt/vol); the exceptions were ethanol (20 ml/liter), glycerol (20 ml/liter), and lactate (16 ml/liter). Growth was assayed turbidimetrically at 570 nm after 2, 4, and 6 days. Histamine contents in cultures were determined by high-performance liquid chromatography (HPLC) as described previously (30). Vibriostatic compound 0/129 (2,4-diamino-6,7-diisopropyl pteridine phosphate) was applied as a filter paper disk (Daiichi Seiyaku). The test was performed with 50% ASW-PMYG agar. All other phenotypic tests were performed by using standard protocols as described previously (22, 31).

DNA base composition and DNA-DNA homology. Genomic DNA was extracted and purified by the method of Marmur (20). DNA base composition (guanine-plus-cytosine [G+C] content) was determined by the HPLC method of Katayama-Fujimura et al. (14). *Escherichia coli* DNA (G+C content, 51.6 mol%) was obtained from Sigma Chemical Co., St. Louis, Mo., and was used as the standard. DNA-DNA pairing studies were performed by the quantitative dot blot hybridization method with photobiotin labeling and colorimetric detection (6, 12).

16S rRNA gene sequencing and phylogenetic analysis. The 16S rRNA gene was amplified by the PCR from protease-digested cell lysates, using a commercial PCR kit, *Taq* DNA polymerase, and a pair of eubacterial consensus primers homologous or supplementary to positions 8 to 27 and 1492 to 1510 (29) (all positions indicated for 16S rRNA molecules are positions in the *E. coli* numbering system). The PCR products were subjected to chloroform-phenol treatment and ethanol precipitation and were purified by agarose gel electrophoresis. Detailed information concerning the PCR procedures used has been given elsewhere (11, 13). PCR-amplified, double-stranded 16S rDNA was sequenced directly by the cycle sequencing method modified for automated fluorescence detection, and the reaction mixtures were analyzed with a Pharmacia model A.L.F. laser fluorescent DNA sequencer, as previously described (11, 13). Sequences were determined from overlapping sequence data by using the GENETYX computer program (Software Development Co., Tokyo, Japan). Nucleotide substitution rates (K_{nuc} values) (17) were determined and a distance matrix tree was constructed by the neighbor-joining method (23), using the CLUSTAL V program (10). Alignment gaps and unidentified base positions were not taken into consideration for the calculations.

Nucleotide sequence accession numbers. The 16S rDNA sequences determined in this study have been deposited in the DDBJ, EMBL, GSDB, and NCBI nucleotide sequence databases under accession numbers D25308 to D25310. The accession numbers of the sequences used as references are as follows: *Aeromonas hydrophila*, X60404; *E. coli*, J01859; *Listonella anguillara* (*Vibrio anguillarum*), X16895; *Photobacterium damsela* (*Listonella damsela*), D11179, D11228, and

FIG. 1. Electron micrograph of a negatively stained cell of strain C-8^T. Bar = 1 μ m.

D11277; *Plesiomonas shigelloides*, M59159; *Shewanella putrefaciens*, D11190, D11239, and D11288; *Vibrio alginolyticus*, X56576; *Vibrio diazotrophicus*, X56577; *Vibrio fischeri*, X74702; *Vibrio hollisae*, X56583; and *Vibrio parahaemolyticus*, X56580.

RESULTS

Morphology and cultural characteristics. The cells of all of the strains were gram-negative, asporogenous, motile, short rods that were 1.0 to 1.2 μ m wide and 2.2 to 2.5 μ m long (Fig. 1). Cells grown on solid media had one to three polar flagella which were not enclosed in a sheath, unlike the flagella of *Vibrio* species such as *V. alginolyticus* and *V. fischeri*. PHB granules accumulated in the cells, but fewer granules were found than are found in *Photobacterium leiognathi* ATCC 25521^T.

Colonies on 50% ASW-PMYG agar plates after 24 h of incubation at 30°C were entire, smooth, semitranslucent, ivory, and approximately 1 to 1.2 mm in diameter. After 48 h of incubation, the colonies were 2.0 to 2.5 mm in diameter; they were not bioluminescent.

Physiological and biochemical characteristics. The 23 strains which we studied were uniform in their physiological characteristics, with few exceptions. A summary of the phenotypic properties is shown in Table 1. All 23 strains required Na⁺ for growth (the optimum NaCl concentration was about 2%) and were not able to grow in media lacking added NaCl. Most of the 23 strains were able to grow at 40°C but not at 45°C. All of the strains failed to grow at 4°C. Optimal growth occurred at temperatures between 30 and 35°C. All strains grew well at pH 4.5 to 9.0; the pH optima were between 6.0 and 7.0. Acid and gas were formed from D-glucose. All isolates produced H₂S in SIM medium, although in very small quantities. All strains formed histamine.

DNA base composition and DNA-DNA hybridization. Table

TABLE 1. Characteristics of the histamine-producing bacteria

Characteristic	23 isolates ^a	Strain C-8 ^T
Gram stain reaction	—	—
Rod-shaped cells	+	+
Spores	—	—
Motility	+	+
Sheathed polar flagella	—	—
Acid produced from D-glucose	+	+
Gas produced from D-glucose	+	+
Catalase	+	+
Oxidase	+	+
Reduction of NO ₃ ⁻ to NO ₂ ⁻	+	+
Accumulation of PHB coupled with the inability to utilize β -hydroxybutyrate	+	+
Production of 2,3-butanediol	+	+
Production of H ₂ S	+	+
Hydrolysis of:		
Starch	+	+
Chitin	+	+
Alginate	—	—
Gelatin	—	—
Luminescence	—	—
Susceptibility to vibriostatic agent 0/129	+	+
Na ⁺ required for growth	+	+
Growth in the presence of:		
0% NaCl	—	—
2% NaCl	+	+
6% NaCl	+	+
8% NaCl	—	—
Growth at:		
4°C	—	—
20°C	+	+
30°C	+	+
40°C	d	+
Utilization of:		
Acetate, succinate, fumarate, D-ribose, D-fructose, D-glucose, D-mannose, D-galactose, maltose, cellobiose, melibiose, trehalose, L-aspartate, L- α -alanine, L-glutamate, L-proline, L-serine, L-threonine, glycerol, hippurate	+	+
Valerate, isovalerate, DL-lactate, pyruvate, DL-malate, L-tartrate, citrate, malonate, D-gluconate, D-glucuronate, D-xylose, L-arabinose, L-rhamnose, lactose, salicine, sucrose, D-galacturonate, D-mannitol, D-sorbitol, ethanol, <i>n</i> -propanol, D- α -alanine, β -alanine, L-leucine, L-arginine, γ -aminobutyrate, L-citrulline, glycine, L-histidine, L-ornithine, betaine	—	—
G+C content of DNA (mol%)	41.3 ~ 41.8 ^b	41.6

^a +, all strains are positive; —, all strains are negative; d, 11 to 89% of the strains are positive.

^b Data for strains C-3, C-8^T, and C-41.

2 shows the DNA base compositions and levels of DNA-DNA relatedness among the organisms studied. The G+C contents of the isolates (strains C-3, C-8^T, and C-41) ranged from 41.3 to 41.8 mol%, values which are similar to the G+C contents of some previously described *Photobacterium* species. Genomic DNA hybridization experiments performed with labeled DNAs from strain C-8^T and *Photobacterium phosphoreum* ATCC 11040^T revealed that the new isolates were closely related to each other but exhibited relatively low levels of hybridization (less than 40%) with the three previously described species *Photobacterium* species, *Photobacterium phosphoreum*, *Photobacterium angustum*, and *Photobacterium leiognathi*. Among the strains of previously described species used for comparison, *Photobacterium leiognathi* ATCC 25521^T exhibited the highest level of homology to strain C-8^T.

16S rDNA sequence comparisons. The PCR-amplified 16S rDNA sequences of one of our isolates, strain C-8^T, and the three previously described *Photobacterium* species were determined to obtain more information about the interspecies relationships of these organisms. The rDNA fragments used, excluding the PCR primer-annealing regions, consisted of continuous nucleotide stretches from positions 28 to 1491 that were 1,470 to 1,476 residues long. The 16S rDNA sequences of the test organisms were compared with the sequences of eight reference species belonging to the gamma subclass of the *Proteobacteria*. Table 3 shows the evolutionary distance (K_{nuc}) values which we calculated for the 1,367 positions of all of the sequences which could be aligned. The corrected distance values obtained for strain C-8^T and the previously described *Photobacterium* species ranged from 0.0271 to 0.0446. The evolutionary distances between strain C-8^T and the other species compared were more than 0.0541. On the basis of the K_{nuc} values shown in Table 3, a distance matrix tree was reconstructed by the neighbor-joining method (Fig. 2).

DISCUSSION

We found that the new isolates which we studied are members of a phenotypically homogeneous group that can be regarded as a single species. These organisms are halophilic, facultatively anaerobic chemoheterotrophs that have gram-negative, nonsporeforming, rod-shaped cells which are motile by means of unsheathed polar flagella. They are also oxidase positive, accumulate PHB, utilize glucose but not mannitol as a carbon source, and have genomic DNA G+C contents of ca. 41 mol%. On the basis of these characteristics, the isolates should be considered members of the genus *Photobacterium* (3). Two characteristics of the isolates, unsheathed polar flagella and PHB accumulation, distinguish them from members of a morphologically and physiologically similar genus, the genus *Vibrio*. Also, our isolates are clearly differentiated from

TABLE 2. Levels of DNA-DNA reassociation between the histamine-producing strains and three *Photobacterium* species

Source of unlabeled DNA	G+C content of DNA (mol%)	% Relatedness to labeled DNA from:			
		C-8 ^T	ATCC 11040 ^T	ATCC 25521 ^T	ATCC 25915 ^T
Histamine-producing strains					
C-8 ^T	41.6	100	29	40	19
C-3	41.8	99	30	NT ^a	NT
<i>Photobacterium phosphoreum</i> ATCC 11040 ^T	40.8	31	100	NT	NT
<i>Photobacterium leiognathi</i> ATCC 25521 ^T	41.9	36	32	100	NT
<i>Photobacterium angustum</i> ATCC 25915 ^T	41.0	34	40	NT	100

^a NT, not tested.

TABLE 3. Evolutionary distance (K_{nuc}) values for 16S rDNAs and rRNAs of *Photobacterium* species, strain C-8^T, and related organisms

Organism	K_{nuc} value with:											
	<i>Photobacterium</i> <i>phosphoreum</i>	<i>Photobacterium</i> <i>angustum</i>	<i>Photobacterium</i> <i>leignathi</i>	Strain C-8 ^T	<i>Vibrio</i> <i>fischeri</i>	<i>Vibrio</i> <i>alginolyticus</i>	<i>Vibrio</i> <i>parahaemolyticus</i>	<i>Listonella</i> <i>anguillara</i>	<i>Vibrio</i> <i>diazotrophicus</i>	<i>Vibrio</i> <i>hollisae</i>	<i>Plesiomonas</i> <i>shigelloides</i>	<i>Aeromonas</i> <i>hydrophila</i>
<i>Photobacterium angustum</i>	0.0154											
<i>Photobacterium leiognathi</i>	0.0263	0.0249										
Strain C-8 ^T	0.0439	0.0446	0.0271									
<i>Vibrio fischeri</i>	0.0527	0.0527	0.0468	0.0563								
<i>Vibrio alginolyticus</i>	0.0571	0.0490	0.0461	0.0541	0.0424							
<i>Vibrio parahaemolyticus</i>	0.0591	0.0578	0.0563	0.0636	0.0512	0.0198						
<i>Listonella anguillara</i>	0.0636	0.0600	0.0622	0.0702	0.0475	0.0322	0.0410					
<i>Vibrio diazotrophicus</i>	0.0622	0.0600	0.0607	0.0680	0.0578	0.0351	0.0424	0.0337				
<i>Vibrio hollisae</i>	0.0629	0.0644	0.0563	0.0549	0.0753	0.0490	0.0541	0.0636	0.0600			
<i>Plesiomonas shigelloides</i>	0.0849	0.0892	0.0871	0.0878	0.0819	0.0849	0.0922	0.0929	0.0863	0.0966		
<i>Aeromonas hydrophila</i>	0.0966	0.1024	0.0944	0.0966	0.0995	0.0988	0.1053	0.0958	0.1039	0.0995	0.0856	
<i>Escherichia coli</i>	0.0900	0.0922	0.0922	0.0966	0.0892	0.0885	0.0980	0.0988	0.0914	0.0973	0.0593	0.0922

members of the genera *Aeromonas* and *Plesiomonas* by their salt requirement, DNA base ratios, and other properties.

However, phenotypic and genotypic studies revealed that the new isolates cannot be assigned to any of the previously described species of the genus *Photobacterium*. The new isolates differ from *Photobacterium phosphoreum* in growing at 35°C but not at 4°C, producing amylase, and utilizing acetate and L-proline. They differ from *Photobacterium angustum* in producing gas and 2,3-butandiol from glucose and utilizing cellobiose, melibiose, and hippurate but not xylose, pyruvate, or gluconate. And they differ from *Photobacterium leiognathi* in producing gas from glucose and utilizing pyruvate, gluconate, and lactate. In addition, the new isolates are characterized by their ability to produce large amounts of histamine. Also, the new isolates exhibit levels of genomic DNA hybridization with the previously described *Photobacterium* species of less than 40%. The hybridization values which we obtained are sufficient to separate these organisms from *Photobacterium phosphoreum*, *Photobacterium leiognathi*, and *Photobacterium angustum* at the species level in light of the species concept standardized on the basis of DNA-DNA reassociation values (28). Recently, *Listonella damsela* was transferred to the genus *Photobacterium* as *Photobacterium damsela* (25) on the basis of phenotypic data. Since *Photobacterium damsela* was not included as a reference organism in this study, we could not

compare our isolates with this species genotypically. However, the information available for *Photobacterium damsela* indicates that there are some phenotypic differences (e.g., amylase production, utilization of lactate and pyruvate) between this species and our isolates.

The results of phylogenetic analyses performed with 16S rRNA gene sequence information support the conclusions described above and further clarify the taxonomic and phylogenetic position of the new isolates among members of the genus *Photobacterium* and related genera. The phylogenetic tree in Fig. 2 shows that strain C-8^T forms a cluster with all previously described members of the genus *Photobacterium* except *Photobacterium damsela*, but is located in a deeply branched, separate lineage within this cluster. Recent research on the phylogeny of marine enterobacteria inferred from partial 16S rRNA sequences has shown that *Photobacterium damsela*, together with *Vibrio costicola* and *V. hollisae*, belongs to a lineage different from the *Photobacterium* cluster (18). We compared the 16S rDNA sequence of strain C-8^T with the partial sequence of *Photobacterium damsela* and found that there were 10 base differences in the 535 positions compared, corresponding to a level of similarity of 98.1%. This value is low enough to warrant placement of the two organisms in separate species. Since the new isolates are similar to a marine bacterium belonging to the gamma subclass, *Shewanella putrefaciens*, in sulfide production, the 16S rDNA sequence of strain C-8^T was also compared with the available partial sequence of that species. The level of similarity between the two sequences was only 89.2% (532 positions were compared), suggesting that these organisms belong to different genera. A previous report has shown that the position of *S. putrefaciens* is far from the position of the *Photobacterium* cluster on the 16S rRNA sequence-based phylogenetic tree (18). On the basis of phenotypic, genotypic, and phylogenetic data, it is logical to conclude that the isolates which we studied should be classified as members of a new species of the genus *Photobacterium*. We propose the name *Photobacterium histaminum* sp. nov. for our isolates because of their ability to produce histamine in scombroid fish.

Description of *Photobacterium histaminum* sp. nov. *Photobacterium histaminum* (his.ta.mi'num. N.L.n. *histaminum*, histamine). Gram-negative, asporogenous, short, rod-shaped cells with rounded ends that are 1.0 to 1.2 µm wide and 2.2 to 2.5

FIG. 2. Unrooted distance matrix tree showing the phylogenetic positions of strain C-8^T and members of the gamma subclass of the *Proteobacteria*. Bar = 0.02 K_{nuc} .

μm long. Motile by means of one to three polar flagella. PHB accumulates in cells. Colonies on ordinary nutrient agar having an ASW base are entire, smooth, semitranslucent, nonluminescent, and ivory; they are 1.0 to 1.2 mm in diameter after 24 h of incubation at 30°C. Halophilic and mesophilic. Best growth occurs at an NaCl concentration of ca. 2%. No growth occurs in the absence of NaCl. The optimal temperature for growth is between 30 and 35°C. No growth occurs at 4 or 45°C. Growth occurs at pH 4.5 to 9.0, and the optimum pH is 6.0 to 7.0. Facultatively anaerobic chemoorganotroph having both respiratory and fermentative types of metabolism. Oxidase and catalase positive. Acid and gas are produced from D-glucose and many other carbohydrates. Nitrate is reduced to nitrite. Amylase and chitinase are produced. Neither gelatin nor alginate is hydrolyzed. Histamine is produced. Susceptible to vibriostatic agent 0/129 (2,4-diamino-6,7-diisopropyl pteridine). The following compounds are utilized as sole carbon and energy sources: acetate, succinate, fumarate, D-ribose, D-fructose, D-glucose, D-mannose, D-galactose, maltose, cellobiose, melibiose, trehalose, glycerol, L-aspartate, L-α-alanine, L-glutamate, L-proline, L-serine, L-threonine, and hippurate. The following compounds are not utilized: valerate, isovalerate, DL-lactate, pyruvate, DL-malate, L-tartrate, citrate, malonate, D-gluconate, D-glucuronate, D-xylose, L-arabinose, L-rhamnose, lactose, salicine, sucrose, D-galacturonate, D-mannitol, D-sorbitol, ethanol, *n*-propanol, D-α-alanine, β-alanine, L-leucine, L-arginine, γ-aminobutyrate, L-citrulline, glycine, L-histidine, L-ornithine, and betaine. The G+C content of the DNA ranges from 41.3 to 41.8 mol%.

Habitat: seawater and marine fish.

The type strain is strain C-8, which has been deposited in the Japan Collection of Microorganisms as strain JCM 8968.

ACKNOWLEDGMENT

We thank R. Yoguchi (Department of Food Science and Technology, Tokyo University of Fisheries) for earlier contributions to this study.

REFERENCES

- Allen, R. D., and P. Baumann. 1971. Structure and arrangement of flagella in species of the genus *Beneckea* and *Photobacterium fischeri*. J. Bacteriol. **107**:295–302.
- Baumann, P., and L. Baumann. 1981. The marine Gram-negative eubacteria, p. 1302–1331. In M. P. Starr, H. Stolp, H. G. Trüper, A. Balows, and H. G. Schlegel (ed.), The prokaryotes, vol. 2. Springer-Verlag, New York.
- Baumann, P., and L. Baumann. 1984. Genus II. *Photobacterium*, p. 539–545. In N. R. Krieg and J. G. Holt (ed.), Bergey's manual of systematic bacteriology, vol. 1. The Williams & Wilkins Co., Baltimore.
- Bullock, G. L. 1961. A schematic outline for the presumptive identification of bacterial disease of fish. Prog. Fish-Cult. **23**:147–151.
- Burdon, K. L. 1946. Fatty material in bacteria and fungi revealed by staining dried, fixed slide preparations. J. Bacteriol. **52**:665–678.
- Ezaki, T., S. Desjirilert, H. Yamamoto, N. Takeuchi, S. Liu, and E. Yabuuchi. 1988. Simple and rapid genetic identification of *Legionella* species with photobiotin-labeled DNA. J. Gen. Appl. Microbiol. **34**:191–199.
- Ferncik, M. 1970. Formation of histamine during bacterial decarboxylation of histidine in the flesh of some marine fishes. J. Hyg. Epidemiol. Microbiol. Immunol. (Prague) **14**:52–60.
- Havelka, B. 1967. Role of the *Hafnia* bacteria in the rise of histamine in tuna fish meat. Cesk. Hyg. **12**:343–352.
- Hidaka, T. 1965. On the specificity of mineral requirement of marine bacteria. Mem. Fac. Fish. Kagoshima Univ. **14**:127–128.
- Higgins, D. G., A. J. Bleasby, and R. Fuchs. 1993. CLUSTAL V: improved software for multiple sequence alignment. Comp. Appl. Biosci. **8**:189–191.
- Hiraishi, A. 1992. Direct automated sequencing of 16S rDNA amplified by polymerase chain reaction from bacterial cultures without DNA purification. Lett. Appl. Microbiol. **15**:210–213.
- Hiraishi, A., Y. Hoshino, and T. Satoh. 1991. *Rhodoferrax fermentans* gen. nov., sp. nov., a phototrophic purple nonsulfur bacterium previously referred to as the “*Rhodocyclus gelatinosus*-like” group. Arch. Microbiol. **155**:330–336.
- Hiraishi, A., Y. K. Shin, Y. Ueda, and J. Sugiyama. 1994. Automated sequencing of PCR-amplified 16S rDNA on “Hydrolink” gels. J. Microbiol. Methods **19**:145–154.
- Katayama-Fujimura, Y., Y. Komatsu, H. Kuraishi, and T. Kanako. 1984. Estimation of DNA base composition by high performance liquid chromatography of its nuclease P1 hydrolysate. Agric. Biol. Chem. **48**:3169–3172.
- Kawabata, T., K. Ishizuka, T. Miura, and T. Sasaki. 1956. Studies on the food poisoning associated with putrefaction of marine products. VIII. An outbreak of allergy-like food poisoning caused by “sashimi” of *Parathurus mebachii* and the isolation of causative bacteria. Bull. Jpn. Soc. Sci. Fish. **22**:41–47.
- Kimata, M., and A. Kawai. 1953. A new species of bacterium which produces a large amount of histamine on fish meats found in spoiled fresh fish. Mem. Res. Inst. Food Sci. Kyoto Univ. **6**:1–2.
- Kimura, M. 1980. A simple method for estimating evolutionary rates of base substitution through comparative studies of nucleotide sequences. J. Mol. Biol. **16**:111–120.
- Kita-Tsakamoto, K., H. Oyaizu, K. Nanba, and U. Simidu. 1993. Phylogenetic relationships of marine bacteria, mainly members of the family *Vibrionaceae*, determined on the basis of 16S rRNA sequences. Int. J. Syst. Bacteriol. **43**:8–19.
- Lee, J. V., M. S. Hendrie, and J. M. Shewan. 1979. Identification of *Aeromonas*, *Vibrio* and related organisms, p. 151–166. In F. A. Skinner and D. W. Lovelock (ed.), Identification methods for microbiologists. Academic Press, London.
- Marmur, J. 1961. A procedure for the isolation of deoxyribonucleic acid from micro-organisms. J. Mol. Biol. **3**:208–218.
- Niven, C. F., M. B. Jeffery, and D. A. Corlett. 1981. Differential plating medium for quantitative detection of histamine-producing bacteria. Appl. Environ. Microbiol. **41**:321–322.
- Okuzumi, M., S. Okuda, and M. Awano. 1981. Isolation of psychrophilic and halophilic histamine-forming bacteria from *Scomber japonicus*. Bull. Jpn. Soc. Sci. Fish. **47**:1591–1598.
- Saitou, N., and M. Nei. 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol. Biol. Evol. **4**:406–425.
- Skerman, V. B. D. 1967. A guide for the identification of the genera of bacteria. The Williams & Wilkins Co., Baltimore.
- Smith, S. K., D. C. Sutton, J. A. Fuerst, and J. L. Reichelt. 1991. Evaluation of the genus *Listonella* and reassignment of *Listonella damsela* (Love et al.) MacDonell and Colwell to the genus *Photobacterium* as *Photobacterium damsela* comb. nov. with emended description. Int. J. Syst. Bacteriol. **41**:529–534.
- Taylor, S. L., L. S. Guthertz, M. Leatherwood, and E. R. Leiber. 1979. Histamine production by *Klebsiella pneumoniae* and an incident of scombroid fish poisoning. Appl. Environ. Microbiol. **37**:274–278.
- Taylor, S. L., and M. W. Speckhard. 1983. Isolation of histamine-producing bacteria from frozen tuna. Mar. Fish. Rev. **45**:35–39.
- Wayne, L. G., D. J. Brenner, R. R. Colwell, P. A. D. Grimont, O. Kandler, M. I. Krichevsky, L. H. Moore, W. E. C. Moore, R. G. E. Murray, E. Stackebrandt, M. P. Starr, and H. G. Truper. 1987. Report of the Ad Hoc Committee on Reconciliation of Approaches to Bacterial Systematics. Int. J. Syst. Bacteriol. **37**:463–464.
- Weisburg, W. G., S. M. Barns, D. A. Pelletier, and D. J. Lane. 1991. 16S ribosomal DNA amplification for phylogenetic study. J. Bacteriol. **173**:697–703.
- Yamanaka, H., and M. Matsumoto. 1989. Simultaneous determination of polyamines in red meat fishes by high performance liquid chromatography and evaluation of freshness. J. Food Hyg. Soc. Jpn. **30**:396–400.

31. **Yoguchi, R.** 1990. Ph.D. thesis. Tokyo University of Fisheries, Tokyo.
32. **Yoguchi, R., M. Okuzumi, and T. Fujii.** 1990. Seasonal variation in numbers of mesophilic and halophilic histamine-forming bacteria in inshore of Tokyo Bay and Sagami Bay. *Nippon Suisan Gakkaishi* **56**:1467–1472.
33. **Yoguchi, R., M. Okuzumi, and T. Fujii.** 1990. Seasonal variation in numbers of mesophilic and halophilic histamine-forming bacteria on marine fish. *Nippon Suisan Gakkaishi* **56**:1473–1479.
34. **Yoshinaga, D. H., and H. A. Frank.** 1982. Histamine-producing bacteria in decomposing skipjack tuna (*Katsuwonus pelamis*). *Appl. Environ. Microbiol.* **44**:447–452.