Vibrio penaeicida sp. nov., a Pathogen of Kuruma Prawns (*Penaeus japonicus*)

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Six similar strains which were isolated as pathogens of cultured kuruma prawns (*Penaeus japonicus*) in Japan had characteristics of the genus *Vibrio*. These organisms were distinguished from previously described *Vibrio* species by their positive results in tests for gelatinase and lipase activities and their negative reactions in tests for arginine dihydrolase and lysine and ornithine decarboxylase activities, growth in the presence of 6% NaCl and at 35°C, acid production from sucrose, utilization of D-glucuronate and propionate, and luminescence. The G+C contents of the DNAs of these organisms were 46.2 to 47.0 mol%. The levels of DNA relatedness among these six strains were 87 to 99% (as determined by the membrane filter method), while the levels of DNA relatedness between prawn pathogen strain KH-1^T (T = type strain) and members of 28 previously described *Vibrio* species were less than 18%. The name *Vibrio penaeicida* sp. nov. is proposed for the prawn-pathogenic strains. The type strain is strain KH-1 (= IFO 15640 = JCM 9123).

Penaeus japonicus (kuruma prawns) is one of the most valuable cultured aquatic animal species in Japan. Vibriosis in cultured kuruma prawns has been observed since 1980, and there have been considerable losses in the prawn industry (28). The prawns affected are characterized by the formation of brown spots (nodules) in the lymphoid organs and gills. The causative bacterium was first isolated by Takahashi et al. in 1982 (29) from diseased prawns in Yamaguchi and Kumamoto Prefectures and was classified as a member of the genus *Vibrio*, but the strains isolated by Takahashi et al. have been lost. Later, we isolated the pathogen which causes vibriosis of kuruma prawns in specimens from various districts in Japan. The pathogen, which we isolated, tentatively designated *Vibrio* species PJ (11), was studied to determine its morphological, biochemical, pathological and serological characteristics (12), and we believe that this organism is the same species described by Takahashi et al. (29). The pathogen was also isolated from apparently healthy prawns and water samples obtained from prawn culture ponds (11). In this study we determined the taxonomic position of the prawn pathogen. Below we propose the name *Vibrio penaeicida* sp. nov. for this organism.

MATERIALS AND METHODS

Bacterial strains. The six *V. penaeicida* strains listed in Table 1 were isolated from kuruma prawns obtained in prefectures of western Japan. These strains were isolated and subcultured on ZoBell's 2216e agar plates (1) at 25°C and were maintained in semisolid ZoBell's 2216e medium containing 0.5% agar at 20°C; they were subcultured every 6 months. Untreated aged seawater obtained from the Inland Sea of Japan was used to prepare ZoBell's 2216e medium.

The reference strains used in DNA hybridization experiments are listed in Table 1. *Vibrio cholerae* HUPF 5004 (= NIH 35A3) is not the type strain of *V. cholerae* but was found to be closely related (level of similarity, 98%) to type strain ATCC 14035 by DNA-DNA hybridization (7).

Morphological observations. Gram staining (Hucker modification [8]) was carried out by using 1-day cultures grown on ZoBell's 2216e agar. Cell form and flagellum arrangement were determined by using an electron microscope to observe cells that had been grown on ZoBell's 2216e agar for 2 days at 25°C. The cells were suspended in 2% ammonium acetate, negatively stained with 1% uranyl acetate containing 0.4% sucrose (3), and examined with a Hitachi model

H-600A transmission electron microscope operated at an accelerating voltage of 75 kV.

Physiological and biochemical characterization. Cells from a 1-day culture on ZoBell's agar were used in all tests. The NaCl concentration was adjusted to 2% (wt/vol) in all test media except those containing seawater or those that were used to determine the range of NaCl concentrations for growth. All of the inoculated test media except the media used to determine the temperature range for growth were incubated at 25°C.

Motility was determined by the hanging drop method (9). Luminescence was observed on ZoBell's 2216e agar and luminous medium plates (26). After 8, 16, 24, and 48 h of incubation, luminescence was determined in a dark room after the investigator's eyes had adapted to the dark for 5 min. Pigmentation was determined after 7 days of incubation on ZoBell's 2216e agar. Susceptibility to vibriostatic agent O/129 was determined with 10- and 150-µg discs on nutrient agar (Difco) after 24 h of incubation. O/129 phosphate was obtained from Sigma Chemical Co., and the discs were prepared as described by Lee et al. (20). Fermentation of glucose was determined in Hugh-Leifson medium (Eiken Chemical Co. Ltd., Tokyo, Japan) containing 1% glucose after 7 days of incubation. Cytochrome oxidase activity was determined by the Kovács method by using test strips (Nissui Pharmaceutical Co., Ltd., Tokyo, Japan) that included tetramethyl-*p*-phenylenediamine dihydrochloride. The cultures were smeared on strips that had been wetted with distilled water, and the appearance of a purple color was determined after 30 s. Catalase activity was detected by the slide method. Cells were transferred to a clean glass slide, and 3% hydrogen peroxide was added. Bubble formation was determined immediately and after 5 min. Indole production was determined in 1% (wt/vol) tryptone (Difco) after incubation for 2 days. Indole was detected by the presence of a red color after 1.0 ml of Kovács reagent (9) was added to the medium. Nitrate reduction was determined by using 1% (wt/vol) Bacto Peptone (Difco) and 0.1% (wt/vol) potassium nitrate. The results were recorded as described by MacFaddin (22) after 2 and 5 days of incubation. The Voges-Proskauer test (Barritt's test) was performed by using 1% (wt/vol) Bacto Peptone (Difco) and 1% (wt/vol) D-glucose; the results were judged by using the procedures described by MacFaddin (22) after 2 and 5 days of incubation. The production of hydrogen sulfide was determined by observing the blackening of SIM medium (Eiken) every day for 4 days. Lysine and ornithine decarboxylation and arginine dihydroxylation were determined in Møller decarboxylase base (Difco) as described by MacFaddin (22) by examining preparations every day for 7 days. Hydrolysis of *o*-nitrophenyl-β-D-galactopyranoside was determined with *o*-nitrophenyl-β-D-galactopyranoside discs (Nissui) containing *o*-nitrophenyl-β-D-galactopyranoside, peptone, and dipotassium hydrogen phosphate in 2% (wt/vol) NaCl after 12 h of incubation. Gelatin hydrolysis was determined in ZoBell's 2216e broth containing 20% (wt/vol) gelatin; preparations were observed for liquefaction for 7 days. ZoBell's 2216e agar containing 0.1% (wt/vol) soluble starch was flooded with Lugol's solution for Gram staining after 2 and 5 days of incubation. The formation of clear zones under and around colonies was recorded as a positive result. ZoBell's 2216e agar media containing 1% (wt/vol) sodium alginate, 1% (wt/vol) colloidal chitin prepared as described by West and Colwell (34), or 1% (wt/vol) Tween 80 were used to determine hydrolysis of alginate, chitin, and Tween 80. Alginase activity was determined after a preparation was overlaid with ethanol after 7 days of incubation. Hydrolysis of alginate and hydrolysis of chitin were indicated by clear zones around colonies.

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TABLE 1. Levels of DNA relatedness between *V. penaeicida* KH-1^T and other *V. penaeicida* strains and strains belonging to other *Vibrio* species and related genera

Species	Strain ^a	Origin or source of strain ^a	% DNA reassociation with strain KH-1 ^T
<i>Vibrio penaeicida</i>	KH-1 ^T (= IFO 15640 ^T = JCM9123 ^T) ^b	Kuruma prawn, Kagoshima Prefecture	100
	KA-13 (= IFO 15641 = JCM 9125) ^b	Kuruma prawn, Yamaguchi Prefecture	97
	KO-1 (= JCM 9194) ^b	Kuruma prawn, Oita Prefecture	97
	PO-1 (= JCM 9193)	Kuruma prawn, Hiroshima Prefecture	87
	PD-A (= IFO 15642 = JCM 9124)	Kuruma prawn, Hiroshima Prefecture	90
	ESV-8701 (= JCM 9192)	Kuruma prawn, Ehime Prefecture	99
<i>V. aestuarianus</i>	ATCC 35048 ^T	ATCC	10
<i>V. alginolyticus</i>	HUFP 9107 ^T (= ATCC 17749 ^T)	U. Simidu	14
<i>V. anguillarum</i>	HUFP 5001 ^T (= ATCC 19264 ^T)	OSU	10
<i>V. campbellii</i>	HUFP 9109 ^T (= ATCC 25920 ^T)	U. Simidu	14
<i>V. carchariae</i>	HUFP 9110 ^T (= ATCC 35084 ^T)	U. Simidu	10
<i>V. cholerae</i>	HUFP 5004 (= NIH 35A3)	M. Nishibuchi	6
<i>V. cincinnatiensis</i>	ATCC 35912 ^T	ATCC	8
<i>V. damsela</i>	ATCC 33539 ^T	ATCC	4
<i>V. diazotrophicus</i>	HUFP 9301 ^T (= ATCC 33466 ^T)	H. Kawasaki	8
<i>V. fischeri</i>	ATCC 7744 ^T	ATCC	5
<i>V. fluvialis</i>	HUFP 9302 ^T (= NCTC 11327 ^T)	H. Kawasaki	9
<i>V. gazogenes</i>	HUFP 9303 ^T (= ATCC 29988 ^T)	H. Kawasaki	6
<i>V. harveyi</i>	HUFP 9111 ^T (= ATCC 14126 ^T)	U. Simidu	14
<i>V. logei</i>	HUFP 9310 ^T (= ATCC 15382 ^T)	H. Kawasaki	7
<i>V. mediterranei</i>	ATCC 4331 ^T	ATCC	4
<i>V. natriegens</i>	ATCC 14048 ^T	ATCC	9
<i>V. navarrensis</i>	ATCC 51183 ^T	ATCC	10
<i>V. nereis</i>	HUFP 9112 ^T (= ATCC 25917 ^T)	U. Simidu	12
<i>V. nigrapulchritudo</i>	ATCC 27043 ^T	ATCC	18
<i>V. ordalii</i>	ATCC 33509 ^T	ATCC	10
<i>V. orientalis</i>	ATCC 33934 ^T	ATCC	15
<i>V. parahaemolyticus</i>	HUFP 9114 ^T (= ATCC 17802 ^T)	U. Simidu	13
<i>V. pelagius</i>	HUFP 9115 ^T (= ATCC 25916 ^T)	U. Simidu	12
<i>V. proteolyticus</i>	HUFP 9307 ^T (= NCMB 1326 ^T)	H. Kawasaki	6
<i>V. salmonicida</i>	ATCC 43839 ^T	ATCC	5
<i>V. splendidus</i>	HUFP 9117 ^T (= ATCC 33125 ^T)	U. Simidu	11
<i>V. tubiashii</i>	HUFP 9118 ^T (= ATCC 19109 ^T)	U. Simidu	11
<i>V. vulnificus</i>	HUFP 5002 ^T (= ATCC 27562 ^T)	M. Nishibuchi	13
<i>Photobacterium phosphoreum</i>	HUFP 9311 ^T (= ATCC 11040 ^T)	H. Kawasaki	6
<i>Escherichia coli</i>	K-12		4

^a ATCC, American Type Culture Collection, Rockville, Md.; HUFP, Hiroshima University Fish Pathology Laboratory Culture Collection, Higashihiroshima-shi, Hiroshima, Japan; IFO, Institute for Fermentation Osaka, Osaka, Japan; JCM, Japan Collection of Microorganisms, The Institute of Physical and Chemical Research (RIKEN), Wako-shi, Saitama, Japan; NCTC, National Collection of Type Cultures, London, England; OSU, Oregon State University, Corvallis.

^b Strains KH-1^T, KA-13, and KO-1 were used in G+C content determinations.

Tween 80 hydrolysis was detected by the presence of turbidity around colonies. Hydrolysis of chitin and hydrolysis of Tween 80 were observed daily for 7 days. Accumulation of poly-β-hydroxybutyrate in cells was determined by observing cells with a phase-contrast microscope daily for 4 days as described by Baumann et al. (4). Growth at different temperatures was determined in 1% (wt/vol) Bacto Peptone (Difco) (pH 7.5) after 14 days of incubation. The range of NaCl concentrations at which organisms grew was determined in 1% (wt/vol) Bacto Peptone (Difco) containing different concentrations of NaCl after 7 days. Semisolid basal medium (Eiken) was used for acid and gas production tests. Each sugar solution was autoclaved separately and added to the basal medium at a concentration of 1% (wt/vol). The cultures were observed every day for 7 days. Simmons medium (Eiken) was used for the citrate utilization test. Utilization of carbon sources was determined in the basal medium of Baumann et al. (4) by using 96-well microplates. Bacterial cell suspensions in basal medium were added to filter-sterilized carbon compounds, and the preparations were placed in wells. Each well contained 200 µl of basal medium containing 0.1% (wt/vol) carbohydrate and about 10³ organisms, and growth was examined visually for 7 days.

The chemicals used in this study were obtained from Nacalai Tesque, Inc., Kyoto, Japan, unless indicated otherwise.

Determination of DNA base composition and DNA-DNA hybridization. DNAs were extracted from cells grown at 25°C for 1 day on heart infusion agar (Difco) prepared with 50% seawater and were purified by using the methods described by Saito and Miura (27) and Marmur (23). When we used the method of Marmur, we modified it slightly by mixing the chloroform-isoamyl alcohol reagent with an equal volume of phenol before use. G+C contents of DNAs were determined by the high-performance liquid chromatography method described by Kumagai et al. (19). DNA-DNA hybridization was performed by the membrane filter method as described previously (2). Briefly, reference DNA was

labeled with tritium by nick translation, using a nick translation kit (Takara Shuzo Co., Ltd., Kyoto, Japan). About 10 µg (the exact amount used was recorded) of unlabeled single-stranded DNA immobilized on a nitrocellulose membrane and 0.1 µg of labeled reference DNA were reassociated in 0.1% sodium dodecyl sulfate-2× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate). After incubation for 40 h at 62°C, the filters were washed and dried. The radioactivity was measured with an Aloca model LSC-3500E liquid scintillation counter.

RESULTS AND DISCUSSION

The strains which were tentatively identified as members of *Vibrio* species in a previous study (12) were gram-negative, facultatively anaerobic, straight or slightly curved rod-shaped organisms. Characteristics of the six strains studied, including type strain KH-1, are shown in Table 2. We observed a single polar flagellum and no lateral flagella under the conditions which we used (Fig. 1). The strains were oxidase positive and required NaCl to grow. The G+C contents of three representative strains were quite similar, ranging from 46.2 to 47.0 mol%; the G+C content of strain KH-1^T (T = type strain) was 47.0 mol%. The strains were susceptible to O/129 (inhibition zones were 2 to 3 mm wide when 10-µg discs were used). On the basis of the characteristics described above, we confirmed

TABLE 2. Biochemical and physiological characteristics of six *V. penaeicida* strains^a

Characteristic	% of strains positive	Reaction of strain KH-1 ^T
Fermentation of glucose (4 days)	100	+ ^b
Gas produced from glucose (4 days)	0	—
Oxidase	100	+
Catalase	100	+
Indole production (5 days)	50	—
Nitrate reduction (2 days)	100	+
Voges-Proskauer test (5 days)	0	—
Hydrogen sulfide production (7 days)	0	—
Arginine dihydrolase (7 days)	0	—
Lysine decarboxylase (7 days)	0	—
Ornithine decarboxylase (7 days)	0	—
ONPG hydrolysis ^c	100	+
Alginase (7 days)	17	+
Amylase (2 days)	83	+
Chitinase (7 days)	100	+
Gelatinase (3 days)	100	+
Lipase (3 days)	100	+
PHB accumulation (4 days) ^d	0	—
Growth at:		
4°C	0	—
20°C	100	+
30°C	100	+
35°C	0	—
40°C	0	—
Growth in the presence of:		
0% NaCl	0	—
0.5% NaCl	0	—
1% NaCl	100	+
3% NaCl	100	+
5% NaCl	50	+
6% NaCl	0	—
Acid production (7 days) from:		
Adonitol	0	—
L-Arabinose	0	—
D-Cellobiose	100	+
Dextrin	100	+
Dulcitol	0	—
Fructose	100	+
D-Galactose	0	—
D-Glucose	100	+
Glycerol	0	—
Glycogen	50	+
myo-Inositol	0	—
Lactose	50	—
Maltose	100	+
D-Mannitol	0	—
D-Mannose	100	+
Melibiose	0	—
Raffinose	0	—
L-Rhamnose	0	—
Salicin	0	—
D-Sorbitol	0	—
Sucrose	0	—
Trehalose	100	+
D-Xylose	0	—

Continued

that *Vibrio* species PJ belongs to the genus *Vibrio*. Although there were some variations in physiological and biochemical characteristics among the six strains of *Vibrio* species PJ (Table 2), all of the strains were distinct from other *Vibrio* species (Table 3). The levels of DNA relatedness between five strains of *Vibrio* species PJ and type strain KH-1 were 87% or more; this indicated that the strains which we studied are closely related (Table 1). The levels of DNA relatedness between

TABLE 2—Continued

Characteristic	% of strains positive	Reaction of strain KH-1 ^T
Utilization as a sole carbon source (7 days) of:		
γ-Aminobutyrate	33	—
D-Cellobiose	100	+
Citrate (Simmons)	67	+
L-Citrulline	33	—
Ethanol	0	—
D-Gluconate	83	+
D-Glucuronate	0	—
β-Hydroxybutyrate	83	+
L-Leucine	0	—
DL-Malate	100	+
D-Mannitol	83	+
Propionate	0	—
Putrescine	0	—
Sucrose	0	—
D-Xylose	0	—

^a All tests except the temperature range test were performed at 25°C.^b +, positive; —, negative.^c ONPG, *o*-nitrophenyl-β-D-galactopyranoside.^d PHB, poly-β-hydroxybutyrate.

strain KH-1^T and type or reference strains of 28 previously described *Vibrio* species were significantly lower (less than 18%).

The following seven species were not used in DNA-DNA hybridization experiments: *Vibrio costicola*, *Vibrio furnissii*, *Vibrio hollisae*, *Vibrio marinus*, *Vibrio metschnikovii*, *Vibrio mimicus*, and *Vibrio mytili*. *V. costicola*, *V. furnissii*, *V. hollisae*, and *V. marinus* differ from the prawn pathogen in their DNA G+C contents and phenotypic characteristics. In addition to the characteristics shown in Table 3, these organisms could be differentiated by the following test results: *V. furnissii* produces gas from D-glucose and acid from L-arabinose and uses ethanol and putrescine (5); *V. hollisae* produces acid from L-arabinose (17); and *V. marinus* can grow at 4 and 30°C (5). It has been reported that the type strain of *V. mimicus* (ATCC 33653) exhibited a high level of DNA relatedness to the type strain of *V. cholerae* (67%) (10). However, the representative strain of the prawn pathogen, type strain KH-1, exhibited only 6% DNA relatedness to *V. cholerae* HUF 5004, which is closely related genetically to the type strain of *V. cholerae* (see above). Therefore, *V. mimicus* and the prawn pathogen can be differentiated from each other genetically. The remaining two species not used in DNA-DNA hybridization experiments could be distinguished clearly from the prawn isolates on the basis of phenotypic characteristics (Table 3). In addition, *V. metschnikovii* is Voges-Proskauer positive and negative for oxidase activity and nitrate reduction (5); and *V. mytili* pro-

FIG. 1. Electron micrograph of a negatively stained cell of *V. penaeicida* KH-1^T, showing the single polar flagellum. Bar = 1 μm.

TABLE 3. Characteristics useful for distinguishing *V. penaeicida* from previously described *Vibrio* species^a

Taxon	Susceptibility to O/129 (10 µg)	Arginine dihydro-lase	Lysine decarbox-ylase	Ornithine decarbox-ylase	Growth in the presence of 6% NaCl	Growth at 35°C ^b	Gelati-nase	Lipase	Acid produced from sucrose	Lumines-cence	Utilization of ^b :		G+C content (mol %) ^c
											D-Glucu-ronate	Propio-nate	
<i>Vibrio penaeicida</i>	+ ^d	—	—	—	—	—	+	+	—	—	—	—	46-47
<i>V. aestuarianus</i> ^e	+	+	—	—	ND	+	+	ND	+	—	ND	ND	43-44
<i>V. alginolyticus</i>	—	—	+	+	+	+	+	+	+	—	—	+	45-47
<i>V. anguillarum</i>	+	+	—	—	+	+	+	+	+	—	—	+	44-46
<i>V. campbellii</i>	—	—	+	—	+	+	+	+	—	—	—	+	46-48
<i>V. carchariae</i> ^f	—	—	+	+	+	+	+	+	+	—	ND	—	46
<i>V. cholerae</i>	+	—	+	+	v	+	+	+	+	v	—	+	47-49
<i>V. cincinnatiensis</i> ^g	—	—	+	—	+	+	—	—	+	—	—	ND	45
<i>V. costicola</i>	—	+	—	—	+	+	+	v	+	—	—	+	50
<i>V. damsela</i> ^h	ND	+	—	—	+	+	—	—	—	—	—	—	43
<i>V. diazotrophicus</i> ⁱ	ND	+	—	—	+	+	—	—	+	—	+	v	46-47
<i>V. fischeri</i>	+	—	+	—	+	v	—	+	—	+	—	—	39-41
<i>V. fluvialis</i>	—	+	—	—	+	+	+	+	+	—	+	+	49-51
<i>V. furnissii</i>	—	+	—	—	+	+	+	+	+	—	—	+	50
<i>V. gazogenes</i>	+	—	—	—	+	+	+	+	+	—	—	+	47
<i>V. harveyi</i>	—	—	+	+	+	+	+	+	v	v	+	+	46-48
<i>V. hollisae</i> ^j	ND	—	—	—	v	+	—	—	—	—	ND	ND	50
<i>V. logei</i>	v	—	+	—	ND	—	—	v	v	+	—	—	40-42
<i>V. marinus</i>	—	—	+	—	—	—	v	+	v	—	—	—	42
<i>V. mediterranei</i> ^k	ND	—	v	—	ND	ND	—	+	+	v	ND	ND	42-43
<i>V. metschnikovii</i>	+	+	v	—	+	+	+	+	+	—	—	—	44-46
<i>V. mimicus</i> ^l	v	—	+	+	v	+	+	+	v	—	+	—	ND
<i>V. mytili</i> ^m	ND	+	—	—	+	+	—	+	+	—	—	ND	45-46
<i>V. natriegens</i>	—	—	—	—	+	+	+	+	+	—	v	+	46-47
<i>V. navarrensis</i> ⁿ	ND	—	—	—	+	+	+	ND	+	—	—	—	45-47
<i>V. nereis</i>	—	+	—	—	+	+	v	—	+	—	—	+	46-47
<i>V. nigripulchritudo</i>	—	—	—	—	—	—	+	+	—	—	+	+	46-47
<i>V. ordalii</i>	+	—	—	—	+	—	+	—	+	—	—	—	44-46
<i>V. orientalis</i> ^o	ND	+	ND	ND	ND	+	+	+	ND	+	—	—	45-46
<i>V. parahaemolyticus</i>	—	—	+	+	+	+	+	+	—	—	v	+	46-47
<i>V. pelagius</i> biovar I	+	—	—	—	+	+	—	+	+	—	—	+	45-47
<i>V. pelagius</i> biovar II	+	—	—	—	+	+	+	+	+	—	—	+	45-47
<i>V. proteolyticus</i>	—	+	+	—	+	+	+	+	—	—	—	—	51
<i>V. salmonicida</i> ^p	ND	—	ND	ND	—	—	—	—	ND	ND	ND	ND	42
<i>V. splendidus</i> biovar I	+	+	—	—	v	v	+	+	v	+	+	+	45-46
<i>V. splendidus</i> biovar II	+	—	—	—	v	—	+	+	—	—	—	+	45-46
<i>V. tubiashii</i> ^q	v	+	—	—	v	+	+	+	+	—	—	—	43-45
<i>V. vulnificus</i> biovar I	+	—	+	+	+	+	+	+	—	—	+	+	46-48
<i>V. vulnificus</i> biovar II ^r	ND	—	+	+	ND	+	+	+	—	—	ND	ND	46-47

^a Data from reference 34, except as indicated below.^b Data from reference 18.^c Data from reference 5.^d +, positive for at least 90% of the strains; —, negative for at least 90% of the strains; v, variable among strains; ND, no data available.^e Data from reference 31.^f Data from reference 14.^g Data from reference 6.^h Data from reference 21.ⁱ Data from reference 15.^j Data from reference 17.^k Data from reference 24.^l Data from reference 33.^m Data from reference 25.ⁿ Data from reference 32.^o Data from reference 35.^p Data from reference 13.^q Data from reference 16.^r Data from reference 30.

duces acid from L-arabinose, D-galactose, and D-xylose and gas from D-glucose (25).

V. penaeicida can be differentiated from 35 other *Vibrio* species, even species with similar G+C contents, by the following characteristics: arginine dihydrolyase activity, lysine and ornithine decarboxylase activities, growth in the presence of 6% NaCl and at 35°C, production of acid from sucrose, utilization of D-glucuronate and propionate, and luminescence

(Table 3). In addition to the differential features shown in Table 3, other *Vibrio* species can be differentiated from *V. penaeicida* as follows: *Vibrio mediterranei* produces acid from D-mannitol and D-sorbitol (24); *Vibrio nigripulchritudo* produces a black pigment (5); *Vibrio salmonicida* is negative for production of indole and chitinase and nitrate reduction (13); and *Vibrio splendidus* biovar II does not produce acid from D-mannose (5).

On the basis of the results described above, we propose the name *Vibrio penaeicida* sp. nov. for *Vibrio* species PJ.

Description of *Vibrio penaeicida* sp. nov. *Vibrio penaeicida* (pe.nae.i.ci'da. L. n. *Penaeus*, genus name of kuruma prawns; L. v. *caedo*, to kill; L. adj. *penaeicida*, *Penaeus* killer) cells are gram-negative short rods (1.5 to 2 by 0.5 to 0.8 µm) that are straight or slightly curved and motile by means of a single polar flagellum when the cells are grown on solid media. No lateral flagella are formed on solid media, and there is no swarming. Does not form spores. Facultatively anaerobic. Susceptible to 2,4-diamino-6,7-diisopropylpteridine (vibriostatic agent O/129) at a concentration of 10 µg per disc.

After incubation for 24 h at 25°C colonies on ZoBell's 2216c agar are low convex, entire, circular, cream colored, and translucent. Neither pigmentation nor luminescence is observed. Biochemical and physiological characteristics are shown in Table 2.

The G+C contents of the DNAs range from 46.2 to 47.0 mol% in the three strains that have been tested.

It has been demonstrated that there is a major common O antigen in all six strains tested (12). *V. penaeicida* can be differentiated serologically from fish-pathogenic vibrios such as *Vibrio anguillarum* (nine serovars), *Vibrio alginolyticus*, *V. cholerae* (non-O:1), *Vibrio ordalii*, and *Vibrio vulnificus* (12).

V. penaeicida is the causative agent of vibriosis of kuruma prawns in Japan (12).

The type strain is strain KH-1 (=IFO 15640 = JCM 9123).

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