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Isolation and Characterization of *Vibrio damsela* Infectious for Cultured Shrimp in Taiwan

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A *Vibrio* infection has been found to be associated with spontaneous mortality among cultured tiger shrimp (*Penaeus monodon*) in Taiwan. Affected shrimp display nonspecific signs, including poor growth rate, anorexia, rough shell, and milky musculature. Large numbers of short, curved bacterial rods were observed in the hepatopancreas, giving the infected tissue a pale and atrophic appearance. Melanized granulomatous ulcerations encapsulated by hemocytes and connective tissues in the hepatopancreas were seen by histopathological examination. Twelve isolates of *Vibrio* were isolated and examined for 47 characteristics based on their morphology, physiology, and biochemistry from March to October 1988. They were found to represent a homogeneous group of bacteria thought to belong to a single species. DNA relatedness to reference strain ATCC33539 confirmed that the isolates were *V. damsela*. Experimental infections in both anal intubation and intramuscular injection with *V. damsela* produced mortality in tiger shrimp. It was shown that 10^5 bacteria were able to induce shrimp hepatopancreatitis and death using anal intubation. The hepatopancreatic tubules were necrotic, and cell debris was observed in the lumen. The melanized cells were also found around the foci and inflammatory sinuses were infiltrated with hemocytes. No death, however, existed in the group as a result of water-borne infection. This suggested that *V. damsela* was one of the etiological agents associated with shrimp mortality under stressed conditions.

KEY WORDS: ***Vibrio damsela*; tiger shrimp (*Penaeus monodon*); experimental infection; anal intubation; pathogenicity.**

INTRODUCTION

Vibriosis is one of the most serious problems in marine fish, shellfish, and shrimp. Several species of *Vibrio*, to date, have been described as pathogens for aquacultural shrimp: *V. alginolyticus*, *V. anguillarum* (Lightner, 1983), *V. fisheri* and *V. fluvialis* (Sakata and Taruno, 1987b), *V. parahaemolyticus* (Lightner, 1983), and *V. vulnificus* (Song *et al.*, 1990).

V. damsela was first isolated from naturally occurring skin ulcers of damselfish (*Chromis punctipinnis*) (Love *et al.*, 1981). This organism has also been isolated from captive sharks (Grimes *et al.*, 1984), octopus (Hanlon *et al.*, 1984), oyster (Rodrigues and Hofer, 1987), turtle (Obendorf *et al.*, 1987), dolphin (Fujioka *et al.*, 1988), yellow tail (*Seriola quinqueradiata*) (Sakata *et al.*, 1989), cultured turbot (*Scophthalmus maximus*) (Fouz *et al.*, 1991), and stressed juvenile seabream (*Sparus aurata*) (Vera *et al.*, 1991), as well as from human wounds (McGarey *et al.*, 1990).

Heavy mortality has been found among the cultured shrimp in Taiwan. Those mortalities usually occurred 1 or 2 months after postlarva were transferred into outdoor ponds. A survey was made on cultured tiger shrimp (*Penaeus monodon*) to determine the relationships between *V. damsela* and this host. This study has describes the identification of *V. damsela* isolated from the hepatopancreas of moribund tiger shrimp. Experimental infections have been performed in order to assess the potential pathogenic capability of these *V. damsela* strains.

MATERIALS AND METHODS

Bacterial strains. Twelve strains of *V. damsela* were characterized in this study (Table 1). They were isolated from the hepatopancreas of moribund shrimp by inoculating on thiosulfate–citrate–bile salts–sucrose agar (TCBS, Difco) supplemented with 2.0% NaCl. All bacterial incubations were carried out in a medium containing 3.0% NaCl at 28°C unless otherwise stated. Type strain *V. damsela* ATCC33539 was purchased from the Culture Collection and Research Center, Taiwan.

Morphology and growth study. Colony morphology was observed on TCBS agar after 24 hr. Cell morphology was determined from light microscopic observations of Gram-stained smear preparations. A drop of bacterial broth was suspended in 20% sucrose solution, maintaining bacterial osmolarity; it was then stained

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TABLE 1
Strains of *V. damsela* Isolated from Tiger Shrimp (*P. monodon*)

Strain designation	Location	External appearance and nose	Appearance of hepatopancreas
TG617	Pingtung	Normal	Pale, atrophic
LG711	Pingtung	Normal	Pale, atrophic
L3G716	Tainan	Normal	Pale, normal
I1G720	Ilan	Normal	Pale, atrophic
I2G720	Ilan	Rough shell	Pale, atrophic
PG801	Tainan	Rough shell	Pale, atrophic
J3G801	Tainan	Normal	Pale, atrophic
MIG831	Kaoshiung	Gill rot	Normal, atrophic
M2G831	Kaoshiung	Gill rot	Normal, atrophic
A3G911	Tainan	Foul	Pale, atrophic
A7G911	Tainan	Normal	Pale, atrophic
PG919	Tainan	Foul	Pale, atrophic

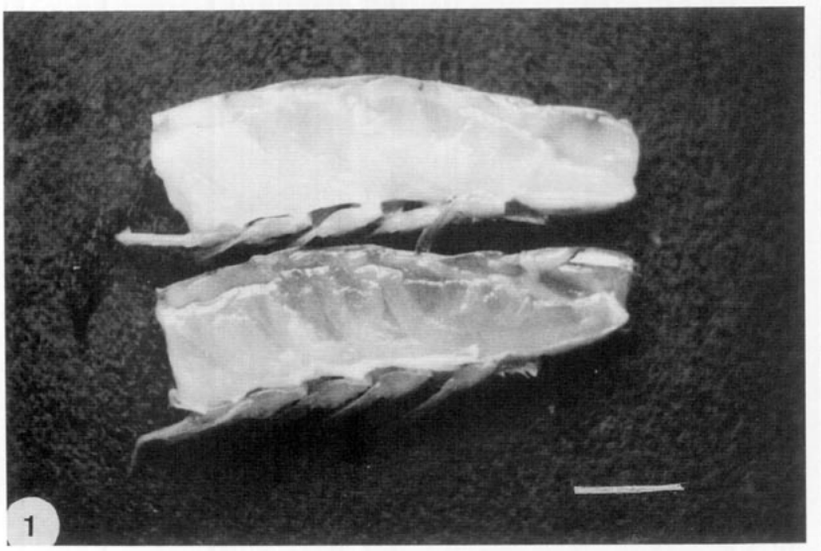
with phosphotungstic acid (2%) for 30 sec. The presence of flagella and pili was observed using a transmission electron microscope. Motility was tested by the hanging-drop method after passage of test organisms through a tryptic soy broth (TSB, Difco). The ability of the strains to grow in TSB at selected temperatures was observed from 1 day to 2 weeks. Tolerance of NaCl was determined by the addition of NaCl to TSB, and cultures were examined for growth after 3 days.

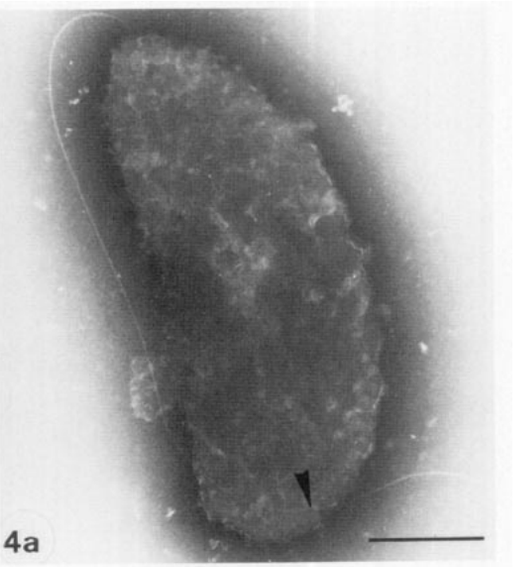
Biochemical characteristics. Test cultures were grown on tryptic soy agar (TSA, Difco) for approximately 18 hr before inoculation into test media. The methods used for phenotypic characterization of each strain have been previously described (Song *et al.*, 1988, 1990). Briefly, TSA supplemented with 7.5% skim milk was used for the examination of hydrolysis of casein. TSA supplemented with 12% gelatin was used for testing gelatinase production. Urea agar base (Difco) was prepared for testing the presence of urease. The decarboxylase basal medium (Difco) combined with 1% L-form lysine, arginine, or ornithine was used to differentiate the ability of test organisms to decarboxylate amino acids. Methods for detection of starch hydrolysis were described in the "Manual of Microbiological Methods" (Society of American Bacteriologists, 1957). Chitin utilization was determined using a method described by Stainer (1947). A modification of the Hugh–Leifson (1953) procedure was used to test for the production of acid from glucose. Both Koser (1923) citrate broth and Simmons (1926) citrate agar were used to examine citrate utilization. Production of acids from carbohydrates was determined by the method of Leifson (1963). Methyl red and Voges–Proskauer (MR-VP) tests were performed by using MR-VP medium (Difco). The ability of the test organisms to reduce nitrate anaerobically was tested according to MacFaddin's (1980) procedure. Hydrogen sulfide production was tested using triple sugar iron agar (Difco). The decomposition of tyrosine was examined using a method described by Lewin and Lounsbery (1969). Ehrlich's reagent (Isenberg and Sundheim, 1958) was used to test for the production of indole from tryptophan. Cytochrome oxidase test strips (Oxoid) were used to detect the presence of this enzyme. Hemolysis of sheep erythrocytes was tested on blood agar base supplemented with 10% fresh blood. Sensitivity to 2,4-diamino-6,7-diisopropyl pteridine phosphate (0/129, 150 μg, Creative Microbiologicals Taiwan), was tested by the method of Seidler *et al.* (1980). Sensitivity to novobiocin, chloramphenicol, tetracycline, kanamycin, and vancomycin was tested on TSA treated with antibiotic discs (30 μg, BBL).

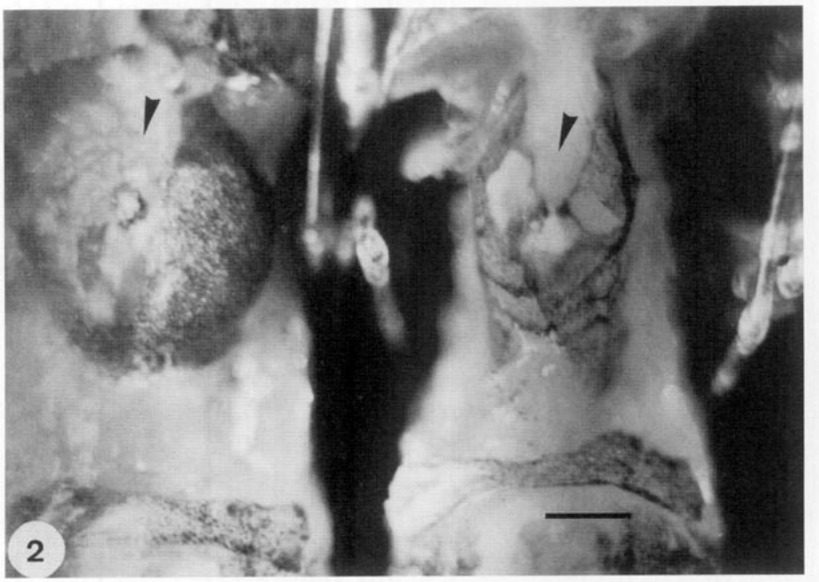
DNA relatedness. DNA hybridization experiments were performed by the hydroxylapatite free solution method (Johnson, 1981). Purified tritium-labeled DNA had a specific activity of 9000/μg DNA. Vials containing DAN were incubated at 61°C (T_m − 25°C) and 73°C (Tm − 13°C) for 23 hr. The amount of DNA renaturation was determined by counting radioactivity for 30 sec in a Beckman Model LS 5801 liquid scintillation counter. Calf thymus DNA (Sigma), having no homology to *V. damsela* DNA, was used as a control in determining the amount of self-renaturation of the labeled fragments.

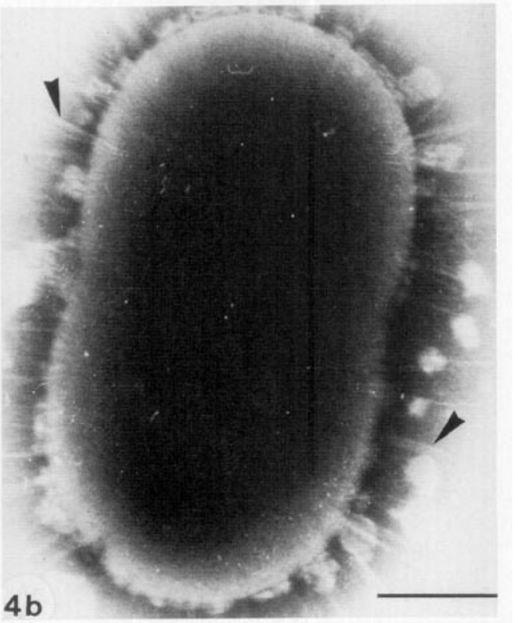
*Rabbit anti-*V. damsela *serum.* Immunogen of *V. damsela* (ATCC 33539) was prepared by inactivation of whole cells at 65°C for 1 hr. The concentration of immunogen suspension was adjusted to 10^9 cells/ml. Then, it was mixed 1:1 with Freund's complete adjuvant, and 1 ml was injected into a New Zealand white rabbit subcutaneously between the scapulae. At 2-week intervals, boosters were administered by injection of the immunogen mixed 1:1 in Freund's incomplete adjuvant. Final bleeding was done when agglutination titers of antiserum reached 1:1024. The antiserum was lyophilized and stored at −20°C for future use.

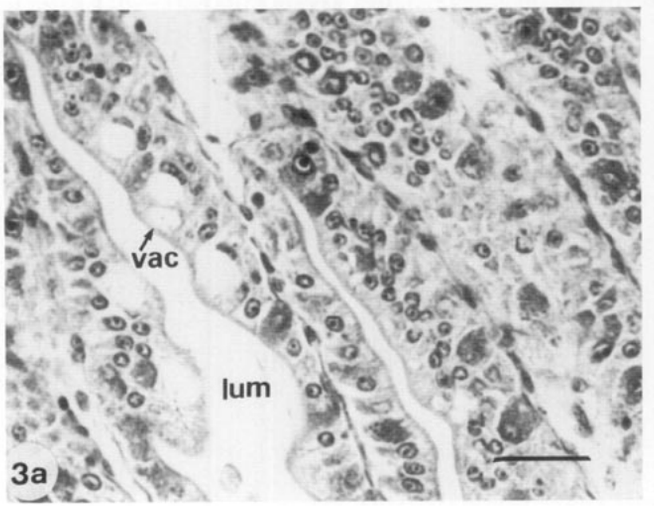
Experimental infections. V. damsela (strain M2G831) was grown on TSA for 24 hr. Colonies were scraped and dissolved in a 0.85% NaCl solution, with the concentration of *V. damsela* being adjusted to 10^9 CFU/ml. Tenfold serial dilutions of bacterial suspension were made. Fifty-microliter bacterial suspensions of different concentrations were used in the anal intubation and the intramuscular infections. However, 1-liter bacterial suspensions of different concentrations were used in the water-borne infection. Five or 10 shrimp per group were inoculated through the anus using a microsyringe (Hamilton, Nevada). Four shrimp were inoculated intramuscularly between the third and the fourth abdominal segments from the ventral side. Water-borne infection was accomplished by scarifying the gills and then immersing 10 shrimp into











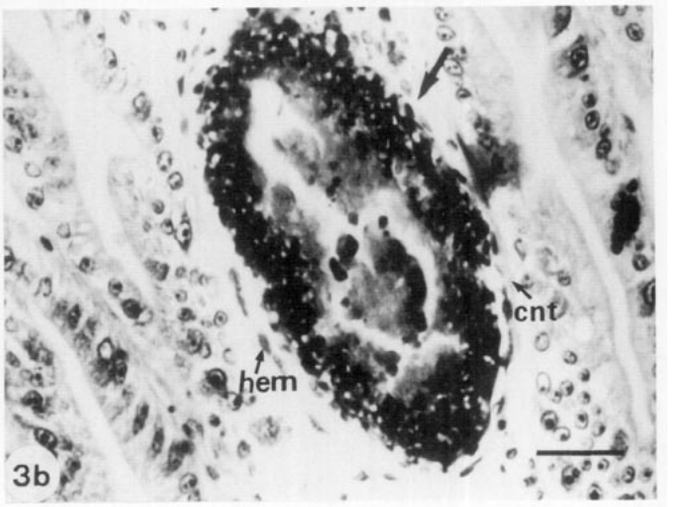


FIG. 1. Milky musculature of spontaneously infected shrimp (top) compared with normal musculature of healthy shrimp (bottom); ×1.5; bar = 1 cm.

FIG. 2. Hepatopancreas (arrow) of spontaneously infected shrimp showing pale and atrophic appearance (right) compared with that of healthy shrimp (left); ×6; bar = 0.2 cm.

FIG. 3. Longitudinal section of the hepatopancreatic tubules. (a) Healthy shrimp. The lumen (lum) and secretory vacuoles (vac) are seen. (b) Spontaneously infected shrimp showing melanized granulomatous ulceration (arrow) which is encapsulated by hemocytes (hem) and connective tissue (cnt). H&E stain; Davidson's fixative; ×400; bar = 30 μm.

FIG. 4. Electron micrographs of *V. damsela* (Strain M2G831) showing (a) a single polar flagellum (arrow) (negative stain with 2% phosphotungstic acid; ×50,000; bar = 0.3 μm) and (b) pili (arrow) (negative staining with 2% phosphotungstic acid; ×57,000; bar = 0.3 μm).

the bacterial suspensions for 10 min. Control shrimp were injected or immersed with a sterile 0.85% NaCl solution in the same manner. After the infection experiments, shrimp were held in a 6-liter round plastic container supplied with a constant flow of recirculated brackish water the salinity of which was adjusted to 2.5% while the temperature was kept at 25°C. Shrimp were fed three times a day and observed for 1 month. The number of moribund shrimp postinfections was recorded, with bacteria isolated from the lesions and categorized with the rabbit anti-*V. damsela* serum using the slide agglutination test (Roberson, 1990). Briefly, one or more bacterial colonies were resuspended in a small amount of saline. With capillary pipets, one drop of bacterial suspension was added to a slide. Another drop of antiserum diluted 1:10 was added to the bacterial suspension and the slide was rocked gently to mix. The slide was examined macroscopically at 10 min for clumping of bacteria. The hepatopancreases of moribund shrimp were fixed in Davidson's solution. The fixed specimens were embedded in paraffin wax and cut into 3.5-μm sections. These were stained with hematoxylin and eosin (H&E).

RESULTS

Vibrio infections have been found to be associated with spontaneous mortality among the cultured tiger shrimp in Taiwan. Affected shrimp display nonspecific signs, including poor growth rate, anorexia, rough shell, and milky musculature (Fig. 1). Large numbers of short, curved bacterial rods were observed in the hepatopancreas, giving the infected tissue a pale and atrophic appearance (Fig. 2). Melanized granulomatous ulcerations encapsulated by hemocytes and connective tissues in the hepatopancreas were seen by histopathological examination (Fig. 3).

Twelve strains of *Vibrio* were isolated from cultured shrimp from March to October 1988 (Table 1). They were examined for 47 characteristics based on their morphology, physiology, and biochemistry. These bacteria were short rod, motile, and Gram-negative (Table 2). A single polar flagellum and numerous pili were observed (Fig. 4). Green colonies were developed on TCBS agar at 28 and 37°C. The NaCl range for growth was from 0.5 to 5.5% (Table 2). All isolates were positive for oxidase and arginine dihydrolase, but not for ornithine decarboxylase and tyrosinase. Only 3 of the 12 strains possessed urease activity, and gas was fermentatively produced from glucose. They were not able to utilize citrate as a sole carbon source, although they were able to utilize some carbohydrates, i.e., fructose, galactose, maltose, mannose, cellobiose, trehalose, and chitin, but not rhamnose, arabinose, lactose, melibiose, sucrose, or starch. They were not able to utilize mannitol, inositol, sorbitol, arbutin, amygdalin, or salicin. All strains were proteolytic by degradation of casein but not gelatin. Nitrate was reduced to nitrite under anaerobic conditions. Hemolytic activity for sheep RBC was observed. The growth of cells was inhibited by the presence of antibiotics such as novobiocin, chloramphenicol, and tetracycline, and also by Vibrio-static agent 0/129. It was, however, not inhibited by kanamycin or vancomycin. All isolates were positive for both Voges–Proskauer and methyl red tests, but negative for tryptophanase, evidenced by the failure to produce indole. No hydrogen sulfide was produced (Ta-

TABLE 2

Morphological and Physiological Characteristics of the 12 Strains Isolated from Diseased Tiger Shrimp (*P. monodon*) Compared to Reference Strains

Properties	*P. monodon* strains (n = 12)	*V. damsela* ATCC 33539	Grimes *et al.* (1984)	Fujioka *et al.* (1988)	Sakata *et al.* (1989)
Gram stain	−	−	−	−	−
Bacterial shape	SR	SR	SR	SR	SR
Colony color on TCBS agar	Gr	Gr	Gr	Gr	Gr
Motility	+	+	+	+	+
NaCl tolerance (%)					
0.5	+	ND	ND	ND	+
1.0	+	+	ND	ND	+
2.5	+	+	ND	ND	+
3.0	+	+	+	+	+
5.5	+	+	ND	ND	ND
6.0	ND	+	−	−	ND
8.0	−	−	−	−	−
10.5	−	−	−	−	−
Growth at (°C)					
5	−	−	ND	ND	ND
28	+	+	ND	ND	ND
37	+	+	ND	+	ND

Note. SR, short rod; ND, not determined; Gr, green.

ble 3). DNA relatedness to the reference strain of *V. damsela* ATCC33539 was, respectively, from 64 to 93% at 61°C (T_m − 25°C) and from 60 to 92% at 73°C (T_m − 13°C) (Table 4).

Experimental infections by both anal intubation and intramuscular injection with *V. damsela* produced shrimp hepatopancreatitis and death. That 10^5 bacteria were able to induce mortality using anal intubation was shown in this study. A 10-fold higher concentration of bacteria was required to induce mortality using intramuscular injection. The mortality of shrimp increased when the infectious dosage of bacteria increased; mortality onset, as well, occurred earlier (Table 5). Death on or beyond the 4th day postinfection was accompanied by a pale and atrophic hepatopancreas. Survivors showed similar signs. Experimentally infected shrimp show necrotic tubules with cell debris being observed in the lumen. The melanized cells were also found around the foci and inflammatory sinuses were infiltrated with hemocytes (Fig. 5). No death occurred in the water-borne infection group, however. Hepatopancreas in survivors was normal.

TABLE 3

Biochemical Characteristics of the 12 Strains Isolated from Diseased Tiger Shrimp (*P. monodon*) Compared to Reference Strains

Test	*P. monodon* strains (n = 12)	*V. damsela* ATCC 33539	*V. damsela* Grimes *et al.* (1984)	*V. damsela* Fujioka *et al.* (1988)	*V. damsela* Sakata *et al.* (1989)
Oxidase	+	+	+	+	+
O/F test	+/+G	+/+G	+/+G	+/+G	+/+G
Nitrate reduction	+	+	+	+	+
Indol (SIM)	−	−	−	−	−
Methyl red	+	+	+	ND	+
Voges–Proskauer	+	+	+	+	+
Citrate utilization	−	−	−	−	−
Hydrogen sulfide production (TSI)	−	−	−	−	−
Arginine dihydrolase	+	+	+	+	+
Lysine decarboxylase	d(11)	−	−	+	−
Ornithine decarboxylase	−	−	−	−	−
Urease	d(3)	ND	+	+	ND
Gelatin liquefaction	−	−	+	−	ND
Casein hydrolysis	+	ND	−	ND	−
Starch hydrolysis	−	ND	+	+	+
Chitin hydrolysis	+	ND	+	−	+
Tyrosine decomposition	−	ND	−	ND	ND
Hemolysis	+(SRBC)	ND	ND	ND	+(tilapia RBC)
Acid production from					
D-Fructose	+G	ND	ND	ND	+G
D-Galactose	+G	ND	ND	ND	+G
Inositol	−	−	−	−	−
Maltose	+G	+G	ND	ND	+G
Mannose	+G	+G	+G	+G	+G
Mannitol	−	−	−	−	−
Rhamnose	−	−	ND	−	−
Sorbitol	−	−	ND	−	−
L-Arabinose	−	ND	−	−	−
Arbutin	−	ND	−	ND	ND
Cellobiose	dG(10)	−	+	−	+G
Lactose	−	−	−	−	−
Melibiose	−	−	ND	−	−
Sucrose	−	−	−	−	−
Trehalose	dG(9)	−	+	ND	−
Amygdalin	−	ND	ND	ND	ND
Salicin	−	−	−	ND	−
Inhibited by					
0/129 (150 μg)	+	ND	+	+	+
Novobiocin (30 μg)	+	ND	ND	ND	ND
Chloramphenicol (30 μg)	+	ND	ND	ND	+
Tetracycline (30 μg)	+	ND	ND	ND	+
Kanamycin (30 μg)	−	ND	ND	ND	−
Vancomycin (30 μg)	−	ND	ND	ND	ND

Note. Number in parenthesis represents strains which reacted positively. ND, not determined; G, gas production.

TABLE 4
DNA Relatedness of *V. damsela* (ATCC 33539) to Local Strains

Strain	Percentage of reassociation	
	T_m – 25°C	T_m – 13°C
ATCC 33539	100	100
TG617	67	77
LG711	74	92
P3G716	64	76
I1G720	81	69
I2G720	79	75
PG801	93	72
J3G801	75	60
M1G831	85	76
M2G831	92	78
A3G911	77	80
A7G911	75	70
E. coli	3	19

DISCUSSION

V. damsela has been experimentally shown to be pathogenic for damselfish, shark, and mouse cell line, as well as yellow tail and eel (Sakata *et al.*, 1989). Fujioka *et al.* (1988) also reported that *V. damsela* is the primary bacterium causing wound infections in dolphins. These conclusions, however, were not in agreement with the conclusion of Vera *et al.* (1991) that *V. damsela* isolated from seabream (*Sparus aurata*) was not virulent by itself. Stress conditions which occurred in a natural disease outbreak might facilitate the disease process. Water-borne infection of shrimp with *V. damsela* in this study induced neither hepatopancreatitis nor mortality. Experimental infections of shrimp with either anal intubation or intramuscular injection, however, induced histopathology and high mortality. Lethal dose 50 value (LD_{50}) of *V. damsela* to the host, although, was not shown in this study. However, it was calculated by Jiang (1991) to be 2.5×10^6 cfu/10 g body wt of tiger shrimp using intramuscular injection. Both techniques are believed here to have caused some degree of stress, which in turn mediated the disease infection.

Under normal conditions, no change of pond water takes place during the first month of stocking because of the limitation of shrimp size. Meanwhile, the local farmers are used to supplementing the rearing pond water with underground water or seawater instead of changing the pond water. Infection of shrimp probably occurs because feces, excess feed, body debris, molded shells, and crowded shrimp constitute a favorable environment for chitinovorous *V. damsela* (Sakata and Taruno, 1987a). On the other hand, in invertebrates, such as molluscs, that possess open circulatory systems, certain environmental factors have been known to reduce immunocompetence and render such hosts more susceptible to parasitism and the expression of pathogenicity by protists (Cheng, 1987). Thus, the polluted nearshore seawater and aged ponds without drainage of waste from the bottom of the pond, leading to poor water quality, probably suppress host resistance and facilitate disease progression.

It is possible that *V. damsela* did not successfully attach or invade wounded body surfaces, since water-borne infection did not produce disease. Indirect evi-

TABLE 5
Experimental Infection of Tiger Shrimp (*P. monodon*) with *V. damsela*

Route of infection	Bacterial dose (CFU/shrimp)	Number of deaths									Death ratio (%)
		2	4	8	12	24 (hr)	48	72	120	144	
	5 × 10E7	1[a]		1		1		1			4/5[d] (80)
	5 × 10E6					1	1				2/5 (40)
	5 × 10E5					1					1/5 (20)
	0										0/5 (0)
Anal intubation	5 × 10E7		1[b]	1	1	1					4/5 (80)
	5 × 10E6			1		1	1				3/5 (60)
	5 × 10E5									1	1/5 (20)
	0										0/5 (0)
	5 × 10E7				1[c]	2		1			4/10 (40)
	5 × 10E6									2	2/10 (20)
	5 × 10E5						1				1/10 (10)
	0										0/10 (0)
Intramuscular injection	5 × 10E7	2[b]				1		1			4/4 (100)
	5 × 10E6	1	1			1					3/4 (75)
	5 × 10E5										0/4 (0)
	0										0/4 (0)

[a] Body wt, 13–15 g.
[b] Body wt, 6–7 g.
[c] Body wt, 3–4 g.
[d] Sample size.

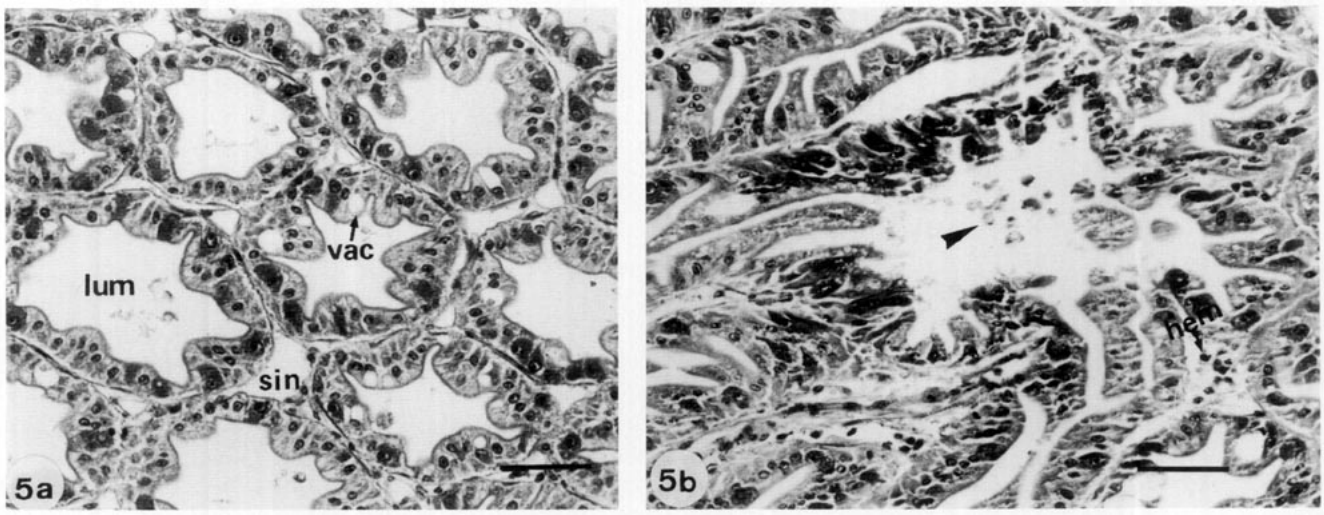


FIG. 5. Cross section of the hepatopancreatic tubules. (a) Healthy shrimp. The lumen (lum) and secretory vacuoles (vac) are seen. Between tubules are hemal sinuses (sin). (b) Experimentally infected shrimp showing slightly necrotic tubules (arrow) and inflammatory sinus infiltrated with hemocytes (hem). H&E stain; Davidson's fixative; ×250; bar = 50 μm.

dence came from (1) the failure here to isolate *V. damsela* from muscle of naturally infected shrimp and (2) the fact that the dosage of bacteria required to induce shrimp hepatopancreatitis and death was 10-fold higher with the method of intramuscular injection in comparison to that of anal intubation. These phenomena show the possibility that the transmission of *V. damsela* occurs through the gastrointestinal tract. This conclusion was also proposed by Lavilla–Pitogo *et al.* (1990), as the infection of tiger shrimp cultured in the Philippines by the luminous *V. harveyi,* the other shrimp pathogen, was initiated through an oral route.

Hemolytic strains of *V. damsela* have been shown to be associated with pathogenecity (Kreger, 1984). *V. damsela* strains isolated from the cultured shrimp in this study have been shown to be hemolytic for sheep red blood cells. The presence of pili (which have been responsible for colonization) in *V. damsela* strains might also account for the virulence.

This has been the first report of *V. damsela* isolated and characterized from cultured tiger shrimp associated with mortality under stress conditions.

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REFERENCES

Cheng, T. C. 1987. Some cellular mechanisms governing self and nonself recognition and pathogenicity in vertebrates and invertebrates relative to protistan parasites. *Aquaculture* 67, 1–14.

Fouz, B., Larsen, J. L., and Toranzo, A. E. 1991. *Vibrio damsela* as a pathogenic agent causing mortalities in cultured turbot (*Scophthalmus maximus*). *Bull. Eur. Assoc. Fish Pathol.* 11, 80–81.

Fujioka, S. R., Greco, S. B., Cates, M. B., and Schroeder, J. P. 1988. *Vibrio damsela* from wounds in bottlenose dolphins (*Tursiops truncatus*). *Dis. Aquat. Org.* 4, 1–8.

Grimes, D. J., Stemmler, J., Hada, H., May, E. B., Maneval, D., Hetrick, F. M., Jones, R. T., Stoskopf, M., and Colwell, R. R. 1984. *Vibrio* species associated with mortality of sharks held in captivity. *Microb. Ecol.* 10, 271–282.

Hanlon, R. T., Forsythe, J. W., Cooper, K. M., Dinuzzo, A. R., Folse, D. S., and Kelly, M. T. 1984. Fatal penetrating skin ulcers in laboratory-reared octopuses. *J. Invertebr. Pathol.* 44, 67–83.

Hugh, R., and Leifson, E. 1953. The taxonomic signification of fermentative versus oxidative metabolism of carbohydrates by various Gram-negative bacteria. *J. Bacteriol.* 66, 24–26.

Isenberg, H. D., and Sundheim, L. H. 1958. Indole reactions in bacteria. *J. Bacteriol.* 75, 682–690.

Jiang, S. C. 1991. "Study of Pathogenicity of Four *Vibrio* Species to Culture Giant Tiger Prawn (*Penaeus monodon*) and Kuruma Prawn (*Penaeus japonicus*)." Master's thesis, Natl. Taiwan University.

Johnson, J. L. 1981. Genetic characterization. *In* "Manual of Methods for General Bacteriology" (P. Gerhardt, R. G. E. Murray, R. Costilow, E. W. Nester, W. A. Wood, N. R. Krieg, G. B. Phillips, Eds.), pp. 450–472. American Society for Microbiology, Washington, DC.

Koser, S. A. 1923. Utilization of the salts of organic acids by the colon-aerogenes group. *J. Bacteriol.* 8, 493–520.

Kreger, A. S. 1984. Cytolytic activity and virulence of *Vibrio damsela*. *Infect. Immun.* 44, 326–331.

Lavilla-Pitogo, C. R., Baticados, M. C. L., Cruz-Lacierda, E. R., and de la Pena, L. D. 1990. Occurrence of luminous bacterial disease of *Penaeus monodon* larvae in the Philippines. *Aquaculture* 91, 1–13.

Leifson, E. 1963. Determination of carbohydrate metabolism of marine bacteria. *J. Bacteriol.* 85, 1183–1184.

Lewin, R. A., and Lounsbery, D. M. 1969. Isolation, cultivation and characterization of *Flexibacteria*. *J. Gen. Microbiol.* 58, 145–170.

Lightner, 1983. Diseases of cultured penaeid shrimp. *In* "CRC Handbook of Mariculture," Vol. 1, "Crustacean Aquaculture" (J. P. McVey, Ed.), pp. 289–320. CRC Press, Boca Raton, FL.

Love, M., Teebken-Fisher, D., Hose, J. E., Farmer, J. J., III, Hick-

man, F. W., and Fanning, G. R. 1981. *Vibrio damsela*, a marine bacterium, causes skin ulcers on the damselfish (*Chromis punctipinnis*). *Science* **214**, 1139–1140.

McFaddin, J. F. 1980. "Biochemical Tests for Identification of Medical Bacteria," 2nd ed., pp. 236–244. Williams & Wilkins, Baltimore.

McGarey, D. J., Kraxberger, T. B., Alberts, V. A., Strake, D., and Lim, D. V. 1990. Investigations of potential microbial pathogens associated with ulcerative disease syndrome (UDS) of Florida fish. *In* "Pathology in Marine Science," (F. Perkins and T. Cheng, Eds.), pp. 65–75. Academic Press, San Diego.

Obendorf, D. L., Carson, J., and McManus, T. J. 1987. *Vibrio damsela* infection in a stranded leatherback turtle (*Dermochelys coriacea*). *J. Wildl. Dis.* **23**, 666–668.

Roberson, B. S. 1990. Bacterial agglutination. *In* "Techniques in Fish Immunology" (J. S. Stolen, T. C. Fletcher, D. P. Anderson, B. S. Roberson, W. B. van Muiswinkel, Eds.), pp. 81–86. SOS Publications, New Jersey.

Rodrigues, D. D. P., and Hofer, E. 1987. *Vibrio* species from the water–oyster ecosystem of Sepetiba Bay in Rio de Janeiro State Brazil. *Rev. Microbiol.* **71**, 332–338.

Sakata, T., Matsuura, M., and Shimokawa, Y. 1989. Characteristics of *Vibrio damsela* isolated from diseased yellowtail *Seriola quinqueradiata*. *Nippon Suvian Gakkaishi* **55**, 135–141.

Sakata, T., and Taruno, N. 1987a. Ecological studies on microflora in the digestive tract of prawns *Penaeus japonicus* I. *Suisan Zoshoku* **35**, 147–151.

Sakata, T., and Taruno, N. 1987b. Ecological studies on microflora in the digestive tract of prawns *Penaeus japonicus* II. *Suisan Zoshoku* **35**, 153–160.

Seidler, R. J., Allen, D. A., Colwell, R. R., Joseph, S. W., and Daily, O. P. 1980. Biochemical characteristics and virulence of environmental Group F bacteria isolated in the United States. *Appl. Environ. Microbiol.* **40**, 715–720.

Simmons, J. S. 1926. A culture medium for differentiation organisms of typhoid-colon aerogenes groups and for isolation of certain fungi. *J. Infect. Dis.* **39**, 209–214.

Society of American Bacteriologists. 1957. "Manual of Microbiological Methods." McGraw–Hill, New York.

Song, Y. L., Cheng, W., Shen, C. H., Ou, Y. C., and Sung, H. H. 1990. Occurrence of *Vibrio vulnificus* infections in cultured shrimp and eel in Taiwan. *In* "Proceedings, ROC–JAPAN Symp. Fish Dis.," pp. 172–179.

Song, Y. L., Fryer, J. L., and Rohovec, J. S. 1988. Comparison of gliding bacteria isolated from fish in North America and other areas of the Pacific Rim. *Fish Pathol.* **23**, 197–202.

Stanier, R. Y. 1947. Studies on non-fruiting myxobacteria. *J. Bacteriol.* **53**, 297–315.

Vera, P., Navas, J. I., and Fouz, B. 1991. First isolation of *Vibrio damsela* from seabream (*Sparus aurata*). *Bull. Eur. Assoc. Fish Pathol.* **11**, 112–113.