

Characterization and Pathogenicity Studies on *Vibrio* Bacteria Isolated from Freshwater Prawn (*Macrobrachium rosenbergii*) Hatcheries

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Abstract

Phenotypic identification of fifty *Vibrio* bacterial strains, isolated from diseased and healthy larval prawn as well as larval rearing water in prawn hatcheries, together with twenty-two reference strains were investigated by Euclidean distance with unweighted average linkage clustering. Comparison based on forty-seven phenotypic characters showed that these isolates mainly clustered in five groups of which four were equated with the well known *Vibrio* species, *V. alginolyticus*, *V. carchariae*, *V. cholerae* and *V. mimicus*. For the remaining group comprising five isolates, it was more difficult to establish any relationship with known species when compared with reference strains.

All isolates were sensitive to Gentamicin, Tetracyclin, Chloramphenicol, Nalidixic Acid, Chlorotetracyclin, Neomycin and Oxytetracyclin and most were sensitive to Kanamycin (49/50), Streptomycin (40/50), Carbenicillin (42/50) and Oleandomycin (33/50). A large number of strains were resistant to Penicillin (49/50), Vancomycin (45/50) and Polymyxin-B (32/50).

Strains were also subjected to pathogenicity study in prawn larvae at the concentration of 10^4 to 10^8 cells ml^{-1} by immersion method. Experimental challenges resulted in significant mortalities of larvae and post larvae of *Macrobrachium rosenbergii* within 48 h post immersion challenge.

1. Introduction

Two groups of bacteria cause serious disease in different phases of shrimp culture including *Leucothrix* sp. and several *Vibrio* species (Lavilla-Pitogo, 1995). *Vibrio* bacteria are one of the pathogenic factors, which cause high mortality among economically important species of farmed marine fish and shrimp in Thailand (Ruangpan and Kitao, 1991). *V. alginolyticus* was reported as a pathogen of sea bream (*Sparus aurata*) and grouper (*Epinephelus malabaricus*) (Colorni *et al.* 1981; Lee 1995). Kiiyukia *et al.* (1992) isolated *V. cholerae* non-O1 from ayu (*Plecoglossus altivelis*). *Vibriosis*, especially luminous disease has caused serious loss in prawn hatcheries. *V. harveyi* was reported as the causative bacterium of vibriosis in pearl oyster (*Pinctada maxima*), black tiger prawn (*Penaeus monodon*), kuruma prawn (*Penaeus japonicus*) (Pass *et al.* 1987; Lavilla-Pitogo *et al.* 1990; Karunasagar *et al.* 1994; Liu *et al.* 1996; Leano *et al.* 1998). Larval prawns are particularly susceptible to *V. harveyi* succumbing to what has been termed luminescent bacterial disease (Lavilla-Pitogo *et al.* 1990). This disease has been identified as a major problem in the Philippines, causing severe losses of juvenile prawns in several hatcheries (Lavilla-Pitogo *et al.* 1992). In addition, Austin and Austin (1993) have categorized the seven main *Vibrio* fish pathogen as being *V. alginolyticus*, *V. anguillarum*, *V. carchariae*, *V. cholerae*, *V. damsela*, *V. ordalii* and *V. vulnificus*.

In recent years the shrimp diseases encountered in Vietnam have included vibriosis in larvae and broodstock (Tho and Khang, 1990). Vibriosis was reported in various species of cultured shrimp (Hao *et al.* 1997), however, bacterial disease of freshwater prawn has not been reported yet. Information on vibriosis in prawn hatcheries is therefore essential to support healthy seed supply activity.

This study aimed to identify and characterise bacterial strains isolated from freshwater prawn to obtain information on fish vibriosis with regard to biochemical and physiological characteristics as well as antimicrobial susceptibility. The pathogenic capacity of these isolates was also reported.

2. Materials and Methods

Bacterial strains

Fifty bacterial strains, which were presumptively equated with the genus *Vibrio*, were studied together with nineteen reference strains (Table 1). The strains were isolated from the shell of artemia cyst, rearing water, healthy and diseased freshwater prawn (*M. rosenbergii*) larvae. Samples were collected from prawn larval rearing tanks from 1998 up to now, in which a part of them were collected in a complete cycle of larval rearing (from larval stage 1-11). There are 41 strains were isolated from Can Tho prawn hatchery and the rest were isolated from Long My prawn hatchery. Strains were stored at - 35 °C in brain heart infusion broth (Difco) containing 15% glycerol and supplemented with 1.5 % (w/v) sodium chloride.

Morphological and phenotypic characterization

Colony morphology were recorded after incubation for 2 days at 30 °C on thiosulfate citrate bilesalts sucrose (TCBS, Himedia) agar. Cell morphology was studied in Gram-stained preparations from nutrient agar plates supplemented with 1% (w/v) NaCl according to Hucker's modification method (Barrow & Feltham 1993). Motility in broth (veal infusion broth (Difco) supplemented with 1% (w/v) NaCl) was studied using a drop of overnight culture on a slide and observed under light microscope.

Selection of biochemical and physiological characters (Table 2) followed the diagnostic scheme for *Vibrio* species associated with fish diseases described by Larsen & Pedersen (1999). Examinations of characters were performed according to the principles of Cowan and Steels Manual (Barrow & Feltham 1993) and methods of West & Colwell (1984). The phenotypic characters were coded in a binary format by scoring positive and negative character as 1 and 0 respectively. The data were examined using Euclidean distance with unweighted average linkage (UPGMA) clustering (Priest & Austin 1993) using Statistica computer program.

Antibiotic sensitivity testing

Strains were test for their susceptibility to 16 different antibiotics (Table 3) by the agar diffusion method as described by Pedersen *et al.* (1995).

Pathogenicity studies

Challenge experiments was carried out to determine the pathogenicity of studied strains applying immersion method. Healthy larvae and post larvae of *Macrobrachium rosenbergii* were used for these assays. 25 prawn postlarvae (or 50 prawn larvae) were kept in one-l capacity glass bottle containing aerated, chlorinized and static sea water (12 ‰) at 25 °C. The bottles were covered with loosely-fitting plastic covers with a hole for aeration tubing. No water exchange and aseptic techniques were observed throughout the experiments. There was no water exchange during the challenge test. Bacterial cultures were grown in 10 ml of heat infusion broth at 28 °C, centrifuged at 13,000 r.p.m. for 3 minutes, whereafter pellets were resuspended in 10 ml of sterile 0.9% (w/v) NaCl. The optical density of bacterial cells was estimated using spectrophotometer. Bacterial suspension was added to the challenge bottle at the concentration of 10^5 - 10^7 cell ml⁻¹, control bottles were included. Treatments were tested in three replicates. Prawn were observed at night for luminescence. Survival was determined after 24 and 48 hours post challenge. Before and after challenge water and prawn samples were processed for bacteriological examination. Bacteria recovered from diseased prawn were reidentified to confirm identity with the test strains. Data were analyzed using Statistics computer program.

3. Results

Identification and chracterization of studied isolates

Phenotypic characteristics of studied isolates and reference strains are given in Table 2. A dendrogram showing phenotypic relationship between strains is illustrated in Fig.1. Euclidean distance was transformed into percentage of similarity. The studied isolates formed five groups, of which four groups were identified

as *V. alginolyticus*, *V. carchariae*, *V. cholerae* and *V. mimicus*. The remaining group comprising five isolates could not be assigned to any species by comparing with the reference strains and were subsequently designated *Vibrio* spp. On the basis of phenotypic data, the strains were assigned to species as indicated in Table 1.

Vibrio cholerae

Twenty six isolates clustered together with the *V. cholerae* reference strain (Fig. 1). These strains formed small sized colonies (1-1.5 mm) after 2 days of incubation at 30 °C on TCBS agar. The colonies were circular, entire and low convex. It is greyish on nutrient agar. The surface of the colonies was smooth and shiny. All isolates were positive in lysine decarboxylase but negative in arginine dihydrolase. They grew in 1% peptone broth containing 0, 3, and 6% NaCl but did not grow on 8% and 10%. All strains reduce nitrate to nitrite, gave positive VP reaction, produced indole and acid from glucose, lactose, mannitol and trehalose but not from arabinose, cellobiose, salicin or xylose. Gas was not produced from glucose. They degraded gelatine and hydrolyse starch but gave a negative result for luminescence, and urease. Variable reactions were observed in ornithine decarboxylase, citrate utilization, acid production from galactose, glycerol and sucrose.

Vibrio alginolyticus

Ten isolates clustered together with the *V. alginolyticus* type strain (Fig. 1). These isolates developed swarming growth on nutrient agar plate after 1 day of incubation at 37 °C. All ten isolated strains grew in 1% peptone medium containing 3, 6, 8, 10% NaCl but not growing on 0% NaCl, produced acid from glucose, glycerol, mannitol, sucrose and trehalose but not from arabinose, galactose, lactose, salicin or xylose. All ten strains produced indole, gave positive VP reaction, reduce nitrate to nitrite and were positive in lysine and ornithine decarboxylase. They all hydrolysed gelatinase but tested negative for luminescence, urease and arginine dihydrolase. All of strains do not produced gas from glucose. Variable reactions were observed in citrate utilization, lipase and acid production from cellobiose.

Vibrio carchariae

Five isolates clustered together with the *V. carchariae* type strains (Fig. 1). The strains did not develop swarming growth. They formed large sized (3-3.5 mm) colonies after two days incubation at 20 °C on TCBS agar. The colonies were circular, shiny, smooth, adherent. Strains were positive in lysine and ornithine decarboxylase but negative for arginine dihydrolase. They grew in 1 % peptone medium containing 3, 6 and 8 % sodium chloride but not 0 and 10 %. They produced indole but gave a negative in VP reaction and luminescence. All strains reduced nitrate to nitrite, hydrolysed starch, tween 80 and gelatin, produced acid from glucose, arabinose, cellobiose, galactose, glycerol, lactose, mannitol, salicin, sucrose but not from xylose. Variable reactions were observed in urea and citrate utilization.

Vibrio mimicus

There was four isolates that clustered together with the *V. mimicus* type strain (Fig. 1). The size of the colonies was 3 mm after 2 days of incubation at 20 °C. The colonies were circular, entire and low convex. The surface of the colonies was smooth and shiny. They were positive in lysine and ornithine decarboxylase. They grew in 1 % pepton medium containing 0, 3 and 6 % sodium chloride but not 8 and 10 %, produced indole, utilized citrate, reduced nitrate to nitrite, lipase, gelatinase but were negative in arginine dihydrolase, VP reaction, urease and luminescence. All strain produced acid from glucose, galactose, lactose, mannitol, salicin and trehalose but not from arabinose, sucrose and xylose. All of strains did not produce gas from glucose. Variable reactions were observed in starch hydrolysed, acid from cellobiose and glycerol.

***Vibrio* spp**

Five strains were included in this group (Fig. 1). They did not develop swarming growth. The colonies were circular, entire and low convex. The surface of the colonies was smooth and shiny. All five isolates grew in 3, and 6 % NaCl, citrate utilisation, produced acid from galactose, mannitol, salicin and trehalose.

They did not produce gas from glucose as well as hydrolyse starch but they hydrolysed gelatine. They were negative for luminescence and did not produce gas from glucose. Variable reactions were observed in amino acid degradation (arginine, lysine and ornithine), growth in 0, 8 and 10 % NaCl, urease, indole production, VP reaction, lipase, and produce acid from arabinose, cellobiose, glycerol, lactose and sucrose.

Antibiotics sensitivities test

The results of the antibiotic sensitivity testing are shown in table 3. All isolates were sensitive to Gentamicin, Tetracylin, Chloramphenicol, Nalidix-Sav, Chlortetracylin, Neomycin and Oxytetracylin and most were sensitive to Kanamicin (49/50), Streptomycin (40/50), Carbenicilin (42/50) and Oleandomycin (33/50). A large number of strains were resistant to Penicilin (49/50), Vancomycin (45/50) and Polymycin-B (32/50).

Challenge experiment

Of the 50 isolates, twelve could infect larvae and postlarvae *M. rosenbergii* and cause mortality ranging from 18-80% (Table 4). No mortality was observed for prawn in the control group. Result of pathogenicity tests indicate that exposure to *V. carchariae*, *V. cholerae*, *V. alginolyticus* and *V. mimicus* induced significant (at 0.05 level) mortalities in *M. rosenbergii* larvae and postlarvae within 48 hours.

Weak and moribund larvae became opaque white and settled to the bottom and exhibited intermittent and very weak swimming movements. Densely packed motile bacteria in the hemocoel were observed under light microscope.

4. Discussion

All strains displayed the key phenotypical features of bacteria belonging to the genus *Vibrio*. Thus, all were motile, oxydase and catalase positive, Gram-negative rods which degraded D-glucose fermentatively, reduced nitrate to nitrite, grew on a *Vibrio* selective medium (TCBS) and were sensitive to the vibriostatic agent O/129 using 150 µg discs (West *et al.* 1986). In addition, all strains grew abundantly in 3 % and 6% NaCl. They all produced acid from mannitol and trehalose and gave positive result for indole production.

Alsina & Blanch (1994) recorded that *V. harveyi* and *V. carchariae* were different in their utilization of arabinose as sole carbon source. All five strains within this group gave positive response for arabinose which was also the case for the *V. carchariae* type strain. Urease production has been suggested to be an important trait for virulence of *V. carchariae* (Bryant *et al.* 1986; Pedersen *et al.* 1998). There is one isolate among this group produced urease. They also showed slight differences in citrate utilization compared to type strain.

Ten isolates clustered together with the *V. alginolyticus* type strain and performed typical phenotypic characters of *V. alginolyticus*, which are swarming growth on saline solid media, arginine dihydrolase negative, lysine decarboxylase positive, positive result for VP reaction, acid production from glycerol and sucrose, and growth in the presence of 8 and 10 % sodium chloride but not 0 % (Austin & Austin 1993; Frerichs 1993; Balebona *et al.* 1998).

Twenty six isolates clustered together with the *V. cholerae* type strain. They showed slight differences in lysine decarboxylase and acid production from galactose, glycerol, and sucrose but otherwise, their phenotypic traits were in agreement with the description of *V. cholerae* (Baumann *et al.* 1984).

Strains clustered together with the *V. mimicus* type strain gave different responses in acid production from salicin and slight differences in starch hydrolysis and acid production from cellobiose and glycerol compared to the *V. mimicus* type strain.

Vibrio spp. performed unidentical phenotypic characters and these characters were a mixture between reference strains (Fig. 1).

Different *Vibrio* strains had similar antibiotic resistant profiles. All isolates were sensitive to Gentamicin, Tetracycline, Chloramphenicol, Nalidixic Acid, Chlorotetracycline, Neomycin and Oxytetracycline and most were sensitive to Kanamycin (49/50), Streptomycin (40/50), Carbenicillin (42/50) and Oleandomycin (33/50). This finding indicates that these antibiotics would be effective against tested isolates. A large number of strains were resistant to Penicillin (49/50), Vancomycin (45/50) and Polymyxin-B (32/50) meaning that these antibiotics will have no effect for treatment of these bacteria.

Vibrio species occur widely in aquatic environments and part of the normal flora of coastal seawater. They also exist as normal flora in fish and shellfish but have also been recognized as opportunistic pathogens in many marine animals (Austin and Austin, 1993). The present study has shown that at least 4 species of *Vibrio* are associated with vibriosis in *M. rosenbergii*. Li *et al.* (1999) also showed 7 *Vibrio* species associated with vibriosis in silver sea bream (*Sparus sarba*) of these species *V. alginolyticus*, *V. vulnificus*, *V. parahaemolyticus* are dominant. Sae-oui *et al.* (1987) induced significant mortalities in *Penaeus merguensis* nauplii challenged with 10^8 cells/ml but not with 10^7 cells/ml, levels much higher than those used in the present study. This information will serve as a guide when implementing routine sanitary procedures to reduce the bacterial load of the rearing water. Bacterial build-up to the 10^2 cfu/ml level should be avoided as it was shown that this level leads to significant mortalities within 48 h.

Literature Cited

- Austin, B. & D.A. Austin. 1993. Bacterial fish pathogens, Diseases in farmed and wild fish, 2nd edn. Ellis Horwood Ltd., Chichester.
- Barrow, G.I. & R.K.A. Feltham. 1993. Cowan and Steel's manual for the identification of medical bacteria, 3rd edn. Cambridge University Press, Cambridge.
- Colomi, A., I. Paperna & H. Gordin. 1981. Bacterial infection in gilt-head sea bream *Sparus aurata* cultured at Elat. *Aquaculture* **23**: 257-267.
- Hao, N.V., B.Q. Te, L.T.T. Loan, L.T.P. Yen & L.M. Thanh. 1997. Pathogen in cultured shrimp in Southern Vietnam. Pp. 233-239 in: T. W. Flegel and I.H. MacRae (eds), Diseases in Asian Aquaculture III. Fish Health Section, Asian Fisheries Society, Manila.
- Karunasagar, I., R. Pai, G.R. Malathi & I. Karunasagar. 1994. Mass mortality of *Penaeus monodon* larvae due to antibiotic-resistant *Vibrio harveyi* infection. *Aquaculture* **128**: 203-209.
- Kiiyukia, C., A. Nakajima, T. Nakai, K. Muroga, H. Kawakami & H. Hashimoto. 1992. *Vibrio cholerae* Non-O1 isolated from ayu fish (*Plecoglossus altivelis*) in Japan. *Applied and Environmental Microbiology* **58** (9): 3078-3082.
- Larsen, J.L. & K. Pedersen. 1999. Diagnostic schemes for *Vibrio* species. (in preparation)
- Lavilla-Pitogo CR, Baticados MCL, Cruz-Larcierda ER, de la Pena LD, 1990. Occurrence of luminous bacterial disease of *Penaeus monodon* larvae in the Philippines. *Aquaculture* **91**: 1-13.
- Lavilla-Pitogo CR, Albright LJ, Paner MG, Sunaz NA, 1992. Studies on the source of luminescent *Vibrio harveyi* in *Penaeus monodon* hatcheries. *Disease in Asian aquaculture I*. Fish Health Section, Asian Fisheries Society, Manila. 157-164
- Lavilla-Pitogo CR, 1995. Bacterial disease of *Penaeid* shrimp. *Disease in Asian Aquaculture II*. Fish Health Section, Asian Fisheries Society, Manila. 107-121
- Leano, E.M., C.R. Lavilla-Pitogo & M.G. Paner. 1998. Bacterial flora in the hepatopancreas of pond-reared *Penaeus monodon* juveniles with luminescent vibriosis. *Aquaculture* **164**: 367-374.
- Lee, K.K. 1995. Pathogenesis studies on *Vibrio alginolyticus* in the grouper, *Epinephelus malabaricus*. *Microbial Pathogenesis* **19**: 39-48.
- Li, J., J. Yie, R. W. T. Foo, J. M. L. Ling, H. Xu & N. Y. S. Woo. 1999. Antibiotics resistance and plasmid profiles of *Vibrio* isolates from cultured silver sea bream, *Sparus sarba*. *Marine Pollution Bulletin* **39**: 245-249
- Liu, P., K. Lee, K. Yii, G. Kou & S. Chen. 1996. Isolation of *Vibrio harveyi* from diseased kuruma prawns. *Current Microbiology* **33**: 129-132.

Pedersen, K., T. Tiainen & J.L. Larsen. 1995. Antibiotic resistance of *Vibrio anguillarum*, in relation to serovar and plasmid contents. *Acta vet.scand.* **36**: 55-64.

Pass, D.A., R. Dybdahl & M. M. Mannion. 1987. Investigations into the causes of mortality of the pearl oyster, *Pinctada maxima* (Jamson), in Western Australia. *Aquaculture* **65**: 149-169.

Ruangpan L & T. Kitao. 1991. *Vibrio* bacteria isolated from black tiger shrimp, *Penaeus monodon* Fabricius. *Journal of Fish diseases* **14**. 383-388.

Sae-oui, D., A. Tansutapanit, L. Ruangpan. 1987. *Vibrio harveyi* a causative agent of white shrimp nauplii *Penaeus merguensis*. *Thai Fish. Gaz.*, **40**: 177-182.

Tho, N. H. and L. T. Khang. 1990. Report on Shrimp disease in Vietnam. Proceedings of the SEAADCP Shrimp health Management Workshop 103-106.

West, P.A. & R.R. Colwell. 1984. Identification and classification of Vibrionaceae-an overview. Pp. 285-363 in: R.R. Colwell (edn). *Vibrios in the environment*. John Wiley & Sons, New York.

Table 1. Studied isolates (No. 1-50) and reference strains (No. 51-69) used in the study

Isolate no.	Collection strain no.	Species identification	Source
1	1b	<i>V. carchariae</i>	Prawn larvae
2	2b	<i>V. alginolyticus</i>	Prawn larvae
3	2g	<i>V. cholerae</i>	Prawn larvae
4	2d	<i>V. cholerae</i>	Prawn larvae
5	1e	<i>V. cholerae</i>	Prawn larvae
6	3c	<i>V. cholerae</i>	Prawn larvae
7	IIB3	<i>V. alginolyticus</i>	Prawn larvae
8	8c	<i>V. cholerae</i>	Prawn larvae
9	11b	<i>V. cholerae</i>	Prawn larvae
10	4b	<i>V. cholerae</i>	Prawn larvae
11	3b	<i>V. cholerae</i>	Prawn larvae
12	At4a	<i>V. cholerae</i>	Prawn larvae
13	1fY	<i>V. alginolyticus</i>	Prawn larvae
14	C2a14.6	<i>V. cholerae</i>	Prawn larvae
15	2Ctom H	<i>V. cholerae</i>	Prawn larvae
16	7bG	<i>V. mimicus</i>	Prawn larvae
17	Ld1	<i>Vibrio sp.</i>	Prawn larvae
18	11aY	<i>V. mimicus</i>	Prawn larvae
19	11e	<i>V. cholerae</i>	Prawn larvae
20	163	<i>V. alginolyticus</i>	Prawn larvae
21	5b	<i>V. mimicus</i>	Prawn larvae
22	TCX NTSH	<i>V. cholerae</i>	Prawn larvae
23	990605	<i>V. carchariae</i>	Prawn larvae
24	990607	<i>V. cholerae</i>	Prawn larvae
25	990608	<i>V. cholerae</i>	Prawn larvae
26	990609	<i>V. cholerae</i>	Prawn larvae
27	990610	<i>V. cholerae</i>	Prawn larvae
28	990611	<i>V. carchariae</i>	Prawn larvae
29	990612	<i>Vibrio sp.</i>	Prawn larvae
30	990613	<i>V. cholerae</i>	Prawn larvae
31	990614	<i>Vibrio sp.</i>	Prawn larvae
32	990615	<i>V. cholerae</i>	Prawn larvae
33	Labeled (TCX)	<i>V. mimicus</i>	Prawn larvae
34	L1	<i>V. cholerae</i>	Prawn larvae
35	A1	<i>Vibrio sp.</i>	Shell of artemia cyst

36	A3	<i>V.cholerae</i>	Shell of artemia cyst
37	A6	<i>V. alginolyticus</i>	Shell of artemia cyst
38	A3IIT(a)	<i>Vibrio sp.</i>	Rearing water
39	A3IIT(b)	<i>V.cholerae</i>	Rearing water
40	P1	<i>V. alginolyticus</i>	Rearing water
41	P2	<i>V.cholerae</i>	Rearing water
42	P5	<i>V. carchariae</i>	Rearing water
43	B3a	<i>V.cholerae</i>	Rearing water
44	B3T	<i>V.cholerae</i>	Rearing water
45	B3aT	<i>V. alginolyticus</i>	Rearing water
46	D1	<i>V. alginolyticus</i>	Rearing water
47	D3IIT(a)	<i>V.cholerae</i>	Rearing water
48	D3IIT(b)	<i>V. alginolyticus</i>	Rearing water
49	A1aT	<i>V. carchariae</i>	Rearing water
50	P4G	<i>V. alginolyticus</i>	Rearing water
51	<i>V. anguillarum</i> ATCC 19264 ^T	-	<i>Gadus morhua</i>
52	<i>V. fluvialis</i> NCIMB 2249 ^T	-	Human faeces
53	<i>V. furnissii</i> ATCC 35016 ^T	-	Human faeces
54	<i>V. natriegens</i> ATCC 33898 ^T	-	-
55	<i>V. pelagius</i> biovar I NCIMB 1900 ^T	-	Water
56	<i>V. pelagius</i> biovar II NCIMB 2253 ^T	-	-
57	<i>V. alginolyticus</i> ATCC 33838 ^T	-	-
58	<i>V. carchariae</i> ATCC 43516 ^T	-	-
59	<i>V. cholerae</i> 889, O1, Ogawa	-	-
60	<i>V. harveyi</i> ATCC 33866 ^T	-	-
61	<i>V. mimicus</i> ATCC 33653 ^T	-	Human ear
62	<i>V. tubiashii</i> NCIMB 1340 ^T	-	Larvae of hard clam
63	<i>V. splendidus</i> biovar I NCIMB 1 ^T	-	Fish
64	<i>V. splendidus</i> biovar II NCIMB 2251 ^T	-	Water
65	<i>V. ichthyenteri</i> HWU P 8603	-	-
66	<i>V. hollisae</i> NCTC 11640 ^T	-	-
67	<i>V. penaeicida</i> KH-1 ^T	-	Shrimp
68	<i>V. ordalii</i> ATCC 33509 ^T	-	<i>Oncorhynchus kisutch</i>
69	<i>V. nigripulchritudo</i> LMG 3896 ^T	-	Water

Note:

ATCC: American Type Culture Collection; T: Type strain; NCIMB: National Collection of Industrial and Marine Bacteria, Aberdeen,

UK; HWU: Heriot-Watt University, Edinburgh, UK; NTCC: National Type Culture Collection, Colindale, UK; LMG: Laboratorium voor

Microbiologie, Rijksuniversiteit Gent, Belgium; CIP: Collection of the Pasteur Institute, Paris, France;

(a) Ribotype patterns were given number from 1-11

Table 2. Phenotypic characters of studied isolates and eference strains

Character	<i>V. spp</i> (n = 5)	<i>V. alg.</i> (n = 10)	<i>V. cho.</i> (n = 26)	<i>V. car</i> (n=5)	<i>V. mim.</i> (n =4)	<i>V. alg.</i> ATCC 33838	<i>V. cho.</i> 889, O1, Ogawa	<i>V. car.</i> ATCC 43516	<i>V. mim.</i> ATCC 33653
Swarming	-	+	-	-	-	+	-	-	-
Motility	+	+	+	+	+	+	+	+	+
Catalase	+	+	+	+	+	+	+	+	+
Oxidase	+	+	+	+	+	+	+	+	+
Pellicle	-	-	-	-	-	-	-	-	-
Pigment	-	-	-	-	-	-	-	-	-
Arginine	2	-	-	-	-	-	-	-	-
Lysine	2	+	+	+	+	+	+	+	+
Ornithine	2	+	24	+	+	+	+	+	+
Growth in									
0% NaCl	4	-	+	-	+	-	+	-	+
3%	+	+	+	+	+	+	+	+	+
6%	+	+	+	+	+	+	+	+	+
8%	4	+	-	+	-	+	-	+	-
10%	3	+	-	-	-	+	-	-	-
O test	+	+	+	+	+	+	+	+	+
F test	+	+	+	+	+	+	+	+	+
Urease	2	-	-	1	-	-	-	+	-
TCBS	+	+	+	+	+	+	+	+	+
0/129 150 µg	+	+	+	+	+	+	+	+	+
Luminescence	-	-	-	-	-	-	-	-	-
Citrate utilization	+	6	17	4	+	+	+	+	+
Indole production	3	+	+	+	+	+	+	+	+
VP	1	+	+	-	-	+	+	-	-
Nitrate reduction	+	+	+	+	+	+	+	+	+
Amylase	-	+	+	+	2	+	+	+	-
Gelatinase	+	+	+	+	+	+	+	+	+
Tween 80	4	8	21	+	+	+	+	+	+
Glucose	+	+	+	+	+	+	+	+	+
Gas from glucose	-	-	-	-	-	-	-	-	-
Arabinose	2	-	-	+	-	-	-	+	-
Cellobiose	4	7	-	+	1	-	-	+	+
Galactose	+	-	23	+	+	-	+	+	+
Glycerol	4	+	17	+	2	+	+	+	+
Lactose	3	-	+	+	+	-	+	+	+
Mannitol	+	+	+	+	+	+	+	+	+
Salicin	+	-	-	+	+	-	-	+	-
Sucrose	3	+	24	+	-	+	+	+	-
Trehalose	+	+	+	+	+	+	+	+	+
Xylose	1	-	-	-	-	-	-	-	-

+', All strains positive; '-', all strains negative; numerical values indicate the number of positive strains.

Table 3. Antibiotics sensitivity of studied isolates

Antibiotic	<i>Vibrio sp</i> (n=5)			<i>V. carchariae</i> (n=5)			<i>V. cholerae</i> (n=26)			<i>V. mimicus</i> (n=4)			<i>V. alginolyticus</i> (n=10)		
	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R
Vancomycin		1	4	1		4		1	25	1		3		1	9
Kanamycin	5			5			25	1		4			9		1
Oleandomycin	1	2	2	4		1	21	1	4	3		1	4		6
Gentamicin	5			5			26			4			10		
Tetracilin	5			5			26			4			10		
Streptomycin	4		1	5			20	5	1	4			6	2	2
Chloramphenicol	5			5			26			4			10		
Polymycin-B	1		4	3		2	8		18	1	1	2	2	2	6
Nalidix-sav	5			5			26			4			10		
Chlortetracylin	5			5			26			4			10		
Pennicilin			5	1		4			26			4			10
Neomycin	5			5			26			4			10		
Carbenicilin	3	1	1	5			23		3	4			7		3
Erythromycin	2		3	3		2	10	1	15		1	3	5	1	4
Oxytetracylin	5			5			26			4			10		
Amoxicilin		2	3	4		1	7	4	15		1	3	3	3	4

S: Sensitive; I: Intermediate; R: Resistant; Numerical values indicate the number of strains

Table 4. Pathogenicity of *Vibrio* strains to *Macrobrachium rosenbergii* larvae

Species	Strain no.	Inoculum level (cfu/ml)	Number of prawn tested	Number of dead prawn	Mortality %
<i>V. carchariae</i>	1b	10 ⁵	50	25	50
<i>V. carchariae</i>	990605	10 ⁶	50	35	70
<i>V. cholerae</i>	B3T	10 ⁷	50	19	38
<i>V. cholerae</i>	990615	10 ⁷	50	26	52
<i>V. cholerae</i>	L1	10 ⁷	50	9	18
<i>V. cholerae</i>	11e	10 ⁶	50	12	24
<i>V. mimicus</i>	7bG	10 ⁷	25	20	80
<i>V. mimicus</i>	5b	10 ⁷	25	6	24
<i>V. alginolyticus</i>	1fy	10 ⁷	50	12	24
<i>V. alginolyticus</i>	D1	10 ⁶	50	11	22
<i>V. cholerae</i>	2Ctom H	10 ⁶	50	10	20
<i>Vibrio spp</i>	LD1	10 ⁶	50	10	20
Saline (0.9% NaCl) injection			50	0	0