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Short Note

Characterization and Ecological Implication of Luminous *Vibrio harveyi* Isolated from Tiger Shrimp (*Penaeus monodon*)

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Yen-Ling Song and Sue-Ping Lee (1993) Characterization and ecological implication of luminous *Vibrio harveyi* isolated from tiger shrimp (*Penaeus monodon*). *Bull. Inst. Zool., Academia Sinica* **32**(3): 217-220. Among 602 tiger shrimp examined, 379 (63%) were found to be carriers of the *Vibrio* bacteria and 29% were found to be carriers of *Vibrio harveyi*. The *V. harveyi* strains are morphologically, physiologically, and biochemically described in this paper.

The percentages of shrimp carrying both luminous and non-luminous *V. harveyi* in the Tungkang and Linyuan hatcheries of Taiwan were 51% and 46%, respectively at the time of this study. However, of both types of *V. harveyi*, the percentage of the luminous form in Tungkang strains was 42%, while in the Linyuan strains it was only 1%. Tracking records reveal that shrimp in the Tungkang hatchery started dying shortly after sampling, but that no deaths or diseases were found in shrimp from the Linyuan and three other hatcheries after sampling. It therefore appears that luminous *V. harveyi* is connected to the recorded shrimp deaths.

Key words: *Vibrio harveyi*, Tiger shrimp (*Penaeus monodon*), Luminous bacteria.

The phenomenon of nighttime luminescence in cultured shrimp and pond water followed by significant shrimp mortality has long been observed in Taiwan, the Philippines (Baticados *et al.* 1990), Thailand, Indonesia, and Ecuador. The occurrence of luminous bacteria in penaeid larvae and other crustacean species has been reported by Harvey (1961), Bowman and Phillips (1984), Sunaryanto and Mariam (1986), and Lavilla-Pitogo *et al.* (1990). Huang (1989) surveyed the bacterial flora of diseased shrimp collected from hatcheries located in northern, central, and southern Taiwan between March and October of 1988. *V. harveyi* was shown to be the predominant (36%) bacteria, with its luminous form composing between 11 and 89% of the *V. harveyi* total. Chang (1991) conducted a survey of bacterial flora in cultured shrimp at two hatcheries located in Ilan, Taiwan, between March and July, 1990; although no mortalities or symptomatic diseases were found at the time of that sampling, *V. harveyi* constituted 16% of the total isolated Ilan strains (luminous form 9.4%). Similarly, luminous vibrios –predominantly *V. harveyi*– were consistently isolated from both infected and seemingly uninfected *Penaeus monodon* stocks in the Philippines (Lavilla-Pitogo *et al.* 1990).

Some researchers have reported that this *Vibrio* species is among normal penaeid microflora, none of which have been linked with disease. For example, *V. harveyi*'s natural habitat appears to be the guts of shrimp, and its free-living environment appears to be marine sediments (O'Brien and Sizemore 1979); it has been categorized as an opportunistic pathogen which

causes disease only under conditions which favor them instead of their host (Lightner 1984). However, other evidence has implicated this organism in the mortalities of raft-cultured pearl oysters in Western Australia (Pass *et al.* 1987). It appears to be a possibly lethal terminating agent in light of the severe pathology attributed to it in extensive bacteremia (Anderson *et al.* 1987, Huang 1989). Whether or not *V. harveyi* is associated with shrimp disease epidemics remains uncertain. The present study was designed to determine: (1) the characteristics of luminous *V. harveyi*; and, (2) the connection between the incidence of luminous bacteria and seriousness of subsequent mortalities.

Materials and Methods—A total of 602 tiger shrimp (*Penaeus monodon*) ranging from 0.6 to 23.5 cm in length were sampled from Tainan hatcheries in July and August, 1990, and at Tungkang, Chiatung, and Linyuan in April, 1991 (Table 1). No mortalities or diseases were observed at the time of sampling.

V. harveyi (type strain ATCC14126) was purchased from the Culture Collection and Research Center of Taiwan. Bacteria were isolated from shrimp hepatopancreases and selected on thiosulfate-citrate-bile salt-sucrose agar supplemented with 2% NaCl (TCBS). Both green and yellow colonies were subcultured on tryptic soy agar supplemented with 2.5% NaCl (TSA). Each TSA isolate was then tested for susceptibility to both the *Vibrio*-static agent (2,4-diamino-6,7-diisopropyl pteridine phosphate (0/129, 150 μg, CPM)), and the antibiotic novobiocin (30 μg, BBL). Motility was tested via the hanging-drop method following the passage of test organisms through a tryptic soy broth (Difco). The number of shrimp carrying *Vibrio* bacteria was then recorded. Sensitive colonies were characterized according to Bergey's Manual of Systematic Bacteriology (Baumann *et al.* 1984). The number of shrimp specifically carrying *V. harveyi* in each sample was recorded; luminescent *V. harveyi* strains were observed in a dark-room.

Mortality and disease records from the facilities where sampling occurred were checked shortly after sampling was completed.

Results—Among the 602 shrimp examined, 379 (63%) were carriers of *Vibrio* bacteria; 29% were carriers of *V. harveyi* (Table 1). The *V. harveyi* bacteria were observed to be Gram negative and pleiomorphic in morphology. Both yellow and green colonies were observed on TCBS agar; they were found to be sensitive for both 0/129 and novobiocin. Seventy-eight of the 447 *V. harveyi* strains isolated were luminous. All *V. harveyi* strains were able to grow at 40°C, but not in tryptic soy broth without an NaCl supplement. They were found to be lysine decarboxylase positive, but arginine dihydrolase and ornithine decarboxylase negative. All isolates were proven positive for the methyl-red test, but negative for the Voges-Proskauer test; they were able to utilize chitin, cellobiose, casein, tryosine and serine. However, all tested *V. harveyi* strains failed to decompose *o*-nitrophenyl-β-*D*-galactopyranoside (Table 2).

The percentages of shrimp from the Tungkang and Linyuan hatcheries carrying both luminous and non-luminous *V. harveyi* were 51% and 46%, respectively. However, the percentage of luminous *V. harveyi* in the observed Tungkang strains was 42%, and that in the Linyuan strains only 1% (Table 1). Tracking records show that Tungkang hatchery shrimp started dying shortly after our sampling was completed; however, no shrimp mortalities or diseases were found in the Linyuan or three other hatcheries following our sampling.

Discussion—Larval to adult shrimp were screened for the presence of *V. harveyi*. Our results show that shrimp carry this species of bacteria at all developmental stages. Although the pathogenicity of the

Table 1. Numbers and percentages of shrimp carrying *Vibrio harveyi* bacteria.

Location	Date	No. of collected shrimp	Body length (cm)	No. of shrimp carrying *Vibrio* (%)	No. of shrimp carrying *V. harveyi* (%)	Luminous strains/ Total strains (%)
Tainan	Jul. '90	212	0.6 – 2.1	131 (62)	40 (19)	13/ 87 (15)
Tainan	Aug. '90	135	0.8 – 3.5	118 (81)	47 (35)	9/ 69 (13)
Tungkang	Apr. '91	78	6.5 – 10.5	48 (62)	40 (51)	51/121 (42)
Chiatung	Apr. '91	116	12.2 – 16.3	40 (34)	21 (18)	4/ 43 (16)
Linyuan	Apr. '91	61	19.1 – 23.5	42 (69)	28 (46)	1/127 (1)
Total		602		379 (63)	176 (29)	78/447 (17)

Table 2. Characteristics of *Vibrio harveyi* strains isolated from cultured tiger shrimp (*Penaeus monodon*).

Characteristic	Local strains (n = 447)	*V. harveyi* ATCC14126
Gram stain	–	–
Morphology	pleiomorphism	straight rod
Motile	+	+
Colony on TCBS agar	Y(60)/G(387)[a]	Y
0/129 sensitivity	+	+
Novobiocin sensitivity	+	+
Luminescence	d(78)[b]	+
Growth at 40°C	+	+
Growth in TSB without NaCl	–	–
Arginine dihydrolase	–	–
Ornithine decarboxylase	–	–
Lysine decarboxylase	+	+
Methyl-red	+	+
Voges-Proskauer	–	–
Chitin degradation	+	+
Cellobiose utilization	+	+
Casein degradation	+	+
Tyrosine utilization	+	+
Serine utilization	+	+
ONPG	–	–

a: Colonies of 60 strains show yellow color; colonies of 387 strains show green color
b: Number of luminous strains of *V. harveyi*
TCBS: Thiosulfate-citrate-bile salt-sucrose
TSB: Tryptic soy broth
ONPG: *o*-nitrophenyl-*β*-*D*-galactopyranoside

luminous bacterium to shrimp was not tested in this study, luminous *V. harveyi* has previously been proven to be pathogenic to tiger shrimp and another Chinese penaeid (*P. orientalis*) via both injection and water-borne infections (Huang 1989, Liu *et al.* 1989, Lavilla-Pitogo *et al.* 1990). The percentages of shrimp in the Tungkang and Linyuan hatcheries carrying both luminous and non-luminous *V. harveyi* were very close (51% and 46%); also, tracking records show that Tung-kang hatchery shrimp started dying shortly after our sampling, but that no mortalities or diseases were found in Linyuan or three other hatchery shrimp after our sampling. It appears that luminous *V. harveyi* connected in some way to the observed shrimp epidemic, although our evidence is purely circumstantial. A study on changes in the number of shrimp carrying luminous *V. harveyi* over sequential time before the onset of mortalities is needed to support our conjecture.

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草蝦（*Penaeus monodon*）螢光弧菌*Vibrio harveyi*的鑑定及生態上的意義

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602隻採樣草蝦中，有379隻（63%）帶有弧菌，176隻（29%）帶有弧菌*Vibrio harveyi*，這些*V. harveyi*菌株已從形態、生理及生化方面定性。東港及林園養蝦場的採樣中，發現草蝦帶有*V. harveyi*菌株（包括螢光株及非螢光株）之比例爲51%及46%，但是東港菌株中有42%而林園菌株中僅有1%爲螢光株。採樣後追踪記錄顯示東港場不久即有蝦陸續死亡，而林園及其它3個蝦場卻未發現草蝦死亡或出現症狀，這種情況似乎暗示螢光弧菌*V. harveyi*與草蝦之發病有關。