

A Comparative Study of *Vibrio* Infections in Healthy and Diseased Marine Finfishes Cultured in Floating Cages near Penang, Malaysia

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Abstract

A total of 497 healthy and diseased marine finfishes cultured in floating cages near Penang, Malaysia, were examined for bacteria in the kidney and spleen. The fishes examined were juvenile greasy grouper (*Epinephelus malabaricus* (Bloch and Schneider)) (12 healthy; 87 diseased); juvenile silver seabass (*Lates calcarifer* (Bloch)) (119; 126); and juvenile and marketable-sized golden snapper (*Lutjanus johnii* (Bloch)) (81; 72). Most of the bacteria isolated from both healthy and diseased fishes were identified as *Vibrio* spp.

Five of 12 healthy (42%) and 69 of 87 diseased (79%) juvenile grouper were infected with bacteria. Seven of a total of 13 isolates (54%) from healthy grouper vs. 69 of 156 total isolates (58%) from diseased fish were identified as vibrios.

For juvenile seabass, 45 of 119 (38%) healthy and 102 of 126 (81%) diseased fish were infected. Forty-one of 109 total isolates (38%) from healthy seabass vs. 156 of 257 total isolates (61%) from diseased seabass were identified as vibrios.

Thirty-four of 81 (42%) healthy marketable-sized and 55 of 72 (76%) diseased juvenile snapper were infected. Of the 65 isolates from healthy snapper, 39 (62%) were vibrios, as were 71 of 96 total isolates (74%) from diseased fish.

The species of vibrios obtained from the healthy fishes differed from those occurring in diseased fishes. Diseased fishes harbored predominately Group I vibrios: grouper (42%), seabass (56%) and snapper (62%), whereas fewer Group I vibrios were found in healthy seabass (12%) and snapper (3%) and none were isolated from grouper. Healthy fishes harbored a more heterogeneous group of vibrios, which belonged predominantly to Groups IV and V.

Introduction

Three species of marine finfishes, greasy grouper (*Epinephelus malabaricus* (Bloch and Schneider)), silver seabass (*Lates calcarifer* (Bloch)) and golden snapper (*Lutjanus johni* (Bloch)), are commonly cultured in floating cages in the vicinity of Penang, Malaysia.

The fry of these species are either collected locally or are imported at high cost from neighbouring countries like Thailand and the Philippines for stocking into the cages. Since the introduction of this method of fish culture in Malaysia in 1973, the development of the industry has been hampered by many disease outbreaks, due mainly to farm mismanagement (e.g., fouling of net cages; rough handling of fish during sorting). Quite often, poor water quality at the culture sites also contributes to these outbreaks. These two factors have created favorable environments for multiplication of pathogens and their spread within and between farms.

Bacteria are suspected to be the main cause of high mortality of these cage-cultured fishes (Chua and Teng 1980; Wong and Leong 1987). However, there have been very few systematic studies to establish the identities of bacterial pathogens and their prevalences in either healthy (symptomless) or diseased fishes. This paper presents the results of part of our efforts to provide this information.

Materials and Methods

Normal healthy and moribund fishes were obtained from cage culture sites in Penang and Perak, West Malaysia. The body surface was swabbed with 70% ETOH after removing the scales. The abdominal cavity was aseptically opened and tissues taken from the anterior kidney and spleen were inoculated onto five culture media as previously reported (see Wong and Leong 1986). Plates were incubated at room temperature ($30^\circ \pm 2^\circ\text{C}$) for 24-48 hours and colonies of various morphology were selected and transferred to tryptic soy agar (TSA) slants for further testing.

Isolates were examined for Gram's reaction, Huge-Leifson test, oxidase test, catalase test, motility, sensitivity to vibriostatic agent (0/129) at 10 μg and 150 μg and novobiocin at 10 μg , MR-VP test, salt tolerance test (0%, 3%, 6%, 8% and 10% NaCl), decarboxylation of amino acids (L-arginine, L-lysine and L-ornithine), gelatine liquefaction, nitrate reduction, and growth at 42°C according to the methods described by West and Colwell (1984). Keys for the identification of gram-negative aerobic bacteria as given by Buchanan and Gibbons (1974) were used in the initial identification, while further identification of bacteria within the family of Vibrionaceae was done according to the scheme described by West and Colwell (1984) with slight modification. Vibrios were grouped into six groups

with respect to the decarboxylation of amino acids (L-ornithine, L-lysine and L-arginine): Group I vibrios ($O^+ L^+ A^-$) include *V. parahaemolyticus*, *V. alginolyticus*, *V. harveyi* and *V. vulnificus*; Group II vibrios ($O^- L^+ A^-$) include *V. campbellii*, *V. fischeri*, *V. logei* and *V. marinus*; Group III vibrios ($O^- L^- A^-$) include *V. anguillarum* II, *V. natriegens*, *V. nigripulchritudo*, *V. pelagius* I and II and *V. splendidus* II; Group IV vibrios ($O^- L^- A^+$) include *V. anguillarum* I, *V. costicola*, *V. fluvialis*, *V. nereis* and *V. splendidus* I; Group V vibrios include those showing $O^- L^+ A^+$ reaction, which are not included in the scheme of West and Colwell (1984); and Group VI vibrios include those *Vibrio* isolates that do not conform to the above reactions. Further identification of individual isolates to species level is still in progress.

Results

A total of 119 healthy (symptomless) and 126 diseased juvenile seabass showing fin and tail rot and body lesions were examined for bacterial infection. Forty-five of the healthy fish (38%) were infected, while 102 of the diseased seabass juveniles (81%), which showed a very much higher prevalence of infection, were infected. Forty-one out of a total of 109 isolates (38%) obtained from healthy juvenile seabass and 156 of 257 isolates (61%) obtained from the diseased fish were identified as vibrios, indicating a higher prevalence of *Vibrio* infection in diseased than healthy juveniles. A detailed study of the types of vibrios infecting the fish showed that healthy juvenile seabass were infected by a heterogeneous group of vibrios, with the highest prevalence of vibrios coming from Group IV (22%). However, diseased juveniles were infected mainly by Group I vibrios (56%), as indicated in Table 1.

Table 1 also shows the prevalences of infection and frequencies of *Vibrio* infections in healthy and diseased juvenile grouper. Five out of 12 (42%) healthy and 69 of 87 (79%) diseased juveniles were infected, indicating a higher prevalence of bacterial infection in diseased than in healthy grouper, as was the case for seabass. However, the numbers of isolates identified as vibrios did not differ much between the healthy and diseased juvenile grouper, which showed 54% and 58% *Vibrio* infection, respectively. Detailed analysis of the types of *Vibrio* infection indicated that healthy grouper harbored Group II, Group IV and Group VI vibrios, while diseased

Table 1. Prevalence of *Vibrio* infection in healthy and diseased silver seabass, greasy grouper and golden snapper.

	Seabass			Grouper			Snapper	
	Healthy	Diseased	Healthy	Diseased	Healthy	Diseased		
No. of fish infected/	46/119 (38%)	102/126 (81%)	5/12 (42%)	69/87 (79%)	34/81 (42%)	55/72 (76%)		
No. examined								
No. of <i>Vibrio</i> isolates/	41/109 (38%)	158/257 (61%)	7/13 (54%)	91/156 (68%)	39/65 (62%)	71/86 (74%)		
Total isolates								
Type of <i>Vibrio</i> isolates:								
Total <i>Vibrio</i> isolates:								
Group I	5/41 (12%)	87/156 (58%)	0/7 (0%)	39/91 (43%)	1/39 (3%)	44/71 (62%)		
Group II	3/41 (7%)	19/156 (12%)	3/7 (43%)	11/91 (12%)	0/39 (0%)	2/71 (3%)		
Group III	2/41 (5%)	9/156 (6%)	0/7 (0%)	11/91 (12%)	6/39 (15%)	5/71 (7%)		
Group IV	9/41 (22%)	10/156 (6%)	0/7 (0%)	2/91 (2%)	15/39 (38%)	7/71 (10%)		
Group V	5/41 (12%)	8/156 (5%)	3/7 (43%)	8/91 (9%)	4/39 (10%)	2/71 (3%)		
Group VI (Other vibrios)	17/41 (41%)	23/156 (15%)	1/7 (14%)	21/91 (23%)	12/39 (33%)	11/71 (15%)		

juveniles were mainly infected by Group I vibrios (42%), as was the case for diseased juvenile seabass.

A study of healthy marketable-sized snapper (600-1,000 g) and diseased juvenile snapper (10-20 g) showing fin and tail rot and body lesions also showed lower prevalence of bacterial infection in healthy than in diseased fish (42% vs 76%), as shown in Table 1. Thirty-four of 81 (42%) healthy fish and 55 of 72 (76%) diseased snapper were infected. The prevalence of *Vibrio* infection was also slightly higher in the diseased fish. Seventy-one of 96 isolates (74%) from the diseased fish and 39 of 65 isolates (62%) from healthy fish were identified as vibrios. Detailed analysis of the types of *Vibrio* infection showed a slightly higher prevalence of Group IV *Vibrio* infection (38%) in the healthy fish, while diseased snapper were mainly infected by Group I vibrios (62%).

Discussion

The results of this study show that the prevalences of bacterial infection in diseased fishes, whether juveniles or larger-size fish, were much higher than those found in healthy fishes for all three species of marine finfishes studied. This is in accord with the study of Ojala (1968), who showed 83% of diseased and 49% of healthy freshwater fishes infected with bacteria. Chung and Kou (1973, 1974) also reported lower prevalence of bacterial infection (42%) in healthy as compared with diseased (96.3%) Japanese eels (*Anguilla japonica* (Temminck and Schlegel)). This is also in agreement with our early reports (Wong and Leong 1986) on the bacterial flora of seabass fry.

The high prevalences of *Vibrio* infection in both healthy and diseased seabass, grouper and snapper are also in agreement with our previous findings for seabass fry, where we reported a higher prevalence of *Vibrio* infection in diseased fish (Wong and Leong 1986). However, differences in the prevalence of *Vibrio* infection between healthy and diseased juvenile grouper (54% vs 58%) and healthy and diseased snapper (62% vs. 74%) are not so distinctive as that seen in juvenile seabass (38% vs. 61%). This may be due to the nature of the fishes studied, as juvenile seabass are normally more homogeneous as they are produced in hatcheries, while grouper and snapper are collected from the wild and are more genetically heterogeneous. The high prevalence of vibrios in the three species of fishes studied probably reflects the environmental conditions of the

cage culture. In the tropics these facilities are located mainly in estuaries or coastal waters where pollution has favored the growth of vibrios (see Yap 1980; Ong 1984).

A comparison of the types of vibrios infecting the three species of cultured marine finfishes reveals that healthy fishes are normally infected with a heterogeneous assemblage of vibrios, with slightly higher prevalences of infection for Groups IV and V. On the other hand, for all three species studied, diseased fish had much higher prevalences of Group I vibrios (42-62%), which includes the species *V. parahaemolyticus* and *V. alginolyticus*, considered to be pathogenic to fish in this region. Our study therefore supports the view that *V. parahaemolyticus* and *V. alginolyticus* are probably the cause of many of the disease outbreaks which occur in cage farms in Southeast Asia (see, for example, Wong et al. 1979 and Chong et al. 1983). The higher prevalences of Group I vibrios in the diseased fishes may be related to their ability to invade the tissues of the host when the host is under stress, in contrast to the other groups of vibrios, which may not have the ability to do so. It will therefore be interesting to compare the virulence of the Groups IV and V vibrios isolated from healthy fishes with that of the Group I vibrios obtained from diseased fishes. This work is now in progress in our laboratory.

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