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editormvzcordoba@gmail.com
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Albuquerque C, Renata; S, Giselle Cristina; Lima A, Rayza; RC, Edirsana Maria; SFV, Regine Helena
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ORIGINAL

Microbiota of *Vibrio* sp. in the hepatopancreas of cultured white pacific shrimp (*Litopenaeus vannamei*)

Vibrio sp., microbiota en el hepatopáncreas del camarón blanco del Pacífico (*Litopenaeus vannamei*)

Renata Albuquerque C,[1,2*] Ph.D, Giselle Cristina S,[2] M.Sc, Rayza Lima A,[1] B.Sc, Edirsana Maria RC,[2] M.Sc, Regine Helena SFV,[1,2] Ph.D.

[1]Federal University of Ceará, Fish Engineering Department. Fortaleza-Ceará, Brazil. [2]Federal University of Ceará, Sea Sciences Institute. Fortaleza-Ceará, Brazil. *Corresponding author: renata.albuq@gmail.com

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ABSTRACT

Objective. The present study aimed to investigate the presence of vibrios in the hepatopancreas of cultured shrimp. **Materials and methods**. Vibrios from the hepatopancreas of fifteen samples of five specimens each, of apparently healthy Pacific white shrimp (*Litopenaeus vannamei*) were isolated, identified and quantified. **Results**. The vibrio density ranged from 430 to 2,400 MPN g^{-1} (r_s MPN cm^{-1}=-0.114; r_s MPN g^{-1} = 0.211). Thirty isolations were obtained, most of which belonged to the species *V. cholerae* (n=11) and *V. parahaemolyticus* (n=7). **Conclusions**. The outcomes of the present study suggest that, even in the absence of symptoms of vibriosis, the microbiota of the hepatopancreas of cultured shrimp may include sucrose positive and negative vibrios.

Key words: Hepatopancreas, *Vibrio*, white shrimp (*Source:OED*).

RESUMEN

Objetivo. El presente estudio tuvo como objetivo investigar la presencia de vibrios en el hepatopáncreas del camarón de cultivo. **Material y métodos**. En este estudio, fueron aislados, identificados y cuantificados los vibrios del hepatopáncreas de 75 camarones blancos del Pacífico (*Litopenaeus vannamei*), aparentemente sanos, oriundos de un cultivo en la región nordeste de Brasil. Quince muestras, cada una consta de cinco camarones, se pusieron a prueba. **Resultados**. La densidad de *Vibrio* varió de 430 a 2.400 NMP g^{-1} (r_s NMP cm^{-1} = -0.114; r_s NMP g^{-1} = 0.211). Treinta aislamientos fueron obtenidos, la mayoría de los cuales pertenecían a la especie *V. cholerae* (n=11) y *V. parahaemolyticus* (n=7). **Conclusiones**. Los hallazgos del presente estudio sugieren que, incluso en ausencia de síntomas de la vibriosis, la microbiota de lo hepatopáncreas del camarón de cultivo puede incluir vibrios sacarosa positivos y negativos.

Palabras clave: Camarón blanco, hepatopáncreas, *Vibrio* (*Fuente:OED*).

INTRODUCTION

Vibrios are ubiquitous in marine environments (1), and are part of the natural microbiota of aquatic invertebrates. They may colonize the exoskeleton, gills and intestines of penaeids (2), the hemolymph of crustaceans (3) and the hepatopancreas of shrimp (4).

Vibrio colonization of the digestive tract of aquatic organisms can be beneficial to the host. Sawabe et al (5) suggests that the populations of *V. halioticoli* present in the intestines of different species of abalone contribute to nourish the host by fermenting algal polysaccharides, converting alginic acid into acetic acid.

However, because *vibrio* are part of the indigenous microbiota of both marine invertebrates and their environment, and because of the opportunistic character of infections caused by vibrios (6) they represent a permanent potential source of infection for livestock.

According to Sung et al (7), the establishment of vibriosis in cultured shrimp is associated with increased levels of pathogenic vibrios in the environment, though not necessarily for the total vibrio population. *V. harveyi*, one of the species most frequently implicated in vibriosis, is known to cause severe infections in penaeid livestock (8).

Vibriosis in shrimp farms has also been shown to be related to the vibrio density in the animals' hepatopancreas. In an analysis of the hepatopancreas and hemolymph of infected shrimp, Soto Rodriguez et al (9) estimated the vibrio density by inducing vibriosis, but vibrio levels have not yet been established for healthy shrimp. Thus, the objective of the present study was to evaluate the vibrio sp., microbiota of apparently healthy shrimp farmed in Ceará (Northeastern Brazil) by isolating, identifying and quantifying (MPN) vibrios found in the hepatopancreas.

MATERIAL AND METHODS

Sampling. The study included 75 adult shrimp (*Litopenaeus vannamei*) cultured at the Center for Research on Coastal Environments (CEAC/Labomar/UFC) in the state of Ceará, Northeastern Brazil. Sampling was done in May 2009, which corresponds to the rainy season. The water quality conditions measured were: temperature (29°C), pH (6.58) and salinity (15%). The shrimp farmed in tanks were collected with nets and transported live in 30-L containers with culture water. The time from collection to bacteriological analysis did not exceed two hours.

Sample preparation. Thermal shock was used as euthanasia technique by immersion in a mixture of water and ice (6:1) during 10 minutes. The shrimp were washed with 70% (v/v) alcohol and weighed. Then the carapace was opened and the hepatopancreas (HP) was removed with sterilized tweezers. Each sample consisted of HP from five specimens (total: 15 samples) diluted in 0.85% (w/v) saline in a proportion of 1:9 (w/v). Serial decimal dilutions were prepared from 10^{-1} to 10^{-4}.

Quantification and isolation of vibrios. Vibrios were quantified with the MPN method (MNP.g^{-1}), following the recommendations of Kaysner and DePaola Jr (10). Presumptive tests (PT) were performed with 1 ml of each dilution inoculated in alkaline peptone water (pH 8.5) and incubated at 35°C for 24 hours. For the confirmatory test, aliquots from positive PT tubes were inoculated, spread-plated in duplicates, in thiosulfate citrate bile sucrose (TCBS) agar and incubated at 35°C for 18 hours. The MPN of vibrios was calculated from the critical series resulting from the combination of PT-positive tubes and sucrose-positive and/or negative colonies in TCBS. The corresponding value was read from the table cited by Blodgett (11), multiplied by the average dilution and divided by 100. Results were expressed in MPN g^{-1}. For the purpose of isolation, two sucrose-positive or negative colonies were selected from each sample and seeded in trypticase soy agar (TSA) containing 1% (w/v) NaCl.

Identification of vibrios. Pure colonies isolated in TSA (1% w/v NaCl) were submitted to biochemical identification (12) and morphotype characterization by Gram staining.

Statistical analysis. The relation between variation in MPN g^{-1} and average sample weight and size was tested with Spearman's correlation coefficient (r_s).

RESULTS

Vibrio density ranged from 430–2,400 MPN g^{-1} (Table 1), and the higher loads were those determined in the samples with the larger specimens (average weight: 11.7 and 11.4 g). The r_s MPN cm^{-1} was -0.114 and r_s MPN g^{-1} was 0.211 (Table 1).

Out of the five species identified, the most frequent was *V. cholerae* (n=11; 36.7%), followed by *V. parahaemolyticus* (n =7; 23.3%), *V. neptunius* (n= 5; 16.7%), *V. xuii* (n=4; 3.3%) and *V. coralliilyticus* (n= 3; 10.0%).

Table 1. Most probable number (MPN) of vibrios in the hepatopancreas of shrimp (*Litopenaeus vannamei*) cultured at the Center for Research on Coastal Environments (CEAC/Labomar/UFC). State of Ceará, Northeastern Brazil.

Sample	Average size (cm)	Average weight (g)	MPN g^{-1}
1	9.3	7.8	930
2	10.4	11.7	2.400
3	9.1	8.1	930
4	9.7	8.3	930
5	8.7	7.7	430
6	9.2	8.4	930
7	8.7	8.6	930
8	10.7	7.8	430
9	9.3	11.4	2.400
10	9.5	8.2	930
11	9.2	7.9	930
12	9.0	6.2	430
13	9.4	7.3	430
14	9.2	7.2	430
15	9.3	7.5	430
Average/S	9.4±0.5	8.3±1.5	926±644.8
r_s (MPN cm^{-1})		-0.114	
r_s (MPN g^{-1})		0.211	
(r_s MPN g^{-1} 0.211)			

*S: standard deviation. r_s: Spearman's correlation coefficient

DISCUSSION

There was no significant correlation between morphometric parameters (size and weight shrimp) and quantification of *Vibrio* (r_s MPN cm^{-1}=-0.114; r_s MPN g^{-1}=0.211) In the present study. The relationship between weight and microbiota of *Vibrio* in shrimp hepatopancreas has been reported by Soto Rodríguez et al (9). In Shrimp Diseases, the authors cited found that vibrio density in hepatopancreas individuals weighing 8–12 g had a greater bacterial loads than individuals weighing 0.26–4.0 g.

The observation of vibrios in the HP of healthy shrimp (Table 1) confirms the findings of Gomez-Gil et al (4) who studied the natural microbiota in penaeid shrimp with a mean weight of 10.66 g, and concluded that a large variety of vibrios in the HP of healthy shrimps are not necessarily an indication of disease. The authors added that the tissues of *L. vannamei* display a diversified microbiota, including saccharose-fermenting bacteria in the intestinal tract.

Leãnos et al (13) found similar total vibrio loads in healthy and infected shrimp during a complete 60-day culture cycle. However, sick shrimp presented an increase in luminescent vibrios suggesting that infection involves the multiplication of a specific population of pathogens. Considering that the HP of healthy shrimp may be colonized by vibrios, the authors proposed a safety level of 10^4 CFU/HP for the prevention of disease outbreaks during the first 30 days of culture.

The bacterial load in shrimp HP is often quantified by standard plate count (7,13). In contrast, the method used in this study (MPN) does not provide a direct bacterial count, but determines the density of viable microorganisms in the sample and is therefore indicated for samples with an expected bacterial concentration of less than 10 UFC g^{-1} (14). Thus, the mean vibrio density ($9.26x10^2$ MPN g^{-1}) observed in the present study is not comparable to the indexes (1.30×10^4 and 10^5 CFU g^{-1}) reported by Gomez-Gil et al (4) for vibrios in healthy *L. vannamei.* The lack of comparability makes it difficult to say whether our MPN g^{-1} values are high or low.

The presence of *V. cholerae* in the HP of apparently healthy penaeids matches results published by Suzita et al (15). According to the latter, *V. cholerae* occurs in salt and brackish water, freely or associated with zooplankton and algae, and may adhere strongly to the digestive tract of marine organisms.

In contrast with these findings, Sung et al (16) failed to isolate *V. parahaemolyticus* from the HP of asymptomatic shrimp. In any case, colonization by *V. parahaemolyticus* of the HP of apparently healthy shrimp represents a potential hazard.

Even if *V. cholerae* and *V. parahaemolyticus* (represented in this study by 60% of the isolates) were parts of the indigenous microbiota of shrimp HP, both species have been implicated in vibriosis outbreaks on shrimp farms (17). Aside from risk to shrimp culture, the presence of V. choleare and V. parahaemolyticus in seafood have been associated with food borne diseases (18).

On the other hand, vibrio colonization of the HP is not the only possible source of vibriosis in cultured shrimp. Vibrios can penetrate the epithelium and shrimp tissues may be colonized in several different ways, such as via contaminated food or carapace injury (19).

The other vibrios identified in this study, such as *V. neptunius* and *V. xuii*, were first described by Thompson et al (20) based on isolates from aquaculture environment (bivalves, fish, rotifers and shrimp). Likewise, the species *V. coralliilyticus* was described only recently by Ben-Haim et al (21) based on strains isolated from infected coral (*Pocillopora damicornis*) and larvae of *Crassostrea gigas* and *Nodipecten nodosus*. Since some of the

strains used to describe *V. neptunius* and *V. xuii* have also been isolated from marine organisms occurring or farmed in Northeastern Brazil, the presence of these vibrio species in the HP of *L. vannamei* cultured in Ceará should come as no surprise.

The findings of the present study suggest that, even in the absence of symptoms of vibriosis, the microbiota of the hepatopancreas of cultured shrimp may include sucrose-positive and negative vibrios.

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