CHITINOCLASTIC BACTERIA ASSOCIATED WITH SHELL DISEASE IN Penaeus SHRIMP AND THE BLUE CRAB (Callinectes sapidus) *

Authors: COOK, DAVID W., and LOFTON, SANDRA R.

Source: Journal of Wildlife Diseases, 9(2) : 154-159

Published By: Wildlife Disease Association

URL: https://doi.org/10.7589/0090-3558-9.2.154

CHITINOCLASTIC BACTERIA ASSOCIATED WITH SHELL DISEASE IN *Penaeus* SHRIMP AND THE BLUE CRAB *(Callinectes sapidus)**

DAVID W. COOK and SANDRA R. LOFTON, Microbiology Section, Gulf Coast Research Laboratory, Ocean Springs, Mississippi, 39564, U.S.A.

Abstract: The occurrence of shell disease in three species of penaeid shrimp is reported. Chitinoclastic bacteria isolated from lesions on these shrimp and from lesions on the blue crab were classified as members of the genera *Beneckea, Vibrio,* and *Pseudomonas.* One type of *Beneckea* was present in all cases of shell disease encountered, making this organism suspect of being the causative agent.

INTRODUCTION

Shell disease, burned spot disease and rust disease all refer to necrotic lesions in the exoskeleton of crustaceans. Commercially important marine crustaceans reported to be infected by this disease are the lobster *(Homarus americanus),* the blue crab *(Callinectes sapidus)* and the king crab *(Paralithodes camtschatica* and *Paralithodes platypus).*[1,3]

The causative agent of shell disease in marine crustaceans has not been conclusively established, although chitinoclastic bacteria are suspected, being frequently isolated from necrotic material. The only attempt to classify these chitinoclastic bacteria was made by Hess,[4] who indicated that his isolates from lobster were similar to *Bacillus chitinovorous (Beneckea chitinovora*[2]*).*

Chitinoclastic bacteria are ubiquitous to the marine environment and are a normal part of the flora on marine crustaceans. Therefore, it appears that mechanical damage to the shell is required to provide a portal of entry for the infecting bacteria. Rosen[6] reported that shell degredation in crabs was limited to the exocuticle and the calcified endocuticle. The infecting bacteria did not penetrate into the soft tissue underlying the shell and infected specimens overcame the disease by molting.

In natural populations of crustaceans, the incidence of shell disease is very low. When crustaceans are crowded together in holding pens or shedding boxes, the incidence of the disease increases; probably in response to the increased incidence of mechanical damage. Foreseeable problems may, therefore, be expected to occur when rearing crustaceans in high densities. As Rosen[7] pointed out, additional studies are needed to determine "the identity of the groups of organisms found in the lesions, their inter-relationship and their exact role in the disease." This information may help provide better prophylactic or even therapeutic treatment.

On several occasions the senior author has collected crabs and shrimps from trawl samples and has received specimens of shimp from other research institutions which had soft brown necrotic areas on the exoskeleton. The findings reported here describe the chitinoclastic bacteria from lesions on these specimens and attemps to experimentally infect crabs with shell disease.

MATERIALS AND METHODS

The source of specimens used in this study was as follows: white shrimp, *(Penaeus setiferus),* laboratory reared from the postlarval stage at the Gulf

*This study was conducted in cooperation with the Department of Commerce, NOAA, National Marine Fisheries Service, under Public Law 88-309, Project No. 2-85-R.

Coast Research Laboratory; white shrimp, reared in ponds at the Texas Parks and Wildlife Department, Marine Fisheries Research Station, Palacios, Texas; white shrimp, reared in ponds at the Louisiana Wild Life and Fisheries Commission, Marine Biological Laboratory, Grand Terre Island, Louisiana; white shrimp collected from Biloxi Bay, Mississippi; pink shrimp *(Penaeus duoraum)* held in tanks at School of Marine and Atmosperic Sciences, Miami, Florida; brown shrimp *(Penaeus aztecus)* collected from Biloxi Bay, Mississippi; blue crab *(Callinectes sapidus)* collected from Biloxi Bay, Mississippi; blue crab collected near Grand Terre Island, Louisiana; and blue crab from holding tanks at the Gulf Coast Research Laboratory.

Isolation of chitinoclastic bacteria was accomplished by removing a small portion of the diseased shell with a sterile knife and streaking this material onto plates of Difco-marine agar (MA) supplemented with precipitated chitin.[9] With non-diseased shrimp, a segment of the shell was removed with forceps and placed in an enrichment medium of Difco-marine broth and precepitated chitin. After 5 days of incubation, the enrichments were streaked onto plates of the MA-chitin medium. Chitinase-positive colonies, as indicated by clearing of the chitin from the medium around the colony, were restreaked for purity and then maintained on MA slants until they could be identified.

Isolates were identified using standard bacteriological methods.[9,10] Bacteriological culture media were prepared with 75% aged sea water or by using MA as the base for solid media. Requirements for NaCl by the bacteria were determined by comparing growth on a 0.5% peptone-0.3% yeast extract medium prepared with and without 2.5% NaCl. Flagella stains were made on young, agar-grown cultures using Difco-flagella stain and the method of Leifson.[5] Sensitivity tests were performed by placing antibiotic sensitivity discs (COLAB) and a few crystals of the vibriostat compound 0/129 upon the surface of a MA plate inoculated with the test bacterium. Clear zones around discs or crystals of vibriostat 0/129 after 24 hr. incubation were considered as positive results. Identification procedures were carried out at 25 C.

The bacteria were classified according to Bergey's Manual[2] and Baumann.[1]

In experimental infection studies with crabs, the shell was mechanically damaged by rasping small patches on the ventral side of the carapace with a file. Disinfection of the crab shell and the file with 70% alcohol was performed prior to rasping and care was taken not to penetrate the shell. Areas under study along with undamaged control areas were swabbed with a pure culture of the test bacterium. Damaged control areas were not inoculated with the bacteria. All crabs were held in the same circulating sea water system[4] at about 25 C.

RESULTS

Typical necrotic lesions observed on crabs and shrimp are shown in Figures 1 and 2. Microscopic examination of material from these lesions indicated the presence of numerous motile, rod-shaped bacteria. Fungi were observed in only a few samples from badly decomposed areas on the crab exoskeleton.

Chitinoclastic bacteria were isolated from the necrotic lesions on all the crabs and shrimp examined. Morphological, physiological and biochemical tests (Table 1) were used to classify these bacteria as members of the genera *Beneckea, Vibrio* and *Pseudomonas.* More specific identification was not attempted.

Beneckea type I was isolated from all diseased and non-diseased specimens of crabs and shrimp examined (Table 2). These specimens were obtained from four states and included wild specimens, pond reared specimens and laboratory reared specimens. Three species of *Penaeus* shrimp and one species of crab was involved.

Attempts to experimentally produce the shell disease in crabs met with mixed results. In all cases where the exoskeleton was mechanically damaged, necrosis was observed after a few weeks. This included both the areas inoculated with *Beneckea* type I and the uninoculated control

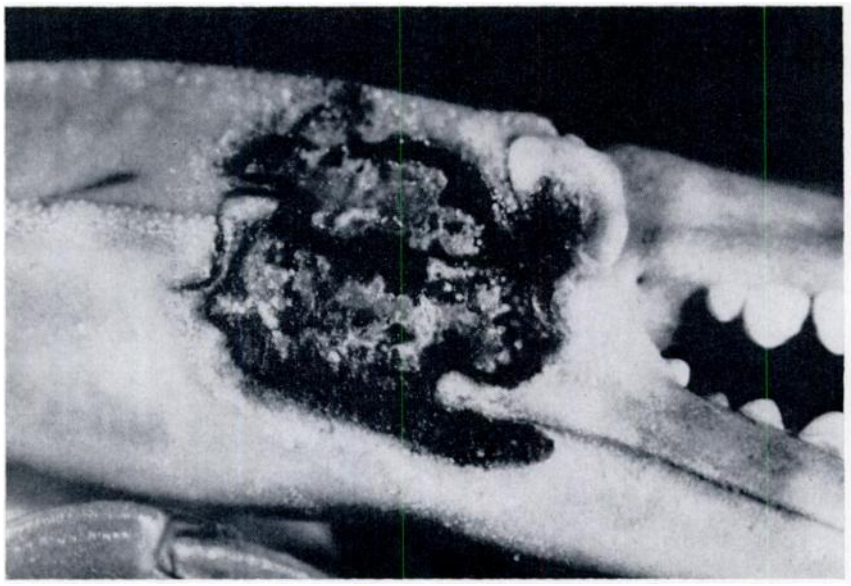

FIGURE 1. Necrotic lesion on the claw of a blue crab.

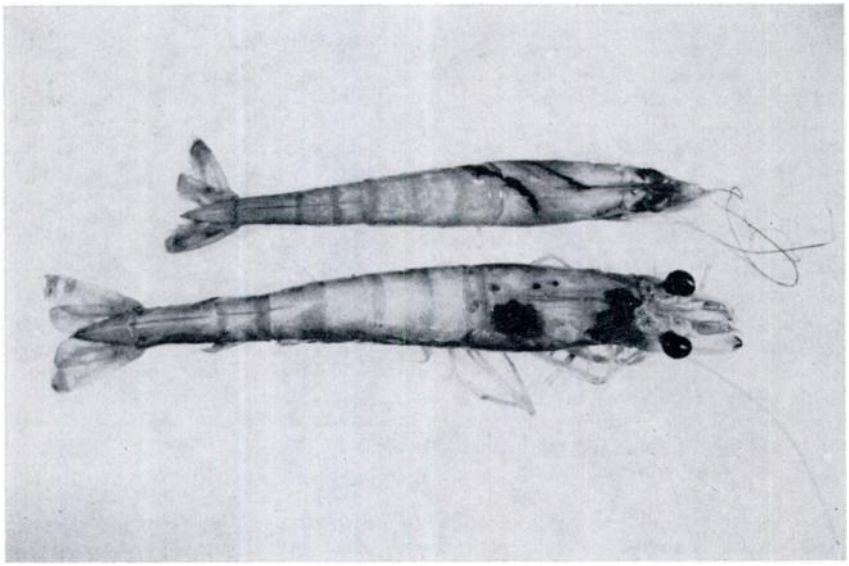

FIGURE 2. Necrotic lesions on the exoskeleton of brown shrimp.

areas as shown in Figure 3. Areas not mechanically damaged, but inoculated, failed to develop necrotic lesions. *Beneckea* type I was isolated from the necrotic areas on all crabs.

DISCUSSION

This report adds another group of crustaceans, the penaeid shrimp, to the list of marine *Crustacea* susceptible to shell disease.

TABLE 1. Characteristics of Chitinoclastic Bacteria Isolated from Shrimp and Crabs.

	Beneckea Type					*Vibrio* Type			*Pseudomonas*
	I	II	III	IV	V	I	II	III	
Morphology	Rod	Rod	Rod	Rod	Rod	Rod	Rod	Rod	Rod
Gram Reaction	—	—	—	—	—	—	—	—	—
Motility	+	+	+	+	+	+	+	+	+
Flagellation	Per	Per	Per	Per	Per	Pol	Pol	Pol	Pol
Pigment	—	—	—	—	—	—	—	—	Yel
Oxidase Reaction	+	+	+	+	+	+	+	+	+
Catalase Reaction	+	+	+	+	+	+	+	+	+
Attact on Glucose	F	F	F	F	F	F	F	F	—
Chitin Hydrolysis	+	+	+	+	+	+	+	+	+
Agar Hydrolysis	—	—	—	—	—	—	—	—	—
Starch Hydrolysis	+	+	+	+	+	+	+	+	+
Gelatin Hydrolysis	+	+	+	+	+	+	+	+	+
Lipid Hydrolysis	+	+	+	+	—	+	—	—	+
Indol	+	—	+	—	—	+	+	—	—
Methyl Red	+	+	+	+	—	+	+	+	—
Voges—Proskauer	—	—	—	—	—	—	—	—	—
Simmons Citrate	+	+	+	+	—	+	+	—	+
Nitrate Reduction	+	+	+	+	+	+	+	+	—
NH_3 from Peptone	+	+	+	+	+	+	+	+	+
H_2S Production	+	+	—	—	—	+	+	+	—
Hemolysis of RBC	+	+	+	+	+	+	+	+	+
Acid produced from									
Sucrose	14	+	—	+	+	+	+	—	—
Glycerol	+	+	+	+	+	+	+	—	—
Lactose	—	—	—	—	—	—	—	—	—
Glucose	+	+	+	+	+	+	+	+	—
Added salt required for growth	+	+	+	—	+	—	+	+	+
Sensitivity test									
Vibriostat 0/129	20	—	—	—	+	+	+	+	—
Chloramphenicol 30 mcg	+	+	+	+	+	+	+	+	+
Penicillin-G 10 Units	2	—	—	—	+	—	+	+	
Dihydrostreptomycin 10 mcg	24	+	+	+	—	+	+	+	+
Tetracycline 30 mcg	21	+	+	+	+	+	+	+	
Number of cultures tested	28	2	1	1	1	3	3	1	2

+ All positive; — all negative; numbers indicate positive results if less than all; Per. — pertrichous flagellation; Pol. — polar flagellation; Yel. — yellow; F — fermentative.

TABLE 2. Chitinoclastic Bacteria Isolated from Shrimp and Crabs.

Source	Number Specimens Examined	Types of Bacteria Isolated	
DISEASED SHRIMP			
Pink Shrimp, School of Marine and Atmospheric Sciences	1	*Beneckea* type I	(1)*
		Pseudomonas	(1)
White Shrimp, Texas Parks and Wildlife Department	1	*Beneckea* type I	(1)
Brown Shrimp, Biloxi Bay, Miss.	2	*Beneckea* type I	(2)
White Shrimp, Gulf Coast Research Laboratory	4	*Beneckea* type I	(9)
		Beneckea type V	(1)
DISEASED CRABS			
Blue Crab, Grand Terre Island, La.	1	*Beneckea* type I	(1)
Blue Crab, Biloxi Bay, Miss.	2	*Beneckea* type I	(2)
		Vibrio type III	(1)
Blue Crab, Gulf Coast Research Laboratory Holding tanks	3	*Beneckea* type I	(3)
		Vibrio type I	(3)
		Vibrio type II	(3)
NON DISEASED SHRIMP			
White Shrimp, Biloxi Bay, Miss.	1	*Beneckea* type I	(2)
White Shrimp, Louisiana Wild Life and Fisheries Shrimp Ponds	7	*Beneckea* type I	(7)
		Beneckea type III	(1)
		Beneckea type IV	(1)
		Beneckea type II	(2)
		Pseudomonas	(1)

* Number of cultures isolated from each source.

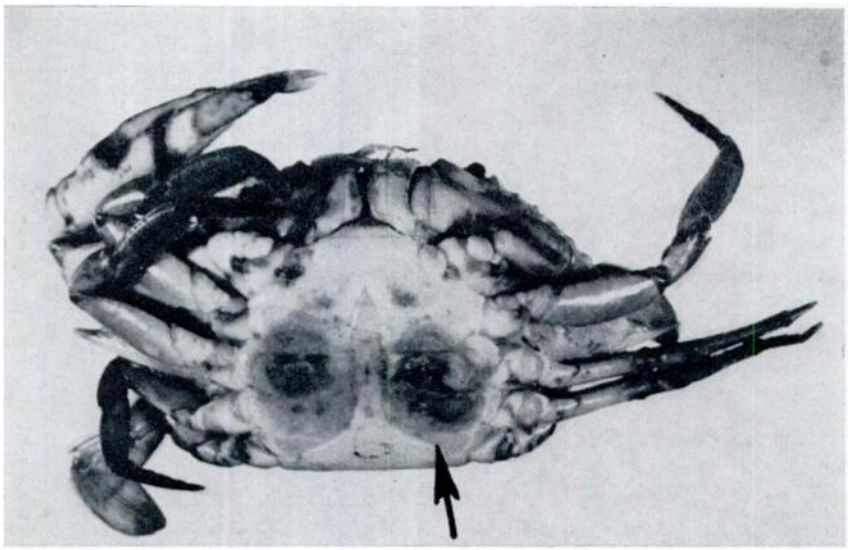

FIGURE 3. Shell disease induced by mechanical damage to the exoskeleton of a blue crab. Arrow indicates area inoculated with chitinoclastic bacteria.

The presence of chitinoclastic bacteria in necrotic areas on the exoskeleton of both *Penaeus* shrimp and blue crab suggests that bacteria are responsible for the shell disease of these crustaceans. The presence of fungi in only the old, badly deteriorated areas indicated that they were secondary invaders. These results are in agreement with Rosen[6] who did not find fungi associated with shell disease in crabs.

The isolation of a bacterium designated as *Beneckea* type I from all cases of shell disease encountered makes this organism suspect of being the causative agent. Chitinoclastic bacteria other than *Beneckea* type I were found in some of the cases examined, but *Beneckea* type I was always present. No attempts were made to identify any bacteria other than the chitinoclastic bacteria from the necrotic lesions because the possibility seemed unlikely that they would be active in this disease for two reasons. First, the shell disease involves the degradation of chitin and only bacteria with the chitinase enzyme are known to degrade chitin. Second, members of the genus *Beneckea* do not require accessory growth factors and are quite active biochemically, therefore, symbiotic relationships with other bacteria would probably not be required to produce growth factors for the *Beneckea* or to degrade organic material other than the chitin within the shell.

Although the experimental infection studies were inconclusive, it was noted that only mechanically damaged areas on the crab shell became necrotic. Therefore, if the *Beneckea* type I isolated from these areas was responsible for the necrosis, it appears that this bacterium does not have the ability to penetrate the epicuticle but functions as an opportunist when damage occurs. Future research, however, may provide isolates of more virulent strains with the ability to breach that defense barrier.

Acknowledgements

The authors wish to express their thanks to the following groups for providing them with specimens: Eco-Physiology Section, Gulf Coast Research Laboratory; Texas Parks and Wildlife Department, Marine Fisheries Research Station; Louisiana Wild Life and Fisheries Commission, Marine Biological Laboratory and the University of Miami, School of Marine and Atmospheric Science.

LITERATURE CITED

1. BAUMANN, PAUL, *et al.* 1971. Taxonomy of marine bacteria: The Genus *Beneckea.* Journal of Bacteriology. 107: 268-294.
2. BREED, R. S., E. G. D. MURRAY, and N. R. SMITH. 1957. *Bergey's Manual of Determinative Bacteriology,* 7th ed., Williams and Wilkins Co., Baltimore.
3. COOK, D. W. 1972. A circulating seawater system for experimental studies with crabs. The Progressive Fish-Culturist. 34: 61-62.
4. HESS, E. 1937. A shell disease in lobster *(Homarus americanus)* caused by chitinovorous bacteria. J. Biol. Board Canada. 3: 358-362.
5. LEIFSON, E. 1960. *Atlas of Bacterial Flagellation.* Academic Press Inc., New York.
6. ROSEN, BARUCH. 1967. Shell Disease of the Blue Crab, *Callinectes sapidus.* J. Invert. Pathol. 9: 348-353.
7. ROSEN, BARUCH. 1970. "Shell Disease of Aquatic Crustaceans" in A *Symposium on Diseases of Fishes and Shellfishes,* special publications No. 5, American Fisheries Society, Washington, D.C.
8. SINDERMANN, C. J., and AARON ROSENFIELD. 1967. Principal diseases of commercially important marine bivalve Mollusca and Crustacea. Fishery Bulletin. 66: 335-385.
9. SKERMAN, V. B. D. 1967. *A Guide to the Identification of the Genera of Bacteria.* Williams and Wilkins Co., Baltimore.
10. SOCIETY OF AMERICAN BACTERIOLOGIST. 1957. *Manual of Microbiological Methods.* McGraw-Hill Book Co., New York.

Received for publication November 14, 1972