

A Research Note

Hot Sauce: No Elimination of *Vibrio vulnificus* in Oysters

YI SUN and JAMES D. OLIVER*

Department of Biology, University of North Carolina at Charlotte, Charlotte, North Carolina 28223

(MS # 94-180, Received 2 August 1994/Accepted 9 November 1994)

ABSTRACT

Either Tabasco® sauce or a horseradish-based seafood cocktail sauce was placed on freshly shucked oysters which were incubated for 10 min on the half shell. Oysters were then assayed for numbers of *Vibrio vulnificus* cells present on the surface and within the oyster tissue, and the results compared to control oysters to which no sauce was added. Results indicated that Tabasco® sauce, but not the cocktail sauce, was highly effective in reducing the number of *V. vulnificus* cells present on the oyster meat surface. However, little reduction in the numbers of *V. vulnificus* cells present within the oysters was observed with either sauce. Our results suggest that hot sauces are not capable of significantly reducing the overall numbers of *V. vulnificus* cells associated with oysters, and that persons who are at risk for infection with this bacterium should continue to avoid the consumption of raw seafood, especially raw oysters.

Key words: *Vibrio vulnificus*, hot sauce, oysters

Vibrio vulnificus is an estuarine bacterium which is part of the normal microflora of oysters (3,4,7,8). The bacterium is of special concern due to its ability to rapidly produce fatal infections in individuals with certain underlying diseases (6). Ingestion of raw oysters is the major source of infection, and this single bacterium is responsible for 95% of seafood-borne deaths in the United States (1). In October of 1993, researchers at the Louisiana State University Medical Center in New Orleans announced in a press release that such "hot sauces" as Tabasco® sauce were able to kill cells of *V. vulnificus*. The present study was undertaken to investigate in more detail the bacteriocidal effects of "hot sauces" against *V. vulnificus* when present on the surface of, and within, raw oysters.

MATERIALS AND METHODS

Strain CVD713 of *V. vulnificus* was employed in these studies. This strain carries the transposon, *TnphoA*, which imparts kanamycin resistance to the cells. In addition, cleavage by the alkaline phosphatase encoded by the *TnphoA* of a chromogenic substrate, 5-bromo-4-chloro-3-indolyl phosphate (BCIP), present in the plating medium results in the production of brilliant blue colonies by this strain. The use of this substrate allows rapid and specific monitoring of the presence of this strain of *V. vulnificus*

in oysters (2,5). Cells were routinely grown in a marine broth overnight at room temperature to stationary phase.

Louisiana oysters, *Crassostrea virginica*, obtained from a local distributor, were housed in 55 gal tanks of artificial seawater (ASW). After acclimatization, cells of *V. vulnificus* were added to the tanks to a final density of ca. 8×10^4 CFU/ml, and oysters were allowed to take up the cells for 1 hour. Following uptake, oysters were removed from the tanks and their exteriors washed. Oysters (ca. 10 g each) were shucked, taking care not to disrupt the oyster tissue, and individually transferred to 50 ml sterile containers containing 10 ml ASW. After shaking, the ASW in each container was sampled to determine the number of *V. vulnificus* cells present on the exterior of each oyster. Oysters were then blended in ASW to release bacterial cells present within the oysters.

A second group of shucked oysters was left on the half-shell, and placed on ice. Sauce, either Tabasco® or horseradish-catsup-based, was placed over the oyster, and was left for 10 min before being removed by sterile ASW rinsing. Individual oysters were then placed into 50 ml containers and sampled for both external and internal microbial flora, as described above.

Numbers of *V. vulnificus* cells were obtained by plating on a kanamycin- and BCIP-containing medium which is highly selective for strain CVD713 (2,5). Total numbers of bacteria were estimated on heart infusion agar. In all cases, 4-5 oysters were individually examined for each sample point, and the data shown here represent the average of these replicates. Results were statistically analyzed using the unpaired *t* test.

RESULTS

Levels of bacteria present on the meat surfaces of the oysters were examined (Table 1). Untreated (control) oysters had levels of both *V. vulnificus* and total bacteria typical of those observed in summer months. Oysters to which the horseradish-catsup-based cocktail sauce was added showed no statistically significant reduction in the levels of either *V. vulnificus* or total bacteria. In contrast, Tabasco® sauce resulted in a dramatic and significant decrease (to undetectable levels) of *V. vulnificus*. The effect of Tabasco® sauce on the total microbial flora on the oyster meat surface was also statistically significant.

In Table 2 the levels of bacteria present within the oyster meat are shown. Untreated (control) oysters had an average of 15 times more total bacteria, and 20 times more

TABLE 1. Numbers of *V. vulnificus* and total bacteria present on oyster meat surface. Average bacterial loads in CFU/oyster ($n = 4-5$).

Oyster Treatment	<i>V. vulnificus</i>	Total Bacteria
Control	1.3×10^4	5.9×10^5
Cocktail sauce	1.6×10^4	1.9×10^6
Tabasco® sauce	$<10^*$	$1.6 \times 10^{4**}$

* Significantly different (unpaired *t* test) from control.

TABLE 2. Numbers of *V. vulnificus* and total bacteria present within oyster meats. Average bacterial loads in CFU/oyster ($n = 4-5$).

Oyster Treatment	<i>V. vulnificus</i>	Total Bacteria
Control	2.7×10^5	9.2×10^6
Cocktail sauce	1.5×10^5	2.9×10^7
Tabasco® sauce	3.1×10^4	1.9×10^7

V. vulnificus cells, inside compared to outside the oysters. The horseradish-catsup-based cocktail sauce again had no statistically significant effect on either bacterial population. However, and in contrast to its effects on *V. vulnificus* cells on oyster meat surfaces, the addition of Tabasco® sauce to the oysters resulted in no significant decrease in this pathogen within the oysters, and had no effect on the total bacterial population.

DISCUSSION

Our data suggest that the addition of Tabasco® sauce to oysters has little effect on the presence of *V. vulnificus* within oysters. It could be argued that the methodology employed in this study, including the washing away of the hot sauces, is not representative of the "natural" ingestion of oysters on the half-shell. However, neither the swallowing of whole oysters, nor the chewing of oysters prior to ingestion, is likely to place a large number of *V. vulnificus* cells in contact with the hot sauce for a significant length

of time. Further, the 10 min contact time employed for the hot sauces in this study would generally exceed that used by consumers.

We do not know, nor have we attempted to discover, what component of Tabasco® sauce results in the toxicity to *V. vulnificus*. Tabasco® sauce consists solely of peppers, vinegar, and water and it is possible that the low pH alone contributes to its toxicity. It is also quite possible that some constituent of the peppers employed is bacteriocidal for *V. vulnificus*.

We conclude that hot sauces do not remove the public health hazard presented by *V. vulnificus* to at-risk individuals, and that such individuals should continue to refrain from consuming raw or undercooked seafood.

ACKNOWLEDGMENT

This study was supported by funds from the North Carolina Sea Grant Program (R/MRD-24).

REFERENCES

1. Food and Drug Administration. 1989. Estimates of relative risk of food borne illness due to chicken, fish, and shellfish. Food and Drug Administration, Washington, D.C.
2. Groubert, T. N. and J. D. Oliver. 1993. Interaction of *Vibrio vulnificus* and the Eastern oyster, *Crassostrea virginica*. J. Food Prot. 57:224-228.
3. Kaysner, C. A., C. Abeyta, Jr., M. M. Wekell, A. DePaola, Jr., R. F. Stott and J. M. Leitch. 1987. Virulent strains of *Vibrio vulnificus* isolated from estuaries of the United States West Coast. Appl. Environ. Microbiol. 53:1349-1351.
4. Kitaura, T., S. Doke, I. Azuma, M. Imaida, K. Miyano, K. Harada and E. Yabuuchi. 1983. Halo production by sulfatase activity in *V. vulnificus* and *V. cholerae* O1 on a new selective sodium dodecyl sulfate-containing medium: A screening marker in environmental surveillance. FEMS Microbiol. Lett. 17:205-209.
5. Murphy, S. E. and J. D. Oliver. 1992. Effects of temperature abuse on *Vibrio vulnificus* in oysters. Appl. Environ. Microbiol. 58:2771-2775.
6. Oliver, J. D. 1989. *Vibrio vulnificus*. pp. 569-600. In M. P. Doyle (ed.), Food borne Bacterial Pathogens. Marcel Dekker, Inc., New York, NY.
7. Oliver, J. D., R. A. Warner and D. R. Cleland. 1983. Distribution of *Vibrio vulnificus* and other lactose-positive vibrios in the marine environment. Appl. Environ. Microbiol. 45:985-998.
8. Tamplin, M., G. E. Rodrick, N. J. Blake and T. Cuba. 1982. Isolation and characterization of *Vibrio vulnificus* from two Florida estuaries. Appl. Environ. Microbiol. 44:1466-1470.