Effect of ionizing radiation on *Vibrio* bacteria in *Crassostrea virginica* (American oyster)

Effet d'une irradiation ionisante sur les bactéries Vibrio chez l'huître Crassostrea virginica *(huître américaine)*

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Abstract

The presence of *Vibrio* bacteria in raw shellfish poses a significant health hazard to the consuming public. These human pathogens are natural, seasonal components of the microbial flora of shellfish which can result in primary septicemic or diarrheal fatal infections. Existing shellfish depuration neither targets nor eliminates the *Vibrio* bacteria. For this reason, additional purification processes are necessary. In this study, the effect of low dose gamma radiation on the inactivation of *Vibrio cholerae* (01 and non 01) and *vulnificus* is investigated. Log decrement (D_{10}) values ranging only to a high of 0.09 KGy in the *Vibrio* strains support the hypothesis that food irradiation technology can provide an additional effective and efficient shellfish sanitation method.

Keywords: *Vibrio*, shellfish, depuration, gamma irradiation, food irradiation.

Résumé

La présence de *Vibrio* dans les coquillages crus pose un risque sanitaire important pour les consommateurs. Ces pathogènes humains constituent des composants naturels et saisonniers de la flore microbienne des coquillages, et peuvent entraîner des infections septicémiques primaires ou diarrhéiques mortelles. Les méthodes actuelles de purification ne visent ni n'éliminent les bactéries de type *Vibrio*. C'est pourquoi des procédés supplémentaires de purification sont nécessaires. Cette étude examine l'effet d'une irradiation gamma à faible dose sur l'inactivation de *Vibrio cholera* (01 et non-01) et de *Vibrio vulnificus*. Des taux d'abattement d'un logarithme (D_{10}) obtenus pour des niveaux d'irradiation de 0,09 kGy sur des souches de *Vibrio* confortent l'hypothèse que les techniques d'irradiation alimentaire peuvent fournir des méthodes de décontamination supplémentaires et efficaces pour les coquillages.

Mots-clés : *Vibrio*, purification, irradiation gamma, irradiation alimentaire.

INTRODUCTION

Incidences of shellfish-borne *Vibrio* infection have been reported with increasing frequency in the American hemisphere and throughout the world (Stahr *et al.*, 1989; Nip-Sakamoto and Pien, 1989; Farmer *et al.*, 1991; Molero, 1989; Garay *et al.*, 1985; CDC, 1991). Currently employed depuration practices are of questionable effectiveness in the elimination of naturally occurring *Vibrios* from contaminated shellfish stock (O'Neill *et al.*, 1991). Clearly, a more reliable and efficient method is needed, specifically to resolve *Vibrio* sanitation problems impacting commercial shellfish harvests.

The objective of this study was to investigate the effectiveness of low-dose gamma food irradiation applications on the survival of several Vibrio strains experimentally inoculated into mantle fluid from the American oyster (*Crassostrea virginica*) and to investigate the inactivation of Vibrios found naturally colonising shellfish stock.

Materials and methods

The UMass-Lowell/U.S.D.O.E. 0.5 Megacurie ^{60}Co source was used to irradiate samples in a dry environment and dosimetry was monitored with a Far West Technology Inc. (Galeta, Ca.) opti-chromic dosimetry system.

Commercially obtained oysters were shucked, and the mantle fluid was collected, pooled, and centrifuged at 1,000 x g for 10 minutes. The supernatant was divided into 50 ml aliquots, frozen, and stored at -70°C for later use.

Cultures of *Vibrio cholera* non-01(ATCC # 35971), *V. cholera* 01 (ATCC # 14035), *V. vulnificus* (ATCC # 33817) and *V. vulnificus* (Jackson Estuarine Laboratory, University of New Hampshire, Durham N.H.) were maintained on thiosulfate citrate bile salts sucrose agar (TCBS) and grown overnight in nutrient broth at 37.5°C (supplemented with 1% NaCl for cultures of *V. vulnificus*). One ml of an overnight culture was inoculated into 100 ml of nutrient broth and grown for 7 hours at 37.5°C in a shaking water bath (100 rpm). A 1:100 dilution of this culture was made with mantle fluid (4°C), dispensed into 19 screw capped test tubes in 5 ml aliquots, irradiated at selected doses, serially diluted into 0.85% NaCl, and plated in triplicate onto TCBS agar. All plates were incubated at 35°C overnight, colonies were counted, and log decrement (D_{10}) values were calculated by least squares regression analysis.

The radiation doses, in kiloGrays (kGy), administered to each culture were :

V. cholerae non-01	0.00, 0.05, 0.09, 0.22, 0.41, 0.62 kGy
V. cholerae 01	0.00, 0.05, 0.10, 0.24, 0.37, 0.61 kGy
V. vulnificus (ATCC)	0.00, 0.02, 0.04, 0.07, 0.09, 0.14 kGy
V. vulnificus (N.H.)	0.00, 0.02, 0.05, 0.07, 0.11, 0.15 kGy

After determination of the D_{10} range for the four Vibrio strains, two dozen commercially obtained oysters were divided into control and irradiated groups which received a dose of 0.85 kGy. Following irradiation, both groups were separately homogenized and plated onto TCBS plates. The plates were incubated overnight at 35°C and examined for colony formation.

Result and discussion

Log decrement (D_{10}) values for *V. cholerae* non 01, 01 and the two *V. vulnificus* were determined to be 0.06 kGy, 0.04 kGy, and 0.07 kGy, and 0.04 kGy, respectively (figures 1-4). In figure 5, the data obtained in all *V. vulnificus* studies are combined, and the D_{10} value obtained is 0.08 kGy. These inactivation values are extremely low when compared to D_{10} values cited for other bacterial species. (Bandekar *et al.*, 1987), determined the D_{10} value for *V. parahaemolyticus* to be 0.10 KGy. Comparative inactivation values stated for *E. coli,*

Table I: Radiation inactivation of microbial contaminants in foods

Microbial species	Medium	Di_{10}	Author
Aéromonas hydrophila	Chilled Fish	0.16 kGy	Palumbo *et al., 1985*
Campylobacter jejuni	Raw Beef	0.15 kGy	Tarkowaki *et al.*, 1984
Clostridium perfringens	Reduced buffer	0.37 kGy	Gombas and Gomez, 1985
Escherichia coli	Minced Clam Meat	0.37 kGy	Mallett *et al.*, 1985
Salmonella typhimurium	Minced Clam Meat	0.51 kGy	Mallett *et al.*, 1985
Shigella dysenteriae	Frozen Shrimp	0.22 kGy	Mossel, 1985
Shigella flexneri	Frozen Shrimp	0.41 kGy	Mossel, 1985
Staphylococcus aureus	Minced Clam Meat	0.42 kGy	Mallett *et al.*, 1985
Streptococcus faecalis	Minced Clam Meat	0.97 kGy	Jonsson, 1986
Vibrio cholerae (El Tor)	Oyster Liquor	0.04 kGy	This Study
Vibrio cholerae (Non-01)	Oyster Liquor	0.06 kGy	This Study
Vibrio parahaemolyticus	Frozen Shrimp	0.10 kGy	Bandekar *et al.*, 1987
Vibrio vulnificus	Oyster Liquor	0.07 kGy	This Study
Yersinia enterocolitica	Phosphate Buffer	0.10 kGy	El-Zawahry and Grecz, 1981
Hepatitis A Virus	Live Oyster	2.02 kGy	Mallett *et al.*, in press
Poliovirus I	Live Quahog	3.30 kGy	Mallett *et al.*, in press

S. typhimurium, S. aureus, and *S. faecalis* are 0.37, 0.51, 0.42 and 0.97 kGy, respectively table I.

These preliminary studies with commercially obtained shellfish confirmed that a dose of 0.85 KGy was sufficient to completely eliminate *Vibrio* bacteria from naturally colonized oysters. Homogenates of unirradiated oysters were found to have 10^5 CFU/ml, after irradiation, whole oysters were found to have non-detectable level of *Vibrio*. Previous studies performed, have demonstrated that doses of up to 2 kGy can be administered to American oysters (*Crassostrea virginica*) while preserving the market qualities of shelflife, appearance, odor, taste, and texture (Mallett *et al.*, 1991). Therefore, the application of food irradiation techniques would be a very welcomed addition to Vibrio shellfish sanitation. Due to the extreme sensitivity of *Vibrio sp.*, low doses of ionizing gamma irradiation could provide a very effective additional method of resolving the Vibrio problem. Doses as low as 1.0 kGy could produce as much as a 10 log decrease in numbers of Vibrio, virtually eliminating the presence of the pathogen from shellfish. Irradiation of shellfish should not be considered as an exclusive method of sanitation to replace current practices, nor should it be used to effect a "clean-up" of substandard or improperly handled products. Shellfish to be irradiated should be harvested from certified clean waters and should meet guidelines of good manufacturing practice (GMP) as exemplified by the U.S. National Shellfish Sanitation Program (NSSP, 1990). Radiation should be regarded as an additional purification procedure to provide an increased measure of safety in the elimination of *Vibrio sp.* (which are not removed by existing procedures). Vibrio are ideal targets for food irradiation due to their extreme sensitivity to ionizing radiation and low level gamma application may ultimately prove to be an ideal solution to the vexing problem of Vibrio contamination in shellfish.

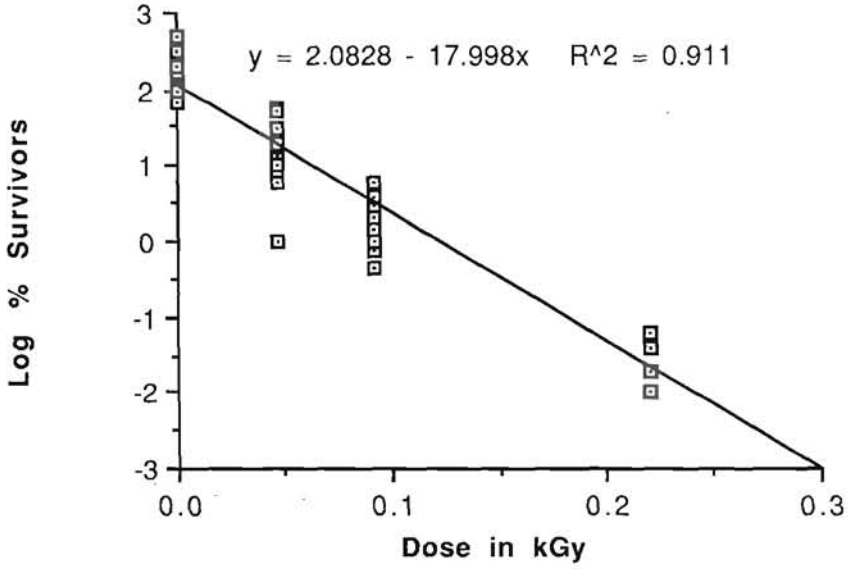

Figure 1: Demonstrates the log decrement (D_{10}) plot on an irradiated culture of *Vibrio cholerae* non-01

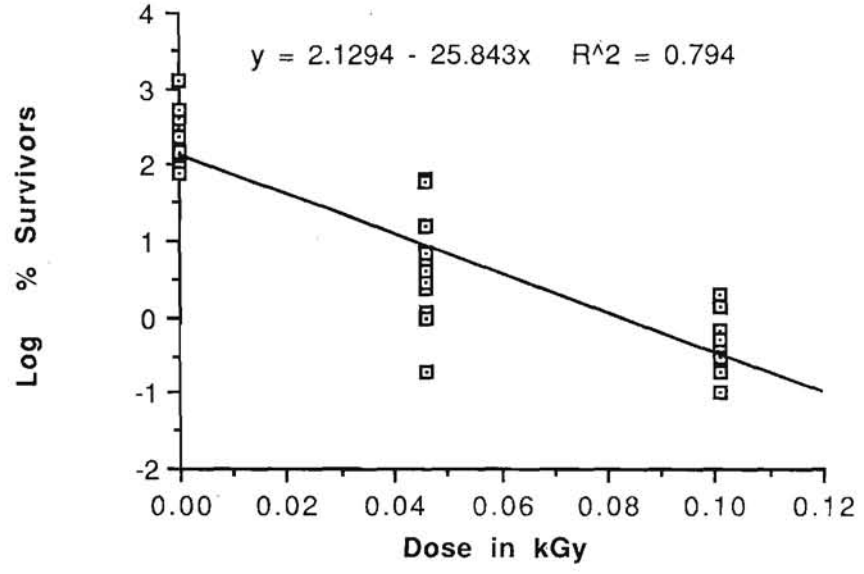

Figure 2: Demonstrates the log decrement (D_{10}) plot on an irradiated culture of *Vibrio cholerae* 01 El tor

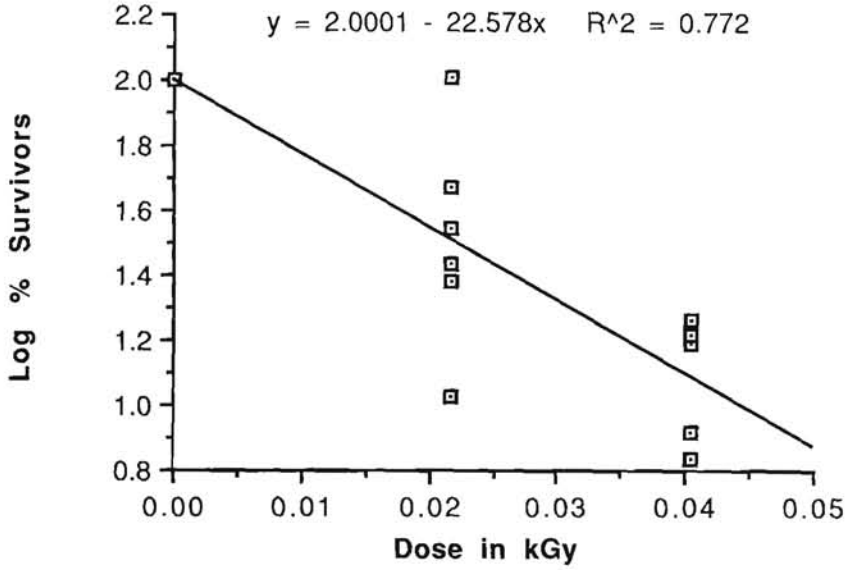

Figure 3: Demonstrates the log decrement (D_{10}) plot on an irradiated culture of *Vibrio vulnificus* (ATCC - 33817)

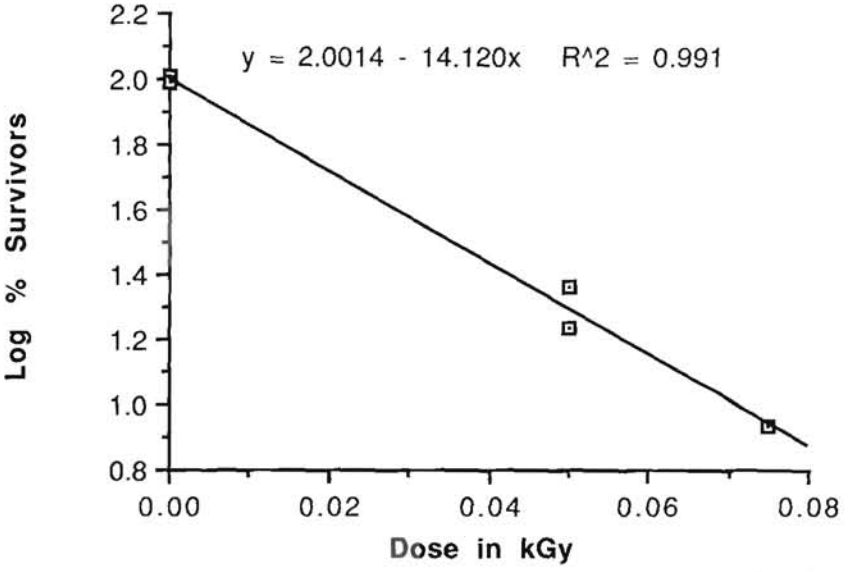

Figure 4: Demonstrates the log decrement (D_{10}) plot on an irradiated culture of *Vibrio vulnificus* (UNH)

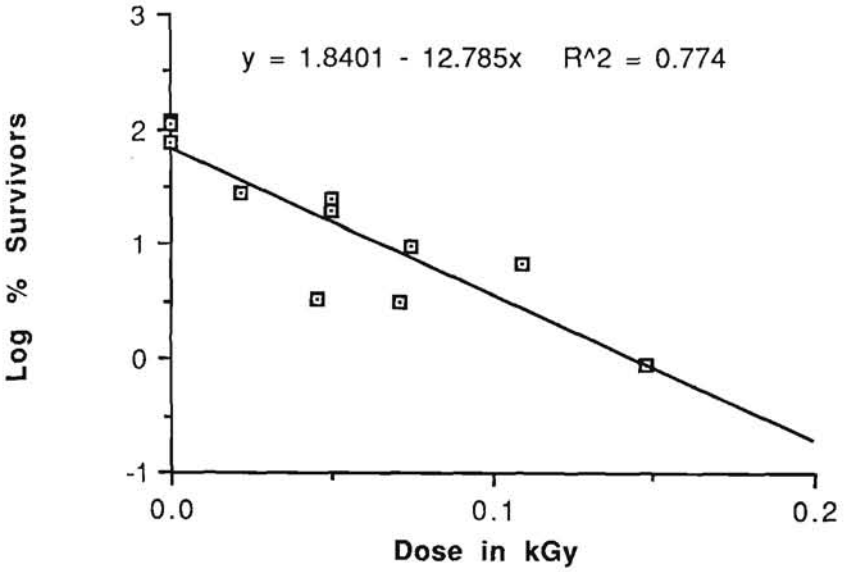

Figure 5: Demonstrates the log decrement (D_{10}) plot on an irradiated culture of *Vibrio vulnificus* composite

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