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Behavior of Pathogenic Bacteria in the Oyster, *Crassostrea commercialis*, During Depuration, Re-laying, and Storage

NGUYEN THI SON AND GRAHAM H. FLEET*

School of Food Technology, University of New South Wales, Kensington, New South Wales, Australia 2033

Oysters (*Crassostrea commercialis*) harvested from major cultivation areas within the state of New South Wales, Australia, were commonly contaminated with low levels of the food-poisoning organisms *Bacillus cereus*, *Clostridium perfringens*, and *Vibrio parahaemolyticus*. *Salmonella* was found in oysters on only one occasion. These bacteria were cleansed from oysters during oyster purification by re-laying in a non-polluted waterway. Oysters were laboratory contaminated to levels in excess of 1,000 cells per g with either *B. cereus*, *C. perfringens*, *V. parahaemolyticus*, *Salmonella typhimurium*, or *S. senftenberg*. These species were cleansed from such oysters during purification in a laboratory depuration unit that used ultraviolet light for sterilizing the depuration water. *Escherichia coli* was also cleansed from oysters under the same re-laying or depuration conditions so that its measurement alone could be used to indicate the cleansing of the above pathogenic species. The levels of these bacteria were also measured during the storage of oysters under conditions that occur during marketing. While *B. cereus* counts remained relatively stable during storage, the *Salmonella* spp. gradually decreased in numbers and *C. perfringens* rapidly died off. *V. parahaemolyticus* counts increased slightly during the first 4 days of storage, after which decreases occurred.

Outbreaks of typhoid, cholera, dysentery, hepatitis, and various forms of gastroenteritis have been linked to the consumption of oysters (4, 7, 11, 38). Pathogenic microorganisms isolated from oysters include *Salmonella* and *Shigella* species, *Vibrio cholerae*, *V. parahaemolyticus*, *Clostridium perfringens*, *C. botulinum*, *Yersinia enterocolitica*, and numerous enteric viruses (1, 3, 34, 38). Being filter feeders (22), oysters accumulate microorganisms from their environment so that their microbiological safety as a human food is directly related to the quality of the waterways in which they are cultivated. Their cultivation in waterways that have become polluted with human sewage has been the basis for many outbreaks of enteric disease and food poisonings (4, 38). Their safety as a food is also related to the potential of contaminating bacterial species to multiply to infective levels during marketing and retailing operations.

Polluted oysters may be rendered safe for human consumption by a process of purification. This is achieved by allowing the oyster to actively feed in microbiologically clean water for a short period just prior to sale. Microorganisms accumulated in the alimentary tract of the animal by previous feeding activities are eventually discharged as part of the faecal material and the oyster is then considered to have become microbiologically cleansed or purified. Purification may be accomplished by re-laying oysters in natural waterways that have been determined to be pollution free or by a process called depuration where the oysters are held in tanks of sea water that has been sterilized by physical or chemical means (15, 27, 38). The technology of oyster depuration has been well studied (9, 15, 23, 26, 29, 30, 37) and reviewed (8, 10), and most countries have chosen to cleanse their oysters in depuration plants rather than by re-laying. Ultraviolet irradiation is widely used to sterilize the sea water for depuration (21, 37), although ozone is used in France (17).

The microbiological quality of an oyster is commonly determined by testing for the presence of indicator bacteria such as coliforms, fecal coliforms, or *Escherichia coli*, and a number of countries have adopted microbiological standards that are based on these tests (18, 38). A widely accepted standard is 2.3 *E. coli* cells per g of oyster; oysters containing less than this level of *E. coli* are considered safe for human consumption.

The efficiency of oyster purification, by either re-laying or depuration, is monitored by the extent to which indicator bacteria, such as *E. coli*, have been cleansed. When indicator levels have been cleansed to acceptable standards, it is considered that any pathogenic contaminants, such as *Salmonella*, will have been equally

cleansed. The assumption made is that all pathogenic bacterial species likely to contaminate oysters will be eliminated or discharged at a rate comparable to that of the indicator organism. However, experimentation to check the validity of this assumption by specifically measuring the rates of cleansing of individual pathogenic bacteria from oysters is lacking. In a properly functioning depuration plant, polluted oysters may be cleansed to below an *E. coli* standard of 2.3 cells per g within 36 to 48 h, and most commercial operations now work on a 2-day depuration cycle (29, 38). Significantly, Janssen (20) has noted the persistence of *Salmonella typhimurium* in oysters after depuration for the standard 48 h. This observation is of considerable public health significance and challenges the long-accepted assumptions correlating the cleansing of indicator bacteria with the cleansing of specific pathogenic bacteria.

This paper examines the cleansing of the pathogens *S. typhimurium*, *S. senftenberg*, *Bacillus cereus*, *V. parahaemolyticus*, and *C. perfringens* from the Sydney rock oyster *Crassostrea commercialis* during re-laying and depuration.

In Australia, it is the commercial practice to distribute and retail oysters in the unopened state at ambient temperatures. These marketing operations may take up to 2 weeks, and during this time potential exists for the multiplication of pathogenic bacteria that may have contaminated the oyster during its cultivation. A second aspect of this paper examines the fates of the above pathogenic species during the storage of unopened oysters at ambient temperature.

MATERIALS AND METHODS

Microorganisms. The following control or reference strains were used throughout the study: *S. typhimurium*, *S. senftenberg*, *B. cereus* (School of Food Technology, University of New South Wales collection), *V. parahaemolyticus* (ATCC 17802), *C. perfringens* (NCTC 2932), and *C. perfringens* (NCTC 10240). All cultures were checked for purity and identity by the procedures recommended by the American Public Health Association (APHA) (31).

Microbiological examination of oysters. Oysters were washed and scrubbed under running tap water to remove surface mud and marine life and then surface sterilized by dipping in 70% ethanol. After the oysters were dried, they were opened aseptically and the flesh was transferred to a sterile blender jar and blended for 60 s. A sample of 10 pooled oysters was used for each analysis. When necessary, oyster homogenates were diluted in sterile 0.1% peptone water or 0.1% peptone water containing 3% NaCl.

Total plate counts were performed by spread plating 0.1-ml samples of homogenate on to nutrient agar (Oxoid) containing 3% NaCl and by examining for colony development after incubation at 30°C for 48 h.

E. coli levels were measured with a three-tube most-probable-number procedure. Tubes of MacConkey broth (Oxoid) were inoculated with homogenate and incubated at 37°C for 48 h. Tubes displaying positive acid and gas production were subcultured into tubes of brilliant green bile broth (Oxoid) and incubated at 44.5°C for 48 h. Tubes positive for gas production were confirmed for the presence of *E. coli* by plating onto eosin methylene blue agar (Oxoid) and by determining indole production at 44.5°C (19).

Salmonella levels were measured by the most-probable-number enrichment of 10-, 1-, 0.1-, and 0.01-g samples of homogenate in selenite brilliant green sulphonamide broth (GIBCO Diagnostics) at 37°C for 24 h followed by plating onto brilliant green agar (Oxoid). Plates were incubated at 37°C for 24 h and then examined for typical *Salmonella* colonies. Representative colonies were restreaked onto nutrient agar for purity verification and then confirmed as *Salmonella* according to the following tests: Gram stain; reactions in triple sugar iron agar (Oxoid) and *O*-nitrophenyl-β-D-galactopyranoside broth; production of urease, oxidase, and lysine decarboxylase; utilization of Simmon citrate agar; fermentation of Hugh-Leifson O/F medium; and agglutination using *Salmonella* specific O- and H- polyvalent antisera (Wellcome Research Laboratories) (19, 31).

B. cereus was enumerated by spread-plating 0.1-ml samples of oyster homogenate onto phenol red-egg yolk-polymyxin agar (19). After incubation at 30°C for 24 h, typical *B. cereus* colonies were counted and confirmed by the tests listed by the American Public Health Association (31). Gram-positive, spore-forming isolates that were positive for acetoin, gelatinase, and nitrate reductase and utilized glucose anaerobically were confirmed as *B. cereus*.

V. parahaemolyticus was enumerated by spread-plating 0.1-ml samples of oyster homogenate onto thiosulfate-citrate-bile salts-sucrose agar (Oxoid). After incubation at 37°C for 24 h, typical blue-green colonies of *V. parahaemolyticus* were counted and confirmed by the following biochemical tests: indole, acetoin, oxidase, and lysine decarboxylase production; reaction on triple sugar iron agar (Oxoid); growth in the absence of salt; growth in the presence of 8 and 11% salt; and growth at 42°C (19, 31).

C. perfringens was enumerated by spread-plating 0.1-ml samples of oyster homogenate onto tryptose-sulfite-cycloserine agar and overlaying with 10.0 ml of the same medium without the egg yolk (19). Plates were incubated anaerobically at 37°C for 24 h, after which presumptive colonies were counted and confirmed by the tests recommended by the American Public Health Association (31). Gram-positive, nonmotile isolates that hydrolyzed gelatin and fermented lactose were considered as positive for *C. perfringens*.

Survey of oysters. Oysters were harvested from commercial leases throughout the state of New South Wales (Fig. 1) and were examined within 24 h of harvesting.

Depuration of oysters. Oyster depuration was carried out on a laboratory scale in glass or Perspex tanks (67 by 30 by 30 cm). By using an Eheim 1818 centrifugal pump, water (20 liters) in the tanks was

circulated through an ultraviolet light water sterilizing system and a glass heat-exchanger unit that was immersed in a constant-temperature water bath. All the components in the circulation cycle were joined by PVC tubing. Water sterilization was effected by two aquarium-scale ultraviolet light units (Oliphant Pty Ltd., Adelaide, South Australia) connected in series. Each unit contained one 30-cm ultraviolet lamp (G8T15N) that generated 20 mW/cm^2 at 1 m. The tank water was maintained at a temperature of 18 to 22°C and was circulated at a rate of 60 liters/h. Water for depuration was collected from the estuary where the oysters were grown and was circulated within the system for 12 to 24 h before each trial. Oysters were collected from commercial leases, washed by high-pressure water sprays to remove surface mud, and used in depuration studies within 12 h of collection. Oysters were arranged as a single layer on the bottom of the depuration tank at a density not exceeding four oysters per liter of tank water. Dissolved oxygen in the circulating water remained at levels in excess of 70% saturation so that aeration was not necessary. Samples of oysters for microbiological examination were removed from the tanks as a function of depuration time. The operational characteristics of this laboratory-scale depuration system have been described in detail by Souness and Fleet (30).

Re-laying of oysters. Oysters collected from sampling sites in the Georges River were re-layed on trays in the designated pollution-free waters of Quibray Bay (Fig. 1). After 2 and 6 days, samples of the re-layed oysters were collected for microbiological examination.

Laboratory contamination of oysters. To obtain artificially high levels of oyster contamination with *Salmonella*, *B. cereus*, *V. parahaemolyticus*, and *C. perfringens*, it became necessary to contaminate the oyster in the laboratory. This was done by allowing the oysters to feed for periods of up to 6 h in laboratory aquaria that had been inoculated with cells from actively growing cultures.

Microbiological changes in oysters during storage. Oysters that were freshly harvested from leases within the Georges River or those that had been laboratory contaminated were stored out of water and unopened at temperatures between 20 and 25°C. Samples were removed for microbiological analyses at intervals during storage.

RESULTS

Methods for the enumeration of specific pathogenic bacteria in oysters. Numerous methods have been proposed for the specific enumeration of *Salmonella*, *B. cereus*, *V. parahaemolyticus* and *C. perfringens* in foods (19, 31). The successful application of any particular method is ultimately dependent upon the food under examination. The methods used in this study for enumerating organisms in oysters were chosen after evaluation of a number of recommended methods by determining recoveries of known levels of test organisms that had been inoculated into oyster homogenates. The results of these trials form the basis of another communication but, for the present study, it is pertinent to note the following conclusions in relation to those methods finally chosen and described in Materials and Methods. *Salmonella* levels as low as 1 cell per g could be recovered from oyster homogenates and greater than 85% of *Salmonella* cells inoculated into an oyster homogenate were quantitatively recovered by the most-probable-number selective enrichment and plating procedure described. Greater than 85% of the cells of a reference strain of *B. cereus* that had been inoculated into oyster homogenates could be recovered by direct plating of homogenate samples onto phenol red-egg yolk-polymyxin agar. Approximately 70% of *V. parahaemolyticus* cells inoculated into oyster homogenates could be recovered by direct plating onto thiosulfate-citrate-bile salts-sucrose agar. Recovery of *C. perfringens* cells from oyster homogenates by direct plating onto tryptose-sulfite-cycloserine agar was always low and averaged around 50%. This was not a factor of the medium being used but, as will be discussed later, was probably due to factors within the oyster homogenate that were unfavorable to the survival of *C. perfringens*. Knowing the capabilities of these methods, we were able to draw valid conclusions from the following studies.

Occurrence of pathogenic bacteria in New South Wales oysters. Oysters obtained from a number of geographically diverse locations throughout the state of New South Wales (Fig. 1) were examined for the microorganisms shown in Table 1. Although a majority of the samples would conform to an *E. coli* standard of less than 2.3 cells per g and a total plate count standard of less than 10^5 cells per g, all samples showed the presence of *B. cereus*, *V. parahaemolyticus*, and *C. perfringens*. *C. perfringens* occurred at levels less than 50 cells per g, but five out of the six samples contained *B. cereus* at levels exceeding 100 cells per g and two samples showed *V. parahaemolyticus* exceeding this level. *Salmonella* was not detected in any of the oyster samples tested in this survey, although the samples from one area had been exposed to heavy fecal pollution as indicated by an *E. coli* count of 46 cells per g.

Elimination of bacteria from oysters on re-laying. Oysters obtained from polluted areas within the Georges River were re-layed in the nonpolluted waters of Quibray Bay which is located at the mouth of the river (*E. coli* counts of the water from where the oysters were harvested averaged 30 cells per 100 ml, while the waters of Quibray Bay showed *E. coli* counts of less than 3 cells per 100 ml.) The reductions in bacterial counts as a consequence of re-laying are presented in Table 2. Oysters which were

unacceptably polluted on the basis of high *E. coli* counts were cleansed to acceptable levels of less than 2.3 *E. coli* cells per g after 2 days; after 6 days no *E. coli* could be detected in any of the re-layed oyster samples. *Salmonella* was found in one of the oyster samples before re-laying, but was not detected after re-laying. This isolate was serotyped as *S. singapore*. *B. cereus* levels as high as 440 cells per g of oyster were cleansed to values of 50 to 60 cells per g after re-laying. Re-layed oysters never reached zero counts for *B. cereus*, as the waters of Quibray Bay were found to contain this organism at levels of around 45 cells per 100 ml. *V. parahaemolyticus* and *C. perfringens* cells were also eliminated from oysters on re-laying and could be cleansed to undetectable levels. Three further re-laying trials were conducted, and similar results were obtained in each case.

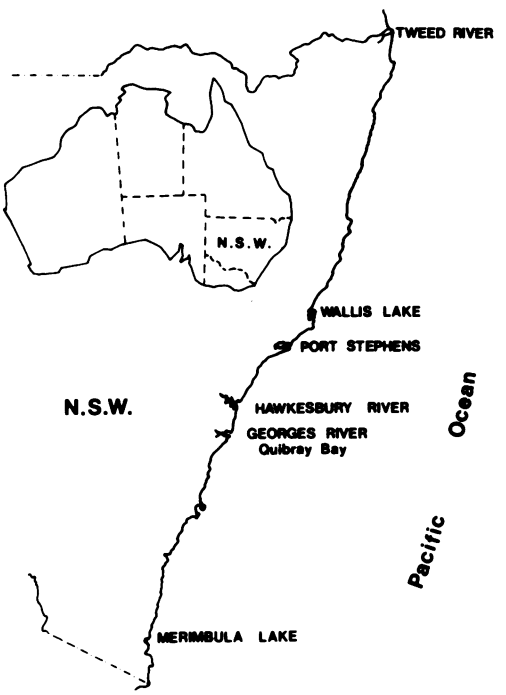

FIG. 1. *Map of oyster sampling sites within the state of New South Wales, Australia.*

TABLE 2. *Elimination of bacteria from polluted oysters on re-laying in a pollution-free waterway*

Oyster sample	Re-laying time (days)	Total plate count	Cell counts/g of oyster homogenate				
			E. coli	*Salmonella*	*B. cereus*	*V. parahaemolyticus*	*C. perfringens*
1	0	5×10^4	5.90	0.36[a]	440	18	18
	2	2×10^4	0.08	ND[b]	68	5	5
	6	4×10^4	ND	ND	60	5	ND
2	0	5×10^4	6.0	ND	230	18	18
	2	1×10^4	0.18	ND	65	3	8
	6	3×10^4	ND	ND	53	ND	ND

[a] Isolated and serotyped as *S. singapore*.
[b] ND, Not detected.

Elimination of bacteria from oysters during depuration. Table 3 summarizes the results of a series of experiments conducted to examine the cleansing of specific bacteria from oysters during depuration. Separate depuration trials were conducted for each organism. Trials for *Salmonella*, *B. cereus*, *V. parahaemolyticus*, and *C. perfringens* used oysters that had been laboratory contaminated since it was not possible to obtain oysters that were naturally contaminated with high levels of these organisms.

As measured by the reduction of *E. coli* numbers from 100 cells per g to undetectable levels, the laboratory depuration system gave very effective oyster cleansing within 48 h. Total plate counts decreased by 10-fold over this period, but rarely went below a value of 10^4 cells per g of oyster. The failure of total plate counts to de-

TABLE 1. *Levels of bacteria found in oysters harvested from a number of locations throughout New South Wales*[a]

Origin of sample	Total plate count	Cell count/g of oyster homogenate				
		E. coli	*Salmonella*	*B. cereus*	*V. parahaemolyticus*	*C. perfringens*
Georges River 1	1.7×10^4	4.3	ND[b]	475	33	30
Georges River 2	1.3×10^4	46	ND	490	33	15
Port Stephens	6.5×10^4	0.91	ND	190	87	7
Tweed Heads	1.0×10	0.36	ND	250	425	15
Merimbula Lake	9×10^3	0.91	ND	87	17	5
Wallis Lake	3×10^4	0.36	ND	450	155	7

[a] The results presented are the means of duplicate oyster samples. Oysters were harvested during the months of April and May.
[b] ND, Not detected.

crease below this value is not a reflection of inadequacies in the depuration system itself, but, rather, is related to the maintenance of an indigenous microbial flora within the oyster (30). Both *S. typhimurium* and *S. senftenberg* were rapidly eliminated from heavily contaminated oysters and by 2 days, 300- to 1,000-fold reductions in initial counts had been achieved. Since *Salmonella* is of major public health concern, its cleansing from the oysters was studied for different initial contaminating loads. Longer depuration times were required for the more heavily contaminated oysters (Table 3), and oysters with initial *Salmonella* levels in excess of 10^3 cells per g still exhibited low levels of contamination after 3 days of depuration. Oysters with lower initial loads of *Salmonella* showed less than 1 cell per g after 1.5 days depuration and, significantly, eventually cleansed to undetectable levels. Both *B. cereus* and *V. parahaemolyticus* were readily cleansed from oysters during depuration, and by 2 days initial contaminating loads had been reduced by higher than 1,000-fold to levels generally less than 20 cells per g. Reduction of *C. perfringens* during depuration was even more rapid with initial levels of 10^3 to 10^5 cells per g being reduced to less than 10 cells per g in 2 days (Table 3).

Changes in bacterial counts of oysters during storage. Table 4 shows the changes in bacterial counts of oysters during storage in the unopened form at 20 to 25°C. Separate storage trials were conducted for each organism. Total plate counts fluctuated during storage, and after 14 to 16 days the counts were generally around 10^6 cells per g. These fluctuations in total plate counts were noted in all storage trials. In a storage trial not shown in Table 4 an initial total count of 3×10^4 cells per g had increased to 2×10^5 cells per g after 12 days of storage. Since

TABLE 3. *Elimination of bacteria from oysters during laboratory depuration*[a]

Depuration time (days)	Total plate count	Cell counts/g of oyster homogenate								
		E. coli	*S. typhimurium*		*S. senftenberg*		*B. cereus*	*V. parahaemolyticus*	*C. perfringens*	
0	2×10^5[b]	1×10^2[b]	1×10^5	9×10^2	2×10^3	4×10^2	1×10^4	9×10^5	1×10^5[c]	1×10^3[d]
0.5	—[e]	—	—	15	—	4.3	—	—	—	—
1	8×10^4	2.0	7×10^3	—	40	—	60	1×10^2	1.8×10^2	10
1.5	—	—	—	0.23	—	0.93	—	—	—	—
2	2×10^4	ND[f]	3×10^2	—	1.8	—	7	20	5	—
2.5	—	—	—	0.09	—	ND	—	—	—	—
3	—	ND	59	—	0.36	—	—	8	—	—
3.5	—	—	—	ND	—	—	—	—	—	—

[a] Results are the means of duplicate oyster samples.
[b] Initial levels represent natural contamination.
[c] Strain 2932.
[d] Strain 10240.
[e] —, Not assayed.
[f] ND, Not detected.

TABLE 4. *Changes in the bacterial counts of oysters stored unopened at 20 to 25°C*

Storage time (days)	Total plate count	Cell counts/g of oyster homogenate					
		S. typhimurium	*S. senftenberg*	*B. cereus*	*V. parahaemolyticus*	*C. perfringens*	
0	1.2×10^5	2.4×10^4[a]	2.4×10^4[a]	430	65	20	1.5×10^5[b]
1	—[c]	1.0×10^4	2.4×10^4	—	—	—	1.4×10^3
2	5×10^4	—	—	250	6×10^2	20	6.0×10^2
3	—	1.5×10^3	4.3×10^3	—	—	—	—
4	9×10^4	—	—	340	1.5×10^3	ND[d]	10
6	6×10^5	2.4×10^3	4.3×10^2	380	30	—	—
9	—	2.9×10^3	4.6×10^2	—	—	—	—
12	7×10^4	2.4×10^2	1.5×10^2	350	65	—	—
16	8×10^5	—	—	380	100	—	—

[a] Laboratory contaminated.
[b] Laboratory contaminated, strain 2932.
[c] —, Not assayed.
[d] ND, Not detected.

it was not possible to find oysters that were naturally contaminated with *Salmonella*, storage trials were conducted with oysters that had been laboratory contaminated with these bacteria. It is evident from Table 4 that there was no tendency for either *S. typhimurium* or *S. senftenberg* to increase in cell numbers during oyster storage, and there was a tendency for these cells to slowly die off.

After 12 days of storage, initial *Salmonella* counts had decreased by about 100-fold. Similar *Salmonella* data were also obtained during the storage of other batches of oysters. *B. cereus* levels remained relatively stable during storage, showing no dramatic trends to increase or decrease in cell number. On the other hand, *V. parahaemolyticus* exhibited definite increases (around 20-fold) in cell numbers during the first 4 days, after which significant decreases and further slight increases were noted. These trends were also noted during the storage of other batches of oysters. Natural contaminants of *C. perfringens* died off during oyster storage. This was further tested with oysters that had been laboratory contaminated with either control strain 2932 or strain 10240. Both strains rapidly died off after oyster contamination and subsequent storage. As seen in Table 4, *C. perfringens* loads of 1.5×10^5 cells per g had decreased by 100-fold within 1 day of storage and after 4 days only 10 cells per g could be recovered.

DISCUSSION

A limited survey of oysters harvested from some major production sites within Australia has established the ubiquitous occurrence of the pathogenic species *B. cereus*, *V. parahaemolyticus*, and *C. perfringens*, thereby demonstrating a need to assess the behavior of these organisms in oysters during purification and marketing practices.

Oysters are not normally examined for the presence of *B. cereus*, but since this organism was reported as being responsible for an outbreak of food poisoning involving oysters (16), it was included in this study. *B. cereus* was found in oysters at levels between 100 and 500 cells per g and, in view of the high infective dose (10^7 to 10^8 cells) required by this species, it was considered unlikely that these low levels of contamination would pose any direct risk to public health. Furthermore, there was no tendency for these low numbers to multiply to higher levels within the oyster under conditions that might arise during oyster distribution and marketing.

C. perfringens is a common contaminant of estuarine waters and sediments (25), but its occurrence in oysters has received little attention. It has been reported in British oysters at levels generally less than 5 cells per g (2). All oysters examined in the present study showed the presence of *C. perfringens*, but the levels noted were less than 50 cells per g. Even allowing for possible underestimation by counting on tryptose-sulfite-cycloserine agar, it is unlikely that any samples would have exceeded levels of 100 *C. perfringens* cells per g. As for *B. cereus*, these low levels were considered unlikely to pose any direct risk to public health. Interestingly, *C. perfringens* rapidly died off during oyster storage, suggesting that conditions or factors within the oyster may be unfavorable to its survival. This may explain the low numbers found in freshly harvested oysters.

Since both *B. cereus* and *C. perfringens* are spore formers, closer public health consideration should be given to cooked oyster products such as oyster soups, gravies, and sauces. In these cases, spores may survive the cooking process and later germinate and multiply to infective levels if such foods are inadequately stored. A more careful assessment of the public health risks associated with the presence of *B. cereus* and *C. perfringens* in cooked oyster products is warranted.

The association of *V. parahaemolyticus* with oysters is well documented and contamination levels of around 100 cells per g are commonly reported (3, 35). Contamination levels of the same order have previously been found in Australian oysters (32) and were also noted in the present study. The public health risk associated with the presence of these low numbers of *V. parahaemolyticus* in oysters is related to their ability to increase during commercial handling operations. In this investigation, *V. parahaemolyticus* exhibited a definite tendency to multiply within oysters during the first few days of storage at 25°C, after which time the counts decreased to less than 100 cells per g. The public health significance of this observation is questionable since the highest levels reached before reductions ocurred were around 1,500 cells per g. Similar increases and decreases in *V. parahaemolyticus* numbers have been noted during storage of the American oyster *Crassostrea virginica* (21, 33), and it was suggested that *Pseudomonas* species, which predominate in stored oysters, eventually inhibit *V. parahaemolyticus* development (12).

A more extensive survey may reveal a higher incidence of *Salmonella* in New South Wales oysters than found in this study, it being detected on only one occasion and at the low level of 0.36 cells per g (Table 2). A previous, larger survey of New South Wales oysters also failed

to reveal the presence of this pathogen (R. B. Qadri, Ph.D. thesis, University of New South Wales, Australia, 1974). Nevertheless, its occurrence in American oysters has been noted on a number of occasions, although quantitative levels have not been reported (1, 28). With respect to public health, the presence of salmonellae in oysters is considered intolerable, any hazards becoming magnified if these organisms were able to grow in oysters during storage. However, *S. typhimurium* and *S. senftenberg* showed no tendency to grow in oysters during storage, and their counts gradually decreased. This behavior is consistent with an earlier observation on the survival of *Salmonella* in *C. virginica* (24).

The total plate count of the oysters increased by 5- to 10-fold after 12 days storage, but fluctuations in counts were noted during this period. The final counts did not exceed 10^6 cells per g. Hoff et al. (14) recorded somewhat higher (10- to 20-fold) increases in counts during similar storage of the oysters *C. gigas* and *Ostrea edulis* and also noted count fluctuations. They suggested that the increases were attributable to bacterial multiplication in the oyster digestive tract and shell liquid.

Bacteriological examinations of oysters have shown the predominant flora to consist of *Pseudomonas, Vibrio, Aeromonas, Moraxella, Acinetobacter, Flavobacterium,* and *Cytophaga* species, and it has been suggested that oysters might maintain an indigenous gut flora (6, 36). This would imply mechanisms for selectively retaining some microbial species in the alimentary tract while eliminating others with the feces. This concept is supported by the fact that total bacteriological counts of oysters do not decrease to zero levels when oysters are allowed to feed for extended periods in sterilized water, as is the case during depuration (30, 36). As found elsewhere (29, 30) and also in the present study, total counts of depurated oysters were generally around 10^4 cells per g. This ability of oysters to selectively retain some microbial species while eliminating others has profound public health implications when considering the microbiological purification of oysters by either depuration or re-laying and where the elimination of an indicator organism, such as *E. coli*, is used to judge the elimination of other pathogenic species.

It has been clearly established for the oyster species *C. virginica, C. gigas, C. commercialis, Ostrea lurida,* and *O. edulis* that coliforms, and more specifically *E. coli*, are not selectively retained in the gut and are readily eliminated with the oyster feces during oyster purification (9, 26, 30, 37). The present investigation has now established that, for *C. commercialis* at least, *S. typhimurium, S. senftenberg, B. cereus, V. parahaemolyticus* and *C. perfringens* are not specifically retained in the gut of the oyster and are readily cleansed from the oyster under conditions which promote cleansing of *E. coli*. This was demonstrated for oysters that were naturally contaminated with these pathogens and for oysters that had been contaminated in the laboratory. Although no attempt was made to study the relative rates of cleansing of these different bacteria, it is evident from Table 3 that differences in these rates may exist. On laboratory depuration, counts for *B. cereus, V. parahaemolyticus,* and *C. perfringens* were reduced by factors in excess of 1,000-fold within 2 days. Under the same conditions, 2 to 3 days were required for similar reductions in *Salmonella* counts. Also, it appears for all organisms that the greatest reductions in counts occur during day 1 of depuration, the rate of cleansing being significantly reduced after this time. No explanations for these differences can be given, but a more detailed study into the kinetics of elimination of individual bacterial species from oysters would be worthwhile. Such studies should also examine possible differences between the rates of cleansing of naturally contaminated oysters and laboratory-contaminated oysters (13).

The large reductions in counts of *S. typhimurium* and *S. senftenberg* noted in the present depuration studies are at variance with the data reported by Janssen (20). Using laboratory-contaminated oysters and a laboratory-scale ultraviolet light depuration system, he reported initial *S. typhimurium* counts of around 10^4 cells per g of oyster to remain largely unchanged after 3 days of depuration. Although his studies were conducted with a different oyster species, *C. virginica*, it seems that the oysters may not have been fully active in his depuration system, and this may account for the discrepant observations.

The time required for the purification of polluted oysters depends upon the initial level of contamination, more heavily contaminated oysters requiring longer cleansing times (8, 9, 10, 26). This is also evident from the data on *Salmonella* in Table 3. Commercial experiences in a number of countries have shown that a depuration cycle of 36 to 48 h consistently yields oysters that meet current *E. coli* standards (<2.3 *E. coli* cells per g) and have a good public health record (29, 38). The data obtained in this study suggest that this depuration time would also be sufficient to give acceptable cleansing of the contaminating levels of *Salmonella, B. cereus, V. parahaemolyticus,* and *C. perfringens* that are likely to be found in oysters under natural conditions. Although Table 3 does not show

complete elimination of *Salmonella* from oysters by 48 h, large reductions in counts were achieved by this time and it is unlikely that the high initial levels of contamination used in those trials would ever be encountered under natural conditions. Oysters containing 900 cells per g of *S. typhimurium* were cleansed to less than 1 cell per g by 2 days and to undetectable levels by 3.5 days, whereas those with 400 cells per g of *S. senftenberg* showed no *Salmonella* after 2.5 days.

In Australia some oysters are also cleansed by re-laying and, provided the re-laying zone is not subject to pollution, this is an acceptable means of achieving oyster purification (Table 2). Although acceptable cleansing may be achieved within 2 days, a minimum re-laying period of 7 days is adopted to account for possible variations in oyster feeding rates (hence cleansing rates) that might arise through environmental disturbances such as temperature fluctuations and water movements. It should be noted that in Australia oysters are cultivated on trays or sticks in such ways that they are only submerged and feeding during the high tide, and this must be taken into consideration when calculating re-laying times.

Although this study has established that some bacterial pathogens are cleansed from oysters at a rate comparable to the cleansing of *E. coli*, this might not be the case for other bacterial pathogens such as *Y. enterocolitica* (34) or enteric viruses (11), and further research in those areas is required.

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