Significance of Coliforms and Enterococci in Fish Products

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Coliforms, more recently fecal coliforms, and enterococci are often used to assess the hygienic quality of foods. Although these bacteria serve rather successfully as an index of fecal pollution of water supplies, their usefulness in measuring fecal pollution in foods is less than satisfactory. The ratio and correlation of coliforms and enterococci in fish-fillet and lobster-meat samples led us to conclude that these organisms originated from improperly sanitized working surfaces, where they survive and multiply, and not from fecal pollution. Therefore, the presence of coliforms and enterococci in fish fillets and lobster meat reflects the quality of fish-plant sanitation and not the direct fecal pollution of these products.

The production of foods of good sanitary quality is necessitated by aesthetic considerations as well as by considerations of public health. Such foods can be produced in a controlled sanitary environment, and hygienic processing necessarily involves quality control through the application of standards. For a long time, bacteriological techniques have been successfully employed for the assessment of the sanitary quality of water. Water quality has been determined by relating coliform bacteria densities to the magnitude of fecal pollution. This suggested the use of coliform bacteria to indicate the fecal contamination of foods. Thus, it is of little surprise that these organisms were applied and are frequently being applied to foods processed under different conditions. The use of coliform bacteria as an index of fecal pollution has often been criticized mainly because organisms in this group may originate from nonfecal sources. Therefore, the use of Escherichia coli type I as an indicator was suggested by several workers (3), since this organism has a higher fecal specificity. Similar arguments were advanced in favor of enterococci, since easy and reliable means of identifying these organisms have been found (8).

According to Bonde (1), the estimation of fecal pollution is essentially a diagnostic and statistical problem. The densities of indicator bacteria must be related to the extent of actual fecal pollution before a realistic diagnosis of pollution can be arrived at. When indicator bacteria multiply in a given environment, the interpretation of their sanitary significance becomes complicated (9). Therefore, it is essential to evaluate the sanitary significance of indicator bacteria in a given circumstance where these bacteria are used to indicate fecal pollution.

Before the densities of E. coli (fecal coliform) and enterococci can be related to the extent of fecal contamination, it is necessary to determine their relative numbers in human feces. Buttiaux and Mossel (2) reported that 1 g of human fecal matter usually contains about 40 million coliforms, 4 million E. coli, and 4 million enterococci. The ratio of these bacteria to each other is about 10:1:1, respectively. Accordingly, 10 E. coli cells per gram of food would be equivalent to about 2.5 µg of fecal contamination. Litsky et al. (8) found a 1:7.6 ratio and a significant 70.5% correlation between fecal coliforms and enterococci in polluted river water. It is evident that if human fecal pollution is alone responsible for the presence of fecal coliforms and enterococci in foods, the ratio and correlation of these bacteria should be similar to those in fecal matter and in polluted water. Fish caught in unpolluted seawater were not found to carry fecal coliforms (12) or enteric pathogens. Griffiths (4) concluded that E. coli was not a normal inhabitant of the intestinal tract of marine fish, although this organism could be present in fish caught in polluted water. Thus, the indicator bacteria in fish products must originate from the processing environment. Fish products were often found to contain large numbers of fecal coliforms, particularly enterococci (11), but subsequent analysis for salmonellae invariably produced negative results. This finding is supported by Liston's
statement (7) that fish products generally have enjoyed good public health records in the past. The inconsistency between high apparent fecal contamination and good public health records necessitated the evaluation of indicator bacteria as an index of fecal pollution of fish products.

In this paper, the significance of indicator bacteria in lobster and fillet meat will be discussed.

**Materials and Methods**

From each of 10 lobster meat plants located in the Canadian maritime provinces, five samples of lobster meat were selected at random from the end of the packing line. Each sample was aseptically placed in a sterile jar, refrigerated in crushed ice, and shipped to the laboratory without delay. Fish fillet samples were taken and handled similarly. In the laboratory, 55 g of lobster meat and 100 g of fish fillet from each sample were measured into separate sterile Waring Blender jars. After sterile distilled water was added to the samples in a ratio of 1:2, they were blended for 2 min. The resulting homogenates were tested for fecal coliforms, enterococci, and salmonellae.

Fecal coliforms were detected in Lactose Broth and confirmed in E C Broth incubated at 45.5 C for 24 hr (5). Densities were estimated by the most probable number procedure (6).

Enterococci were detected in modified Azide Dextrose Broth and confirmed in Ethyl Violet Azide Broth (BBL) incubated at 37 C (8). Densities were estimated by the most probable number technique (6).

The test for salmonellae was carried out by use of Dulcitol Selenite Cystine Broth (10), incubated at 43 C (13), and Tetrathionate Broth, incubated at 37 C. These enrichment media were employed in parallel. Enrichment cultures were streaked on SS Agar, Brilliant Green Agar, and MacConkey's Agar. Non-lactose-fermenting colonies from the agar plates were purified and transferred to Triple Sugar Iron Agar and Urea Agar slants. After incubation, suspicious cultures were tested with poly "O" antisera.

The geometric average of five most probable number counts from each plant was calculated. The data for fecal coliforms and enterococci were plotted, and the regression correlation values were calculated by the least sum of squares method.

The potential of indicator bacteria to multiply in a fish processing plant was measured by the generation time of these bacteria in fish-meat extract incubated at 25 C. The meat extract was prepared by blending four parts of physiological saline with one part of meat. The resulting homogenate was centrifuged at high speed, and the extract was filter sterilized.

The ability of indicator bacteria to survive in the environment was assessed by measuring their resistance to disinfection. The test was carried out by the "use dilution" technique (Official Methods of Analysis of the Association of Official Agricultural Chemists, 1960, 9th ed.).

**Results**

Figure 1 shows the regression correlation of fecal coliforms and enterococci obtained from lobster meat. There is no significant correlation between these bacteria at a 95% level of significance. Enterococci outnumbered fecal coliforms 334 to 1. Similar results obtained from fillet samples are shown in Fig. 2. Lack of significant correlation between fecal coliforms and enterococci can also be observed. Enterococci outnumbered fecal coliforms 122 to 1. Figures 3 and 4 show that lobster and fish meat are good media for the growth of indicator bacteria. Thus, if the
Fish plants in the Canadian maritime area, the source of our samples, usually use 50 ppm of chlorine for disinfection. Table 1 shows the efficiency of chlorine solutions in killing bacteria absorbed onto metal surfaces. It is obvious that a solution containing 50 ppm of chlorine is not adequate to kill the nonsporeforming bacteria absorbed onto metal surfaces in fish plants.

Bacteria belonging to the genus Salmonella were not detected in the meat samples or in the processing environment.

**DISCUSSION**

The ratio and correlation between fecal coliforms and enterococci are significantly different in lobster and fish-fillet meat than in human feces. Therefore, these organisms must originate from sources other than human fecal pollution. The most likely sources of these bacteria are the improperly sanitized working surfaces where they could survive and proliferate. The ratio and correlation of fecal coliforms and enterococci from working surfaces, rather than from feces, account for their rate of multiplication and their resistance in the processing environment. The indicator bacteria (Fig. 3, 4) can multiply on working surfaces. This evidence alone should disqualify these bacteria as an index of fecal pollution in lobster and fillet meat. However, the indicator bacteria can be used as an index of plant sanitation. The higher resistance of enterococci to disinfection accounts for their predominance over the fecal coliforms. Finally, the lack of salmonellae in seafoods can be attributed to the minimal contamination of these products with human fecal matter.

**LITERATURE CITED**


6. **Hoskins, J. K. 1934.** Most probable numbers for the evaluation of coli-aerogenes test by fer-

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**TABLE 1. Effectiveness of chlorine on bacteria absorbed onto metal surfaces**

<table>
<thead>
<tr>
<th>Cultures</th>
<th>Chlorine in medication mixture (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td><em>Streptococcus faecalis</em></td>
<td>4/4*</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>4/4</td>
</tr>
</tbody>
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* Positive cultures/tubes inoculated.

Temperature in the processing area is high enough, indicator bacteria can proliferate on the working surfaces, as well as in the meat itself, at a considerable rate.

**FIG. 3. Growth curves of bacteria cultures in lobster-meat-extract medium incubated at 25 C.**

**FIG. 4. Growth curves of bacteria cultures in fillet-meat-extract medium incubated at 25 C.**