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A Research Note

Recovery of Coliphages from Chicken, Pork Sausage and Delicatessen Meats

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ABSTRACT

Coliphages were recovered from 18 of 18 fresh chicken and pork sausage samples as well as from 2 of 6 processed delicatessen meat samples employing a rapid technique using EC medium as both an eluent and as a modified phage assay plating medium. Coliphage recoveries ranged from approximately 3.3 to 4.4 $\log_{10}$, 1.2 to 3.5 $\log_{10}$ and zero to 2.7 $\log_{10}$ plaque forming units per 100 g in fresh chicken, fresh pork sausage and roast turkey breast, respectively. High coliphage levels generally reflected high fecal coliform counts, particularly for fresh meat samples. These data indicate that coliphages can be readily enumerated in foods within 16 h.

Coliphages have received increased attention in recent years as simple and inexpensive indicators of fecal contamination in water and wastewater (*9,10,12-14,26,30,31*). Coliphage analyses offer an alternative to conventional analysis of water for coliforms and/or *Escherichia coli*, and have also shown promise for quantitatively evaluating the efficiency of bacterial and viral removal in water and wastewater treatment (*2,3,14,18*). Coliphage models have also been used to evaluate the effect of thermal processing and low temperature storage on virus survival in shellfish (*6,7*). Highly significant correlations between coliphage numbers and both total and fecal coliforms in various water systems using simple and rapid (6 to 24 h) coliphage assay techniques were reported in recent, comprehensive studies (*12,31*).

Despite the continuing interest in development of a coliphage indicator system for water and wastewater analysis, coliphages have not been examined as potential indicators of fecal contamination in foods other than shellfish. The recovery of bacteriophages infecting the coli-typhoid-paratyphoid group of bacteria from milk was reported as early as 1937 (*17*). A high degree of correlation between coliphage numbers and coliform MPN counts in oysters from polluted water has been reported (*13*). The need for development and adoption of more rapid and inexpensive alternatives for enumeration of specific fecal indicators, i.e., *E. coli*, in foods which would have the same sensitivity as the time-consuming AOAC procedures (*29*), has been addressed in recent years (*1,8,11,23,28*). Although the incidence of animal viruses in foods and the development of associated recovery methodology has been studied extensively (*4,5,15*), information concerning techniques for quantitative recovery of coliphages from various foods or the occurrence of coliphage in foods is lacking. This study was undertaken to investigate the feasibility of enumerating coliphages in foods. The occurrence and numbers of coliphages as well as total and fecal coliforms in retail samples of fresh chicken, fresh pork sausage and cooked delicatessen meats were determined.

MATERIALS AND METHODS

Three separate samples of fresh chicken breasts, chicken thighs, chicken gizzards with hearts and three different brands of fresh pork sausage for a total of 9 fresh poultry and 9 fresh pork sausage samples were anlayzed for aerobic plate counts, presumptive coliforms, presumptive fecal coliforms and coliphages. In addition, three separate samples each of delicatessen roast turkey and corned beef sliced at time of purchase were also examined. All samples were transported to the laboratory in insulated containers and held at 5°C until the time of analysis (6 to 12 h). For each sample, a 50-g subsample was analyzed for aerobic plate counts (35°C, 48 h) using plate count agar according to standard methodology (*29*). Presumptive (unconfirmed) coliforms and fecal coliforms were enumerated using Tryptic soy agar and violet red bile agar (VRB) as described by Powers and Latt (*23*) for rapid estimation of stressed coliforms and fecal coliforms.

In preliminary studies (data not presented), various proteinaceous eluents, such as nutrient broth, gram-negative broth and EC medium, were more effective in extraction of coliphage from fresh meats than phosphate buffer. EC medium was selected as both the eluent and as the basal medium for the subsequent phage assay as it appeared to be most effective for recovery of coliphages from fresh meats in preliminary investigations. Phage assay medium containing bile salts appeared to suppress growth of most interfering bacteria and allow rapid growth of *E. coli* hosts before proliferation of indigeneous bile-tolerant bcteria

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could interfere with resolution of coliphage plaques on the host lawn. Chloroform treatment of clarified food extracts to eliminate interfering bacteria on phage assay plates was not completely effective, was time-consuming and reduced phage titers as compared to assaying directly in media containing bile salts. EC medium used for coliphage plating medium without pretreatment of samples with chloroform was also more effective than the conventional procedure, using nutrient agar and chloroform pretreatment, for assaying coliphage in sewage effluent and natural water (manuscript in preparation).

E. coli carrying a sex factor (F) was selected as a host for coliphage analyses in this study due to the superior recovery of coliphages in preliminary studies (data not presented) with food, water and wastewater using *E. coli* C as compared to *E. coli* B or a mixed host of *E. coli* B and C. In addition, ecological studies recently reviewed by Seeley and Primrose (*27*) suggest that although no universal plating host for coliphages exists, an *E. coli* C host carrying a sex factor would be an especially useful coliphage host since male specific coliphages are widespread in natural water and *E. coli* C does not have DNA restriction and modification systems which many *E. coli* strains possess and which may significantly reduce phage titers. Although the possibility of false-positive coliphage recoveries due to plaquing of *E. coli* host by polyvalent phages capable of lysing host cells of closely related genera exists, ecological studies of coliphages in natural water suggest that the incidence of such polyvalent phages is low (*24*).

For coliphage analyses in this study, 100 g of sample were blended at ca. 6,000 RPM with 200 ml of sterile EC broth for 5 min. The resulting suspension was filtered through sterile glass wool (5 g), which had been pretreated with 50 ml of EC broth analogous to the clarification techniques for virological analysis of food described by Larkin et al. (*16*). After filtration for ca. 15 min, the clarified food filtrate was used for subsequent coliphage assay. For each coliphage assay plate, 2.0 ml of sample filtrate, 0.5 ml of *E. coli* C host (6-h culture in nutrient broth) and 3.0 ml of molten EC medium containing 0.75% agar (45°C) were mixed and overlaid upon a prepoured plate of EC medium containing 1.5% agar. Fifteen plates were prepared in this manner for each sample filtrate to obtain a sensitivity of ca. 10 coliphages per 100 g of food.

Coliphage plaques were counted after 16 to 18 h of incubation at 35°C. Although plaques were observed after 6 h at 35°C, plaque counts were considerably higher after 16 h of incubation for most samples. One large sample of chicken gizzards with hearts was analyzed in triplicate as indicated to determine the variation due to recovery technique. Percent standard deviations between replicate runs were approximately 40, 7, 12 and 13 for aerobic plate counts (APC's), coliforms, fecal coliforms and coliphages, respectively. All culture media used were obtained from Difco Laboratories (Detroit, MI).

RESULTS AND DISCUSSION

Ranges, means and standard deviations of $\log_{10}$ values of coliphages, coliforms, fecal coliforms and APC's of each sample type and overall groups of samples are presented in Tables 1 and 2, respectively. Coliphages were recovered in all fresh chicken and pork sausage samples (18 of 18) as well as in 2 of 3 delicatessen roast turkey samples. Coliphage levels ranged from approximately 3.3 to 4.4 $\log_{10}$ counts per 100 g in fresh chicken and from approximately 1.2 to 3.5 $\log_{10}$ counts per 100 g in fresh pork sausage. Mean fecal coliform and coliphage levels were relatively consistent for each type of chicken product but varied between different brands of pork sausage.

Coliphages were recovered in four pork sausage samples in the absence of fecal coliforms and in one sausage sample in the absence of fecal or total coliforms. A coliphage count of ca. 590 plaque-forming units (PFU) per 100 g was observed for the sausage sample containing no

TABLE 1. *Aerobic plate counts, coliforms, fecal coliforms and coliphage counts from fresh chicken, fresh pork sausage and delicatessen meats.*

Samples	APC	Coliforms	Fecal coliforms	Coliphages
Chicken breasts				
(Mean ± S.D.)[a]	5.67 ± 1.13	4.15 ± 1.66	2.93 ± 0.52	4.12 ± 0.24
(Range)	4.47 – 6.72	2.34 – 5.61	2.41 – 3.44	3.84 – 4.26
Chicken thighs				
(Mean ± S.D.)	5.63 ± 1.03	3.88 ± 1.28	2.88 ± 1.23	3.70 ± 0.36
(Range)	4.44 – 6.29	2.40 – 4.64	2.11 – 4.31	3.30 – 4.01
Chicken gizzards				
(Mean ± S.D.)	7.16 ± 0.93	4.97 ± 0.70	2.95 ± 0.24	4.14 ± 0.40
(Range)	6.16 – 7.98	4.22 – 5.61	2.72 – 3.21	3.68 – 4.39
Pork sausage A				
(Mean ± S.D.)	4.45 ± 1.29	2.55 ± 2.02	0.39 ± 0.68	1.95 ± 0.67
(Range)	2.95 – 5.24	0.70 – 4.71	NR[b] – 1.18	1.20 – 2.50
Pork sausage B				
(Mean ± S.D.)	3.42 ± 0.23	1.07 ± 1.11	0.72 ± 1.25	2.68 ± 0.23
(Range)	3.20 – 3.65	NR – 2.22	NR – 2.16	2.42 – 2.86
Pork sausage C				
(Mean ± S.D.)	5.33 ± 0.68	2.39 ± 0.25	1.29 ± 0.55	3.10 ± 0.39
(Range)	4.55 – 5.83	2.15 – 2.64	0.70 – 1.40	2.83 – 3.54
Roasted turkey breast				
(Mean ± S.D.)	5.23 ± 0.76	3.48 ± 0.69	2.55 ± 1.67	1.43 ± 1.37
(Range)	4.38 – 5.84	3.05 – 4.27	0.70 – 3.95	NR – 2.73
Roast corned beef				
(Mean ± S.D.)	7.68 ± 0.02	3.02 ± 1.25	1.13 ± 0.99	NR
(Range)	7.66 – 7.70	2.02 – 4.42	NR – 1.88	

[a]Values represent means of $\log_{10}$ counts per g (bacteria) or per 100 g (coliphage) of three separate samples (n = 3).
[b]NR, none recovered.

TABLE 2. *Summary of aerobic plate counts, coliforms, fecal coliforms and coliphage counts from fresh chicken and pork sausage.*

Samples	APC	Coliforms	Fecal coliforms	Coliphages
Chicken				
(Mean ± S.D.)[a]	6.15 ± 1.17	4.33 ± 1.21	2.92 ± 0.68	3.99 ± 0.37
(Range)	4.44 − 7.98	2.34 − 5.61	2.11 − 4.31	3.30 − 4.39
Pork sausage				
(Mean ± S.D.)	4.40 ± 1.11	2.00 ± 1.36	0.80 ± 0.86	2.58 ± 0.65
(Range)	2.95 − 5.83	NR[b] − 4.71	NR − 2.16	1.20 − 3.54
Overall				
(Mean ± S.D.)	5.28 ± 1.43	3.17 ± 1.73	1.86 ± 1.32	3.29 ± 0.89
(Range)	2.95 − 7.98	NR − 5.61	NR − 4.32	1.20 − 4.39

[a]Values represent means of $\log_{10}$ counts per g (bacteria) or per 100 g (coliphage) of 9 samples for chicken or pork sausage (n = 9) or of 18 samples overall (n = 18).
[b]NR, none recovered.

fecal or total coliforms, whereas coliphage levels in pork samples having total, but not fecal, coliforms ranged from 16 to 720 PFU per 100 g. Coliphages were recovered in 2 of 3 roast turkey samples at levels of 40 and 540 PFU per 100 g; corresponding fecal coliform levels were approximately 3.0 and 4.0 $\log_{10}$ counts per g, respectively. The roast turkey sample in which no coliphage was recovered had 0.7 $\log_{10}$ fecal coliforms per g. No coliphages were recovered in delicatessen corned beef samples, although presumptive fecal coliforms were recovered in 2 of 3 samples at levels of 1.5 and 1.9 $\log_{10}$ counts per g.

The lack of any apparent relationship between coliphage and fecal coliform recovery in some samples, particularly delicatessen meats, may be attributed in part to the rapid coliform procedure used in that presumptive fecal coliform colonies were not identified or confirmed. It should be noted that levels of fecal coliforms and particularly total coliforms in refrigerated foods may reflect proliferation of psychrotrophic species of these groups rather than initial fecal contamination (*20,22*) and the proportions of *E. coli* and confirmed fecal coliforms among colonies recovered on VRB medium can vary markedly with the food product being examined (*19-22,25*).

Correlations between coliphages and more specific fecal indicators, i.e., *E. coli*, may be more definitive than correlations between coliphage and coliforms for refrigerated foods. There was generally less variation among coliphage counts than among fecal coliform and particularly total coliform counts for fresh chicken or poultry samples, as evidenced by the respective standard deviations. These observations may also be attributed to the potential for proliferation of psychrotrophic coliform bacteria in these refrigerated food samples. Whether coliphages recovered from delicatessen meats reflected post-processing contamination of the products or the ability of some coliphages to survive thermal processing could not be ascertained from these data. Coliphage T4 has been reported to survive normal cooking procedures used for crabs (*7*) as well as survive extended periods of refrigerated storage in cooked crabs (*6*).

Although correlations between coliphage levels and levels of fecal and total coliform should be regarded with caution, considering both the limited numbers of samples examined and the presumptive identity of these bacterial groups, high coliphage counts generally reflected high presumptive fecal and total coliform counts (Table 1). For all 18 fresh meat samples, correlation coefficients between coliphages vs. fecal coliforms, coliphages vs. coliforms, and coliphages vs. APC's were 0.77, 0.64 and 0.72, respectively, whereas correlation coefficients between fecal coliforms vs. coliforms and coliforms vs. APC's were 0.67 and 0.82, respectively. All of these correlation coefficients were significant at the 0.01 level.

These data indicate that coliphages can be readily enumerated in fresh and processed meat samples within 16 h and that coliphages may offer a promising alternative as rapid indicators of fecal contamination in foods. Studies directed toward characterizing the ecological relationship of coliphages to classical bacterial indicator organisms, enteric pathogens and sources of fecal contamination in foods and the behavior of coliphages under various physical and chemical conditions encountered in food processing and storage are needed to substantiate the utility of coliphages as indicator organisms in foods.

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