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Selected Enrichment Broths for Recovery of *Campylobacter jejuni* from Foods

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We attempted to shorten the required time for enrichment broth culture for the isolation of *Campylobacter jejuni*. Enrichment broths described by Doyle and Roman and Park and Stankiewicz and one developed during this study were compared for ability to isolate *C. jejuni* from raw chicken carcasses. Our medium was a modification of that of Doyle and Roman with the addition of filter-sterilized FBP (0.2% ferrous sulfate, 0.025% sodium metabisulfite, 0.05% sodium pyruvate), 0.1% sodium lauryl sulfate, and 0.075% agar. Initially, laboratory strains were employed in the development of this medium. Subsequently, an indigenous load of *C. jejuni* obtained from chickens was used to compare media. Isolation rate comparisons were as follows: direct plating, 40%; Doyle and Roman broth, 45% at 7 h and 61% at 16 h; Park and Stankiewicz broth, 53% at 7 h and 60% at 16 h; our broth, 48% at 7 h and 50% at 16 h. In addition to having the highest isolation rate, the enrichment broth of Doyle and Roman showed greatest selectivity. Our inoculation method of indigenous bacteria provided a controlled means for comparison of isolation procedures.

Increasing evidence suggests that *Campylobacter jejuni* (5) is a major cause of foodborne gastroenteritis. Skirrow (6) reported the isolation of the pathogen from 7.1% of patients with diarrhea. A study by Pai and co-workers (4) indicated that *Campylobacter* spp. were isolated from 4.3% of the fecal specimens from a group of diarrheic children. Other researchers (1, 11) have indicated that the isolation of *C. jejuni* from patients with diarrhea is at least as common as the isolation of *Salmonella* spp.

Rapid and sensitive methods for recovering *C. jejuni* cells from foods would be useful for both epidemiological work and routine examination of food sources. The main drawback associated with numerous available enrichment procedures is the length of time needed for enrichment. Enrichment culture incubation ranges from 16 to 48 h before plating on selective media, which then requires an additional 24 to 48 h for isolation. This 3- to 4-day procedure is difficult to reconcile with rapid marketing strategies while maintaining interest in the public health.

This study describes an attempt to develop an enrichment broth requiring only 7 h of incubation and the comparison of our enrichment broth with those described by Doyle and Roman (DR) (2) and by Park and Stankiewicz (PS) (Abstr. Assoc. Off. Anal. Chem., Annu. Meet., 1982, 10, p. 3) in isolating *C. jejuni* from broiler chickens. It was concluded that an enrichment broth requiring 7 h of incubation could not be developed with the tested conditions and that DR broth was superior.

MATERIALS AND METHODS

Enrichment broth development. (i) Preparation of inocula. The following *C. jejuni* strains were used: USN509 (human clinical isolate obtained from J. C. Coolbaugh, Naval Medical Research Institute, Bethesda, Md.), FRI-CF-3 (human clinical isolate obtained from M. P. Doyle, Food Research Institute, Madison, Wis.), KC1609 (human clinical isolate obtained from R. W. Weaver, Centers for Disease Control, Atlanta, Ga.), ATCC 29428 (American Type Culture Collection, Rockville, Md.), L-7 (beef liver isolate), Calf-3 (calf fecal isolate), L49-3B (lamb carcass isolate), FM-3 (lamb flank meat isolate), CL-1 (chicken liver isolate), and Turk-2 (turkey fecal isolate). Stock cultures were maintained in 10 ml of fluid thioglycollate medium (Difco Laboratories, Detroit, Mich.) at 5°C. Transfers of 1 ml were made into fresh media each month. Newly transferred cultures were incubated at 42°C for 48 h followed by storage at 5°C. One milliliter of the stock culture was transferred into 25 ml of brucella broth (Difco) supplemented with 0.2% ferrous sulfate–0.025% sodium metabisulfite–0.05% sodium pyruvate (FBP) (3). Flasks containing the inoculated broths were incubated at 42°C in a shaker water bath (Precision Scientific; model 50), at 100 oscillations per min for 24 h. A 1-ml portion of this culture was transferred into flasks containing 25 ml of fresh brucella broth with FBP and incubated at 42°C. The overnight culture contained between 10^7 and 10^9 organisms per ml. Culture was serially diluted in Cary-Blair (C-B) transport medium without agar (0.09 g of calcium chloride, 1.1 g of disodium hydrogen phosphate, 1.5 g of sodium thioglycollate, 5.0 g of sodium chloride per liter of distilled water) (10).

(ii) Preparation of enrichment broths. The control enrichment broth has been described by Doyle and Roman (2). To 99 ml of DR enrichment broth, 1 ml of the diluted culture inoculum was added, yielding between 10^2 and 10^4 *C. jejuni* cells per ml. The headspaces of 250-ml Erlenmeyer flasks containing broth were evacuated and replaced twice with a gas mixture of 5% O_2–10% CO_2–85% N_2 (hereafter referred to as microaerobic conditions). Flasks were incubated at 42°C in a shaker water bath at 100 oscillations per min. Enumeration was made initially, after 8 h, and after 16 h. Appropriate serial dilutions were made in C-B diluent before being spread plated on a selective medium (VPTK) containing blood agar base no. 2 (Oxoid Ltd., London, England) plus 5% lysed horse blood, vancomycin (10 μg/ml), polymyxin B (2.5 IU/ml), trimethoprim (5 μg/ml), and cephalothin (15 μg/ml) (6) (Bakte Bennett Laboratory Product Litera-

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TABLE 1. Average $\log_{10}$ increase in CFU of *C. jejuni* (from duplicate flasks)

Medium and supplement	Incubation time (h)	Avg $\log_{10}$ increase in CFU of strains:										Avg of all strains
		USN509	CF-3	L-7	ATCC 29428	Calf-3	L49-3B	FM-3	KC 1609	CL-1	Turk-2	
Control[a]	8	1.69	1.90	1.76	0.06	1.42	0.16	0.37	1.21	0.42	−1.07	0.79
	16	4.39	4.63	4.77	−0.02	4.25	−0.05	−0.14	3.84	2.57	−1.76	2.25
0.1% SLS	8	1.94	1.88	2.72	1.26	1.88	1.01	1.22	1.31	1.50	0.56	1.53
	16	4.80	5.08	5.20	4.17	5.04	5.17	3.71	4.81	3.93	3.85	4.58[b]
FBP	8	1.90	1.74	1.91	0.55	1.62	0.94[c]	1.18	1.15	1.29	0.42	1.27
	16	4.76	4.25	5.22	3.20	4.63	4.69	4.02	3.45	3.01	1.16	3.84
0.075% Agar	8	1.46	1.45	1.48	−0.42	1.79	1.23[b]	0.16	1.87	0.18	−0.90	0.83
	16	3.47	4.86	4.68	−1.42	4.30	4.45[b]	−0.01	3.87	1.45	−1.23	2.44

[a] Base enrichment broth as described in reference 2.
[b] Statistical significance ($P < 0.05$) for a difference with control.
[c] Entry indicates the $\log_{10}$ increase enumerated from a single flask.

ture, Berkeley, Calif.). Plates were incubated at 42°C for 48 h in the prescribed microaerobic atmosphere. A glycerol-impregnated filter paper was placed in each anaerobic jar to reduce swarming of *Campylobacter* colonies (9).

The modifications of the control medium included adding FBP with incubation of flask contents under aerobic conditions, adding 0.1% sodium lauryl sulfate (SLS) with incubation under prescribed microaerobic conditions, and supplementing with 0.075% agar with incubation under microaerobic conditions. The control and modified broths were evaluated by duplicate determinations of the 10 *C. jejuni* strains listed. Analysis of variance (7) was used to test the effect of enrichment broth modifications.

Formulation of enrichment broth. Broiler carcasses obtained from local stores provided a uniform load of indigenous *C. jejuni* for each enrichment flask. Two split carcasses were each rinsed in 250 ml of C-B. The rinsings were combined, filtered through a double layer of cheesecloth, and centrifuged (Ivan Sorvall, Inc., Norwalk, Conn.; model RC-5B) at 16,000 × *g* for 10 min. The supernatant was poured off, and the pellet was resuspended in 15 ml of C-B. A 1-ml portion of this suspension was used to inoculate 99 ml of the following enrichment broth formulations: (i) DR control broth plus FBP–0.1% SLS–0.075% agar and incubation under aerobic conditions; (ii) DR control broth plus FBP–0.1% SLS–0.075% agar and incubation under microaerobic conditions; (iii) DR control broth plus 0.1% SLS and incubation under microaerobic conditions; (iv) DR control broth plus FBP and incubation under microaerobic conditions; and (v) DR control broth and incubation under microaerobic conditions.

Duplicate flasks of each formulation, inoculated with the suspension, were incubated at 42°C in a shaker water bath (Brunswick; model RW 650) at 160 oscillations per min. Enrichment cultures were plated after 7 and 16 h with VPTK plates and C-B diluent. Characteristic *Campylobacter* colonies (8) were counted, and phase-contrast microscopical examination was made on selected colonies. The inoculating suspension containing the indigenous flora was also directly enumerated on VPTK medium with C-B dilution blanks.

Enrichment broth comparison. The DR enrichment medium (2) and the PS medium (Abstr. Assoc. Off. Anal. Chem. Annu. Meet. 1982) were chosen for comparison because each is reported to be capable of recovering small numbers (0.1 cells per g) of *C. jejuni* from food containing large numbers (ca. 10^6 to 10^7 cells per g) of competing organisms. We also compared our most promising modified enrichment broth (PJR) which contained the DR broth with FBP–0.1% SLS–0.075% agar. Duplicate flasks of the three media were inoculated with chicken carcass washings and incubated at 42°C under microaerobic conditions in a shaker water bath (160 oscillations per min). Dilutions of each enrichment culture were plated on VPTK after 7- and 16-h incubations in addition to direct plating of the inoculating suspension. Resulting characteristic *Campylobacter* colonies were counted, and randomly selected ones were examined by phase-contrast microscopy.

RESULTS AND DISCUSSION

Results show the average increase in numbers of *C. jejuni* cells after 8 and 16 h of incubation in the four broths (Table 1). A larger number would suggest a faster growth rate and, therefore, a more productive medium. At time zero, the control and broths supplemented with SLS, FBP, and 0.075% agar contained average $\log_{10}$ numbers of 3.08, 2.84, 3.06, and 3.75, respectively, per ml. The control medium produced an average $\log_{10}$ increase of 0.79 after 8 h and 2.25 after 16 h of incubation. Compared with the control broth, each of the three modified media yielded higher numbers, and the broth containing 0.1% SLS appeared best. Statistical analysis revealed significant differences in numbers of *C.*

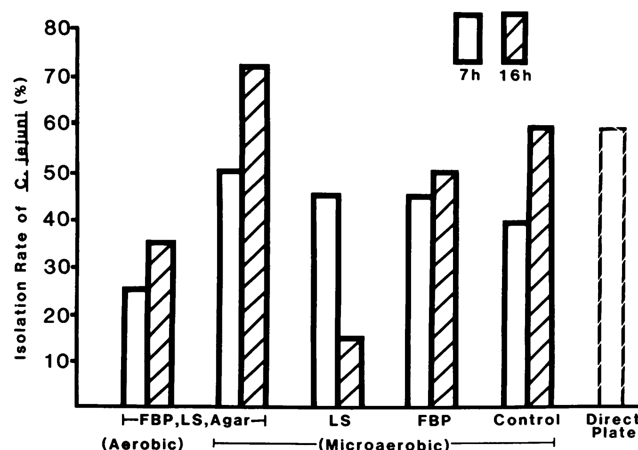

FIG. 1. Isolation rates of indigenous *C. jejuni* from 10 composite broiler chicken samples with five enrichment media (containing FBP, SLS, and agar) and a direct plating procedure. See text for details of formulation.

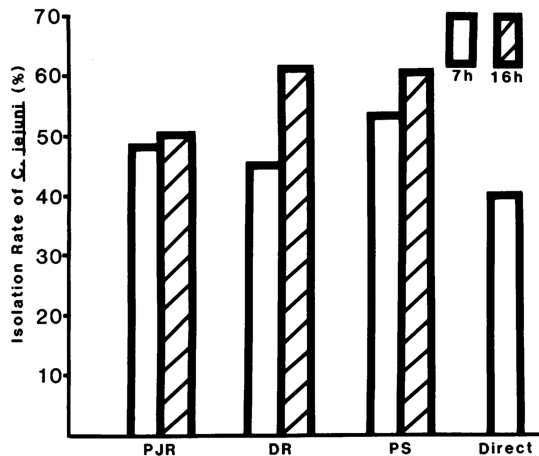

FIG. 2. Isolation rates of indigenous *C. jejuni* from 20 broiler chickens with three enrichment media and a direct plating procedure. See text for details of formulation.

jejuni only in the control and the broth containing SLS. Variability existed among the growth responses for individual *C. jejuni* strains. Although the component modifications were not significant, we thought that a combination might produce a significantly improved enrichment broth.

The increase in number of single *C. jejuni* CFU present in the enrichment broth could be explained by the SLS dissolving the extracellular viscous material produced by the organism (9) and thereby reducing the number of clumping cells. As a result, more single *C. jejuni* cells might be present and thus more colonies formed. Although this does not explain an increase in growth rate, it does give a reason for the apparent increase in the number of *C. jejuni* cells.

Enrichment broth formulations were inoculated with an average of 310 indigenous cells of *C. jejuni* per flask. Isolation rates for the five media are presented in Fig. 1. The highest isolation rate obtained after 7 h of enrichment was produced by medium B, containing FBP, SLS, and agar incubated under microaerobic conditions. After 16 h of enrichment, medium B again produced the highest rate. We hypothesize that the increase in isolation rate was due to the FBP supplement more completely reducing the oxygen tension of the medium. SLS may also have increased the number of isolated cells of *C. jejuni* present.

The modified 7-h enrichment procedure did not seem promising after examining the data presented in Fig. 1. The highest isolation rate obtained with a 7-h enrichment was considerably lower than the highest produced with a 16-h enrichment. Even direct plating yielded a higher isolation rate. Since the procedure yielding the highest isolation rate should be used, the 16-h enrichment with medium B was the method of choice.

Inoculation of indigenous *C. jejuni* cells by washing and pelleting the natural flora of broiler chickens delivered a uniform load of the organism for comparison of these experimental parameters. Uniformity was confirmed by comparing the number of *C. jejuni* cells in duplicate enrichment flasks receiving the same inoculum. The use of indigenous *C. jejuni* in formulating an enrichment broth has advantages over the use of pure cultures in an inoculated sample. The medium can be evaluated in terms of selectivity by observing suppression of other indigenous organisms. The naturally occurring *C. jejuni* strains may respond to enrichment differently than laboratory-passed strains. This method produces a realistic load of organisms for use in testing enrichment media.

Each of the three enrichment broths received an average initial inoculum of 280 cells. Average isolation rates from retail broilers for the three media (duplicate flasks) are found in Fig. 2. After 7 h of enrichment, the PS broth produced the best results with a rate of 53% (21/40). Direct plating of the inoculating suspension yielded isolations in 40% (8/20) of the samples. A comparison of the media after 16 h of enrichment gave a slightly different picture. In this case, the DR and PS broths produced similar isolation rates. Based solely on isolation rates, the optimal media were the PS broth with 7-h enrichment and the DR broth with 16-h enrichment.

Because isolation rates of the three media were similar, the additional factor of selectivity was considered in characterizing the best enrichment broth. The degree of selectivity was highly variable among the three broths, and this factor greatly influenced the ease with which *C. jejuni* was isolated. Quantitative data concerning contamination of the broths with non-*Campylobacter* organisms were not obtained due to the nature of its growth on selective agar plates. Quite often competing flora exhibit a confluent growth which makes enumeration difficult. Instead, qualitative observations were recorded as to the degree of non-*Campylobacter* contamination on the selective agar plates. Generally, after 7 h of enrichment, little or no contamination with these organisms was found. After incubation for 16 h, the growth of competing organisms was often heavy, and this potentially masked any *Campylobacter* colonies present. Overall the DR broth was the most selective against non-*Campylobacter* organisms, whereas the PJR and PS broths showed progressively less selectivity. Aerobic plate counts were performed on broiler samples concurrently with analyses for *C. jejuni* cells. The average $\log_{10}$ numbers of *C. jejuni* and aerobic bacteria were 1.21 and 7.16, respectively, per carcass. A weak, inverse correlation ($r = -0.578$) was encountered and seemed of limited interest.

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