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Comparison of Basal Media for Culturing *Campylobacter jejuni* and *Campylobacter coli*

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Four strains of *Campylobacter jejuni* and four strains of *Campylobacter coli* were used to compare the quantitative growth of *Campylobacter* cells on blood agar base no. 2 (Oxoid), brucella agar (BBL Microbiology Systems and Difco Laboratories), campylobacter agar base (Difco), Columbia blood agar base (Difco and Oxoid), and Mueller-Hinton agar (Difco and Oxoid). Columbia blood agar base and blood agar base no. 2 were inhibitory to most of the strains tested, as evidenced by reduced (10- to 1,000-fold) colony counts compared with other basal media. One of the brucella agars was inhibitory to two of the *C. coli* strains. The inhibitory effect of these media could be eliminated by addition of FBP (0.05% each ferrous sulfate hydrate, sodium metabisulfite, and sodium pyruvate) or 7% defibrinated sheep blood. However, addition of FBP or blood to brucella agar, campylobacter agar base, or Mueller-Hinton agar did not significantly affect the count, indicating that supplements are not required in these media for growth of *Campylobacter* in pure culture.

Several basal media with various supplements are used for the isolation and growth of *Campylobacter jejuni* and *Campylobacter coli*, such as blood agar base no. 2 (6, 19), brucella agar (1, 5, 21, 23), Columbia blood agar base (7, 13), Mueller-Hinton agar (16, 17), and thioglycolate agar medium (8). Common supplements include horse blood (5 to 7%) for use when trimethoprim is incorporated as a selective agent (4, 6) or sheep blood (5 to 15%) (6, 10, 16, 19, 20) or FBP (a mixture of ferrous sulfate, sodium bisulfite, and sodium pyruvate), which is added to increase the aerotolerance of the organisms (7, 11, 12), or both. A combination of antibiotics is usually added to inhibit other bacteria present in clinical and environmental samples.

For studies of *Campylobacter* in pure culture, the use of antibiotic inhibitors is not necessary, and cultures can be incubated at 37°C instead of 42°C (5, 14, 16). For enumeration of campylobacters, it may be necessary to increase levels of agar up to 3% to reduce swarming (19, 22). Blood is not generally preferred as a supplement to media in research studies because it is undefined and could cause differences in results between batches. We used eight strains of *C. jejuni* and *C. coli*, of different serotypes, to determine the ability of these organisms to grow on selected basal media, with or without added blood or FBP.

MATERIALS AND METHODS

Preliminary study. Several *Campylobacter* species, including *C. jejuni*, *C. coli*, *C. fetus*, and *C. laridis*, were grown in modified K broth (22) containing the following: tryptic soy broth (Difco Laboratories), 10 g; special peptone (Oxoid), 5 g; yeast extract (Oxoid), 5 g; Tris buffer, 0.75 g; and sodium pyruvate, 5 g in 1 liter of distilled water; 5 ml of filter-sterilized 3% 1,4-dithiothreitol was added per liter. These cultures were incubated at 37°C for 24 h in a modified atmosphere containing 7% CO_2. Appropriate dilutions of these cultures, giving 30 to 300 CFU per plate, were spread onto brucella agar (Difco) and brucella agar prepared according to BBL Microbiology Systems and Oxoid formulations, using appropriate ingredients (BBL and Oxoid); Columbia blood agar base (Oxoid); Columbia blood agar base supplemented with 10% defibrinated sheep blood, with 1.5 or 3% agar; and Mueller-Hinton agar (Difco). Inoculated plates were incubated at 37°C in a 7% CO_2 atmosphere and counted after 48 and 72 h of incubation.

Stock cultures. Four strains of *C. jejuni*, serotypes 4, 5, 7, and 17, and four strains of *C. coli*, serotypes 8, 20, 45 and 55, determined by Lior heat-labile antigenic factors (16), were obtained from H. Lior (Laboratory Centre for Disease Control, National Health and Welfare, Ottawa, Ont., Canada). All eight strains were human isolates, representing some of the most common serotypes causing human gastroenteritis (15, 16). Cultures were maintained on Columbia blood agar base (Oxoid) with 10% added defibrinated sheep blood at 37°C in 7% CO_2 and subcultured twice weekly.

Cultural conditions. All cultures were incubated at 37°C in an atmosphere containing 7% CO_2. For use in the growth studies, a 48-h stock culture on Columbia blood agar with 10% sheep blood was inoculated into 5 ml of modified K broth. Stock cultures were also grown in brucella broth (Difco) for comparison with growth in modified K broth.

Plate counts. Appropriate dilutions of the 24-h cultures grown in modified K broth were prepared in 0.85% saline and surface plated in triplicate onto the following basal media: brucella agar (BBL and Difco), blood agar base no. 2 (Oxoid), campylobacter agar base (Difco), Columbia blood agar base (Difco and Oxoid), and Mueller-Hinton agar (Difco and Oxoid). The composition of the basal media is summarized in Table 1. The basal media were also prepared either with addition of FBP (0.05% each ferrous sulfate hydrate, sodium metabisulfite, and sodium pyruvate) or with 7% defibrinated sheep blood. Plates with FBP were prepoured and held at room temperature (ca. 21°C) to dry overnight. Some media with added sheep blood required additional agar to prevent swarming. The increased agar levels were as follows: 2% agar in brucella agar (Difco), campylobacter agar base (Difco), and Mueller-Hinton agar (Oxoid) and 2.5% agar in Columbia blood agar base (Oxoid). All prepoured and inoculated plates were held in the dark (12). A 0.1-ml sample of appropriate dilutions, to give 30 to 300 CFU per plate, was inoculated onto each medium. Inoculated

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TABLE 1. Composition of basal media used for growth of *Campylobacter* spp.

Ingredient	g/liter				
	Brucella agar (BBL and Difco)	Blood agar base no. 2 (Difco and Oxoid)[a]	Columbia blood agar (Difco and Oxoid)[a]	Mueller-Hinton agar	
				Difco	Oxoid
Peptone[b]	20.0	15.0	23.0		
Yeast extract	2.0	5.0			
Casein hydrolysate[c]				17.5	17.5
Meat infusion				300[d]	6.0
Liver digest		2.5			
Dextrose	1.0				
Starch			1.0	1.5	1.5
NaCl	5.0	5.0	5.0		
Sodium bisulfite	0.1				
Agar	15.0	12.0	15/10[e]	17.0	10.0
pH	7.0	7.4	7.3	7.4	7.4

[a] Difco campylobacter agar base is a standardized blood agar no. 2.
[b] Brucella agar (BBL) contains 20 g of polypeptone peptone; brucella agar (Difco) contains 20 g of peptamine. Columbia blood agar base (Difco) contains 10 g of peptone, 10 g of bitone, and 3 g of tryptic digest of beef heart; that from Oxoid contains 23 g of special peptone.
[c] Mueller-Hinton agar (Difco) contains technical Casamino Acids; that from Oxoid contains casein hydrolysate.
[d] Mueller-Hinton agar (Difco) contains infusion from 300 g of beef.
[e] Columbia blood agar (Difco) contains 15 g of Bacto-Agar that from Oxoid contains 10 g of agar no. 1.

plates were dried in a laminar-flow hood for 15 min and incubated at 37°C in a 7% CO_2 atmosphere for 48 and 72 h for enumeration of the colonies. The experiment was done in duplicate.

Data analysis. Media were compared by using $\log_{10}$ transformed data for analysis of variance, using a BMDP statistical package (Biomedical Computer Programs, P-series, University of California, 1983). Duncan's multiple range test was used, where appropriate, to measure differences among medium means.

RESULTS

In the preliminary study, the two *C. laridis* strains grew poorly on Mueller-Hinton agar (Difco) and on brucella agar (Oxoid). All of the strains tested grew poorly on Columbia blood agar base (Oxoid), which produced a 10- to 1,000-fold reduction in CFU per milliliter compared with other media. Analysis of variance of the data, excluding the data for Columbia blood agar base and *C. laridis*, revealed a statistically significant difference attributable to media ($P = 0.013$). Duncan's multiple range test was used at the 95% confidence level to measure differences among medium means. The results indicated that brucella agar (Difco) supported significantly lower CFU per milliliter than other media. However, the difference in the mean counts was less than fivefold, which is not of practical significance. Furthermore, there was no difference in counts observed on Columbia blood agar base containing 10% defibrinated sheep blood, using the standard 1.0% agar (Oxoid agar no. 1) or with the agar concentration increased to 3%. Colonies on the 1.0% agar medium were flat, large, and spreading, whereas on the 3% agar colonies were very small and difficult to count after 48 h of incubation. Brucella and Mueller-Hinton agars (basal media without added blood) gave counts equivalent to those on Columbia blood agar base with 10% defibrinated sheep blood.

Consequently, the possibility of using basal media without FBP or 7% sheep blood supplement was assessed for quantitative growth of *C. jejuni* and *C. coli*. All strains of *C. jejuni* and *C. coli* grew to 10^8 CFU/ml within 24 h at 37°C in modified K and brucella broths. No change in count was observed after 48 h of incubation. However, after 48 h, phase-contrast microscopy revealed that some coccoid forms were present. Coccoid forms are believed to be a degenerative form of *Campylobacter* cells (6). As a result, all strains in this study were subsequently grown in modified K broth. This was done to preclude the degenerative coccoid forms and any possibility that prior growth in brucella broth would give brucella agars an advantage over other basal media. Log_{10} mean counts and the standard deviations for growth of four strains of *C. jejuni* and four strains of *C. coli* on basal and supplemented media are shown in Table 2. These data are based on duplicated experiments of the eight cultures on each medium. An analysis of variance for three grouping factors and repeated measures (BMDP-2V) was used to compare the growth of the eight test cultures on the eight media and the effect of added FBP or blood supplements. This was done on the mean $\log_{10}$ transformed counts obtained after 48 and 72 h of incubation. A summary of the analysis of variance is shown in Table 3. Significant effects were attributable to media and supplements. There were also significant interaction effects between culture and supplement and medium and supplement. The interaction effect between medium and supplement was probably due to the fact that not all basal media require growth supplements for optimum recovery of the test cultures under the conditions of this experiment. There was a statistically significant increase ($P < 0.001$) in the count at 72 versus 48 h. Some strains of *C. jejuni* and *C. coli* formed only very small colonies on the basal media after 48 h of incubation. After 72 h of incubation, all strains of *C. jejuni* and *C. coli* on all media formed colonies with diameters of between 1.0 and 2.6 mm. As a result, the 72-h count was selected for further analyses.

The data were separated into three groups, based on the use of supplements, for further analysis, using a two-way factorial design (ANOVA, BMDP-2V) (Table 4). With added supplements (FBP or 7% sheep blood) there was no significant difference among media used for growth of the test organisms, and there was no interaction effect between cultures and media. In contrast, there was a significant difference between basal media without supplements, as

TABLE 2. Log_{10} mean counts of duplicate experiments with eight strains of *C. jejuni* and *C. coli* grown on basal media with and without supplements, incubated at 37°C for 48 and 72 h

Medium	Log_{10} mean CFU/ml ± SD					
	Basal medium		With FBP[a]		With blood[b]	
	48 h	72 h	48 h	72 h	48 h	72 h
Blood agar base no. 2 (Oxoid)	7.02 ± 0.97	7.02 ± 0.97	8.53 ± 0.23	8.56 ± 0.20	8.55 ± 0.20	8.56 ± 0.20
Brucella agar (BBL)	8.51 ± 0.23	8.53 ± 0.22	8.53 ± 0.23	8.53 ± 0.23	8.54 ± 0.25	8.55 ± 0.25
Brucella agar (Difco)	7.88 ± 1.24	8.20 ± 0.71	8.33 ± 0.92	8.58 ± 0.21	8.56 ± 0.20	8.56 ± 0.20
Campylobacter agar base (Difco)	8.24 ± 0.91	8.44 ± 0.26	8.56 ± 0.22	8.54 ± 0.22	8.08 ± 1.22	8.46 ± 0.31
Columbia agar (Difco)	7.20 ± 1.11	7.46 ± 0.79	8.55 ± 0.21	8.57 ± 0.21	8.51 ± 0.22	8.53 ± 0.20
Columbia agar (Oxoid)	6.97 ± 1.06	7.15 ± 0.97	8.31 ± 0.91	8.54 ± 0.21	8.06 ± 1.22	8.45 ± 0.29
Mueller-Hinton (Difco)	8.00 ± 1.20	8.40 ± 0.28	8.32 ± 0.91	8.55 ± 0.21	8.27 ± 0.90	8.47 ± 0.27
Mueller-Hinton (Oxoid)	8.50 ± 0.24	8.50 ± 0.24	8.34 ± 0.91	8.56 ± 0.20	8.32 ± 0.91	8.52 ± 0.22

[a] 0.05% each ferrous sulfate hydrate, sodium metabisulfite, and sodium pyruvate.
[b] 7% defibrinated sheep blood.

well as a significant interaction effect between cultures and media. The log_{10} mean counts for the cultures on each of the basal media are shown in Table 5. The lowest colony counts were observed on Columbia blood agar base (Difco and Oxoid) and blood agar base no. 2 (Oxoid). Two of the *C. coli* strains did not grow well on brucella agar (Difco). However, all strains grew well on other basal media, including campylobacter agar base (Difco), which is equivalent to blood agar base no. 2 (9). With the addition of FBP or 7% defibrinated sheep blood supplements to Columbia blood agar base or blood agar base no. 2 (Oxoid), the inhibitory action of these media was averted. Examination of the means indicates that the interaction effect can be attributed to the variable growth response of the cultures on Columbia blood agar base (Difco and Oxoid), brucella agar (Difco), and blood agar base no. 2 (Oxoid). When these media were excluded from the analysis, there was no significant difference between counts at 48 and 72 h or among basal media: Mueller-Hinton agar (Difco and Oxoid), campylobacter agar base (Difco), and brucella agar (BBL). The addition of growth supplements to these media did not significantly change the counts of the test cultures on the media.

TABLE 3. Summary of repeated-measures analysis of variance for growth response of *C. jejuni* and *C. coli* cultures on different growth media after 48 and 72 h of incubation

Source of variation[a]	df	*F* value	Probability
C	7	14.49	<0.001
S	2	67.68	<0.001
M	7	10.17	<0.001
CS	14	3.78	<0.001
CM	49	1.28	0.12
SM	14	10.40	<0.001
CSM	98	0.96	0.59
I	1	20.07	<0.001
CI	7	4.93	<0.001
SI	2	0.21	0.81
MI	7	1.25	0.28
CIS	14	1.60	0.08
CIM	49	0.99	0.50
SIM	14	0.65	0.82
SICM	98	0.59	0.99

[a] C, Culture; S, medium supplements; M, media; I, incubation.

DISCUSSION

Modified K broth supported the growth of all *Campylobacter* spp. used in this study. Cultures grew to 10^8 to 10^9 CFU/ml within 24 h at 37°C. When examined by phase-contrast microscopy, all cells appeared to be in the spiral form, and no degenerative cocci were observed. Hence, differences in counts on the solid growth media are attributed to differences among media. The nutrient composition of the

TABLE 4. Summary of two-way (factorial design) analyses of variance for comparison of growth response of cultures inoculated on basal and supplemented media

Source of variation[a]	df	*F* value	Probability
Basal media			
C	7	13.20	<0.001
M	7	35.10	<0.001
CM	49	2.14	0.002
Media with FBP supplement			
C	7	19.19	<0.001
M	7	0.14	0.99
CM	49	0.14	1.00
Media with 7% sheep blood			
C	7	18.64	<0.001
M	7	1.05	0.41
CM	49	0.44	0.99

[a] C, Cultures, M, media.

TABLE 5. Log_{10} mean count of *C. jejuni* and *C. coli* plated on basal media without supplements

Culture	Log_{10} mean count on given medium[a]							
	DMH	DCB	DBA	DCAMP	OMH	OCB	OBA2	BBA
C. jejuni serotype:								
4	8.33	7.97	8.64	8.37	8.39	6.81	6.98	8.59
5	8.56	7.04	8.54	8.56	8.53	7.76	6.98	8.58
7	8.28	8.16	8.33	8.18	8.28	7.68	7.82	8.27
17	8.36	6.56	8.45	8.44	8.50	7.18	7.33	8.55
C. coli serotype:								
8	8.18	7.68	8.59	8.28	8.57	7.68	7.60	8.49
20	8.14	7.07	7.34	8.26	8.23	6.52	5.71	8.36
45	8.84	8.66	8.81	8.89	8.87	8.18	7.66	8.89
55	8.48	6.52	6.90	8.55	8.59	5.40	6.10	8.50

[a] BBA, DBA = brucella agar (BBL, Difco); DCAMP = camplylobacter agar base (Difco); DCB, OCB = Columbia blood agar base (Difco, Oxoid); DMH, OMH Mueller-Hinton agar (Difco, Oxoid); OBA2 = blood agar base no. 2 (Oxoid).

basal media differs not only between media types but also within types between manufacturers, for example, brucella agar.

The use of a 7% CO_2 atmosphere and incubation at 37°C were satisfactory for growth of stock strains of *Campylobacter*. Incubation time depends on the medium used; 48 h of incubation is sufficient for media with FBP or blood supplements and for four of the basal media in our study, including Mueller-Hinton agar (Difco and Oxoid), campylobacter agar base (Difco), and brucella agar (BBL). For the other four basal media without supplements, 72 h of incubation is necessary for adequate growth of the colonies.

The low colony counts on the basal media, Columbia blood agar base (Difco and Oxoid), blood agar base no. 2 (Oxoid), and brucella agar (Difco), indicated an inhibition of growth of some cells on these media. This inhibitory effect can be eliminated by addition of either FBP or defibrinated sheep blood supplements to these media. Our studies show that brucella agar (BBL), campylobacter agar base (Difco), and Mueller-Hinton agar (Difco and Oxoid) can be used without enriching supplements for growth of *Campylobacter* cells in pure culture. The recovery rate on these basal media was virtually 100% when compared with supplemented media. In contrast, Bolton and Coates (3) reported only a 9% recovery rate as the best result with the basal media they used. In their study, the basal media included blood agar base no. 2 (Oxoid) and Columbia blood agar base (Oxoid), which gave the poorest results in our study.

Some researchers increased the amount of agar in campylobacter media, which reduces swarming of the organisms (19, 22). However, Martin et al. (18) indicated that the use of additional agar (3%) may not be necessary in the media used for isolation purposes. The use of additional agar was necessary for some media supplemented with blood to reduce swarming of the organisms on the agar surface. This further detracts from the use of blood as an enriching supplement for routine media intended for enumeration purposes. The strains used in this study did not swarm on basal media (with or without FBP) with normal amounts of agar (1.0% Oxoid and 1.5% Difco). However, the plates used were dried, as described in Materials and Methods. Hence, adding extra agar to basal media may not be advantageous because increased agar concentration results in reduced colony size.

For isolation of *Campylobacter* spp. from stools and mixed cultures, antibiotics are added to the media (6, 14, 18, 23). The medium chosen may affect the final activity of the antibiotics (6). Mueller-Hinton agar is recommended for antibiotic susceptibility testing (2), and we have shown that Mueller-Hinton agar gives high recovery rates when pure cultures are plated onto this medium. It is likely that Mueller-Hinton agar would be the medium of choice for growth of *C. jejuni* and *C. coli*, especially when antibiotics are included in the medium.

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