JOURNAL OF CLINICAL MICROBIOLOGY, Mar. 1981, p. 526-531
0095-1137/81/03-0526/06$02.00/0
Vol. 13, No. 3

Selective Medium for Isolation of *Clostridium botulinum* from Human Feces

M. DEZFULIAN,† L. M. McCROSKEY, C. L. HATHEWAY, AND V. R. DOWELL, JR.*
Enterobacteriology Branch, Bacteriology Division, Bureau of Laboratories, Centers for Disease Control, Atlanta, Georgia 30333

A selective medium, *Clostridium botulinum* isolation (CBI) agar, was developed for the isolation of *C. botulinum* from human feces. This medium contains cycloserine (250 μg/ml), sulfamethoxazole (76 μg/ml), and trimethoprim (4 μg/ml) as selective inhibitory agents. Qualitative tests indicated complete recovery of *C. botulinum* types A, B, F, and G on CBI medium. It was more difficult to recognize type G colonies on the medium because of their lack of lipase activity. Except for a few species of *Clostridium*, the growth of other obligate anaerobes and of the facultative anaerobes tested on CBI medium was suppressed. Quantitative studies of *C. botulinum* on the selective medium yielded counts comparable to those obtained on egg yolk agar control plates. Isolation of *C. botulinum* types A, B, and F from seeded fecal specimens was easily achieved with CBI medium. The use of CBI agar should aid the rapid isolation of *C. botulinum* from fecal specimens associated with foodborne and infant botulism.

In recent years, coproexamination has been shown to be an invaluable procedure for laboratory confirmation of foodborne and infant botulism (6, 10, 14). The procedure is especially useful for confirmation of infant botulism because botulinal toxin is very rarely detected in the serum of an infant with the illness (1, 13). Coproexamination involves testing an extract of the patient's feces for botulinal toxin with mouse bioassay and toxin neutralization techniques and culturing the feces for *Clostridium botulinum* (1, 6, 10).

Usually, a culture procedure employing a spore selection technique (heat or ethanol treatment to kill vegetative cells) in combination with nonselective liquid enrichment and agar plating media is used for isolating *C. botulinum* (6, 17). One of the major disadvantages of this procedure is that strains of *C. botulinum* which do not sporulate readily (e.g., *C. botulinum* type G [18]) may not be isolated.

It has recently been reported (3) that *C. botulinum* types A and B have a high level of resistance to cycloserine, sulfamethoxazole, and trimethoprim. Also, Swenson et al. (20) have shown that *C. botulinum* types A, B, and G and proteolytic type F are highly resistant to a combination of sulfamethoxazole and trimethoprim. In this paper, we describe the development of an agar medium, *C. botulinum* isolation (CBI) medium, containing cycloserine, sulfamethoxazole, and trimethoprim as selective agents, which allows quantitative as well as qualitative primary isolation of *C. botulinum* from stool specimens.

† Present address: Johns Hopkins University Medical School, Baltimore, MD 21205.

MATERIALS AND METHODS

Organisms. One hundred twenty strains of bacteria were used in the study. These included 48 strains of *C. botulinum*, 35 strains (16 species) of other clostridia, 15 strains (11 species) of nonsporeforming anaerobes, and 22 strains (8 species) of various facultatively anaerobic bacteria. The clostridia, including *C. botulinum*, came from the collection of the Centers for Disease Control (CDC) Anaerobe Section. All of the strains of *C. botulinum* type B were proteolytic. Five of the type F strains were proteolytic, and two (F-5 and F-6) were nonproteolytic. Details regarding long-term and short-term storage and techniques for confirming the identity of clostridia have been reported previously (3, 4). Pure cultures of the facultative anaerobes, previously identified by various reference laboratories at CDC, were obtained from the CDC Antimicrobics Investigations Section.

Medium for isolation of *C. botulinum*. On the basis of previous findings at CDC (3, 20) we decided to cultivate *C. botulinum* on CDC-modified McClung Toabe egg yolk agar (EYA) containing cycloserine (ICN Pharmaceuticals, Cleveland, Ohio), sulfamethoxazole (Wellcome Laboratories, Research Triangle Park, N.C.), and trimethoprim (Wellcome Laboratories) in various concentrations. In preliminary experiments, the effects of various concentrations of cycloserine, sulfamethoxazole, and trimethoprim in EYA on the growth of *C. botulinum* were studied to determine optimal concentrations of the antimicrobial agents to use (see Table 1).

On the basis of data derived from the preliminary study and information from the literature relative to

the antimicrobial action of cycloserine, sulfamethoxazole and trimethoprim on other bacteria, we formulated a medium we call CBI. The medium has the following composition (per liter): Trypticase peptone (BBL Microbiology Systems, Cockeysville, Md.), 40.0 g; Na_2HPO_4, 5.0 g; NaCl, 2.0 g; $MgSO_4$ (5% aqueous solution), 0.2 ml; D-glucose, 2.0 g; agar, 20.0 g; yeast extract (Difco Laboratories, Detroit, Mich.), 5.0 g; distilled water, 900.0 ml; egg yolk suspension (50% in saline; Difco) 100.0 ml; cycloserine, 250.0 mg; sulfamethoxazole, 76.0 mg; and trimethoprim, 4.0 mg.

In preparing the medium, all of the ingredients except the egg yolk suspension and the antimicrobial agents were mixed, dissolved by heating, adjusted to pH 7.4, and then autoclaved at 121°C for 15 min. After autoclaving, the basal medium was cooled to 55°C in a water bath, and the egg yolk suspension (warmed to ambient temperature) and sterile filtered stock solutions of the antimicrobial agents were added aseptically. The cycloserine stock solution was a 1% solution in distilled water. The stock solution of sulfamethoxazole (1.9%) was prepared by dissolving the drug in distilled water and adding enough 10% NaOH to put it in solution (19). The stock solution of trimethoprim (0.1%) was made in the same manner as that described for sulfamethoxazole, except 0.05 N HCl was used instead of 10% NaOH, and the process was carried out in a 55°C water bath. Twenty-five milliliters of the cycloserine, 4 ml of the sulfamethoxazole, and 4 ml of the trimethoprim solutions were added in the preparation of one liter of medium. If there is significant loss of volume due to evaporation during autoclaving the volume must be adjusted by adding sterile distilled water. The medium was mixed thoroughly and dispensed in 20-ml quantities into 15- by 100-mm plastic petri dishes and allowed to solidify. The plates were left at ambient temperature for several hours to avoid excessive moisture on the surface of the medium and then stored in sealed plastic bags at 4°C in a refrigerator. The plates were held in an anaerobic glove box for 4 h before use.

Evaluation of CBI medium. The medium was tested qualitatively by comparing growth obtained on it with that on EYA by using pure cultures of *C. botulinum*, various nonsporeforming anaerobes and *Clostridium* species, and a variety of facultative anaerobes (see Table 2). Bacterial suspensions equivalent to one-half the turbidity of a McFarland number 1 nephelometer standard (21) were prepared in peptone-yeast extract-glucose broth (5, 11) by suspending cells from colonies on EYA which had been incubated anaerobically at 35°C. For the qualitative comparisons, the bacterial suspensions were further diluted 1:10 in peptone-yeast extract-glucose before inoculating the CBI and EYA media. A plate of each medium was inoculated with 0.01 ml of diluted bacterial suspension and streaked for isolation by using disposable plastic loops (A/S Nunc, Roskilde, Denmark). Dilutions were made, and plates were inoculated on the laboratory bench; immediately after inoculation, the plates were placed in a jar flushed with nitrogen until they could be transferred to the anaerobic glove box. Growth on the two media was compared after 48 h of incubation in an anaerobic system at 35°C.

Quantitative recovery of *C. botulinum* strains (types A, B, F, and G) on CBI and EYA media were compared (see Table 3). The bacterial suspension in peptone-yeast extract-glucose (one-half the turbidity of a McFarland no. 1 standard) as described above was diluted in 10-fold increments in peptone-yeast extract-glucose. The 10^3, 10^4, and 10^5 dilutions of the suspension were used for inoculation of media. Either a disposable 0.01-ml plastic loop or a 0.025-ml pipette dropper (Cooke Laboratory Products, Alexandria, Va.) was used for inoculation of the media with the diluted cell suspensions. In the latter case, sterile bent-glass rods ("hockey sticks") were used to spread the inoculum over the surface of the medium. The plates were incubated anaerobically at 35°C for 48 h, and the colonies were counted to obtain the number of organisms recovered on each medium.

Recovery of *C. botulinum* from seeded fecal specimens on CBI medium was compared with that obtained by conventional techniques (spore selection by ethanol, heat treatments, and growth on EYA medium). Seeded fecal samples were prepared by adding samples of *C. botulinum* cell suspensions to fecal specimens obtained from healthy adults (CDC employees). A sample (10 to 20 g) of feces was weighed in a sterile mortar and thoroughly mixed with gelatin phosphate diluent (4), 1 g of feces to 3 ml of diluent, until a uniform suspension was obtained. Then 1 ml of a 10^2 dilution of the *C. botulinum* cell suspension (one-half the turbidity of a McFarland no. 1 standard in peptone-yeast extract-glucose) was mixed with 4 ml of fecal suspension. The seeded fecal sample was then divided equally in two screw-capped test tubes. One portion was mixed with an equal volume of absolute ethanol to kill vegetative cells of bacteria as described previously (4). The other portion was mixed with an equal volume of the gelatin-phosphate diluent. This 10^1 dilution of fecal sample was further diluted in 10-fold increments to a final dilution of 10^3. Plates of the CBI and EYA media were inoculated with samples (0.01 or 0.025 ml) of the diluted feces (10^1, 10^2, or 10^3) and incubated anaerobically at 35°C for 48 h, and the colonies were counted. In some experiments the 10^1 dilution of seeded fecal suspensions was held in an 80°C water bath for 10 min to kill vegetative cells before inoculating the CBI and EYA media (see Table 4).

Toxicity tests. Random colonies from fecal samples exhibiting characteristic lipase activity on CBI and EYA media were picked to tubes of chopped meat-glucose-starch medium (5) and were tested for toxicity. Toxin neutralization tests to identify the toxin type were performed as described previously (3, 4, 10).

RESULTS

Optimal concentrations of antimicrobial agents. Data from a preliminary experiment to determine the quantitative recovery of *C. botulinum* on EYA medium containing various concentrations of cycloserine, sulfamethoxazole, and trimethoprim are shown in Table 1. On the basis of these data and data from several similar experiments it was decided to use 250 mg of

TABLE 1. *Quantitative recovery of C. botulinum on CDC modified McClung-Toabe EYA containing various concentrations of cycloserine (cycl), sulfamethoxazole (SMX), and trimethoprim (TMP)*

Combination	Concn (μg/ml) of:			Viable *C. botulinum* per ml of cell suspension	
	Cycl	SMX	TMP	Type A (A28K)	Type G (G3)
A	500	152	8	4.5×10^6	Microcolonies
B	250	76	4	4.7×10^6	4.0×10^7
C	500	76	4	5.0×10^6	4.8×10^7[a]
D	250	152	8	6.0×10^6	6.8×10^7[a]
E (control)	0	0	0	5.4×10^6	4.3×10^7

[a] Very small colonies.

cycloserine, 76 mg of sulfamethoxazole, and 4 mg of trimethoprim per liter of medium (combination B in Table 1), and this formulation, designated CBI medium, was used in all subsequent experiments to be described. As shown in Table 1, *C. botulinum* type A grew quite well in all of the media with or without the antimicrobial agents, and type G was inhibited and showed only tiny colonies on media prepared with certain combinations of the antimicrobial agents (combinations A, C, and D in Table 1).

Qualitative tests. Growth of 48 strains of *C. botulinum*, 35 strains of other clostridia, 15 strains of gram-positive and gram-negative nonsporeforming anaerobes, and 22 strains of facultatively anaerobic bacteria on CBI medium was compared with that on EYA medium (Table 2). All 14 strains of *C. botulinum* type A, all 21 strains of type B, all 7 strains of type F (proteolytic and nonproteolytic), all 3 strains of type G, and 1 out of 3 strains of type E grew on CBI plates (Table 2).

Among 34 species of obligately anaerobic and facultatively anaerobic bacteria tested, only five species of *Clostridium* (*C. bifermentans*, *C. cadaveris*, *C. perfringens*, *C. sordellii*, *C. sporogenes*) grew on CBI medium. The *C. perfringens* strains were partially inhibited by the selective medium (Table 2). All the organisms tested grew on EYA medium control plates.

Quantitative tests. The recovery rates for 18 isolates of *C. botulinum* (including 8 of type A, 4 of type B, 5 of type F, and 1 of type G) were determined on CBI and EYA media (Table 3). No difference was observed in the recovery of the organisms on CBI versus EYA medium, except the size of the colonies. The colonies appeared slightly smaller in certain cultures grown on CBI as compared with EYA.

Cell morphology of the bacteria grown on CBI plates appeared normal when the gram-stained smears from random colonies were examined under the microscope. As expected, the colonies of all type A, B, and F strains were lipase positive, and the type G colonies were lipase negative.

TABLE 2. *Growth of various species of bacteria on CBI medium*[a]

Species	No. tested	No. growing on:	
		CBI medium	EYA medium
Clostridium botulinum type A	14	14	14
C. botulinum type B	21	21	21
C. botulinum type E	3	1	3
C. botulinum type F	7	7	7
C. botulinum type G	3	3	3
C. bifermentans	3	3	3
C. cadavaris	3	3	3
C. perfringens	2	(2)[b]	2
C. sordellii	3	3	3
C. sporogenes	2	2	2

[a] Strains that grew on the EYA control plate, but not on CBI medium, included: 3 strains each of *Clostridium butyricum*, *C. difficile*, *C. innocuum*, *C. paraputrificum*, *C. septicum*, *C. tertium*, *Bacteroides fragilis*, *Escherichia coli*, and *Proteus vulgaris*; 2 strains each of *Clostridium limosum*, *C. sphenoides*, *C. subterminale*, *Eubacterium limosum*, *Propionibacterium acnes*, *Providencia stuartii*, and *Staphylococcus aureus*; 1 strain each of *Clostridium ramosum*, *Bacteroides distasonis*, *B. eriksonii*, *B. ovatus*, *B. thetaiotaomicron*, *B. uniformis*, *B. vulgatus*, *Eubacterium lentum*, *E. moniliforme*, *Citrobacter freundii*, *C. diversus*, *Bacillus cereus*, and *B. subtilis*; 4 strains of *Proteus mirabilis*; and 5 strains of *Streptococcus faecalis*.

[b] Parentheses indicate partial inhibition.

Recovery of *C. botulinum* from seeded fecal samples. The results of quantitative testing with nine fecal specimens seeded with 16 pure cultures of *C. botulinum* (types A, B, and F) are summarized in Table 4. As expected, the EYA medium was nonselective and yielded bacterial colonies of various types. The *C. botulinum* strains on EYA appeared as flat colonies embedded in the agar and usually were covered with multiple colonies of the fecal flora. The diffused lipase reaction of these colonies differed considerably from the typical reaction characteristic of pure cultures of *C. botulinum* on EYA plates. The CBI medium was clearly more advantageous than EYA medium for isolation of *C. botulinum*. The number of lipase-positive colonies appearing on the CBI plates was comparable to that on EYA medium (Table 4), and the number of (lipase-negative) colonies derived

TABLE 3. *Recovery of C. botulinum strains on CBI and EYA media*

Isolate[a]	Toxin type	No. of organisms recovered/ml of culture on: CBI medium	EYA medium
a-3N	A	6.8×10^6	6.0×10^6
a-1	A	5.4×10^8	4.8×10^8
a-4	A	2.8×10^6	3.1×10^6
A-22	A	6.8×10^6	5.0×10^6
A-24	A	6.4×10^6	7.0×10^6
A-28K	A	4.0×10^7	2.8×10^7
A-29	A	5.4×10^7	4.1×10^7
b-15	B	9×10^5	2.2×10^6
B-21	B	2.4×10^6	2.0×10^6
B-35	B	1.3×10^6	1×10^6
B-23	B	1×10^5	9×10^5
F-1	F	3.2×10^7	2.4×10^7
F-2	F	3.2×10^7	2×10^7
F-3	F	1.6×10^6	4.8×10^5
F-4	F	3.2×10^7	2×10^7
G-3	G	7.6×10^7	7×10^7

[a] Type A and B isolates designated with lower-case letters were isolated from infant botulism cases; all strains used in these experiments were proteolytic.

from fecal flora was reduced approximately 50 to 80%. These lipase-negative colonies were limited to a few colonial types and were mostly pinpoint to 0.5 mm in diameter. The highest dilution of feces used in these experiments was 10^3, so that the small number of *C. botulinum* cells seeded in the fecal specimen could be detected. Obviously, at the higher dilutions, the selectivity of the medium could be improved, although at the expense of its sensitivity. In contrast to the flat colonies of *C. botulinum* on EYA plates, the colonies of this organism on CBI medium were somewhat raised and more discrete due to less competition and exhibited a typical lipase reaction. All of 10 lipase-positive colonies selected at random from CBI plates streaked with seeded fecal specimens were proved to be toxic and type specific by toxin neutralization tests.

The lecithinase-positive colonies, which appeared on most of the EYA plates inoculated with feces, were partially or completely inhibited by CBI medium, and, at least in one case, the lecithinase activity rather than the growth of the organism was completely suppressed.

As shown in Table 4, heat (80°C for 10 min) or alcohol treatments resulted in reduced recovery or complete elimination of *C. botulinum* from the fecal specimens.

Lack of lipase activity makes *C. botulinum* type G distinct from other toxigenic types of this organism. With a dilution technique we obtained an indirect evidence that this type of *C. botulinum* could also be recovered from a mixed population. A fecal specimen was diluted in a 10-fold series (10^1 to 10^6) before or after addition of a small number of *C. botulinum* type G. Plates of CB were then inoculated with 0.01 ml of various dilutions of the two specimens. No colonies resembling type G were recovered from the three highest dilutions (10^4, 10^5, and 10^6) of the unseeded fecal specimen, but 36, 11, and 1 colonies, respectively, were recovered from the same dilutions of the seeded specimen. The characteristics of these colonies were identical

TABLE 4. *Recovery of C. botulinum from seeded fecal specimens on CBI medium*[a]

Fecal specimen	Seed organism[b]	Toxin type	Spore selection method	No. of lipase-positive organisms recovered from: CBI	EYA control
1	a-3N	A	None	2.8×10^6	1.6×10^6
2	A-28K	A	None	1.12×10^7	7.6×10^6
			Alcohol	3.1×10^5	1.0×10^5
3	a-1	A	None	4.8×10^8	2.4×10^8
4	a-4	A	None	8.0×10^5	3.2×10^5
			Alcohol	0	0
5	A-22	A	None	1.0×10^6	4.4×10^6
			Alcohol	0	0
6	A-24	A	None	1.0×10^6	8.4×10^5
			Alcohol	2.4×10^5	8.0×10^4
7	A-29	A	None	2.0×10^7	4.0×10^7
			Alcohol	0	0
8	A-28N	A	None	1.0×10^6	3.0×10^6
			Alcohol	0	0
9	a-15N	A	None	5.6×10^6	1.3×10^7
			Heat	0	0
10	b-2	B	None	2.5×10^6	2.7×10^6
11	B-41N	B	None	3.6×10^6	5.6×10^6
			Heat	0	0
12	F-1A	F	None	7.7×10^6	6.2×10^7
13	F-1	F	None	1.6×10^7	1.6×10^7
14	F-3	F	None	2.4×10^7	1.2×10^7
15	F-2	F	None	8.8×10^6	1.0×10^7
			Alcohol	3.2×10^5	4.8×10^5
16	F-4	F	None	1.2×10^6	4.0×10^5

[a] Lipase-positive colonies were considered as *C. botulinum* since unseeded specimens did not yield any, and all lipase-positive colonies tested produced botulinal toxin.

[b] Type A and B strains designated with lowercase letters were isolated from infant botulism cases; all strains used in these experiments were proteolytic.

with those of the *C. botulinum* type G on EYA medium.

DISCUSSION

A method commonly used for isolation of *C. botulinum* from fecal samples involves cultivation of primary specimen or enriched culture on EYA medium (6, 10). Though differential for lipase-positive organisms (including most toxigenic types of *C. botulinum*), EYA medium is nonselective and allows the growth of a wide variety of bacteria from fecal flora. Spore selection techniques (heat or alcohol treatment) are commonly used for selective isolation of clostridia from fecal specimens (6, 17). Our data, however, indicate that when only a small number of spores are present in the fecal sample, heat or alcohol treatment may lead to the elimination of the organism and thus to a false-negative result (Table 4). Use of CBI medium would allow recovery of *C. botulinum* by direct streaking with a specimen without treatment or enrichment.

The use of cycloserine, sulfamethoxazole, and trimethoprim in CDC modified EYA yielded a medium (CBI) which is both selective and differential for *C. botulinum* types A, B, and F. This selective medium proved superior to the nonselective method of culturing on EYA and the selection method involving heat and alcohol treatment. Whereas the majority of other microorganisms present in feces were inhibited, some did grow on CBI medium. The colonies of these bacteria on CBI medium were mostly small or pinpoint and could easily be distinguished from the lipase-positive colonies of *C. botulinum* types A, B, and F. Due to the inhibitory effect of CBI on the fecal microflora, the colonies of *C. botulinum* on this medium were usually readily apparent and easy to isolate.

A combination of cycloserine, sulfamethoxazole, and trimethoprim was incorporated into the EYA medium with the knowledge that the presence of thymidine in the complex EYA medium might interfere with the bacteriocidal effect of the combination of sulfamethoxazole and trimethoprim (12, 19) and thus with the selectivity of the medium. It has been demonstrated that adding thymidine phosphorylase (present in hemolyzed horse blood) may improve certain media (with limited thymidine) by converting thymidine to thymine (19). Improved selectivity of CBI medium, however, would interfere with the isolation of less resistant type G (Table 1) and nonproteolytic type F (20) strains of *C. botulinum*. Cycloserine alone or in combination with cefoxitin has previously been used for the isolation of *C. difficile* (7, 23). Likewise, trimethoprim combined with sulfamethoxazole (9) or other antimicrobial agents (16) has been employed in various selective media.

Cycloserine in a concentration of 500 μg/ml has been shown to affect the morphology of bacterial cells, converting the gram-positive bacilli of *C. difficile* to filamenteous forms (7). No such effect was observed in the case of *C. botulinum* cells growing on CBI medium (with cycloserine at 250 μg/ml). The morphology and sporulation of the cells, as judged by microscopic examination of gram-stained smears of the colonies on CBI medium remained normal.

Although *C. botulinum* type G can also be recovered on CBI medium, lack of lipase activity makes the differentiation of the colonies of this organism from those of other bacteria more difficult. In cases where the results of clinical and toxigenic studies warrant a search for *C. botulinum* type G, further examination of the lipase-negative colonies on CBI medium is justified. Presumptive identification can be achieved by means of a spot test for "indole-derivatives" and examination of volatile and nonvolatile acids by gas-liquid chromatography (15; M. Dezfulian, unpublished data), and the identity can be confirmed by toxin-neutralization tests in mice (2, 8). The immunodiffusion technique of Ferreira et al. (J. L. Ferreira, M. K. Hamdy, F. A. Zapatka, and W. O. Herbert, Abstr. Annu. Meet. Am. Soc. Microbiol. 1980, P8, p. 190), based on the interaction of toxin and antitoxin to form a "precipitin zone" around the colonies of *C. botulinum* type A, could possibly be extended to the presumptive identification of *C. botulinum* type G colonies on the selective medium. In the present report, we described the use of diluted fecal suspensions cultured on CBI for the recovery of this organism.

Recently, Wilcke et al. (22) described the use of a medium consisting of heart infusion broth supplemented with agar, egg yolk (3%), and kanamycin sulfate (12 μg/ml) in the quantitation and isolation of *C. botulinum* from the feces of four infants with botulism. Although the authors found that the medium did not appreciably inhibit fecal bacteria, they stated that it did suppress the growth of some organisms sufficiently so that the presence of lipase-positive colonies of *C. botulinum* could be easily detected. It is well known that a variety of intestinal bacteria (sporeformers and nonsporeformers) are resistant to high concentrations (e.g., 100 μg/ml) of aminoglycoside antibiotics. Some, such as kanamycin, paromomycin, and neomycin are commonly used for selective isolation of bacteroides, fusobacteria, *C. perfringens*, and a variety of others from clinical materials (4).

Although CBI medium seems to be superior to nonselective EYA medium, the simultaneous use of both media for the isolation of *C. botulinum* from the primary specimen, and if necessary from the enriched culture, is recommended, to avoid missing drug-susceptible strains of *C. botulinum* such as some type E strains (20).

Our data also indicate that CBI medium, with or without some modification, can be used for the isolation of certain other clostridia such as *C. bifermentans*, *C. sordellii*, and *C. cadaveris* (Table 2). The colonies of these lipase-negative organisms are easily distinguished from *C. botulinum* colonies. Another organism which grew on CBI medium, as would be anticipated, was *C. sporogenes*, which can be differentiated from *C. botulinum* by the mouse toxicity and neutralization test.

The CBI medium should be especially useful for selective isolation and presumptive identification of *C. botulinum* associated with infant botulism. This assumption is based on the facts that: (i) the fecal bacterial flora of the human infant is less complex than that of the adult; (ii) only types A, B, and F (all proteolytic) have been associated with infant botulism to date; (iii) these toxin types are resistant to the antimicrobial agents in CBI medium; (iv) all three toxin types of *C. botulinum* exhibit lipase activity on the CBI medium.

LITERATURE CITED

1. **Center for Disease Control.** 1979. Botulism in the United States, 1899-1977. Center for Disease Control, Atlanta, Ga.
2. **Ciccarelli, A. S., D. N. Whaley, L. M. McCroskey, D. F. Gimenez, V. R. Dowell, Jr., and C. L. Hatheway.** 1977. Cultural and physiological characteristics of *Clostridium botulinum* type G and the susceptibility of certain animals to its toxin. Appl. Environ. Microbiol. **34**:843-848.
3. **Dezfulian, M., and V. R. Dowell, Jr.** 1980. Cultural and physiological characteristics and antimicrobial susceptibility of *Clostridium botulinum* isolates from foodborne and infant botulism cases. J. Clin. Microbiol. **11**:604-609.
4. **Dowell, V. R., Jr., and T. M. Hawkins.** 1974. Laboratory methods in anaerobic bacteriology: CDC laboratory manual. Center for Disease Control, Atlanta, Ga.
5. **Dowell, V. R., Jr., G. L. Lombard, F. S. Thompson, and A. Y. Armfield.** 1977. Media for isolation, characterization and identification of obligately anaerobic bacteria. Center for Disease Control, Atlanta, Ga.
6. **Dowell, V. R., Jr., L. M. McCroskey, C. L. Hatheway, G. L. Lombard, J. M. Hughes, and M. H. Merson.** 1977. Coproexamination for botulinal toxin and *Clostridium botulinum*: a new procedure for laboratory diagnosis of botulism. J. Am. Med. Assoc. **238**:1829-1832.
7. **George, W. L., V. L. Sutter, D. Citron, and S. M. Finegold.** 1979. Selective and differential medium for isolation of *Clostridium difficile*. J. Clin. Microbiol. **9**:214-219.
8. **Gimenez, D. F., and A. S. Ciccarelli.** 1970. Another type of *Clostridium botulinum*. Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. 1 Orig. Reihe **215**:221-224.
9. **Gunn, B. A., D. K. Ohashi, C. A. Gaydos, and E. S. Holt.** 1977. Selective and enhanced recovery of group A and B streptococci from throat cultures with sheep blood agar containing sulfamethoxazole and trimethoprim. J. Clin. Microbiol. **5**:650-655.
10. **Hatheway, C. L.** 1979. Laboratory procedures for cases of suspected infant botulism. Rev. Infect. Dis. **1**:647-651.
11. **Holdeman, L. V., E. P. Cato, and W. E. C. Moore.** 1977. Anaerobe laboratory manual, 4th ed. Virginia Polytechnic Institute and State University, Blacksburg, Va.
12. **Koch, A. E., and J. J. Burchall.** 1971. Reversal of the antimicrobial activity of trimethoprim by thymidine in commercially prepared media. Appl. Microbiol. **22**:812.
13. **Midura, T. F.** 1980. Infant botulism. Clin. Microbiol. News Letter **2**:1-3.
14. **Midura, T. F., and S. S. Arnon.** 1976. Infant botulism: identification of *Clostridium botulinum* and its toxin in feces. Lancet **ii**:934-936.
15. **Moss, C. W., C. L. Hatheway, M. A. Lambert, and L. M. McCroskey.** 1980. Production of phenylacetic and hydrophenylacetic acids by *Clostridium botulinum* type G. J. Clin. Microbiol. **11**:743-745.
16. **Skirrow, M. B.** 1977. Campylobacter enteritis: a "new" disease. Br. Med. J. **2**:9-11.
17. **Smith, L.** 1977. Botulism: the organism, its toxins, the disease. Charles C Thomas, Publisher, Springfield, Ill.
18. **Solomon, H. M., and D. A. Kautter.** 1979. Sporulation and toxin production by *Clostridium botulinum* type G. J. Food Protect. **42**:965-967.
19. **Swenson, J. M., and C. Thornsberry.** 1978. Susceptibility tests for sulfamethoxazole-trimethoprim by a broth microdilution procedure. Curr. Microbiol. **1**:189-193.
20. **Swenson, J. M., C. Thornsberry, L. M. McCroskey, C. L. Hatheway, and V. R. Dowell, Jr.** 1980. Susceptibility of *Clostridium botulinum* to thirteen antimicrobial agents. Antimicrob. Agents Chemother. **18**:13-19.
21. **Thornsberry, C., and J. M. Swenson.** 1978. Antimicrobial susceptibility testing of anaerobes. Lab. Med. **9**:43-48.
22. **Wilcke, B. W., Jr., T. F. Midura, and S. S. Arnon.** 1980. Quantitative evidence of intestinal colonization by *Clostridium botulinum* in four cases of infant botulism. J. Infect. Dis. **141**:419-423.
23. **Willey, S. H., and J. G. Bartlett.** 1979. Cultures for *Clostridium difficile* in stools containing a cytotoxin neutralized by *Clostridium sordellii* antitoxin. J. Clin. Microbiol. **10**:880-884.