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Survival of Anaerobic Bacteria in Various Thioglycolate and Chopped Meat Broth Formulations

MARINA C. CLAROS,[1,2] DIANE M. CITRON,[1]* AND ELLIE J. C. GOLDSTEIN[1,3]

R. M. Alden Research Laboratory, Santa Monica Hospital Medical Center, Santa Monica, California 90404[1]; School of Medicine, University of California, Los Angeles, California 90024[3]; and Paul Ehrlich Institute, D-63225 Langen, Germany[2]

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Three commercially available formulations of thioglycolate broth and of chopped meat broth were evaluated for their abilities to maintain the viabilities of 32 strains of anaerobic bacteria during a period of 8 weeks. While thioglycolate broth supported the initial (48-h) growth of all strains tested, approximately half of the strains died off within 4 weeks. Chopped meat broths maintained the viabilities of almost all cultures during the test period.

Clinical laboratories often need to store anaerobic isolates for extended periods of time to perform identification and susceptibility testing if it becomes clinically indicated. Modified thioglycolate broth (TGB) and chopped meat broth (CMB) are commonly used for such storage purposes (5).

While periodically collecting anaerobes from several local hospitals for research studies, we noticed that many isolates grown in TGB were not viable if there was a 1- to 2-week delay before subculture, whereas isolates grown in CMB maintained their viabilities for several months. Therefore, we studied the abilities of various commercially available formulations of TGB and CMB to grow and maintain the viabilities of a variety of clinical isolates of anaerobic bacteria.

Twenty-nine clinical and three American Type Culture Collection (ATCC) reference strains were selected for study on the basis of their growth characteristics and oxygen susceptibilities and their frequent presence in clinical specimens. The species and number of strains tested were as follows: *Bacteroides fragilis* ($n = 1$), *Bacteroides thetaiotaomicron* ($n = 1$), *Porphyromonas asaccharolytica* ($n = 1$), *Prevotella bivia* ($n = 5$), *Prevotella disiens* ($n = 1$), *Prevotella buccae* ($n = 1$), *Fusobacterium nucleatum* ($n = 2$), *Fusobacterium necrophorum* ($n = 2$), *Fusobacterium varium* ($n = 2$), *Clostridium perfringens* ($n = 1$), *Clostridium ramosum* ($n = 2$), *Clostridium clostridioforme* ($n = 2$), *Eubacterium lentum* ($n = 2$), *Actinomyces odontolyticus* ($n = 1$), *Actinomyces israelii* ($n = 1$), *Peptostreptococcus magnus* ($n = 2$), and *Peptostreptococcus anaerobius* ($n = 2$). The isolates were identified by standard procedures (3, 9). *B. fragilis* ATCC 25285, *B. thetaiotaomicron* ATCC 29741, and *C. perfringens* ATCC 13124 were included as standard controls.

The following broth media were tested: (i) enriched thioglycolate medium and cooked meat medium with glucose, hemin and vitamin K_1 (Baltimore Biological Laboratories, Cockeysville, Md.); (ii) thioglycolate medium and chopped meat glucose (Anaerobe Systems, San Jose, Calif.); and (iii) thioglycolate with supplements and cooked meat with iron (Hardy Media, Santa Maria, Calif.). The components of these broths are listed in Table 1 (CMB) and Table 2 (TGB).

The strains were maintained in skim milk at −70°C (9). Prior to the study, they were removed from the freezer and were transferred at least twice on supplemented brucella blood agar (BBA; Anaerobe Systems) to ensure purity and good growth. Several colonies of each isolate were inoculated into duplicate sets of each of the six broths on the bench. They were incubated at 36°C for 48 h, and the visual turbidity was recorded as 1+ to 4+. One set of each of the media was subcultured twice weekly for a period of 8 weeks by gently stirring and then plating a 0.01-ml plastic loopful (Difco Laboratories, Detroit, Mich.) of broth onto BBA. Cultures from the second set of broths were used only if a tube became contaminated during the study period and to verify the loss of viability. The cultures were kept at room temperature in the dark. At the end of the study period (8 weeks), the pHs of all cultures were measured.

Growth was generally more turbid at 48 h in TGB than in CMB, especially for the peptostreptococci and several of the fusobacteria. The peptostreptococci and fusobacteria achieved only 1+ to 2+ turbidity in CMB compared with a 3+ to 4+ turbidity in TGB. Three strains of *P. bivia* did not grow in CMB 3, possibly because of the lack of vitamin K_1 or hemin supplementation. Subcultures of CMB 3 yielded only a few colonies of *P. bivia* during the first 2 weeks, reflecting the presence of

TABLE 1. Components of three commercially available chopped meat broths

Ingredient	CMB no.[a]:		
	1	2	3[b]
Pancreatic digest casein (g)		30	
Casein meat peptone (g)	20		
Glucose (g)	5	3	
Yeast extract (g)	5	5	
Sodium chloride (g)	5		
Potassium biphosphate (g)		5	
Vitamin K (g)	0.001	0.001	
Hemin (g)	0.005	0.005	
Beef heart (g)	454		1[c]
Lean beef (g/tube)		~1	
Iron filings (mg/tube)			0.01
Distilled water (ml)	1,000	1,000	10

[a] Sources: 1, Baltimore Biological Laboratories; 2, Anaerobe Systems; 3, Hardy Diagnostics.

[b] Subsequent to the present study and communication with the manufacturer, the current formulation contains vitamin K, hemin, and yeast extract supplementation.

[c] One pellet per tube.

* Corresponding author. Mailing address: R. M. Alden Research Laboratory, Santa Monica Hospital Medical Center, 1250 16th St., Santa Monica, CA 90404. Phone: (310) 319-4325. Fax: (310) 829-6335.

TABLE 2. Components of three commercially available formulations of TGB

Ingredient	TGB no.[a]:		
	1	2	3
Pancreatic digest casein (g)	17		17
Papaic digest soybean meat (g)	3		3
Trypticase peptone (g)		15	
Animal tissue (g)		3	
Glucose (g)	6	6	6
Sodium chloride (g)	2.5	2.5	2.5
Sodium thioglycolate (g)	0.5	0.5	0.5
Agar (g)	0.7	0.7	0.7
L-Cystine (g)	0.75	0.75	0.75
Sodium sulfite (g)	0.1	0.1	0.1
Hemin (g)	0.005	0.005	0.005
Vitamin K (g)	0.001	0.001	0.001
Sodium bicarbonate (g)		1	
Calcium carbonate chip (no.)		—[b]	1
Distilled water (ml)	1,000	1,000	1,000

[a] Sources: 1, Baltimore Biological Laboratories; 2, Anaerobe Systems; 3, Hardy Diagnostics.

[b] —, subsequent to the present study and communication with the manufacturer, the current formulation contains a marble chip.

the inoculum rather than actual growth. One strain of *P. anaerobius* died after 6 weeks in CMB 2. All other strains remained viable for at least 8 weeks in all CMBs. The average pHs of the CMBs after 8 weeks were 6.22 in CMB 1, 6.50 in CMB 2, and 6.68 in CMB 3.

The time until the loss of viability of the isolates cultured in TGB is presented in Table 3. There was a much more rapid die-off of anaerobes in TGB than in CMB. The average pHs of the TGBs at the end of 8 weeks were 5.66 in TGB 1, 5.86 in TGB 2, and 6.04 in TGB 3. The main difference between TGB 3 and the other two TGB formulations was that it contained a marble chip which acts, in part, as a buffer. TGB 3 maintained the viability of several saccharolytic strains, including *C. ramosum*, *B. fragilis*, and *C. perfringens*, which produced considerably more acid in the other two formulations (TGBs 1 and 2) and, thus, may have contributed to their own demise. However, other strains that are nonsaccharolytic or weakly saccharolytic and that are usually thought of as oxygen susceptible, including *Prevotella* spp. and *Fusobacterium* spp., died rapidly in all of the formulations. (Subsequent to the present study and communicating with the manufacturer, TGB 2 is now prepared with a marble chip.)

TABLE 3. Time until loss of viability of various anaerobes in three formulations of TGB

Week	Organism(s) that lost viability in TGB[a]:		
	1	2	3
2	*C. ramosum*	*C. ramosum*	
3	*B. fragilis* *F. nucleatum* (*n* = 2) *F. necrophorum* (*n* = 2) *P. disiens* *P. anaerobius* *P. bivia*	*P. bivia* (*n* = 3) *F. nucleatum* *F. necrophorum* *P. buccae* *P. anaerobius*	*F. nucleatum* *P. buccae*
4	*P. bivia* (*n* = 2) *P. asaccharolytica* *P. buccae* *E. lentum*	*B. fragilis* (*n* = 2) *B. thetaiotaomicron* (*n* = 2) *P. bivia* *P. asaccharolytica* *P. anaerobius* *F. necrophorum* *P. disiens*	*P. disiens* *P. bivia* (*n* = 3) *A. israelii*
5	*B. fragilis* *B. thetaiotaomicron* (*n* = 2) *A. odontolyticus* *P. bivia* *C. perfringens* (*n* = 2) *C. clostridioforme* *P. anaerobius*	*C. perfringens* *A. israelii* *A. odontolyticus* *E. lentum*	*P. bivia* *E. lentum* *F. nucleatum* *A. odontolyticus*
6		*F. varium* *P. magnus*	*P. asaccharolytica* *C. ramosum* *P. anaerobius* *P. magnus* (*n* = 2)
7	*C. clostridioforme*	*P. bivia* *C. clostridioforme* *F. varium* *C. clostridioforme*	
8	*A. israelii*		

[a] 1, Baltimore Biological Laboratories; 2, Anaerobe Systems; 3, Hardy Diagnostics. Numbers in parentheses are numbers of organisms.

In 1919, Holman (7) wrote, "The cooked meat medium is the most useful medium we have at present for obtaining growth of both aerobic and anaerobic bacteria, for storing mixed cultures for later isolation as well as pure cultures for further investigation." This statement appears to still be true, at least with the broths examined in the present study. The cooked meat medium does not contain cystine or thioglycolate, which can become oxidized and form toxic products. Rather, the meat itself appears to be a reducing and detoxifying substance (2). We observed (3a) that when hydrogen peroxide is added to CMB, but not to TGB, evolution of bubbles occurs, suggesting the presence of catalase or peroxidase, which can neutralize oxygen radicals that are formed when anaerobes attempt to reduce oxygen. These factors may account for the ability of all of the anaerobes tested to survive in the three CMB formulations.

The addition of reducing substances, such as alkaline sulfides, pyruvic acid, cysteine, glutathione, and thioglycolic acid, to broth media to promote anaerobic conditions date back to 1898 (1, 8). Other investigators experimented with semisolid media for the study of motility and also for the improved growth of anaerobes (4, 6). In 1940, Brewer (1) studied a variety of broth formulations that contained various combinations of pork infusion broth, glucose, 0.1% sodium thioglycolate, and 0.05% agar plus methylene blue reduction-oxidation indicator. He found that formulations without agar rapidly oxidized after preparation and lost their ability to support the growth of *Clostridium novyi*. However, all of the formulations containing 0.05% agar were able to support the growth of *C. novyi* and other clostridia for at least 1 month after preparation. The small amount of agar in the broth decreased convection currents, thus excluding the outside atmosphere. He found that this medium did not have to be boiled prior to use, could be handled in a manner similar to that for broths for aerobes, including being mixed, and would still recover its anaerobic state and support the growth of anaerobes.

Modern formulations of TGB have deleted reduction-oxidation indicators but have incorporated additional reducing substances including L-cystine and sodium sulfite (2). In our study, we found that TGB became toxic for some of the more oxygen-susceptible strains. This may be due to the interaction of small amounts of oxygen with the sulfur compounds and the production of toxic oxygen radicals and the lack of neutralizing substances in the medium.

We conclude that although TGB supports initial luxuriant growth of most anaerobes, CMB is superior for the longer-term storage of anaerobic bacteria. Consequently, clinical laboratories that store anaerobic isolates should consider using CMB for this purpose.

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