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Modified Agar Medium for Detecting Environmental Salmonellae by the Most-Probable-Number Method

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Salmonellae in the environment remain a potential source of disease. Low numbers of salmonellae have been detected and enumerated from environmental samples by most-probable-number methods which require careful colony selection from a plated agar medium. A modified xylose lysine brilliant green medium was prepared to control the loss of selectivity caused by heating the brilliant green component. Added agar reduced colony spreading. The medium contained 47 g of xylose lysine agar base per liter; the agar content was adjusted to 2%, autoclaved, cooled to 50°C, and then amended just before pouring to include H_2S indicator and 7 ppm (7 ml of 1:1,000 brilliant green per liter) of unheated brilliant green dye. H_2S-positive salmonellae were easily detected from sewage sludge compost to the exclusion of most other gram-negative bacteria. As a result, fewer non-salmonellae were picked for further most-probable-number analysis, greatly reducing the work load associated with the most-probable-number method. Direct plating was possible for enumerating salmonellae in laboratory composts containing ca. 10^3 or more salmonellae.

The presence of salmonellae in the environment presents a potential health hazard that has been studied for nearly 90 years (5). Technical improvements in the detection of salmonellae have been made over the years, but the basic question of the significance of the presence of salmonellae remains unanswered (16). Recent evidence shows an increase in the isolation of salmonellae from humans since 1977, and age-associated attack rates have been shifting (5). However, during the period from 1966 to 1975, nearly 30% of the outbreaks of salmonellosis were of unknown origin (16). It would seem that many of these outbreaks were due to environmental exposure. Sewage sludge remains a source of potential exposure to salmonellae. Sewage sludge composting, properly monitored, can be effective in reducing numbers of pathogenic bacteria to acceptable levels (4), leaving a safe, humus-like material for soil improvement in gardens and lawns.

While examining samples of compost, we have also evaluated methods of detecting and enumerating salmonellae. Although no standard procedure exists for enumerating or even detecting salmonellae in the environment (1), several methods have been offered with qualifications (1, 8, 9). Since salmonellae are typically found in very low numbers, if at all, in compost (4), the most-probable-number (MPN) technique is the method of choice (15). Additionally, particulate matter interferes with membrane filtration of large samples. In conformance with the concepts of stress-induced injury (2) and the need to avoid selective agents in primary enrichment media (14), peptone water enrichment is currently used (17, 18). This is followed by elevated-temperature incubation in tetrathionate broth (15) or tetrathionate broth with brilliant green (TTBG; 18). Plating on brilliant green (BG) agar (1, 6, 9), bismuth sulfite (BS) agar (1, 9), xylose lysine deoxycholate (XLD) agar, and brilliant green sulfa agar (15) offers an opportunity to select those colonies that are salmonella-like. The suspect colonies can be presumptively tested serologically and, once purified, biochemically identified and serotyped. Theoretically, if only one salmonella is planted into an MPN tube, it can be detected on the agar plates after growth in the selective enrichment steps.

BS agar is routinely used for plate isolation of *Salmonella typhi* (9), a pathogen rarely isolated from humans in the United States (5) and not yet reported (confirmed) from aerated-pile compost. BG agar uses the dye brilliant green (3) to select for gram-negative enteric bacteria and lactose fermentation to indicate various non-salmonellae. XLD agar uses the ability of salmonellae to ferment xylose, decarboxylate lysine, and produce hydrogen sulfide in addition to the selective activity of the bile salt (detergent), deoxycholate. On XLD agar, coliforms and *Proteus* sp. are differentiated by lactose and sucrose fermentation, respectively (9). Although each of these media offers specific advantages, each medium tends to suffer specific drawbacks, and some require modification. For example, there exists some controversy on the need to age BS agar before use (9). On BG agar, many *Proteus* sp. and *Pseudomonas* sp. colonies appear very similar to salmonellae unless sulfadiazine is added (7) and the medium is prepared without excessive heating (6). In environmental samples such as compost, *Proteus* sp. and *Pseudomonas* sp. organisms are expected to occur in great numbers (unlike clinical samples), and on BG agar plates of compost MPN tests, the vast majority of positive cultures consist of colonies of these genera and not salmonellae. XLD agar shows excellent differential properties and is selective for gram-negative organisms. However, when diluted samples of compost were spread plated on XLD agar, motile organisms spread too easily across the detergent-wetted agar surface.

We wanted to examine xylose lysine (XL) agar with 12.5 ppm (12.5 ml of 1:1,000 BG per liter) of brilliant green (XLBG; 17) as a plating medium for the salmonella MPN test and also for direct-plate colony counts of laboratory-manipulated composts. When this amount of BG dye was added to autoclaved and cooled XL agar base (Difco Laboratories), the medium became very dark and was extremely toxic to all

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inocula. Edel and Kampelmacher (6) have reported increased selectivity of BG agar when heating was minimized. Moats et al. (13) have discussed the destruction of BG upon heating in medium. We carry this farther and report the effect of various concentrations of BG dye that has not been heated beyond 50°C. We propose the use of unheated BG dye in XL agar for detecting H_2S-positive salmonellae from environmental samples with low pathogen concentrations.

MATERIALS AND METHODS

Bacterial strains. Reference cultures used in this study are listed in Table 1. Environmental isolates *Proteus mirabilis* AB2-1a and *Salmonella* sp. strain AB2-3a were recovered during this study from compost. Cultures were maintained from −72°C stock cultures in duplicate at 4 and 20°C on tightly capped agar slants containing 0.5% Bacto-Peptone (Difco), 0.1% yeast extract (Difco), 0.5% sodium chloride, 0.21% disodium phosphate, 0.08% sodium dihydrogen phosphate, and 1.5% agar (pH 7.2).

Culture preparation. For direct plating assay with static salmonellae, *Salmonella typhimurium* was grown in tryptic soy broth (Difco), and *Salmonella newport* 413 (antibiotic resistant) was grown in tryptic soy broth with ampicillin (25 μg/ml) and tetracycline (75 μg/ml) at 36°C overnight in 150-ml flasks. These cultures were centrifuged at 17,000 × *g*, washed in sterile, chilled 0.5% saline, centrifuged again, and suspended in sterile distilled water. Optical density at 420 nm was measured, and the suspensions were diluted to ca. 6×10^7 *Salmonella typhimurium* per ml and 3×10^7 *Salmonella newport* per ml. We had determined by standard curves that the number of salmonellae per milliliter of water equaled 2.2×10^8 salmonellae per ml per optical density unit + 5×10^6 salmonellae per ml (for dilutions with optical densities between 0.1 and 0.4). Portions of each culture suspension were combined equally and stored at 4°C for 21 days. Plate-count agar (Difco) with or without antibiotics was used to confirm the total count as 5.6×10^6/ml, and 1.2×10^6/ml were antibiotic resistant. This suspension was diluted in chilled, sterile distilled water for spread plating.

Pure cultures in TTBG were grown for 24 h at 43°C to simulate the culture state during MPN testing. *Salmonella* sp. strain AB2-3a and *Proteus* sp. strain AB2-1a had been recovered from a compost MPN test in which *Proteus* sp. had overrun salmonellae on XLBG containing autoclaved BG dye. *Salmonella typhimurium* was included as a control. To test for effects of aging, the agar media were prepared and inoculated within 6 ± 1 h and also after overnight storage (30 ± 2 h). Fresh TTBG broth cultures were used each day and diluted in distilled water for spread plating.

TABLE 1. Bacterial strains used in study

Organism	Culture no.	Origin (reference)
Escherichia coli	FC-1	Laboratory strain (10)
Salmonella derby	3	Chesapeake Bay (11)
Salmonella newport	413	N. Stern[a]
Salmonella choleraesuis	RS-1	Sakazaki,[b] Kauffman 1350
Salmonella typhimurium	14028	ATCC 14028
Shigella boydii	RS-7	Sakazaki:Ewing
Citrobacter freundii	RS-9	Sakazaki 10010-68
Klebsiella pneumoniae	RS-10	NCTC 8167
Klebsiella oxytoca	RS-13	Sakazaki 1106-75
Enterobacter cloacae	RS-14	NCTC 10005
Enterobacter aerogenes	RS-15	NCTC 10006
Enterobacter hafniae	RS-16	NCTC 9540
Serratia liquefaciens	RS-18	NCTC 10442
Serratia marcescens	DQ-1	Chesapeake Bay (10)
Proteus vulgaris	RS-20	Sakazaki 878-77
Pseudomonas fluorescens	DT-27	Chesapeake Bay (10)

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[b] R. Sakazaki, Enterobacterial Laboratory, National Institute of Health, Shinagawa-Ku, Tokyo.

For morphology and general inhibition studies, cultures were grown in tryptic soy broth at 36°C for 18 to 24 h, then refrigerated overnight. Plates were streaked directly from this culture.

Preparation of test media. BG agar and BS agar (Difco) were prepared by the method recommended by the manufacturer, XL agar base (Difco) was rehydrated as 23.5 g, and 2.5 g of agar was added (increased to 2% agar) in 480 ml of distilled water in a no. 4 (700-ml) Wheaton bottle containing a magnetic stir bar; the mixture was then autoclaved for 15 min at 121°C and promptly cooled to 50°C in a water bath. To each bottle, 10 ml of aqueous 34% sodium thiosulfate and 10 ml of aqueous 4% ferric ammonium citrate (separately autoclaved) were added with stirring. Aqueous 1:1,000 BG dye (Difco, control no. 298080 and 633545) was added as indicated to make XLBG in each test case. When the dye was not autoclaved, the solution was added to 50°C agar just before pouring. For modified XLBG containing 7 ppm of BG, the pH of the solidified agar was found to be 7.0 to 7.4 at 25°C.

Salmonella MPN test for compost. Compost moisture content was estimated by oven drying overnight ca. 10 g at 90 ± 5°C. Screened wet compost [20 g, dry weight] was diluted 1/10 (wt/vol) in buffered peptone water (6) at 4°C in a half-pint mason jar, and mechanically shaken for 15 min. A three- or, preferably, five-tube MPN set was inoculated with 10 ml of the jar contents into sterile tubes, 1 ml into 10 ml of buffered peptone water and 0.1 ml into 10 ml of buffered peptone water. These tubes and the remainder of the jar were also incubated at 36°C for 24 h and vortexed (the jar was shaken), and 1 ml of the mixture was transferred to 10 ml of TTBG broth. These were incubated at 43 ± 0.2°C in a water bath for 24 h. TTBG broths were vortexed and transferred onto plate media (BS, BG, or XLBG agar or both BG and XLBG agars) by streaking a loopful of broth. Salmonella-like colonies with polyvalent O and H antisera (Difco). Positive tubes were used to estimate the salmonella MPN with tables from the American Public Health Association (1). Recovery of *Salmonella* sp. from the jar (but not the MPN tubes) was used as a qualitative index only. Various cultures were biochemically characterized by the Minitek rapid identification system (BBL Microbiology Systems).

Direct plate enumeration of salmonellae. Composts manipulated in the laboratory contained various levels of *S. typhimurium* and *S. newport* up to 10^7/g. Direct plate counts were possible when salmonellae exceed 300/g because in these situations, more uninjured salmonellae existed, and the ratios of salmonellae to competitors were lower. From the 10^{-1} suspension used for the MPN test, decimal dilutions in buffered peptone water were prepared, and 0.1 ml of each dilution was inoculated by spread plate to XLBG in triplicate. These plates were incubated for 18 to 24 h, and dark-centered colonies were counted as salmonellae. *S. newport* was counted with XL agar base with ampicillin (25 mg/ml) plus kanamycin and tetracycline (each 75 mg/ml), which were added to the 50°C agar just before pouring. Direct plate counting was much preferred to the labor-intensive MPN procedure when salmonellae densities permitted.

TABLE 2. Percentage of colonies counted (and titer) after 24 h of incubation on XL agar with BG dye added before or after medium sterilization

BG dye (ppm)	Plate count results[a]			
	Autoclaved dye		Nonautoclaved dye	
	% Recovered	Density (per g)	% Recovered	Density (per g)
25	88	4.9×10^6	0	
20	79	4.4×10^6	NT[b]	
15	84	4.7×10^6	0	
12.5	88	4.9×10^6	1	6×10^4
10	NT		30	1.7×10^6
6	NT		98	5.5×10^6
3	NT		107	6.0×10^6
1.5	NT		100	5.6×10^6
0	100	5.6×10^6	100	5.6×10^6

[a] Total colony count at 24 h averaged 56 for three plates with a standard deviation (n-1) of 12. Inoculum source was a 5.6×10^6/ml mixture of *Salmonella typhimurium* and *Salmonella newport* (3:2) in distilled water stored at 4°C for 21 days.
[b] NT, Not tested.

RESULTS

A mixed culture of *Salmonella typhimurium and Salmonella newport* was stressed by storage in distilled water at 4°C for 3 weeks and used to test the recovery efficiency of BG dye-containing media. Autoclaving BG dye greatly reduced its toxicity. In XL agar, concentration of BG dye recommended by the manufacturer could be doubled without loss of salmonellae if the dye was autoclaved with the medium. In contrast, the dye content suggested by the manufacturer (12.5 ppm) was toxic if added after autoclaving, as was 10 ppm (Table 2). After 18 h of incubation of the mixed cultures, plates with 10 ppm of XLBG supported colonies of *Salmonella newport* only. *Salmonella typhimurium* colonies did not emerge until after 24 h. However, XL agars with 6 through 9 ppm of BG supported the full salmonella population. On all XL agars with up to 10 ppm of BG, *Salmonella typhimurium* colonies were visibly smaller than *Salmonella newport* colonies, which maintained an orange colony color through the 24-h observation. Hydrogen sulfide production was evident for all salmonella colonies examined.

Agars with heated BG dye show poor correlation between dye content and colony count ($r = -0.771$; less than 90% confidence) (Table 2). However, for agars containing up to 15 ppm of unheated dye, a good correlation exists ($r = -0.942$; greater than 99% confidence).

Pure cultures of salmonellae and *Proteus mirabilis* grown in TTBG were spread-plated on XL agar supplemented with 0, 6, 7, 8, 9, or 10 ppm of BG dye (unheated) and on BG agar (Table 3). Each plating was repeated on medium aged an additional 24 h. Very little difference was noted between counts from BG agar and the XL agar base controls. *Proteus mirabilis* AB2-1a failed to grow on XL with greater than 6 ppm of BG. *Salmonella typhimurium* and *Salmonella* AB2-3a produced colonies on all XL media supplemented with unheated dye (Table 3). *Salmonella* AB2-3a appeared more sensitive to the freshly poured agar than to day-old media (Table 3), but this was not observed for *Salmonella typhimurium*, which showed reduced colony size at 24 h as the BG content increased toward 10 ppm. At 48 h, *Salmonella typhimurium* colonies on XLBG agars with 8, 9, and 10 ppm of BG were of equal size to 24-h colonies on XL agar.

XLBG plates containing 6 to 10 ppm of unheated dye were heavily streak-inoculated with *Proteus vulgaris*, *Pseudomo-*

TABLE 3. Effect of BG dye on plate counts of cultures as shown by percent recovery and titer

BG content of agar (ppm)[a]	Age (h)[b]	Plate count results					
		Proteus AB2-1a		*S. typhimurium*		*Salmonella* AB2-3a	
		% Recovered	Density per g	% Recovered	Density per g	% Recovered	Density per g
12.5 (BG agar)	6	63	3.1×10^7[c]	143	1.1×10^7[e]	105	4.5×10^7
	30	ND[d]		79	1.1×10^6[e]	109	1.2×10^7
0 (XL base)	6	100	4.9×10^7	100	7.7×10^6[f]	100	4.3×10^7
	30	100	4.6×10^6	100	1.4×10^6[e]	100	1.1×10^7
6	6	0	$<10^5$	96	7.4×10^6[e]	47	2.0×10^7
	30	0	$<10^5$	93	1.3×10^6[e]	75	8.2×10^7
7	6	0	$<10^5$	65	5.0×10^6	51	2.2×10^7
	30	0	$<10^5$	79	1.1×10^6	91	1.0×10^7
8	6	0	$<10^5$	61	4.7×10^6[e]	47	2.0×10^7
	30	0	$<10^5$	69	9.6×10^5	70	7.7×10^6
9	6	0	$<10^5$	78	6.0×10^6	47	2.0×10^7
	30	0	$<10^5$	63	8.8×10^5[e]	72	7.9×10^6
10	6	0	$<10^5$	31	2.4×10^6[f]	40	1.7×10^7
	30	0	$<10^5$	56	7.9×10^5	79	8.7×10^6

[a] Amount of BG added to XL agar base after sterilization, except for BG agar (Difco) which contains 12.5 ppm of BG and was prepared as recommended by the manufacturer.
[b] Time between agar hardening and inoculation, ± 1 h.
[c] Percentage of "zero" control count (plate count per milliliter; determined from three plates).
[d] ND, No data.
[e] Average of two plates.
[f] One plate was countable.

nas fluorescens, *Salmonella typhimurium*, *Salmonella newport*, and *Escherichia coli*. These grew well on XL agar base. *Escherichia coli* was completely inhibited by 8 ppm of BG, and *Proteus vulgaris* and *Pseudomonas fluorescens* colonies were barely perceptible with 9 and 10 ppm of BG after 24 and 48 h. Salmonellae were easily detected as black centered and red to orange after 24 h.

When 15 cultures (Table 1) were streaked on plates of XL agar with 6 ppm of unheated BG, the growth of *Shigella boydii*, *Escherichia coli*, *Enterobacter hafniae*, *Serratia liquefaciens*, *Serratia marcescens*, *Pseudomonas fluorescens*, and *Proteus vulgaris* was greatly inhibited. The coliforms *Enterobacter cloacae*, *Enterobacter aerogenes*, *Klebsiella pneumoniae*, *Klebsiella oxytoca*, and *Citrobacter freundii* were bright yellow without black centers. The *Klebsiella* spp. and *Enterobacter aerogenes* produced mucoid colonies 3 mm or more in diameter. The salmonellae tested showed black-centered red or orange colonies ca. 2 mm in diameter at 18 to 24 h. No difference was noted when these inoculations were repeated onto XLBG (6 ppm of BG) containing the alternate dye lot.

The increased sensitivity of salmonellae recovery from compost as a result of the suppression of competing organisms when BG was added after autoclaving was strikingly evident. In previous MPN surveys of 19 composts and nine sludges salmonellae (≥0.3/g) were detected in three sludges only when only BS and BG agars were used. From these, 400 salmonella-like colonies were picked for study, but only 28 (7%) were confirmed as *Salmonella* sp. In assays with commercial XLBG (either alone or in combination with BG or BS agar), 78 colonies were picked, and 21 (27%) were confirmed as *Salmonella* sp. With only BG and BS agars the MPN tests from two other samples yielded salmonellae. One sample was sludge. The other was an atypical compost containing 10^7 coliforms and 17,000 salmonellae per g, as well as *Proteus mirabilis* in unusually high numbers. Colony picks from this compost were more frequently confirmed by BG (49 of 52 picks) and BS (36 of 40 picks) agars but not by commercial XLBG (20 of 52 picks). The overall efficiencies for BG and BS agars were 26 and 20%, respectively, from MPN analysis of 28 composts (one was salmonella positive) and 10 sludges (four were salmonella positive). In recent surveys with modified XLBG (7 ppm of BG) as the sole plating medium of 15 composts, three samples (20%) yielded salmonellae (two were detected but had less than 0.3/g, one had 21/g). From these 15 tests, 26 salmonella-like colonies were picked and 21 (81%) were serologically and biochemically confirmed.

DISCUSSION

BG has been used as a selective agent for more than 70 years. In their early studies, Browning et al. (3) obtained nearly pure cultures of salmonellae from feces incubated in peptone water with 3 to 5 ppm of BG. Although they did not specify, we must assume the dye was not heated. The recent observations of Edel and Kampelmacher (6) and Moats et al. (13), that commercial BG agar suffers from autoclaving, demonstrated the need to study the selectivity of BG for salmonellae from environmental samples.

Prepared as recommended by the manufacturers, neither XLBG nor BG agar was adequately selective for *Salmonella* sp. However, when unheated BG (7 ppm) was added to XL agar, only salmonellae grew with black-centered colonies. This was particularly impressive, since *Citrobacter freundii* often mimics *Salmonella* sp. on commercial BG and XLBG agars (unpublished data). The nearly complete inhibition of

TABLE 4. Comparison of plating media used for selective differentiation of salmonellae

Medium	BG dye content (ppm)	Maximum dye temp (°C)	Agar content (%)	Source
BS agar	25	100	2.0	Difco, BBL
BG agar	12.5	121	2.0	Difco, BBL
	5.0	50	1.5 or 1.35	Moats et al. (13)
XLBG agar	12.5	121	1.5	Difco
	12.5	121	1.35	BBL
	6–7	50	2.0	This paper

Proteus vulgaris and *Proteus mirabilis* on the modified XLBG agar greatly helped colony picks for transfer to triple sugar iron agar. The black-centered colonies contrasted clearly enough to allow rapid location of suspect colonies at 18 to 24 h of incubation. This also allowed inoculation with greater amounts of sample, since more background organisms were easily passed over when qualitative assays were done. Thus, some later samples yielded salmonellae from 16 g of compost in qualitative assays but contained less than 0.3/g in MPN assays.

An additional benefit realized was the suitability of the modified XLBG as a direct plating medium for laboratory-manipulated composts. Although we do not propose this as a routine procedure, the results were reassuring for a suspension of static cells (decline rate of 1 log in 3 weeks). On this medium, spread-plate counts of laboratory-stressed salmonellae were quite similar to those on nonselective media (Tables 2 and 3).

MPN methods for salmonella enumeration employ selective enrichment with TTBG. Thus, plated organisms would be presumed to be adapted to BG. In fact, the BG content of the tetrathionate medium used (6) exceeds the amount in the modified XLBG (7 ppm versus 10 ppm). Interestingly, *Proteus* sp. strain AB2-1a grew to a titer equal to or greater than *Salmonella typhimurium* in the TTBG, but *Proteus mirabilis* was completely inhibited on the modified XLBG (Table 3). Clearly, the activity of BG dye was reduced in the tetrathionate broth, perhaps by heat or iodine addition. Additional studies will be required.

Little difference results from changing the BG content from 6 to 9 ppm (Table 3). However, *Salmonella* sp. strain AB2-3a seemed more sensitive to fresh XLBG than to day-old media. The effect was at maximum only twofold (at 10 ppm of BG) and would make little difference for MPN methods in which colony presence, not colony count, is important. However, 6 and 7 ppm of BG in day-old agar gave more consistent recovery. For convenience, we propose preparation of plates a day in advance.

We suggest that our modification to XL agar base (2% agar, and addition of 6 to 7 ppm of unheated BG to the autoclaved medium at 50°C) provides a useful alternative to other plating media for *Salmonella* sp. (Table 4). However, each laboratory should evaluate its needs and determine what medium formulations are required. Other than caution (14), we offer no reason not to increase the BG content of the modified XLBG to 9 ppm in the MPN procedure when TTBG broth enrichment is used.

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LITERATURE CITED

1. **American Public Health Association.** 1976. Standard methods for the examination of water and wastewater, 14th ed. American Public Health Association, Washington, D.C.
2. **Bissonnette, G. K., J. J. Jezeski, G. A. McFeters, and D. G. Stuart.** 1975. Influence of environmental stress on enumeration of indicator bacteria from natural waters. Appl. Microbiol. **29**:186–194.
3. **Browning, C. H., W. Gilmour, and T. J. Mackie.** 1913. Isolation of typhoid bacilli from faeces by means of brilliant green in fluid medium. Hygiene **13**:335–342.
4. **Burge, W. D., D. Colacicco, and W. N. Cramer.** 1981. Criteria for achieving pathogen destruction during monitoring. J. Water Pollut. Control Fed. **53**:1683–1690.
5. **Centers for Disease Control.** 1983. Human *Salmonella* isolates—United States, 1982. Morbid. Mortal. Weekly Rep. **32**:598–600.
6. **Edel, W., and E. H. Kampelmacher.** 1973. Comparative studies on the isolation of "sublethally injured" salmonellae in nine European laboratories. Bull. W.H.O. **48**:167–174.
7. **Foote, C. J.** 1895. A bacteriologic study of oysters, with special reference to them as a source of typhoid infection. Med. News **66**:320–324.
8. **Galton, M. M., J. R. Boring, and W. T. Martin.** 1964. Salmonellae in foods—a review of methods for isolation and a suggested procedure. Communicable Disease Center, Atlanta, Ga.
9. **Galton, M. M., G. K. Morris, and W. T. Martin.** 1968. Salmonellae in foods and feeds. Communicable Disease Center, Atlanta, Ga.
10. **Hussong, D., J. M. Damaré, R. M. Weiner, and R. R. Colwell.** 1981. Bacteria associated with false-positive most-probable-number coliform test results for shellfish and estuaries. Appl. Environ. Microbiol. **41**:35–45.
11. **Kaper, J. B., G. S. Sayler, M. M. Baldini, and R. R. Colwell.** 1977. Ambient-temperature primary nonselective enrichment for isolation of *Salmonella* spp. from an estuarine environment. Appl. Environ. Microbiol. **33**:829–835.
12. **Moats, W. A., and J. A. Kinner.** 1976. Observations on brilliant green agar with an H_2S indicator. Appl. Environ. Microbiol. **31**:380–384.
13. **Moats, W. A., J. A. Kinner, and S. E. Maddox, Jr.** 1974. Effect of heat on the antimicrobial activity of brilliant green dye. Appl. Microbiol. **27**:844–847.
14. **Mossel, D. A. A., G. A. Harrewijn, and C. F. M. Nesselrooy-van Zadelhoff.** 1974. Standardization of the selective inhibitory effect of surface active compounds used in media for the detection of *Enterobacteriaceae* in foods and water. Health Lab. Sci. **11**:260–267.
15. **Russ, C. F., and W. A. Yanko.** 1981. Factors affecting salmonellae repopulation in composted sludges. Appl. Environ. Microbiol. **41**:597–602.
16. **Silliker, J. H.** 1980. Status of *Salmonellae*—ten years later. J. Food Prot. **43**:301–313.
17. **Taylor, W. I.** 1965. Isolation of shigellae. I. Xylose lysine agars; new media for isolation of enteric pathogens. Am. J. Clin. Pathol. **44**:471–475.
18. **Thomason, B. M., D. J. Dodd, and W. B. Cherry.** 1977. Increased recovery of salmonellae from environmental samples enriched with buffered peptone water. Appl. Environ. Microbiol. **34**:270–273.