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Effect of Dyes on Bacterial Growth

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A rapid screening procedure was used to test the effect of 42 dyes on growth of 30 bacteria on solid media. The results indicated that many readily available dyes might have potential application for selective isolation of specific bacterial groups as well as value in differentiating between closely related bacterial taxa. Separation of *Enterobacter* from *Escherichia*, *Salmonella* from *Shigella*, and *Staphylococcus* from *Micrococcus* by selected dyes was also evaluated.

The inhibitory effects of dyes on bacteria were first studied by Churchman (2) and Kline (7), who tested gentian violet (crystal violet) and Brilliant Green on bacterial cultures. Early works in this area were centered on the elucidation of the chemotherapeutic potential of dyes. Petroff and Gump (8) screened 130 dyes against 7 bacterial genera for bacteriostatic and bactericidal effects; they concluded that the dyes tested were of little or no chemotherapeutic value.

The incorporation of dyes into culture media for the purposes of isolation and differentiation of bacteria was described by Endo (3), who used basic fuchsin decolorized with sodium sulfite for the isolation of coliform organisms. Modifications of Endo's medium were developed in subsequent years, and a number of other dyes came into use. These dye-containing media found their greatest application in the field of sanitary microbiology where they were used for the detection of fecal coliforms from milk and water samples and for the isolation and detection of typhoid and other intestinal pathogens from fecal specimens.

Dyes used extensively for their inhibitory and differential properties (especially selective for gram-negative organisms) are basic fuchsin, crystal violet, eosine Y, methylene blue, and Brilliant Green. Acridine orange, ethyl violet, aniline blue, and trypan blue were also used for selectivity for streptococci. Since these dyes are useful in differentiating between certain bacteria or groups of bacteria on the basis of inhibition, it is possible that many other dyes could be as effective or more effective in a similar manner.

This paper describes the results of a survey screening 30 species of bacteria against 42 different dyes in various concentrations to test for inhibitory and differential properties of these dyes against bacterial groups. Differentiation of *Enterobacter* from *Escherichia*, *Salmonella* from *Shigella*, and *Staphylococcus* from *Micrococcus* utilizing selected dyes based on information obtained from the primary screening was also attempted. Some of these results were presented at the annual meeting of the American Society for Microbiology in Minneapolis, Minn., 2-7 May 1971.

MATERIALS AND METHODS

Dyes. Nineteen acidic dyes, 20 basic dyes, and 3 neutral dyes were used in the screening tests. The major classification, sources, lot number (when available), and the respective solvents of the 42 dyes are listed in Table 1.

Bacteria tested. In the primary screening, 14 gram-negative and 16 gram-positive organisms (Table 2), obtained from the culture collection of the Department of Microbiology, The Pennsylvania State University, were used as test organisms. In studying the selectivity of several dyes on closely related bacterial groups additional organisms were used; the named organisms were obtained from The Pennsylvania State University collection, soil and sewage isolates were obtained locally, and clinical isolates were obtained from local hospitals. All bacterial cultures and isolates used in this study were characterized by routine bacteriological procedures, including morphology and gram reaction, cultural characteristics, biochemical tests, and in some cases serological tests to determine their identities. In general, the characteristics of the organisms corresponded to the descriptions found in *Bergey's Manual* (1). All cultures were grown in tryptic soy broth (Difco) at 37 C for 24 h before they were utilized as inocula in the screening tests.

Screening procedure. Stock solutions of each dye were made by dissolving 0.1 g of dye in 10 ml (1:100 dilution) of suitable solvent (Table 1) and were kept in screw-top test tubes until needed. In the primary screening the basal medium into which the dyes were incorporated was tryptic soy agar (Difco); dyes were incorporated into the basal medium at final dilutions

TABLE 1. *Dyes used in this study, together with the solvent used and the source*

Dyes and classification	Solvent	Source[a]
Azo dyes		
Mono-azo dyes		
Janus green-basic	Distilled water	NAC Cert. no. NJ4
Methyl red-acidic	95% Ethanol	NAC Lot no. 6530
Chrysoidine Y-basic	Distilled water	ACC Lot no. 15490
Dis-azo dyes		
Bismarck brown-basic	Distilled water	NAC Lot no. 3312
Trypan blue-acidic	Distilled water	ACC Lot no. 1571p
Thiazole dyes		
Thioflavine TG-basic	Distilled water	ACC Lot no. 12579
Quinonimine dyes		
Thiazines		
Methylene blue-basic	Distilled water	BDH NA
Thionin-basic	Distilled water	NAC Cert. no. NT8
Oxazines		
Brilliant cresyl blue-basic	Distilled water	ACC NA
Nile blue A-basic	Distilled water	ACC Cert. no. NNb4
Resazurin-acidic	Distilled water	ACC Cert. no. $NR_2$24
Azines		
Neutral red-basic	Distilled water	NAC Cert. no. NX6
Safranin O-basic	Distilled water	MC Cert. no. CS16
Safranin Y-basic	Distilled water	FCC Lot no. 780706
Nigrosine-acidic	Distilled water	FCC Lot no. 714104
Phenylmethane dyes		
Diphenyl-methane dyes		
Auramin O-basic	95% Ethanol	ACC NA
Diamino triphenyl-methane		
Brilliant green-basic	Distilled water	NAC Cert. no. NBg6
Malachite green-basic	Distilled water	BCC Lot no. 5-949
Triamino triphenyl-methane		
Aniline blue WS-acidic	Distilled water	ACC Cert. no. NK6
Crystal violet-basic	Distilled water	ACC Cert. no. NC47
Acid fuchsin-acidic	Distilled water	ACC NA
Basic fuchsin-basic	Distilled water	BCC Cert. no. 9-95
p-Rosaniline-basic	95% Ethanol	ACC Lot no. 16416
Methyl green-basic	Distilled water	MC Cert. no. 411224
Methyl violet B-basic	Distilled water	ACC Lot no. 14213
Xanthene dyes		
Fluorane dyes		
Eosine Y-acidic	Distilled water	NAC Cert. no. NE12
Eosine B-acidic	Distilled water	NAC Cert. no. NEb-4
Erythrosine B-acidic	Distilled water	BCC Lot no. 1-682
Rose bengal-acidic	Distilled water	ACC Cert. no. NR67
Phenolphthalein dyes		
o-Cresolphthalein-acidic	95% Ethanol	EKC Lot no. 774
Thymolphthalein-acidic	95% Ethanol	FCC Lot no. 620499
Sulphonphthalein dyes		
Bromocresol purple-acidic	1:5 EtOH:water[b]	FCC Lot no. 482993
Bromophenol blue-acidic	1:5 EtOH:water	ACC Lot no. 14825
Bromothymol blue-acidic	1:5 EtOH:water	MC Lot no. 330055
Cresol red-acidic	95% Ethanol	NAC Lot no. 5702
Chlorophenol red-acidic	95% Ethanol	FCC NA
Phenol red-acidic	1:5 EtOH : water	FCC Lot no. 781059
Acridine dyes		
Acriflavine-acidic	Distilled water	ACC Lot no. 16136

TABLE 1—*Continued*

Dyes and classification	Solvent	Source[a]
Compound dyes		
Wright's stain-neutral	95% Ethanol	ACC Cert. no. NDr23
Jenner stain-neutral	95% Ethanol	NAC Lot no. 3046
Natural dyes		
Indigo tetrasulfonate-acidic	Distilled water	LCP NA
Carmine-neutral	95% Ethanol	NAC Cert. no. NCa-6

[a] ACC = Allied Chemical and Dye Corp.; BCC = J. T. Baker Chemical Co.; BDH = The British Drug Houses, Ltd.; EKC = Eastman Kodak Co.; FCC = Fisher Scientific Co.; LCP = LaMotte Chemical Products Co.; MC = The Matheson Co., Inc.; NAC = National Aniline and Chemical Co., Inc.; NA = Not available.
[b] EtOH = 95% ethanol; water = distilled water.

of 1:1,000, 1:10,000, and 1:100,000 before the media were sterilized (121 C for 15 min). Tryptic soy agar without dyes was used as positive control for growth. Sterile basal medium as well as dye-containing media were poured into large (150 × 15 mm) disposable petri dishes (Falcon Plastic, Oxnard, Calif.), allowed to solidify, and were placed in a 37-C incubator overnight before use.

By use of sterile Pasteur pipettes, 4 drops (approximately 0.2 ml) of actively growing broth cultures of the test organisms were introduced into individual wells of a "U"-shaped sterile Microtiter plate (obtained from the Cooke Engineering Company, Alexandria, Va.) to form a "master plate." From this "master plate" organisms were transferred onto the agar surface of the basal medium as well as the dye-containing media plates by use of a multipoint inoculation device designed to fit the wells of the Microtiter plate (5, 6). Sterilization of the device was by alcohol flaming as described by Fung and Hartman (4). Inoculation of 30 organisms in triplicate onto 10 dye-containing plates (equivalent to 900 inoculations by conventional methods) could be done in less than 30 min. After inoculation, the agar plates were inverted and incubated at 37 C for 24 and 48 h. Agar plates were observed under incandescent as well as ultraviolet light. Growth was recorded as positive or negative after 24- and 48-h incubation periods. Gross colony morphology, color reaction of the agar medium, as well as the color of the colonies themselves, were recorded. Each organism was tested on all dyes at all three dilutions for at least three times. Retesting of all organisms was performed on all those dyes that showed potential for critical separation between groups of organisms. The pH values of these solidified media before and after growth were measured by pressing a microprobe combination electrode (Corning model 5 pH meter; Corning Scientific Instruments, Medfield, Mass.) into the agar.

Salmonella versus Shigella. Basic fuchsin, *p*-rosaniline, and thioflavine TG provided differentiation of *Salmonella* (resistant) from *Shigella* (sensitive). Further study was performed by growing five known cultures, six confirmed clinical isolates of *Salmonella* spp., and four cultures of *Shigella* spp. in varying dilutions of the dyes (Table 5). SS agar (Difco), Brilliant Green agar (Difco), and bismuth sulfite agar (Difco) were also used as basal medium into which these dyes were added to test the differential ability of these modified media.

Enterobacter versus Escherichia. Methyl violet B differentiated between *Enterobacter* (resistant) and *Escherichia* (sensitive) in the primary screening. Further study was performed by growing *Enterobacter aerogenes* 11, *E. aerogenes* 11a, *Enterobacter cloacae*, four local soil isolates of *Enterobacter*, *Escherichia coli*, *E. coli* B, and two local sewage isolates of *E. coli* on basal medium containing varying dilutions (1:1,000, 1:1,125, 1:2,600, 1:1,750, and 1:3,000) of methyl violet B.

Staphylococcus versus Micrococcus. Acriflavine as well as many other dyes showed inhibitory effects on *Micrococcus rhodochrous* while allowing *Staphylococcus aureus* to grow. Further testing was performed by growing *S. aureus* S-6, *S. aureus* 137, *S. aureus* 196E, *S. aureus* 217, *S. aureus* 494, 37 confirmed clinical isolates of *S. aureus*, *Micrococcus freundenreichii*, *Micrococcus flavus*, *Micrococcus varians*, and *M. rhodochrous* on basal medium containing varying dilutions (1:100,000, 1:200,000, and 1:1,000,000) of acriflavine.

RESULTS AND DISCUSSION

All organisms grew well on the tryptic soy agar basal medium. The pH of the agar of both basal medium and dye-containing media remained approximately 7 before and after experimentation. However, the pH of the agar around each colony was not tested. A myriad of colors (too numerous to report) were obtained from the colonies growing on the plates. Cultures such as *Serratia marcescens* and *S. aureus* provided characteristic colors on all dye plates. Only colonies of *Pseudomonas aeruginosa* fluoresced under ultraviolet light.

The effects of acid and neutral dyes on growth of the test organisms are listed in Table 2. Although the final reading was taken at 48 h, no substantial change was observed between 24- and 48-h readings. With few exceptions, bromothymol blue, *o*-cresolphthalein, eosine B, chlorophenol red, rose bengal, bromocresol purple, erythrosine B, eosine Y, and Wright's stain at

TABLE 2. *Effect of acid and neutral dyes at 1:1,000 dilution on bacterial growth*[a]

Organism	Acid dye medium																			Neutral dye medium		
	Brom thymol blue	o-Cresolphthalein	Eosine B	Chlorophenol red	Rose bengal	Brom cresol purple	Erythrosine B	Eosine Y	Cresol red	Phenol red	Methyl red	Resazurin	Trypan blue	Brom phenol blue	Nigrosine	Aniline blue WS	Acid fuchsin	Indigo tetrasulfonate	Thymolphthalein	Wright's stain	Jenners stain	Carmine
Gram-negative organisms																						
Alcaligenes faecalis				−	+	+			+		v											
Enterobacter aerogenes 11a				+	+	+			+		+											
Enterobacter aerogenes 11b				+	+	+			+		+											
Enterobacter cloacae				+	+	+			+		+											
Escherichia coli				+	+	+			+		v											
Proteus vulgaris				+	+	v			+		−											
Pseudomonas aeruginosa				+	+	+			+		+											
Salmonella typhimurium				+	+	+			+		+											
Salmonella typhosa				+	+	+			+		−											
Salmonella paratyphi				+	+	+			+		−											
Salmonella pullorum				+	+	+			+		+											
Salmonella thompson				+	+	+			+		+											
Shigella flexneri				+	+	+			v		−											
Serratia mercescens				+	+	+			+		+											
Gram-positive organisms																						
Bacillus cereus			−			−	−	v	v	−		+	+	+	+	+				−	+	+
Bacillus danicus			−			−	−	−	v	−		+	+	+	+	+				−	+	+
Bacillus polymyxa			−			−	−	−	v	+		+	v	+	+	+				v	+	+
Bacillus subtilis			−			−	−	−	v	−		−	v	+	+	+				−	+	+
Bacillus sulfidus			−			−	v	−	v	−		−	+	+	+	+				−	+	+
Gaffkya tetragena			−			−	−	−	v	+		−	+	+	+	+				−	−	+
Micrococcus rhodochrous			−			−	−	−	−	−		−	v	−	−	−				−	−	−
Sarcina lutea			−			−	−	−	v	−		−	v	−	−	+				−	−	+
Staphylococcus aureus 241b			−			−	−	−	v	−		+	+	+	+	+				−	+	+
Staphylococcus aureus 241c			−			−	−	v	v	−		+	+	+	+	+				+	+	+
Staphylococcus aureus 241f			−			v	−	v	v	v		+	+	+	+	+				−	+	+
Staphylococcus aureus 241g			+			−	−	v	+	v		+	+	+	+	+				v	+	+
Streptococcus bovis			−			−	−	v	+	v		−	+	+	+	+				−	−	+
Streptococcus faecalis			−			v	v	−	v	v		+	+	+	+	+				v	−	+
Streptococcus lactis			−			−	−	v	v	−		−	v	+	v	+				−	−	+
Streptococcus liquefaciens			−			v	v	−	v	+		+	+	+	+	+				v	−	+

[a] Symbols: − = Negative, + = positive (growth), v = variable. Data compiled from three to six replicate tests of each organisms on each dye. Data (24- and 48-hr) are essentially identical. All organisms grew on the basal medium. The dyes are arranged according to their apparent effectiveness in separating between gram negative and gram positive organisms at the lowest dilution of the dyes (1:1,000).

10^{-3} dilution (Table 2) allowed the growth of gram-negative organisms while inhibiting growth of gram-positive organisms. At 10^{-4} dilution (data not shown) only rose bengal and eosine B showed good separation, and at 10^{-5} dilution (data not shown) none of the acidic or neutral dyes had good separation ability. The effect of basic dyes on growth are listed in Tables 3 and 4. At 10^{-3} dilution, Janus Green, methylene blue, safranine O, safranine Y, basic fuchsin, methyl green, nile blue A, and *p*-rosaniline showed good separation between gram-negative and gram-positive organisms. At 10^{-4} dilution (Table 4) Janus Green, safranine O, basic fuchsin, methyl green, *p*-rosaniline, thioflavine TG, methyl violet B, and crystal violet exhibited promising separation ability, whereas at 10^{-5} dilution (data not shown) only Brilliant Green, methyl violet B, and crystal violet showed good separation.

TABLE 3. *Effect of basic dyes at 1:1,000 dilution on bacterial growth*[a]

Organism	Basic dye medium																		
	Janus Green	Methylene blue	Safranin O	Safranin Y	Basic fuchsin	Methyl green	Nile blue A	*p*-Rosaniline	Malachite green	Bismarck brown	Thionin	Thioflavine TG	Chrysoidine Y	Brilliant cresyl blue	Brilliant Green	Neutral red	Methyl violet B	Auromin O	Acriflavine	Crystal violet
Gram-negative organisms	Positive	Positive	Positive	Positive			Positive		Positive	Positive						Positive		Positive		
Alcaligenes faecalis					+	+		+			v	−	v	−	−		−		−	−
Enterobacter aerogenes 11a					+	+		+			+	+	+	+	+		+		+	+
Enterobacter aerogenes 11b					+	+		+			+	+	+	+	−		+		−	+
Enterobacter cloacae					+	+		+			+	−	v	+	+		+		−	+
Escherichia coli					+	+		+			+	v	+	+	−		−		−	−
Proteus vulgaris					+	−		−			v	−	−	−	−		−		−	−
Pseudomonas aeruginosa					+	+		+			+	+	+	+	+		+		+	−
Salmonella typhimurium					+	+		+			+	v	v	−	−		−		−	−
Salmonella typhosa					+	+		+			+	−	v	−	−		−		−	−
Salmonella paratyphi					+	+		−			v	+	+	+	−		−		−	−
Salmonella pullorum					+	+		+			v	−	v	−	−		−		−	−
Salmonella thompson					+	+		+			−	+	+	+	−		+		−	−
Shigella flexneri					−	−		−			−	−	v	−	−		−		−	−
Serratia marcescens					+	+		+			−	+	+	+	+		+		−	−
Gram-positive organisms	Negative	Negative	Negative	Negative	Negative	Negative		Negative				Negative	Negative	Negative	Negative		Negative		Negative	Negative
Bacillus cereus							−		−	+	v					+		+		
Bacillus danicus							−		v	+	+					+		+		
Bacillus polymyxa							+		v	+	+					−		+		
Bacillus subtilis							−		−	v	−					+		+		
Bacillus sulfidus							−		−	v	−					+		+		
Gaffkya tetragena							−		−	v	−					+		+		
Micrococcus rhodochrous							−		−	−	−					−		−		
Sarcina lutea							−		−	−	−					−		−		
Staphylococcus aureus 241b							−		−	−	v					+		+		
Staphylococcus aureus 241c							−		−	v	−					+		+		
Staphylococcus aureus 241f							−		−	v	v					+		+		
Staphylococcus aureus 241g							−			v	v					+		+		
Streptococcus bovis							−		v	v	−					−		+		
Streptococcus faecalis							+		v	+	−					+		+		
Streptococcus lactis							−		−	−	−					v		+		
Streptococcus liquefaciens							+		v	+	−					v		+		

[a] Symbols and data presentation are the same as in legend to Table 2.

These data showed that gram-negative organisms exhibited greater resistance to dyes than gram-positive organisms and that basic dyes are more inhibitory than acidic and neutral dyes at the same concentration. Several dyes not commonly used in diagnostic microbiology showed promising differentiation ability between gram-negative and gram-positive organisms. These data also showed that *M. rhodochrous*, *Gaffkya tetragena*, and *Sarcina lutea* were sensitive to many dyes even in high dilutions (1:100,000). It should be emphasized that only limited species and strains were tested in this screening. However, these data could be used as a guide for further study of the role of these "minor" dyes in separating bacterial groups. Effects of several dyes on a few selected groups of organisms were further tested in this study.

Shigella spp. in general are more sensitive to basic fuchsin, *p*-rosaniline, and thioflavine TG, whereas *Salmonella* grew well in these dye media at appropriate dilutions (Table 5). Basal medium + *p*-rosaniline (1:1,000), basal medium + thioflavine TG (1:3,000), Brilliant Green agar + basic fuchsin (1:500), and SS agar + *p*-rosaniline (1:1,000) provided perfect separation between these two genera. Other combi-

TABLE 4. *Effect of basic dyes at 1:10,000 dilution on bacterial growth*[a]

Organism	Basic dye medium																		
	Janus green	Methylene blue	Safranin O	Safranin Y	Basic fuchsin	Methyl green	Nile blue A	*p*-Rosaniline	Malachite green	Bismarck brown	Thionin	Thioflavine TG	Chrysoidine Y	Brilliant cresyl blue	Brilliant green	Neutral red	Methyl violet B	Auromin O	Acriflavine	Crystal violet
Gram-negative organisms																			
Alcaligenes faecalis												+			−		+		−	+
Enterobacter aerogenes 11a												+			+		+		+	+
Enterobacter aerogenes 11b												+			−		+		+	+
Enterobacter cloacae												+			+		+		+	+
Escherichia coli												+			+		+		+	+
Proteus vulgaris												−			−		−		−	−
Pseudomonas aeruginosa												+			+		+		+	+
Salmonella typhimurium												+			+		+		+	+
Salmonella typhosa												+			−		+		−	+
Salmonella paratyphi												+			+		+		+	+
Salmonella pullorum												+			+		+		+	+
Salmonella thompsom												+			+		+		+	+
Shigella flexneri												+			−		+		v	−
Serratia mercescens												+			+		+		+	+
Gram-positive organisms																				
Bacillus cereus	−	+	−	−	−		+		+	+	+	−	+	+		+		+	−	
Bacillus danicus	−	+	+	+	−		+		+	+	+	−	+	+		+		+	−	
Bacillus polymyxa	v	+	+	+	v		+		+	+	+	+	+	−		+		+	−	
Bacillus subtilis	−	+	−	+	−		+		+	+	+	−	+	−		+		+	−	
Bacillus sulfidus	−	+	−	−	−		+		+	v	+	−	+	−		+		+	−	
Gaffkya tetragena	−	−	−	−	−		−		+	v	−	−	+	−		+		+	−	
Micrococcus rhodochrous	−	−	−	−	−		−		−	−	−	−	−	−		−		−	−	
Sarcina lutea	−	−	−	−	−		−		−	−	−	−	−	−		−		−	−	
Staphylococcus aureus 241b	−	v	−	−	−		−		+	+	+	−	+	−		+		+	v	
Staphylococcus aureus 241c	−	v	−	−	−		+		+	+	+	−	+	+		+		+	v	
Staphylococcus aureus 241f	−	−	−	−	−		−		+	+	+	−	+	−		+		+	−	
Staphylococcus aureus 241g	−	+	−	−	−		+		+	+	+	−	+	−		+		+	−	
Streptococcus bovis	−	−	−	−	−		+		+	v	−	v	+	−		+		+	−	
Streptococcus faecalis	−	−	−	v	−		+		+	+	+	−	+	+		+		+	−	
Streptococcus lactis	−	−	−	−	−		+		+	−	+	−	+	−		−		+	−	
Streptococcus liquefaciens	−	−	−	v	−		+		+	+	+	−	+	−		+		+	−	

[a] Symbols and data presentation are the same as in legend to Table 2.

nations could also be critically tested. Further testing of these dye-containing media may result in finding several media that can prevent the growth of *Shigella* and which allow the growth of *Salmonella*.

Methyl violet B medium inhibited the growth of all *Escherichia* cultures tested, whereas it allowed all *Enterobacter* cultures to grow at 1:1,500 dilution (data not shown). At 1:3,000 dilution both genera grew, whereas at 1:1,000 and 1:1,125 dilutions only *Enterobacter* grew. Since this dye inhibits growth of all gram-positive organisms and allows only a limited number of gram-negative organisms (Table 3) to grow, further investigation of the usefulness of this dye in sanitary microbiology may be fruitful.

M. rhodochrous was found to be very sensitive to many dyes in the primary screening. Acriflavine medium at 1:200,000 dilution (data not shown) permitted growth of five *S. aureus* strains and 37 clinical isolates of *S. aureus* but inhibited all *Micrococcus* species tested. Even at 1:1,000,000 dilution *Micrococcus* spp. either did not grow or grew poorly. This medium may be utilized to differentiate these two closely related genera.

In conclusion, this paper presents evidence

TABLE 5. *Differentiation of Salmonella and Shigella by basic fuchsin, p-rosaniline, and thioflavine TG media*

Medium[b]	Differentiation[a]														
	Salmonella											*Shigella*			
	cholerasuis	*typhimurium*	*paratyphi*	*pullorum*	*thompson*	Isolate 1	Isolate 2	Isolate 3	Isolate 4	Isolate 5	Isolate 6	*flexneri* Boyd	*flexneri* V	*flexneri* W	*sonnei*
Basal + BF 1:500	+	v	+	v	+	+	v	+	v	v	+	−	−	−	−
Basal + BF 1:750	+	+	+	+	+	+	+	+	+	+	+	−	−	−	+
Basal + BF 1:1,000	+	+	+	+	+	+	+	+	+	+	+	−	−	−	+
SS agar + BF 1:500	+	+	+	+	+	+	−	+	+	+	+	−	−	−	−
Bril Green + BF 1:500	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−
Bismuth sulf. + BF 1:500	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−
Basal + Rosa 1:500	+	−	+	−	+	+	−	+	+	+	+	−	−	−	−
Basal + Rosa 1:1,000	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−
Basal + Rosa 1:4,000	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SS agar + Rosa 1:1,000	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−
Bril Green + Rosa 1:1,000	+	+	+	+	+	+	+	+	+	+	+	+	−	−	+
Bismuth sulf. + Rosa 1:1,000	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−
Basal + Thio 1:1,000	+	−	+	−	+	+	+	+	−	+	+	−	−	−	−
Basal + Thio 1:2,000	+	+	+	−	+	+	+	+	+	+	+	−	−	−	−
Basal + Thio 1:3,000	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−
SS Agar + Thio 1:2,000	+	−	+	−	+	+	−	+	−	+	+	−	−	−	−
Bril Green + Thio 1:2,000	+	+	+	−	+	+	+	+	+	+	+	−	−	−	−
Bismuth sulf. + Thio 1:2,000	+	+	+	−	+	+	+	+	+	+	+	−	−	−	−

[a] *Salmonella* isolates were obtained from a local hospital and were confirmed to be *Salmonella*. All known cultures were obtained from the Pennsylvania State University collection. Symbols: + = positive, − = negative, v = variable.

[b] Basal medium = tryptic soy agar (Difco), SS agar = Salmonella-Shigella agar (Difco), Bril Green = Brilliant Green agar (Difco), bismuth sulf. = bismuth sulfite agar (Difco). All organism grew in all 4 media without added dyes, except *Shigella sonnei* which did not grow on bismuth sulfite agar. BF = basic fuchsin, rosa = *p*-rosaniline, thio = thioflavine TG. Many other dilutions of dyes were tested. The ones reported here illustrate the critical differential dilutions.

that many dyes not commonly used could be utilized for development of new selective and differential media. Although detailed study of the mechanisms of inhibition of each dye on each organism has not been performed, these data should be useful as a guideline for further work in developing new media for various purposes in diagnostic microbiology. Also, the miniaturized mass inoculation technique developed in this laboratory was found to increase the efficiency of studies of this nature by 50 to 100 times.

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