APPLIED MICROBIOLOGY, Jan., 1965 Vol. 13, No. 1

Effect of Various Concentrations of Brilliant Green and Bile Salts on Salmonellae and Other Microorganisms[1]

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Received for publication 13 August 1964

ABSTRACT

MILLER, V. RICHARD (Purdue University, Lafayette, Ind.), AND GEORGE J. BANWART. Effect of various concentrations of brilliant green and bile salts on salmonellae and other microorganisms. Appl. Microbiol. 13:77-80. 1965.—A study of the inhibitory effect of 24 different combinations of brilliant green and bile salts concentrations was conducted, using seven species of microorganisms capable of fermenting mannitol. The inhibitory effect of brilliant green decreased as the bile salts concentration was increased. *Staphylococcus aureus* and *Proteus rettgeri* were inhibited by all test media. *Escherichia coli* was inhibited in all but two combinations of brilliant green and bile salts. *Aerobacter aerogenes* generally followed a pattern of growth similar to that of three species of salmonellae. Three of the 24 combinations of brilliant green and bile salts showed little or no inhibition of salmonellae but did inhibit the other organisms studied.

It has been stated that brilliant green and taurocholate are inhibitory to a large variety of gram-positive and gram-negative microorganisms (Stokes and Osborne, 1955). These investigators reported the effect of a medium consisting of a combination of brilliant green and sodium taurocholate on *Salmonella oranienburg*, *Proteus vulgaris*, and *Escherichia coli*. They stated that the selenite was not essential in their proposed medium, but was used to maintain the selectivity of the medium when large amounts of organic matter were added. It was shown that brilliant green proportionately loses its inhibitive effect on bacteria in the presence of varied amounts of organic material (Browning, Gilmore, and Mackie, 1913; Krumwiede and Pratt, 1914; Stark and Curtis, 1936). The following investigation was initiated to show the effective inhibition of selected microorganisms by brilliant green in the presence of bile salts.

MATERIALS AND METHODS

Microorganisms. *S. montevideo* (obtained from M. Woodburn, Home Economics Department, Purdue University), *S. oranienburg* (WRRL 200E), *S. derby* (obtained from M. Woodburn), *E. coli*, *Aerobacter aerogenes*, *Staphylococcus aureus*, and *Proteus rettgeri* were used in this study.

Basal medium. The basal medium contained in a volume of 1 liter: 5 g of Proteose Peptone No. 3 (Difco), 3 g of D-mannitol (BBL), and 0.02 g of bromocresol purple (MCB) ND180.

Brilliant green (1 g; 98% dye content; Difco) was dissolved in 100 ml of distilled water and further dilutions were made as required to add to the basal medium.

Bile salts (Difco) were weighed into those media containing 2 or 4% levels, whereas a 1% solution was dispensed with the basal medium for lower concentrations.

The concentrations of brilliant green and bile salts are shown in Table 1, as is the coding used for the various media. In preliminary trials, a 2.5 mg per liter concentration of brilliant green gave essentially the same results as the 0 mg per liter concentration; also, the media which contained no brilliant green and the varied concentrations of bile salts gave results comparable to the basal medium. Therefore, these media were eliminated from further investigation.

When the higher concentrations of brilliant green and bile salts were added without prior dilution, a precipitate formed during preparation. This difficulty was overcome by adding the brilliant green to the basal medium and diluting to approximately 1 liter with distilled water, then adding the required amount of bile salts, and diluting to 1 liter.

The various media were tubed in 9-ml amounts

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and autoclaved at 15 psi for 15 min. The media were freshly prepared each week and stored in a dark 15 C walk-in cooler until tested.

The cultures were grown in nutrient broth for 24 hr at 37 C. After incubation, 1 ml of each of these cultures was transferred, respectively, to 100 ml of 5% Proteose Peptone No. 3 (Difco). The inoculated peptone was incubated at 37 C for 24 hr. A three-tube most probable number (MPN) technique (Hoskins, 1934) was used to determine the ability of each of the test media to support the growth of the organisms. The organisms were diluted in sterile 0.1% peptone water, and 1 ml of inoculum was added to each of three tubes of media. These inoculated tubes were incubated at 37 C for 24 hr. Color change of the broth was recorded as positive growth. Brain Heart Infusion (BHI) broth (Difco) was used as a control. A plate count was also performed, on Plate Count Agar (Difco), to use as a comparison of the number of microorganisms determined with BHI and the basal medium.

RESULTS

The MPN values with BHI and basal medium are compared to the plate count in Table 2. It can be seen that the basal medium results are very similar to the BHI and plate count results. With *E. coli*, the plate count was higher than the MPN values of either media. It appears that the basal medium supported the growth of these organisms as well as did BHI.

Figure 1 shows the results of series 1 media (5 mg per liter of brilliant green). *E. coli* was inhibited until 4 g per liter of bile salts were added. There was some inhibition of *S. oranienburg* and *S. derby* when no bile salts were present. The addition of as little as 0.25 g per liter of bile salts reduced the inhibition of brilliant green on these salmonellae. *S. montevido* and *A. aerogenes* did not appear to be inhibited in any of these media containing 5 mg per liter of brilliant green.

When the brilliant green was increased to 10 mg per liter, greater inhibition of the organisms was evidenced (Fig. 2). With no bile salts, all of the organisms were inhibited. The salmonellae showed greater growth with only slight inhibition when as little as 0.25 g per liter of bile salts was added, and no inhibition when as little as 0.50 g per liter of bile salts was included in the media. *E. coli* was inhibited by the brilliant green until 4 g per liter of bile salts were added. *A. aerogenes* was severely inhibited by the brilliant green with 0.25 g per liter of bile salts present, but with 2 or

TABLE 1. *Codings used when various concentrations of brilliant green and bile salts were added to the basal medium*

Brilliant green	Bile salts (g/liter)					
	0	0.25	0.50	1.0	2.0	4.0
mg/liter						
5	A1	B1	C1	D1	E1	F1
10	A2	B2	C2	D2	E2	F2
20	A3	B3	C3	D3	E3	F3
40	A4	B2	C4	D4	E4	F4

TABLE 2. *Comparisons of most probable number values in the basal medium to those obtained in Brain Heart Infusion and on plate count agar*

Organisms	MPN		
	Plate count agar	Brain Heart Infusion	Basal medium
Salmonella montevideo	1.54×10^8	2.46×10^8	2.80×10^8
Salmonella oranienburg	1.25×10^8	5.61×10^8	1.30×10^8
Salmonella derby	1.25×10^8	7.30×10^7	1.62×10^8
Escherichia coli	1.4×10^8	7.6×10^6	3.36×10^6
Aerobacter aerogenes	2.73×10^8	3.38×10^8	3.38×10^8

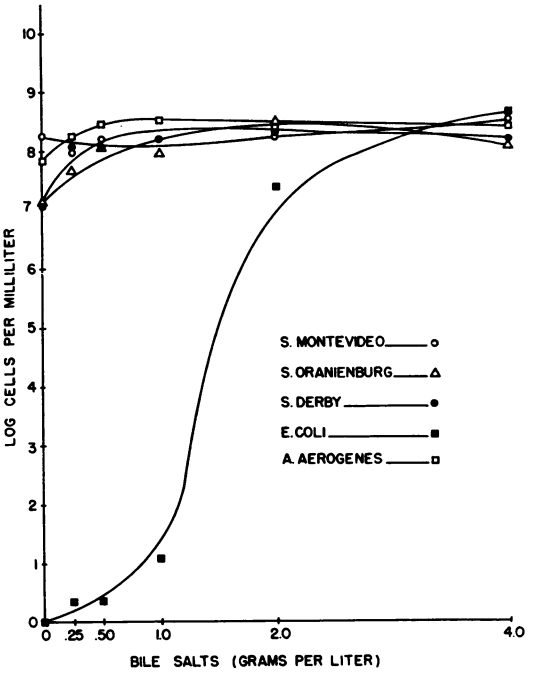

FIG. 1. *Recovery of microorganisms in basal medium containing 5 mg per liter of brilliant green and added bile salts.*

4 g per liter of bile salts added to the media, no inhibition of *A. aerogenes* occurred.

In the series 3 media which contain 20 mg per liter of brilliant green, the salmonellae and *A. aerogenes* were severely inhibited in media with no bile salts, and, except for *S. montevideo*, were inhibited in media with 0.25 g per liter of bile salts (Fig. 3). Even with 1 g per liter of bile salts, *A. aerogenes* and *S. derby* were severely inhibited. *S. derby* was inhibited more than were the other salmonellae. Media with 2 or 4 g per liter of bile salts did not inhibit the salmonellae or *A. aerogenes*. Media with 2 or 4 g per liter of bile salts supported only slight *E. coli* growth. There was no growth of *E. coli* in the media with lesser amounts of bile salts. Also, the microorganisms (Fig. 3) began to express individual variation to the effect of the brilliant green and bile salts.

Figure 4 depicts the effect of 40 mg per liter of brilliant green and varied bile salts concentrations on the microorganisms. It can be seen that each microorganism gave different, although comparable, results. *S. derby* was inhibited more than any of the other salmonellae or *A. aerogenes*. All of the organisms except *E. coli* were recovered successfully in the media containing 4 g per liter of bile salts. Although *E. coli* did show some growth in this medium, it was considerably less

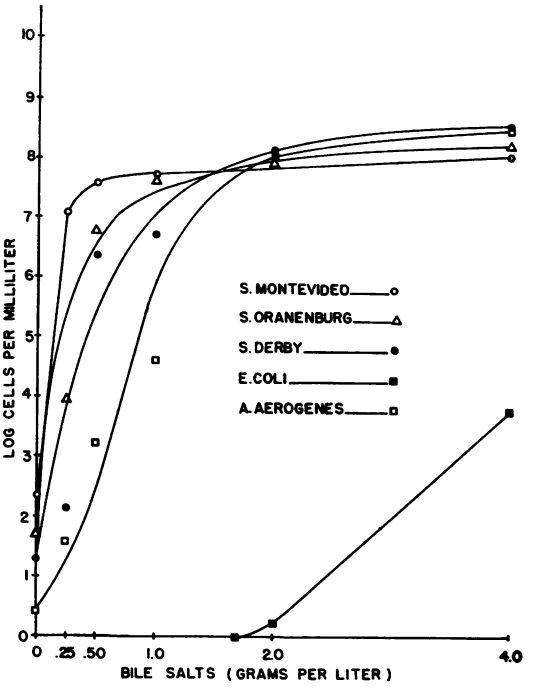

FIG. 3. *Recovery of microorganisms in basal medium containing 20 mg per liter of brilliant green and added bile salts.*

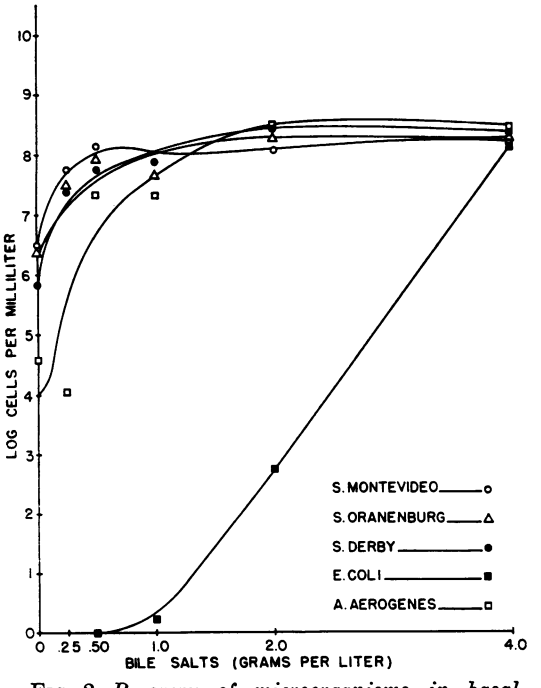

FIG. 2. *Recovery of microorganisms in basal medium containing 10 mg per liter of brilliant green and added bile salts.*

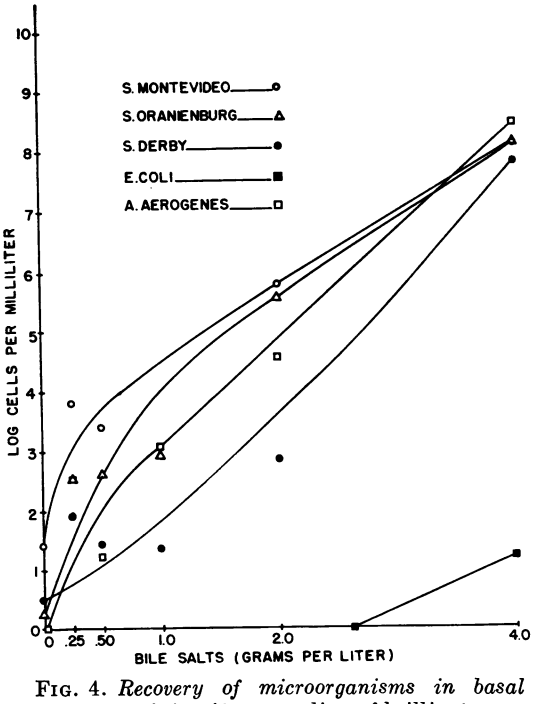

FIG. 4. *Recovery of microorganisms in basal medium containing 40 mg per liter of brilliant green and added bile salts.*

than when lower amounts of brilliant green were present.

The basal medium supported only slight growth of *S. aureus* or *P. rettgeri*. When the various brilliant green and bile salts concentrations were added to the basal medium, complete inhibition was noted; therefore, the *S. aureus* and *P. rettgeri* results were not included in Fig. 1–4.

DISCUSSION

The results of this work depict the effect of an interaction of brilliant green and bile salts on several microorganisms. These results confirm the work of Stokes and Osborne (1955). As the concentration of bile salts was increased, the inhibition of the organisms by brilliant green was decreased. The microorganisms exhibited individual reactions to the various media examined. The effect of the brilliant green and bile salts on *A. aerogenes* was closely associated with that observed on salmonellae. *E. coli* was inhibited by all but two of the media tested. This difference in reactions between the two coliform organisms means that *Aerobacter* species as well as *Escherichia* species should be tested by investigators when developing a selective medium for salmonellae. The more prominent use of *Escherichia* may possibly be due to the fact that *E. coli* is usually considered more closely allied to animals than is *Aerobacter*.

If *Aerobacter* had not been included in this work, all of the media examined, with three exceptions, could be considered excellent selective media for the isolation of salmonellae. Considering the results of *A. aerogenes*, three of the media might warrant further study. These media (B2, C3, and D3) showed complete inhibition of *E. coli* and considerable inhibition of *A. aerogenes*, whereas the salmonellae were not, or only slightly, inhibited. Furthermore, other mannitol-fermenting bacteria, *S. aureus* and *P. rettgeri*, were completely inhibited in these media. *Pseudomonas* species do not ferment mannitol, so would not be confused with the fermentation produced by salmonellae. Stokes and Osborne (1955) reported the effect of adding 1 g of taurocholate to a medium containing 5 mg per liter of brilliant green. This would be comparable to medium D1. Although, as they reported, *E. coli* was inhibited, *A. aerogenes* was not. It would appear that other concentrations of brilliant green and bile salts might be more beneficial than those currently used in selective media for isolating salmonellae.

ACKNOWLEDGMENT

This investigation was supported in part by Public Health Service research grant EF-00416 from the Division of Environmental Engineering and Food Protection.

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