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New Plate Medium for Facilitated Differentiation of *Salmonella* spp. from *Proteus* spp. and Other Enteric Bacteria

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A new agar medium for the differentiation of *Salmonella* spp. from other members of the family *Enterobacteriaceae* is described. This medium exploits a novel phenotypic characteristic of *Salmonella* spp.: the formation of acid from propylene glycol. This characteristic may be used in combination with a chromogenic indicator of β-galactosidase to differentiate *Salmonella* spp. from *Proteus* spp. and the other members of the *Enterobacteriaceae*. Desoxycholate may be included in the plate medium as an inhibitor of gram-positive organisms. Non-*typhi Salmonella* spp. yield distinct, bright red colonies on this medium, allowing facilitated identification and unambiguous differentiation from *Proteus* spp.

Identification of pathogenic *Salmonella* spp. and control of *Salmonella* infections are worldwide problems. Because of the ubiquitous occurrence of *Salmonella* spp. and the high incidence of salmonellosis (2, 5), detection and identification of *Salmonella* spp. in food and water are important in both prevention and control of salmonellosis outbreaks (1, 3).

There are at least eight plate agar media produced commercially for *Salmonella* growth and for differentiation of *Salmonella* spp. from other members of the *Enterobacteriaceae* (6; *The Difco Manual*, Difco Laboratories, Detroit, Mich.). Most of these media rely on one or both of two characteristics—lactose fermentation and hydrogen sulfide production—in combination with various concentrations of inhibitors, for the isolation and identification of *Salmonella* spp. (1, 5, 6; *The Difco Manual*).

The media exploiting these characteristics have several limitations. The use of high concentrations of inhibitors is not desirable for the maximum recovery of contaminating bacteria. Hydrogen sulfide production is a less than ideal trait to use. The detection of hydrogen sulfide production is variable, depending on the sulfide production rate of the bacteria, the oxygen concentration in the colony, pH, and the iron concentration in the media. Measurement of hydrogen sulfide production in combination with tests for the production of acid from lactose is inadequate for the differentiation of *Salmonella* spp. from commensal bacteria. In particular, *Proteus* species which are lactose negative and sulfide positive cannot be differentiated from *Salmonella* spp. by using most commercially available media (*The Difco Manual*).

Additionally, most of the media do not yield distinctly colored, easily identifiable *Salmonella* colonies. The user is required to identify colorless colonies or colonies that are essentially the same color as that of the background media (*The Difco Manual*). This lack of distinct coloration requires close scrutiny and knowledge of colony morphologies and may result in missed *Salmonella* colonies.

Recognizing the limits of the currently utilized phenotypic characteristics, it would appear desirable to develop a medium that would facilitate presumptive identification of *Salmonella* spp. and clearly differentiate *Salmonella* colonies from *Proteus* colonies, as well as from colonies of other members of the *Enterobacteriaceae*. It is also desirable that, because *Salmonella* spp. are the primary organisms of interest, *Salmonella* colonies be distinctly colored to facilitate recognition of colonies.

We found a novel phenotypic characteristic, the formation of acid from propylene glycol, and found that this characteristic could be used in place of hydrogen sulfide production to identify *Salmonella* spp. in plate media. Lactose-utilizing organisms could be identified by using a chromogenic indicator of β-galactosidase, thereby differentiating *Salmonella* spp. from the lactose-utilizing members of the *Enterobacteriaceae*. Desoxycholate was included as an inhibitor of gram-positive organisms, but all compounds were used at concentrations that are generally noninhibitory for enteric pathogens and coliforms.

This new medium was composed of propylene glycol (10 g), peptone (5 g), yeast extract (2 g), sodium desoxycholate (1 g), neutral red (0.03 g), 5-bromo-4-chloro-3-indolyl β-D-galactopyranoside (0.1 g), agar (15 g), and distilled water (1,000 ml). Peptone, yeast extract, sodium desoxycholate, and agar were obtained from KORANO S.A. (Montalieu, France). Propylene glycol, 5-bromo-4-chloro-3-indoxyl β-galactoside, and neutral red were obtained from Sigma Chemical Co. (St. Louis, Mo.).

The biochemical activity of glycols was investigated as follows. A medium was prepared as described above, but without propylene glycol. Twenty milliliters of medium was placed in both 22-mm-diameter tubes and 250-ml flasks. The various glycols were added at a final concentration of 1%, and the medium was inoculated with *Salmonella enterica* AR3001. The cultures were incubated at 37°C for 24 h without agitation. The results are shown in Table 1.

The two conditions, tubes and flasks, were chosen to parallel the conditions of a colony on a plate. In both flasks and tubes, a drop in pH due to the propylene glycol is

TABLE 1. Comparison of medium pH after growth of *Salmonella* isolates for 24 h in desoxycholate media supplemented with various glycols

Supplement	pH of medium in:	
	Tube	Flask
Basic medium alone	7.28	8.68
+ Propylene glycol	5.83	6.06
+ Trimethylene glycol	7.46	8.72
+ 1,2-Butylene glycol	7.48	8.60
+ 1,3-Butylene glycol	7.49	8.67
+ 1,4-Butylene glycol	7.51	8.77
+ 2,3-Butylene glycol	7.46	8.62

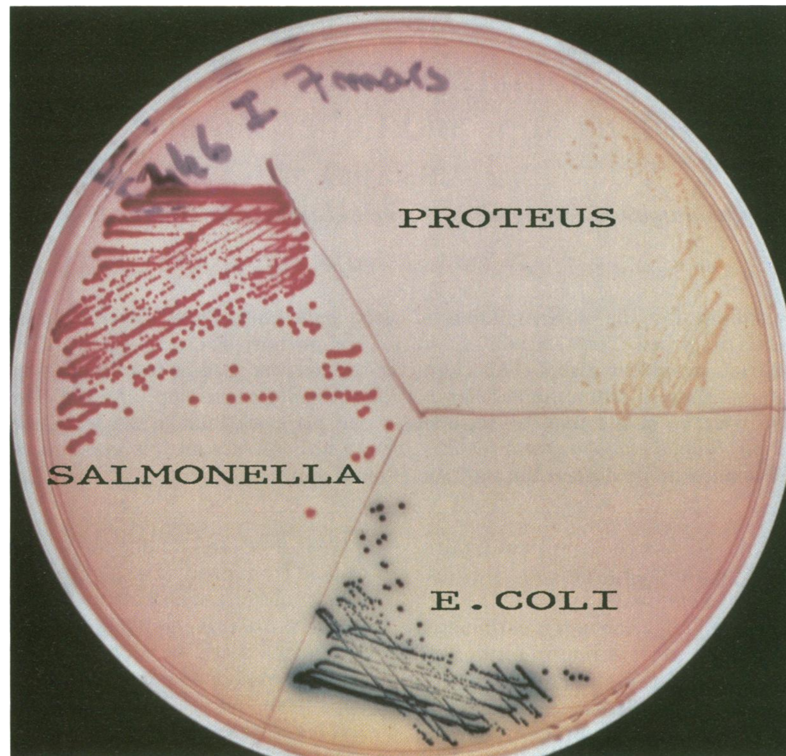

FIG. 1. Distinctive coloration of *Salmonella* colonies on the new medium. Single colonies were picked from peptone medium and streaked on the new medium. The plate was incubated at 37°C for 24 h. *Salmonella* colonies are red, *E. coli* colonies are blue, and *Proteus* colonies are colorless.

obvious. Under the more anaerobic conditions, the medium without propylene glycol remains neutral. Under the more aerobic conditions, the medium without propylene glycol becomes alkaline.

This experiment shows that only propylene glycol produces the effect. The other molecules, which do not react, are chemically similar to propylene glycol. This means that propylene glycol is specifically metabolized in a *Salmonella* culture.

The effect of the propylene glycol, in the desoxycholate-neutral red agar medium described here, is a decrease in pH. Such local acidification is known to precipitate the neutral red in the colonies, yielding a red color.

To find an optimal concentration of propylene glycol, the medium was prepared as described above, but without propylene glycol. Propylene glycol was then added at a concentration of 0.1 to 2%, and the medium was plated and streaked with several *Salmonella* strains.

Color appeared within 24 h and remained stable for 72 h at a propylene glycol concentration of ≥1%. Lower propylene glycol concentrations yielded variable or unstable color formation.

Bacteria were streaked for isolated colonies on the medium and examined after incubation at 37°C for 18 to 24 h. Colonies positive for propylene glycol were bright red. Colonies positive for β-galactosidase were blue. Colonies positive for both characteristics were violet. Colonies positive for neither trait appeared colorless.

The propylene glycol characteristic was tested on 100 non-*typhi Salmonella* isolates. Ninety-seven of the isolates tested were positive for the propylene glycol characteristic, as indicated by the development of a bright red colony. Color appeared within 16 to 24 h. All strains of *S. typhi* tested were negative for this trait and appeared colorless on this new medium.

S. typhi is difficult to identify as a *Salmonella* sp. on plate media because it is typically negative for many important *Salmonella* characteristics, including H_2S production. *S. typhi* is also negative for propylene glycol acidification. *S. typhi* grew on the new medium but was colorless. Therefore, the new medium will have applications similar to those of currently used media, but with facilitated identification of non-*typhi Salmonella* spp.

Strains of *Salmonella enteritidis*, *Salmonella typhimurium*, *Salmonella typhi*, *Escherichia coli*, *Proteus mirabilis*,

TABLE 2. Colony characteristics of members of the *Enterobacteriaceae* on the new medium

Organism (strain[s])	Colony color
Salmonella enteritidis (IP-57.29, IP-81.03, ATCC 13076)	Bright red
Salmonella typhimurium (LT2, ATCC 13311, NCTC 8392)	Bright red
Salmonella spp. (AR3014, AR3015, AR3016, AR3017)	Bright red
Salmonella typhi	Colorless
E. coli (K-12, NCTC 8623, ATCC 4157, IP-A223, IP-52.174)	Blue
Proteus mirabilis IP-60961	Colorless
Proteus morganii AR3023	Colorless
Citrobacter freundii AR3024	Violet

TABLE 3. Comparison of *Salmonella* strain detection in food samples by using brilliant green agar and the new medium

Type of sample (*n*)	No. of samples positive for *Salmonella* strains on:	
	Brilliant green agar	New medium
Meat		
Beef (3)	2	2
Horse (1)	1	1
Pork (2)	2	2
Poultry (1)	0	0
Veal (25)	6	6
Intestinal parts		
Bovine (1)	1	1
Poultry (1)	1	1
Meat products		
Sausage meat (6)	3	3
Fresh sausage (2)	1	1
Merguez (1)	0	0
Cooked foods (5)	0	0
Fish fillet (1)	0	0
Egg products (14)	2	2
Milk powder (2)	2	2
Whey (1)	0	0
Soybean meal (1)	1	1
Pastry flour (3)	0	0
Frog legs (1)	1	1

Proteus morganii, and *Citrobacter freundii* were also tested for growth and color formation on this medium (Table 2).

The distinctive color formation of *Salmonella* strains as compared with *Proteus* and *E. coli* strains can be seen in Fig. 1.

The medium was also supplied to outside laboratories for testing of isolates from contaminated waters and foods. Twenty-three *Salmonella* isolates, seven *Proteus* isolates, and two *E. coli* isolates were tested, and all isolates gave characteristic color identification.

To compare the new medium with a standard one, food samples were inoculated into tetrathionate broth (Difco), incubated for 18 to 24 h at 35°C, and then plated onto both brilliant green agar (Difco) and the new medium and incubated at 35°C for 24 h. The type and number of samples tested and the number of *Salmonella* sp.-containing samples determined with the two media are presented in Table 3.

The data in Table 3 show that all samples contaminated with *Salmonella* strains that were positive on brilliant green agar were also positive on the new medium. Further testing of positive colonies confirmed that bright red colonies were indeed *Salmonella* colonies. No false-positives were found. Technicians quickly learned to recognize the distinctive red color, and *Salmonella* strain-contaminated samples were easily identified.

The medium presented in this paper may prove useful in the detection and prevention of food-borne salmonellosis outbreaks and in veterinary disease control. By using the new positive characteristic, *Salmonella* strains may be more easily differentiated from *Proteus* strains, thereby facilitating early recognition of *Salmonella* contamination.

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