JOURNAL OF CLINICAL MICROBIOLOGY, Oct. 1991, p. 2354–2356
0095-1137/91/102354-03$02.00/0

Vol. 29, No. 10

Use of Rambach Propylene Glycol Containing Agar for Identification of *Salmonella* spp.

RUBEN GRUENEWALD,* RUTH W. HENDERSON, AND SHELIA YAPPOW

Enteric Bacteriology Laboratory, Bureau of Laboratories, New York City Department of Health, New York, New York 10016

Received 1 April 1991/Accepted 11 July 1991

When grown on Rambach Propylene Glycol Containing Agar (Rambach agar), 216 of 230 (93.9%) *Salmonella* organisms isolated from patients and 54 of 62 (87.1%) *Salmonella* stock cultures produced a crimson-colored growth. Of the 14 clinical *Salmonella* isolates which displayed colors other than crimson, 8 were *Salmonella typhi*, 2 were *Salmonella paratyphi* A, and 4 belonged to other commonly isolated serotypes. All eight *Salmonella* stock cultures which failed to produce a crimson color belonged to rarely isolated serotypes. In contrast, of 83 non-*Salmonella* stock cultures distributed among 29 bacterial species, none produced a crimson color. These results suggest that while Rambach agar cannot preidentify *S. typhi* and *S. paratyphi* A, the medium can be used for the presumptive identification and can assist in the definitive identification of the overwhelming majority of *Salmonella* isolates.

The separation of *Salmonella* spp. from other species of the family *Enterobacteriaceae* requires the determination of many biochemical properties, because no one biochemical property alone is sufficient to identify such organisms (2). Thus, *Salmonella* spp. usually do not ferment lactose and most often produce hydrogen sulfide, which is detected by precipitation with ferric compounds, but these two properties are also shared by many *Proteus* and *Citrobacter freundii* isolates. *Salmonella* spp. usually ferment dulcitol, but dulcitol fermentation is also a characteristic of many isolates of *Escherichia coli*, *Citrobacter freundii*, and *Serratia fonticola* (2).

Rambach (5) has described a medium which uses the formation of acid from propylene glycol to differentiate a number of non-typhi *Salmonella* from some of the other species of the family *Enterobacteriaceae*. On this medium the non-typhi *Salmonella* colonies were described as bright red, whereas other organisms were colorless (*Proteus mirabilis*, *Morganella morganii*, *Salmonella typhi*), violet (*Citrobacter freundii*), or blue (*Escherichia coli*). The purpose of the present study was to compare the colors produced by a large number of *Salmonella* isolates grown on Rambach Propylene Glycol Containing Agar (Rambach agar) with the colors produced by other bacterial species to determine whether this medium can be used for the identification of *Salmonella* spp.

Rambach agar, which was obtained from the manufacturer (Technogram, Paris, France) and which was prepared as described by the manufacturer, was boiled for 1 min, cooled to 50°C, resuspended by gentle shaking, and poured into plastic petri dishes (15 by 100 mm; Fisher Scientific Co., Springfield, N.J.). Each plate was inoculated with eight bacterial isolates, each of which was streaked over an area of approximately 25 by 2.5 mm. The plates were incubated for 18 to 24 h at 35°C, and the reactions were recorded.

The somatic and flagellar antigens of *Salmonella* spp., which were isolated from 230 patients, were determined by the methods of Gruenewald et al. (3). When grown on Rambach agar, 216 of 230 *Salmonella* isolates produced a crimson growth. Only eight isolates of *S. typhi*, two each of *S. paratyphi* A and *S. enteritidis*, and one each of *S. hadar*

TABLE 1. Colors displayed by 292 *Salmonella* isolates growing on Rambach agar

Isolate and serotype	No. of isolates	Color(s) (no. of isolates)
Clinical isolates (n = 230)		
Aberdeen	1	Crimson
Abortus-bovis	1	Crimson
Agona	6	Crimson
Berta	5	Crimson
Brandenburg	5	Crimson
Enteritidis	83	Crimson (81), colorless (1), pink (1)
Haardt	5	Crimson
Hadar	15	Crimson (14), colorless (1)
Heidelberg	28	Crimson
Ibadan	1	Crimson
Indiana	4	Crimson
Infantis	7	Crimson
Istanbul	2	Crimson
Johannesburg	1	Colorless
Mbandaka	1	Crimson
Montevideo	1	Crimson
Newport	1	Crimson
Oranienburg	1	Crimson
Paratyphi A	2	Colorless (1), pink (1)
Pomona	1	Crimson
Poona	1	Crimson
Saint-paul	10	Crimson
Senftenberg	2	Crimson
Stanley	1	Crimson
Stanleyville	1	Crimson
Tennessee	1	Crimson
Thompson	6	Crimson
Typhi	8	Colorless (6), pink (2)
Typhimurium	27	Crimson
Virchow	1	Crimson
Worthington	1	Crimson

Continued on following page

* Corresponding author.

TABLE 1—*Continued*

Isolate and serotype	No. of isolates	Color(s) (no. of isolates)
Stock cultures (n = 62)		
Aberdeen	1	Crimson
Adelaide	1	Crimson
Agona	1	Crimson
Alachua	1	Crimson
Anatum	1	Crimson
Arkansas	1	Crimson
Artis	1	Crimson
Bareilly	1	Crimson
Basel	1	Crimson
Betiocky	1	Crimson
Blockley	1	Crimson
Boecker	1	Crimson
Bovis-morbificans	1	Crimson
Brandenburg	1	Crimson
Bristol	1	Crimson
Bunnik	1	Colorless
Carrau	1	Crimson
Cerro	1	Crimson
Champaign	1	Crimson
Dakar	1	Colorless
Derby	1	Crimson
Deversoir	1	Crimson
Dublin	1	Crimson
Dugbe	1	Colorless
Havana	1	Crimson
Houten	1	Colorless
Inverness	1	Crimson
Java	1	Crimson
Javiana	1	Crimson
Litchfield	1	Crimson
Liverpool	1	Crimson
Livingstone	1	Crimson
Locarno	1	Crimson
London	1	Crimson
Luton	1	Crimson
Mbandaka	1	Crimson
Miami	1	Crimson
Mississippi	1	Crimson
Montevideo	1	Crimson
Moscow	1	Pink
Muenchen	1	Crimson
Newington	1	Crimson
Newport	1	Crimson
Ohio	1	Crimson
Oranienburg	1	Crimson
Panama	1	Crimson
Quimbamba	1	Crimson
Reading	1	Crimson
Rostock	1	Colorless
Saint-paul	1	Crimson
San-diego	1	Crimson
Schwarzengrund	1	Crimson
Senftenberg	1	Crimson
Sinstorf	1	Crimson
Tokai	1	Blue
Tranoroa	1	Crimson
Treforest	1	Crimson
Uccle	1	Crimson
Virginia	1	Crimson
Wassennaar	1	Colorless
Waycross	1	Crimson
Weslaco	1	Crimson

TABLE 2. Colors displayed by 83 non-*Salmonella* stock cultures growing on Rambach agar

Organism	No. of isolates	Color(s) (no. of isolates)
Acinetobacter calcoaceticus subsp. *anitratus*	4	Scarlet (2), colorless (2)
Acinetobacter calcoaceticus subsp. *lwoffi*	1	Colorless
Alcaligenes faecalis	1	Colorless
Alcaligenes odorans	1	Pink
Citrobacter diversus	3	Blue
Citrobacter freundii	5	Blue
Enterobacter aerogenes	2	Blue
Enterobacter cloacae	4	Blue
Escherichia coli	11	Blue (9), Blue-green (1), Colorless (1)
Hafnia alvei	2	Blue
Klebsiella oxytoca	2	Blue
Klebsiella ozaenae	1	Blue
Klebsiella pneumoniae	6	Blue
Morganella morganii	2	Colorless
Plesiomonas shigelloides	1	Blue-green
Proteus mirabilis	4	Colorless
Proteus penneri	1	Colorless
Providencia stuartii	1	Colorless
Pseudomonas aeruginosa	5	Scarlet
Pseudomonas stutzeri	1	Pink
Serratia marcescens	3	Blue
Serratia odorifera	1	Blue
Shigella boydii type 5, 6, 13 and 14	4	Colorless
Shigella dysenteriae type 1, 2 and 3	3	Colorless
Shigella flexneri type 1, 2, 4 and 6	4	Colorless
Shigella sonnei	6	Blue
Vibrio cholerae	1	Colorless
Vibrio parahaemolyticus	1	Colorless
Yersinia enterocolitica	1	Blue
Yersinia frederiksenii	1	Blue

and *S. johannesburg* failed to display a crimson color (Table 1). If the eight *S. typhi* and two *S. paratyphi* A isolates are excluded, then 98.2% (216 of 220) of the *Salmonella* isolates produced a crimson color. Use of this medium for the presumptive identification of *Salmonella* spp. should not be compromised by the fact that 8 of the 62 (12.9%) *Salmonella* stock cultures failed to display a crimson color since all 8 organisms belonged to infrequently isolated serotypes. In fact, between 1979 and 1989, only 20 isolates of *S. houten*, 14 isolates of *S. wassennaar*, 5 isolates of *S. rostock*, 2 isolates of *S. dugbe*, and 1 isolate of *S. moscow* were reported to the Centers for Disease Control (Atlanta, Ga.) as having been isolated from human sources in the United States; no isolates of the remaining three serotypes (bunnik, dakar, and tokai) were reported during the same period (1). On the other hand, of 83 non-*Salmonella* stock cultures distributed among 21 species of the family *Enterobacteriaceae* and 8 species in other bacterial families, none produced a crimson color (Table 2). Of these 83 stock cultures, 7 (5 *Pseudomonas aeruginosa* and 2 *Acinetobacter calcoaceticus* subsp. *anitratus*) produced a scarlet-colored growth. Since scarlet is red tinged with yellow and crimson is red tinged with blue, the scarlet-colored organisms were easily distinguished from the crimson-colored *Salmonella* isolates. However, in order to avoid any possible confusion between the scarlet- and crimson-colored organisms, we

recommend that workers who are using Rambach agar for the first time include a crimson color-producing *Salmonella* isolate as well as a scarlet color-producing *Pseudomonas aeruginosa* or *Acinetobacter calcoaceticus* subsp. *anitratus* isolate to serve as controls.

As has been shown in this study and previously (5), Rambach agar cannot be used for the presumptive identification of *S. typhi*, and this also seems to be true of *S. paratyphi* A (4). However, with the exception of these two serotypes as well as eight members of rarely isolated serotypes and four members of commonly isolated serotypes, all of the *Salmonella* spp. produced a crimson-colored growth. The fact that a very high proportion of *Salmonella* isolates produced a crimson color while 83 non-*Salmonella* stock cultures did not strongly suggests that Rambach agar would be an excellent medium for the presumptive identification of *Salmonella* isolates. This property seems to constitute a powerful tool for the identification of these organisms, because it appears to be much more specific for *Salmonella* spp. than their fermentation of dulcitol, the formation of hydrogen sulfide from ferric compounds, or an inability to ferment lactose (2). Indeed, the use of Rambach agar may allow workers to greatly reduce the number of biochemical reagents required for the identification of *Salmonella* isolates.

REFERENCES

1. **Centers for Disease Control.** 1989. Salmonella surveillance—annual summary, 1989. Centers for Disease Control, Atlanta.
2. **Farmer, J. J., B. R. Davis, F. W. Hickman-Brenner, A. McWhorter, G. P. Huntley-Carter, M. A. Asbury, C. Riddle, H. G. Wathen-Grady, C. Elias, G. R. Fanning, A. G. Steigerwalt, C. M. O'Hara, G. K. Morris, P. B. Smith, and D. J. Brenner.** 1985. Biochemical identification of new species and biogroups of *Enterobacteriaceae* isolated from clinical specimens. J. Clin. Microbiol. **21**:46–76.
3. **Gruenewald, R., D. P. Dixon, M. Brun, S. Yappow, R. Henderson, J. E. Douglas, and M. H. Backer.** 1990. Identification of *Salmonella* somatic and flagellar antigens by modified serological methods. Appl. Environ. Microbiol. **56**:24–30.
4. **Rambach, A.** Personal communication.
5. **Rambach, A.** 1990. New plate medium for facilitated differentiation of *Salmonella* spp. from *Proteus* spp. and other enteric bacteria. Appl. Environ. Microbiol. **56**:301–303.