USE OF A MODIFIED MacCONKEY AGAR MEDIUM FOR THE SELECTIVE GROWTH AND ENUMERATION OF *ENTEROBACTERIACEAE*

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Crystal violet neutral red bile lactose agar (violet red bile) media of the type originally suggested by MacConkey (J. Hyg. **5**:333, 1905) has come into general use for the presumptive enumeration of coliform bacteria in dairy products and other foods. It was suggested by Henriksen (Acta Pathol. Microbiol. Scand. **36**:87, 1955) that this and similar lactose-containing media could be used for the detection of virtually all *Enterobacteriaceae* if lactose, which is fermented or assimilated by only a few of the *Enterobacteriaceae*, were replaced by mannitol. We verified this fact (Appl. Microbiol. **5**:379, 1957) and made a MacConkey's agar containing mannitol instead of lactose.

This modified medium has been used in our Institute for about 7 years and has been entirely satisfactory as far as selectivity is concerned. About 2,500 characteristic colonies have been recovered from plates of this agar, after inoculation with suitable dilutions of about 15 different foods, and over 99% have been confirmed as *Enterobacteriaceae*.

However, we have encountered a few false negatives; i.e., certain *Enterobacteriaceae* could not be recovered from food samples, or could be recovered only with difficulty, when using this agar. The reason for this was either that the organisms were more difficult to grow (e.g., salmonellae of the serotype *typhisuis* and, to a lesser extent, the *pullorum-gallinarum* group; *see* Stokes and Bayne, J. Bacteriol. **76**:417, 1958), or they failed to ferment mannitol (e.g., most *Proteus* strains). The failure to ferment mannitol apparently coincided with inadequate utilization of other components of the medium; lactose- and mannitol-negative organisms may grow well in media containing no other added carbon compounds (Taylor, Appl. Microbiol. **9**:487, 1961).

It seemed rational, therefore, to replace the mannitol with glucose, which is utilized by all *Enterobacteriaceae*. The new medium was prepared from commercially available dehydrated Violet Red Bile Agar, to which 1% glucose was added before reconstitution; for the sake of simplicity, this method was preferred to preparation from the original ingredients. The modified medium, which we propose to call MacConkey glucose agar, permitted excellent development of virtually all of the more fastidious *Enterobacteriaceae* referred to earlier. Only one of the cultures examined did not grow on this medium, *viz.*, a variant of *Salmonella pullorum*, which appeared to possess unusual sensitivity to the inhibitory agents in MacConkey glucose agar. Such strains have also been encountered by Smith (Nature **193**:701, 1962). Tests with bacteria not belonging to the *Enterobacteriaceae*, *viz.*, 10 cultures of *Bacillaceae*, 12 of Lancefield group D streptococci, 15 of *Staphylococcus aureus*, 10 of micrococci, 5 of *Achromobacter*, and 5 of *Pseudomonas*, showed that the vast majority of these organisms did not grow; and a few of the *Pseudomonas* strains only gave the typical colonial appearance characteristic of the *Enterobacteriaceae*, after 18 to 20 hr of incubation at 37 C.

The new medium has also been tested extensively in the examination of food and can be recommended for enumeration of *Enterobacteriaceae*. It has also been used as a confirmatory medium for plating presumptive cultures of *Enterobacteriaceae* from agar media and from liquid enrichment cultures. The only organisms which resemble *Enterobacteriaceae* on or in this medium are: (i) the *Aeromonas* group, and an occasional *Pseudomonas*, which can be recognized at once by applying Kovacs' oxidase test (Steel, J. Gen. Microbiol. **25**:297, 1961); and (ii) the *Achromobacter anitratus* group (*Bacterium anitratum;* Schaub and Hauber, J. Bacteriol. **56**:379, 1948). which, although generally inhibited in pour plates, will grow on streak plates. The latter organisms can be differentiated from *Enterobacteriaceae* by their oxidative rather than fermentative attack on freshly deaerated and tubed glucose agar (Mossel and Martin, Ann. inst. Pasteur Lille **12**:223, 1961).