Modified Decarboxylase-Dihydrolase Medium

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A modified base medium for amino acids is described which has advantages over Moeller and Falkow media.

During a survey of cultures, some problems were encountered when strains of Vibrio and related organisms were inoculated into Moeller decarboxylase base medium containing lysine, arginine, or ornithine (2). All strains did not grow. The dark color of the medium imparted by the indicators in it made determination of growth difficult. When growth did occur, the lack of sharp color differences of the indicators resulted in discrepancies in readings when made by different individuals. Falkow base medium (1) with the amino acids used above was easier to read for determination of growth and pH changes, but not all organisms grew and the colors of the indicator faded after 1 day of incubation. For these reasons, studies were undertaken to find a base medium which would overcome the problems noted.

The medium found most suitable contained Trypticase (BBL), 1%; sodium chloride, 1%; dextrose, 0.05%; and, phenol red, 0.005%. The base medium was divided into four portions. Nothing was added to one, which served as the control. To the remaining portions, 1% L(+)-lysine, arginine, or ornithine was added. After adjustment to pH 6.5, each portion was divided into 5-ml amounts in tubes (16 by 120 mm). The medium was sterilized at 121 C for 10 min. The medium can be stored at 4 C for 30 days prior to use.

Table 1. Reactions of routine enteric bacteria on amino acids in different base media*

| Organisms               | Moeller | Falkow | New  |
|-------------------------|---------|--------|------|
|                         | Lysine  | Arginine | Ornithine | Control | Lysine  | Arginine | Ornithine | Control | Lysine  | Arginine | Ornithine | Control |
| *Aeromonas hydrophila*  | K       | K       | A     | A       | (K)     | K+      | A         | A       | A       | (K)     | K+      | A         | A       |
| *Alcaligenes fæcalis*   | K       | K       | K     | K       | K       | K       | K         | K       | K       | K       | K       | K         | K       |
| *Citrobacter*           | A       | (K)     | A     | A       | A       | (K)     | A         | A       | A       | K+      | A       | A         | A       |
| *Comamonas*             | K       | K       | K     | K       | K       | K       | K         | K       | K       | K       | K       | K         | K       |
| *Edwardsiella tarda*    | K       | A       | K     | A       | K       | K       | A         | K       | A       | K       | A       | K         | A       |
| *Enterobacter aerogenes*| K       | A       | K     | A       | K       | A       | K         | A       | K       | A       | K       | A         | A       |
| *E. liquefaciens*       | K       | A       | K     | A       | A       | K       | A         | K       | A       | K       | A       | A         | A       |
| *Escherichia coli*      | K       | (K+)    | (K)  | A       | K       | (K+)    | (K)      | A       | (K)    | K+      | (K)    | A         | A       |
| *Klebsiella pneumoniae* | K       | A       | A     | A       | K       | A       | A         | K       | A       | A       | A       | A         | A       |
| *Plesiomonas shigelloides* | K     | K       | K     | K       | K       | K       | K         | K       | K       | K       | K       | K         | K       |
| *Proteus mirabilis*     | A       | A       | K     | A       | A       | K       | A         | A       | A       | K       | A       | A         | A       |
| *P. morgani*            | A       | A       | K     | A       | A       | K       | A         | K       | A       | A       | A       | A         | A       |
| *P. vulgaris*           | A       | A       | A     | A       | A       | A       | A         | A       | A       | A       | A       | A         | A       |
| *Providencia*           | A       | A       | A     | A       | A       | A       | A         | A       | A       | A       | A       | A         | A       |
| *Pseudomonas aeruginosa*| K       | (K+)    | K     | K       | K       | K       | K         | K       | K       | K       | K       | K         | K       |
| *Salmonella group B*    | K       | K       | A     | K       | (K+)    | K       | A         | (K)    | K+      | K       | A         | K         | A       |
| *Serratia marcescens*   | K       | A       | K     | A       | A       | A       | K         | A       | A       | K       | A       | A         | A       |
| *Shigella flexneri*     | A       | A       | A     | A       | A       | A       | A         | A       | A       | A       | A       | A         | A       |

* Reactions: K, alkaline (violet, violet, and orange for Moeller, Falkow, and new medium, respectively); K+, strongly alkaline (red-violet, deep violet, and magenta, respectively); A, acid (yellow, yellow, and yellow, respectively). Parentheses indicate delayed reaction.
This medium gave good results when tested with several strains of bacteria. However, it was necessary to compare it with the amino acid media used in most laboratories to determine whether the data were comparable. Moeller medium was prepared from the Difco Laboratories dehydrated product, according to the instructions on the bottle. Falkow medium was formulated from the components as described in the Manual of Clinical Microbiology (3). A drop of culture, grown in 1% Trypticase-1% sodium chloride at 37°C overnight, was used as the inoculum for each tube. A 2.0-ml amount of sterile mineral oil was added to each tube, and the cultures were incubated at 37°C. Daily readings were made for 4 days.

The results with the enteric bacteria other than vibrio were identical in all three media (Table 1). Single strains from stock cultures were used. It should be indicated that the difficulties of reading with Moeller and Falkow media were again encountered, and the results with the new medium were obtained without difficulty or doubt. The agreement of results is the first point of significance.

Table 2. Reactions and growth of vibrios and related organisms

| Organisms                    | Lysine | Arginine | Ornithine | Control | No. strains growing in: | New medium only | Total |
|------------------------------|--------|----------|-----------|---------|-------------------------|----------------|-------|
| Aeromonas sp.                | (K) or A | K        | A         | A       | 13                      | 1              | 14    |
| Vibrio parahemolyticus       | K      | A        | K         | A       | 1                       | 36             | 37    |
| Vibrio cholerae              | K      | A        | K         | A       | 10                      | 0              | 10    |
| Unknowns*                    | K      | A        | K         | A       | 9                       | 5              | 14    |
| Total                        |        |          |           |         | 33                      | 42             | 75    |

*Moeller, Falkow, and new media.
* Organisms received as non-cholera vibrios.

In summary, the practical advantages of the proposed new medium over Moeller and Falkow media are: (i) ability of enteric bacteria including vibrios and related organisms to grow in it, (ii) ease of determination of growth and pH changes, (iii) time, and (iv) cost.

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LITERATURE CITED
1. Falkow, S. 1968. Activity of lysine decarboxylase as an aid in the identification of Salmonellae and Shigellae. Amer. J. Clin. Pathol. 29:598–600.
2. Moeller, V. 1964. Simplified tests for some amino acid decarboxylases and for arginine dihydrolase system. Acta Pathol. Microbiol. Scand. 34:158–172.
3. Vera, H. D., and M. Dumoff. 1970. Culture media, p. 648. In J. E. Blair, E. H. Lennette, and J. P. Trauant (ed.), Manual of clinical microbiology. American Society for Microbiology, Bethesda, Md.