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 Vibrion 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% (1+)-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 (K) | K+ A A (K) |
| Alcaligenes fecalis       | K K K K K K K K K K K | K K K K K K K K K | K+ K+ A A |
| Citrobacter               | A (K) A A A (K) A A A K+ A A | A (K) A A A K+ A A |
| Comamonas                 | K K K K K K K K K K K | K K K K K K K K K | K+ K+ A A |
| Edwardsiella tarda        | K A K A K A A K A K A K A A | K A K A K A K A K A |
| Enterobacter aerogenes    | K A K A K A A K A K A K A | K A K A K A K A K A |
| E. liquefaciens           | K A K A K A A K A K A K A | K A K A K A K A K A |
| Escherichia coli          | K (K+) (K) A K (K+) (K) A (K) K+ (K) A (K) | K+ (K) A |
| Klebsiella pneumoniae     | K A A A K A A A K A A A | K A A A |
| Pleisomonas shigelloides  | K K K K K K K K K K | K K K K K K K K |
| Proteus mirabilis         | A A K A A A K A A A K | A A A K |
| P. morgani                | A A K A A A K A A A K | A A A K |
| P. vulgaris               | A A 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 A A |
| Pseudomonas aeruginosa    | K (K+) K K K K K K K+ K | K K K+ K K |
| Salmonella group B        | K K K K K (K+) K A (K) K+ K A | K K K+ K A |
| Serratia marcescens       | K A K A K A K A K A K A | K A K A |
| Shigella flexneri         | A A 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.

Interpretation of results recorded in Table 1 requires comparison of the control tube with the other tubes inoculated with the same culture. A strain which gives an acid reaction in the control tube can be declared positive for activity (decarboxylase or dihydrolase) on the amino acids if these tubes are more alkaline than the control. No differences between the control and amino acid-containing media are recorded as negative. Organisms, such as Alcaligenes or Comamonas, which do not ferment dextrose, exhibit no change in the indicator in any of the tubes and are said to be without action on the amino acids. This is not the same as stating that the organism is decarboxylase or dihydrolase negative. Pseudomonads present a peculiar problem. These organisms can give a strongly alkaline reaction without the control showing acid formation. Such results are considered positive.

A more important consideration is illustrated in Table 2. Seventy-five strains of organisms received by the laboratory with various labels grew in the new medium but only 33 grew in Moeller and Falkow media. An analogous situation could occur in the diagnostic laboratory when one does not know if a freshly isolated strain will grow in the medium being used. This may not be an unusual occurrence if the increased association of non-cholera vibrios and Vibrio parahemolyticus with human diarrheal disease is considered. Time and effort could be lost.

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**

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| Organisms                  | Lysine | Arginine | Ornithine | Control | No. strains growing in: | Total |
|----------------------------|--------|----------|-----------|---------|------------------------|-------|
|                            |        |          |           |         | 3 Media*               |       |
|                            |        |          |           |         | New medium only        |       |
| Aeromonas sp.              | (K) or A | K        | A         | A       | 13                     | 1     |
| Vibrio parahemolyticus     | K       | A        | K         | A       | 1                      | 36    |
| Vibrio cholerae            | K       | A        | K         | A       | 10                     | 0     |
| Unknowns*                  | K       | A        | K         | A       | 9                      | 5     |
| Total                      |        |          |           |         | 33                     | 42    |

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

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Table 2. Reactions and growth of vibrios and related organisms