Acetamide Agar for Differentiation of Nonfermentative Bacteria

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An acetamide agar medium is described for use in the differentiation of nonfermentative gram-negative bacteria. With few exceptions, indicator reactions were rapid, intense, and clear-cut.

Acetamide preparations have been used as selective media for isolation of Pseudomonas aeruginosa from clinical materials (3, 6) or to assist in identification (1, 2, 4, 5, 7). Bühlmann et al. (1) used an acetamide broth medium as a differential test to detect deamidation of acetamide by P. aeruginosa. This medium was a modification of Christensen urea agar; agar was eliminated and 1% acetamide was substituted for glucose and urea. Hedberg (3) described an acetamide medium without the peptone and indicator, but included agar for selective isolation of P. aeruginosa. This medium, a modification of Simmon citrate agar, was developed to determine the suitability of acetamide to serve as a carbon source in the absence of peptone. Recently, Smith and Dayton (6) used the medium of Hedberg without agar to recover P. aeruginosa from rectal swabs.

Assimilation studies by Gilardi (2) and others (7) using basal mineral media showed that acetamide was utilized by a wide variety of nonfermenting organisms. However, reports describing the ability of organisms other than P. aeruginosa (1) to deamidate acetamide are few (4, 5).

An acetamide agar medium was prepared to determine the ability of various nonfermenting gram-negative rods and cocci to deamidate acetamide for purposes of differentiation. Formulation of the medium was as follows (g/liter): NaCl, 5.0; MgSO₄, 0.2; NH₄H₂PO₄, 1.0; K₂HPO₄, 1.0; acetamide, 10; agar, 15; bromothymol blue, 0.08; reagent grade water, 1 liter. The medium was boiled to suspend the agar and cooled to 50°C before adding the indicator. The medium was then dispensed in 10-ml amounts into tubes (16 by 125 mm), sterilized by autoclaving at 121°C for 15 min, and allowed to cool in the slanted position. The pH was adjusted to 6.8 before sterilization.

This report presents the results of studies using strains of nonfermenting rods and cocci referred to this laboratory for identification or confirmation. The acetamide medium was in-

| Organism                      | No. of isolates positive | No. of isolates tested |
|-------------------------------|--------------------------|------------------------|
| Pseudomonas aeruginosa        | 48                       | 50                     |
| P. putida                     | 2                        | 22                     |
| P. fluorescens                | 0                        | 7                      |
| P. cepacia                    | 2                        | 2                      |
| P. pseudomallei               | 2                        | 5^                    |
| P. stutzeri                   | 2                        | 18                     |
| P. maltophilia                | 0                        | 43                     |
| P. putrefaciens               | 0                        | 1                      |
| P. acidovorans                | 11                       | 11                     |
| P. alcaligenes                | 0                        | 6                      |
| P. pseudoalcaligenes          | 0                        | 8                      |
| P. diminuta                   | 0                        | 2                      |
| P. testosteroni               | 0                        | 3                      |
| P. vesiculare                 | 0                        | 3                      |
| Pseudomonas spp.              | 0                        | 13                     |
| Group VE-1 and 2              | 0                        | 4                      |
| Group IIK-1 and 2             | 0                        | 8                      |
| Group III                     | 10                       | 12^                   |
| Group V-C (Alcaligenes denitrificans) | 0               | 2                      |
| Group VI (Alcaligenes faealis) | 1                        | 2                      |
| Alcaligenes odorans           | 5                        | 5                      |
| Group IV-C                    | 0                        | 1                      |
| Group IV-E                    | 0                        | 2                      |
| Flavobacteria                 | 0                        | 20                     |
| Acinetobacter calcoaceticus   | 1                        | 29                     |
| Acinetobacter luofi           | 0                        | 9                      |
| Acinetobacter hemolyticus     | 0                        | 4                      |
| Moraxella spp.                | 0                        | 4                      |

^ Positive in 1 to 2 days.
^ Positive in 7 days.
^ Six isolates positive in 3 to 4 days.
corporated into the routine battery of tests used in this laboratory to identify nonfermenting bacteria. Each slant was inoculated with a drop or loopful of an 18-h triple sugar iron agar culture emulsified in Trypticase soy broth. Slants were incubated at 35°C and observed daily for 4 days and again at 7 days before discarding as negative. Deamidation (alkalinization) of acetamide was indicated by a pronounced blue coloration of the medium. Assimilation of acetamide without deamidation sometimes resulted in an acid (yellow) reaction.

The ability to deamidate acetamide was restricted to a few species of nonfermenting organisms (Table 1). \textit{Pseudomonas acidovorans}, \textit{P. aeruginosa}, Group III (\textit{Achromobacter xylosoxidans}), and \textit{Alcaligenes odorans} were most active on this medium. Although the number of \textit{Pseudomonas cepacia} isolates were small, both deamidated acetamide. The ability of \textit{Pseudomonas stutzeri}, \textit{Pseudomonas putida}, and \textit{Acinetobacter calcoaceticus} strains to deamidate acetamide was limited. The two strains of \textit{Pseudomonas pseudomallei} requiring 7 days for deamidation were each smooth variants of two unrelated cultures included in the table. Some isolates of \textit{Pseudomonas maltophilia} presented an extremely weak reaction after 3 or 4 days of incubation. These were regarded as negative tests since reactions did not increase in intensity even after 7 days of incubation.

The results of this study suggest that the acetamide agar described is suited to assist in the differentiation of nonfermenting organisms. With few exceptions, deamidation reactions were rapid, intense, and clear-cut. Significantly, deamidation of acetamide assisted in differentiating \textit{P. acidovorans} from other non-saccharolytic or weakly saccharolytic pseudomonads. In addition, it was useful in the differentiation of the fluorescent pseudomonads, especially the biochemically aberrant strains, as well as in the separation of some peritrichous organisms from biochemically similar pseudomonads. Since isolates of some species of organisms were few in number, further testing will be needed to better assess the value of the medium.

\textbf{LITERATURE CITED}

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