Urease Activity of Enterobacteriaceae: Which Medium to Choose

A. VUYE AND J. PIUCK

Laboratory for Pharmaceutical Microbiology, State University of Ghent, 9000 Ghent, Belgium

Detection and intensity of urease activity in enterobacteriaceae greatly varies as a function of the media or techniques used, or both. A comparative investigation on several solid and liquid media led us to the following conclusions. (i) Detection of Proteus spp. can be adequately performed with the highly selective solid medium described by Cook (1948), as well as with the different liquid media described (Stuart standard and rapid media; Elek medium). (ii) Detection of Klebsiella should be based upon urease production on solid media with low buffer capacity (Christensen, 1946). (iii) For the identification of Yersinia, either the solid Christensen urea agar or the rapid Elek technique give optimal results.

Urease activity of enterobacteriaceae is routinely determined in the clinical bacteriological laboratory for differentiation and identification purposes. Typical positive results are obtained with Proteus and Klebsiella spp. as well as with non-enterobacteriaceae such as Yersinia, which are now more and more routinely searched for in clinical specimens. Other species, however, show variable urease activities (Table 1). Furthermore, results vary as a function of media or techniques used, or both. In order to clearly standardize procedures and the interpretation of results, a comparative study was made with different solid and liquid media as well as with media of varying buffer capacities.

MATERIALS AND METHODS

Cultures of enterobacteriaceae and Yersinia were obtained from clinical specimens (feces, urine, abscesses, burns, etc.) and identified by using conventional methods (1–3, 7–10). A total of 89 cultures were submitted to the comparative tests, which used different solid and liquid media composed and prepared as in Table 2. Species having no known urease activity were intentionally neglected in this study (i.e., Escherichia, Shigella, Salmonella, etc.). Solid media I, II, and III were inoculated by streaking a loopful of pure culture onto the surface.

Liquid media IV and V were inoculated by adding seven drops of a suspension containing approximately 10⁶ bacteria/ml. For the rapid Elek technique (medium VI), 0.5 ml of medium was inoculated with a loopful of a 24-h culture from a solid medium. Incubation was performed throughout at 37°C, a shaking incubator being used for the liquid media. Incubation time varied as a function of the medium and will be discussed later; urease activity (formation of NH₄ from urea by the action of aminohydrolase) was detected by color changes of the indicator (media I through V), or in absence of indicator (medium V without indicator, and medium VI), by the addition of Nessler reagent.

With the solid media, a quantitative interpretation

| Enterobacteriaceae | Urease activity |
|--------------------|-----------------|
| Tribe I. Escherichiae | 
| Genus I. Escherichia | – |
| Genus II. Shigella | – |
| Tribe II. Edwardsiella | – |
| Genus I. Edwarsdiella | – |
| Tribe III. Salmonelleae | 
| Genus I. Salmonella | – |
| Genus II. Arizona | – |
| Genus III. Citrobacter | Diff (69.4% +; 6.9% d+) |
| Tribe IV. Klebsiellae | 
| Genus I. Klebsiella | + or – |
| K. pneumoniae | 94.5% + |
| K. ozoenae | Diff (9.5% +; 10.3% d+) |
| K. rhinoscleromatis | – |
| Genus II. Enterobacter | + or – |
| E. cloacae | Diff (64.7% +) |
| E. aerogenes | – (2.7% +) |
| E. hafniae | – |
| E. liquefaciens | Diff |
| Genus III. Pectobacterium | Diff |
| Genus IV. Serratia | Diff |
| Tribe V. Proteae | 
| Genus I. Proteus | + (100% +) |
| Genus II. Providencia | – |

* Data were compiled from the literature (1, 8, 9).
* Diff, differing activities.
* d, Delayed activity.
### Table 2. Composition and preparation of test media

| Medium                                      | Components (g) | Preparation                                                                 |
|---------------------------------------------|----------------|-----------------------------------------------------------------------------|
| **Solid medium**                            |                |                                                                             |
| I. Christensen urea agar (4, 7)             |                |                                                                             |
| (a) Concentrate                             | 0.012          | Sterilize (a) by membrane filtration; autoclave (b); cool to 50 C; add 1   |
| (b) Agar solution                           | 0.012          | part (a) to 9 parts (b); distribute 10-ml amounts in tubes; solidify as    |
|                                             | 0.012          | slants                                                                      |
| II. id., without alternative nitrogen source|                | Same as I                                                                   |
| (a) Concentrate                             | 0.012          | Autoclave (a); cool to 50 C; sterilize (b) by membrane filtration; add 1    |
| (b) Agar Solution                           | 0.012          | part (b) to 9 parts (a); distribute 10-ml amounts in tubes and              |
|                                             | 0.012          | let solidify as slants                                                      |
| III. Urease medium (5)                      |                |                                                                             |
| (a) Agar base                               |                |                                                                             |
| (b) Urea solution                           |                |                                                                             |
|                                             |                |                                                                             |
| **Liquid medium**                           |                |                                                                             |
| IV. Stuart standard urease test (11) (0.067 M) | 0.010          | Sterilize by membrane filtration; distribute 3-ml amounts in tubes         |
|                                             | 0.010          | Same as IV                                                                  |
| V. Stuart rapid urease test (0.00067 M)     | 0.010          | Sterilize by membrane filtration                                            |
| VI. Elek technique (6)                       | 0.010          |                                                                             |
| Organism                        | No. of organisms tested | % Positive | Start of positive reaction after (h) | Reaction completed after (days) | % Positive | Start of positive reaction after (h) | Reaction completed after (days) | % Positive | Start of positive reaction after (h) | Reaction completed after (days) | % Positive | Start of positive reaction after (h) | Reaction completed after (days) |
|--------------------------------|-------------------------|-----------|-------------------------------------|-------------------------------|-----------|-------------------------------------|-------------------------------|-----------|-------------------------------------|-------------------------------|-----------|-------------------------------------|-------------------------------|
| Proteus mirabilis               | 11                      | 100       | 1-2                                 | 2                             | 100       | 100                                 | 100                           | 100       | 100                                 | 100                           | 100       | 100                                 | 100                           |
| P. vulgaris                    | 6                       | 60        | 1-2                                 | 2                             | 85        | 4                                   | 85                            | 85        | 5                                   | 85                            | 85        | 5                                   | 85                            |
| P. rettgeri                    | 5                       | 80        | 1-2                                 | 2                             | 80        | 1                                   | 80                            | 80        | 2                                   | 80                            | 80        | 2                                   | 80                            |
| Klebsiella                     | 20                      | 80        | 24-30                               | +5 days                       | 80        | 1                                   | 80                            | 80        | 2                                   | 80                            | 80        | 2                                   | 80                            |
| Enterobacter cloacae           | 8                       | 80        | 9-10                                | +4 days                       | 80        | 1                                   | 80                            | 80        | 2                                   | 80                            | 80        | 2                                   | 80                            |
| E. faecalis                    | 5                       | 80        | 8-30                                | +3 days                       | 80        | 1                                   | 80                            | 80        | 2                                   | 80                            | 80        | 2                                   | 80                            |
| E. coli                        | 8                       | 80        | 8-20                                | +4 days                       | 80        | 1                                   | 80                            | 80        | 2                                   | 80                            | 80        | 2                                   | 80                            |
| P. aeruginosa                  | 3                       | 80        | 8-20                                | +4 days                       | 80        | 1                                   | 80                            | 80        | 2                                   | 80                            | 80        | 2                                   | 80                            |
| P. putrefaciens                | 3                       | 80        | 8-20                                | +3 days                       | 80        | 1                                   | 80                            | 80        | 2                                   | 80                            | 80        | 2                                   | 80                            |
| Serratia                       | 3                       | 80        | 8-20                                | +5 days                       | 80        | 1                                   | 80                            | 80        | 2                                   | 80                            | 80        | 2                                   | 80                            |
| Providencia alcalifaciens      | 4                       | 80        | 8-20                                | +5 days                       | 80        | 1                                   | 80                            | 80        | 2                                   | 80                            | 80        | 2                                   | 80                            |
| Yersinia enterocolitica        | 2                       | 80        | 8-20                                | +4 days                       | 80        | 1                                   | 80                            | 80        | 2                                   | 80                            | 80        | 2                                   | 80                            |
of results (percentage of discoloration; Table 3) was obtained by evaluation of the length of medium reddened after incubation (25, 50, 75, or 100%). To obtain reproducible results, all tests were performed under strict standard conditions (10 ml of media, screw-capped tubes [20 by 160 mm] with deep butt and short slant).

RESULTS

The results obtained with solid media are summarized in Table 3; numbers of each organism tested and percentage of positive reactions are indicated, as well as the time of onset and the time needed for completion of positive reaction. The same table summarizes the percentage of discoloration of the medium after final equilibrium had been reached.

Figure 1 illustrates the evolution of reaction intensity on medium I as a function of time for the most representative urease positive strains. Results obtained with liquid media are summarized in Table 4.

DISCUSSION

It appears from our results that several enterobacteriaceae other than Proteus and Klebsiella possess a more or less intense urease activity, detection of which varies with the sensitivity and buffer capacity of the medium used.

Comparative results obtained on solid media (Table 3) show that media I and II had very similar properties; only Citrobacter seemed to react somewhat more quickly on medium II.

Medium III, which had a higher buffer capacity, had a higher selectivity and should be preferentially used for the identification of Proteus spp. after 24 h of incubation. Detection of urease activity in Klebsiella or Yersinia should preferably be performed on medium I or II.

With the liquid or rapid media, or both, medium IV can be compared with the previous solid media as to reaction speed: after 12 to 18 h of incubation, Proteus spp. reacted positively, and longer incubation (24 to 48 h) gave rise to a positive reaction for Yersinia; Klebsiella remained negative after 24 h of incubation.

Media V and VI allowed rapid urease identification due to their low buffer capacity. When reaction time was standardized to 2 h, clear-cut positive results were obtained for Proteus spp. When ammonia was detected with Nessler reagent (medium V without indicator, and especially medium VI), Yersinia gave a strong positive reaction, as did some Pectobacter spp.

Those species having an irregular urease activity (i.e., Citrobacter, Enterobacter, etc.) should be identified by other biochemical reactions, since the urease criterion is irrelevant.

Summarizing the practical value of this comparative study, we suggest the following procedure: (i) for detection of Proteus, highly selective medium III or any of the rapid liquid media; (ii) for detection of Klebsiella, solid medium with low buffer capacity (medium I or II); and (iii) for detection of Yersinia, solid medium I or liquid medium VI.

In practice, these investigations are of value, for instance, for the rapid differentiation of the genus Proteus from the genus Salmonella (stool examination): suspected gram-negative bacilli which are lactose negative and H$_2$S positive are checked on urease activity in liquid medium VI; if the test is negative, there is possible evidence of Salmonella. This important differentiation

![Fig. 1. Intensity of urease activity in medium 1 as a function of time.](image)
can be performed within 2 h. Furthermore, one can characterize different lactose-positive (and, in certain cases, lactose-negative) organisms by their delayed urease activity on Christensen medium. By using Table 3, where the reaction speeds are indicated, one can, for instance, recognize Klebsiella strains by their moderately delayed urease reaction (onset of reaction after 9 to 10 h as opposed to Proteus spp., which had onset of reaction after 1 to 2 h). Table 3 gives a reliable estimation of reaction speeds of urease-producing enterobacteriaceae other than Proteus spp. The urease test is thus a very useful criterion for identification of these organisms.

**LITERATURE CITED**

1. Bailey, W. R., and E. G. Scott. 1970. Diagnostic microbiology. C. V. Mosby Co., St. Louis.
2. Blair, J. E., E. H. Lennette, and J. P. Truant (ed.) 1970. Manual of clinical microbiology. American Society for Microbiology, Bethesda, Md.
3. Bodily, L. H., E. L. Updyke, and J. O. Mason (ed.) 1970. Diagnostic procedures for bacterial, mycotic and parasitic infections. American Public Health Association, New York.
4. Christensen, W. B. 1946. Urea decomposition as a means of differentiating Proteus and paracolon cultures from each other and from Salmonella and Shigella types. J. Bacteriol. 52:461-466.
5. Cook, G. T. 1948. Urease and other biochemical reactions of the Proteus group. J. Pathol. Bacteriol. 60:171-181.
6. Elek, S. D. 1948. Rapid identification of Proteus. J. Pathol. Bacteriol. 60:183-192.
7. Ewing, W. H. 1962. Enterobacteriaceae. Biochemical methods for group differentiation. In Public Health Service Publ. no. 734, Washington, D.C.
8. Ewing, W. H. 1968. Differential reactions of Enterobacteriaceae. National Communicable Disease Center, Atlanta, Ga.
9. Fife, M. A., W. H. Ewing, and R. R. Davis. 1965. The biochemical reactions of the tribe Klebsiellae. National Communicable Disease Center, Atlanta, Ga.
10. Pijck, J., M. Spoormans-Croux, R. Van den Bossche-Bilo, and A. Vuye. 1972. Identification of Enterobacteriaceae in the clinical microbiology laboratory, p. 390-396, vol. 1. Medikon, European Press.
11. Stuart, C. A., E. Van Stratum, and R. Rustigian. 1945. Further studies on urease production by Proteus and related organisms. J. Bacteriol. 49:437-444.