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Urease Activity of Enterobacteriaceae: Which Medium to Choose

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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.

TABLE 1. *Urease activity of enterobacteriaceae*[a]

Enterobacteriaceae	Urease activity
Tribe I. Escherichieae	
Genus I. *Escherichia*	–
Genus II. *Shigella*	–
Tribe II. Edwardsielleae	
Genus I. *Edwardsiella*	–
Tribe III. Salmonelleae	
Genus I. *Salmonella*	–
Genus II. *Arizona*	–
Genus III. *Citrobacter*	Diff[b] (69.4% +; 6.9% d[c] +)
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*	–

[a] Data were compiled from the literature (1, 8, 9).
[b] Diff, differing activities.
[c] d, Delayed activity.

MATERIALS AND METHODS

Cultures of enterobacteriaceae and *Yersinia* were obtained from clinical specimens (feces, urine, abcesses, 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 10^9 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 NH_3 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

TABLE 2. *Composition and preparation of test media*

Medium	Components (g)												Preparation
	Yeast extract	Peptone	NaCl	Glucose	KH_2PO_4	Na_2HPO_4	Phenol red	Urea	NaOH (0.2 N)	Water	Agar	pH	
Solid medium													
I. Christensen urea agar (4, 7)													Sterilize (a) by membrane filtration; autoclave (b); cool to 50 C; add 1 part (a) to 9 parts (b); distribute 10-ml amounts in tubes; solidify as slants
(a) Concentrate		1	5	1	2		0.012	20		100		6.8	
(b) Agar solution										900	15		
II. id., without alternative nitrogen source													Same as I
(a) Concentrate	0.1		5	1	2		0.012	20		100		6.8	
(b) Agar Solution										900	15		
III. Urease medium (5)													Autoclave (a); cool to 50 C; sterilize (b) by membrane filtration; add 1 part (b) to 9 parts (a); distribute 10-ml amounts in tubes and let solidify as slants
(a) Agar base	0.1				9.1	9.5	0.012			900	20		
(b) Urea solution								20		100		6.8	
Liquid medium													
IV. Stuart standard urease test (11) (0.067 M)	0.1				9.1	9.5	0.010	20		1000		6.8	Sterilize by membrane filtration; distribute 3-ml amounts in tubes
V. Stuart rapid urease test (0.00067 M)	0.1				0.091	0.095	0.010	20		1000		6.8	Same as IV
VI. Elek technique (6)					1.361			4	35 ml	165		7.2	Sterilize by membrane filtration

TABLE 3. *Results of urease activity studies with solid media*

Organism	No. of organisms tested	Medium I			Medium II	Medium III			Final activity (% discoloration of medium at end of reaction—mean values)		
		% Positive	Start of positive reaction after (h)	Reaction completed after	% Positive	% Positive	Start of positive reaction after	Reaction completed after (days)	Medium I	Medium II	Medium III
Proteus mirabilis	11	100	1-2	24 h	100	100	7-8 h	2	100	100	100
P. morganii	6	100	1-2	24 h	100	100	7-8 h	2	100	100	100
P. vulgaris	5	100	1-2	24 h	100	100	7-8 h	2	100	100	100
P. rettgeri	5	100	1-2	24 h	100	100	7-8 h	2	100	100	100
Citrobacter	20	66	24-30	+5 days	70	0			50	80	0
Klebsiella	8	100	9-10	3 days	100	0			100	96	0
Enterobacter cloacae	5	100	8-20	4 days	100	0			100	100	0
E. hafniae	3	33	8-20	+4 days	33	0			75	75	0
E. aerogenes	3	100	8-20	+4 days	50	0			75	100	0
E. liquefaciens	3	100	8-20	+4 days	100	0			75	75	0
Pectobacterium	3	66	8	2 days	66	33	2 days	+4	100	100	88
Serratia	3	100	1-2 days	+5 days	50	0			50	50	0
Providencia alcalifaciens	4	0			0	0			0	0	0
Providencia stuartii	2	0			0	0			0	0	0
Yersinia enterocolitica	8	100	3-5	+4 days	100	50	8-48 h	+4	85	78	81

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.

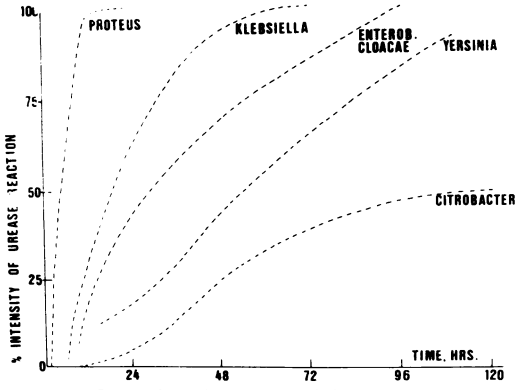

FIG. 1. *Intensity of urease activity in medium I as a function of time.*

TABLE 4. *Results of urease activity studies with liquid media*

Organism	Medium IV	Medium V (after 2 h)	Medium V[b] (after 2 h)	Medium VI[b] (after 2 h)
Proteus sp.	+ (24 h)	+	+	+
Klebsiella	–	Diff	–	–
Citrobacter	–	–	–	–
Enterobacter	–	–	–	–
Serratia	–	–	–	–
Pectobacterium	Diff[a] (48 h)	Diff	–	Diff
Yersinia	+ (48 h)	Diff	Diff	+

[a] Diff, different reaction according to the strain. Some strains were positive or delayed positive; others were negative.

[b] A positive reaction is shown by a dark-brown precipitate, and a negative reaction is shown by a yellow color or slight yellow precipitate. For the actual test, a negative control (e.g., *Escherichia coli* as non-urease producer) and an uninoculated blank should be included.

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; Klebisella* 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 irrevelant.

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_2S positive are checked on urease activity in liquid medium VI; if the test is negative, there is possible evidence of *Salmonella*. This important differentiation

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.

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