Evaluation of the Rapid Decarboxylase and Dihydrolase Test for the Differentiation of Nonfermentative Bacteria

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Received for publication 9 September 1975

A rapid medium for the detection of lysine and ornithine decarboxylase and arginine dihydrolase activity of 439 strains of gram-negative, nonfermenting bacteria was evaluated and compared with Moeller decarboxylase medium. Results were obtained in 4 to 24 h using the rapid medium, whereas Moeller medium often required extended (3 to 7 days) incubation. There was 100% agreement in the lysine tests with both media and almost 100% agreement in the ornithine tests. There was 91% agreement in the arginine tests, with the significance of discrepant results discussed. The sensitivity, specificity, and quick results obtained by the rapid test make it a suitable substitute for Moeller medium for the identification of gram-negative, nonfermenting bacteria.

Tests for lysine and ornithine decarboxylase and arginine dihydrolase have been of considerable value in the differentiation of nonfermenting, gram-negative bacteria (4, 6, 12). At present, the Moeller method (9) is the commonly used test for decarboxylase determinations. Nevertheless, the disadvantage of Moeller medium is that extended incubation is often necessary for positive reactions to be evident (7).

Recently, several methods have been reported describing rapid, practical tests to determine decarboxylase and dihydrolase activity among various organisms. Fay and Barry (3) used a modified decarboxylase medium to detect ornithine decarboxylase activity in the *Klebsiella-Enterobacter-Serratia* group of organisms. The conventional broth medium was modified by omitting glucose, reducing the final pH to 5.3, and decreasing the test volume to 1 ml. Highly reliable results were obtained after only 2 to 4 h of incubation. Using a slight modification of the Fay-Barry medium, Brooker et al. (1) successfully detected lysine decarboxylase in various members of the *Enterobacteriaceae*. Brooks and Sodeman (2) compared the rapid test of Fay and Barry with the Moeller method to test several species of the *Enterobacteriaceae* and gram-negative, nonfermenting bacteria and found close agreement between the two tests.

The purpose of this study was (i) to compare the rapid method described by Fay and Barry with the Moeller method to detect lysine, arginine, and ornithine decarboxylase and dihydrolase activity among a wide variety of gram-negative, nonfermentative bacteria and (ii) to

determine whether this medium would serve as a suitable substitute for Moeller medium.

MATERIALS AND METHODS

Organisms. The 439 organisms used in this study were clinical isolates referred to this laboratory for identification or confirmation. Most organisms were received as "*Pseudomonas* species" or "nonfermenting bacteria." Two strains of *P. mendocina* were kindly provided by Gerald Gilardi. All cultures were identified by using the methods of Weaver et al. (12), as well as the supplemental methods of Gilardi (4), Hugh and Gilardi (6) and Tatum et al. (11). Flagella stains were performed by the method of Leifson (8), and the observations were confirmed by electron micrographs.

Media. The rapid broth medium was prepared according to the formulation of Fay and Barry (3). The medium was divided into four equal amounts, and the equivalent of 10 g of lysine, arginine, and ornithine per liter was added to the respective aliquots, with the fourth portion serving as base control medium. The pH of each portion was adjusted to 5.4 ± 0.1 . On a weekly basis, sufficient 1-ml portions of the respective broths were aseptically dispensed into sterile, snap-cap, disposable plastic tubes (12 by 75 mm). Moeller decarboxylase broth (9) was prepared according to the directions of the manufacturer (Difco) in batch amounts with 5 ml dispensed into tubes (16 by 125 mm).

Test procedure. An emulsion of each test organism was prepared from Trypticase soy agar slants (BBL) using sterile reagent grade water. Each tube of the rapid medium and Moeller medium was heavily inoculated with 1 drop of the emulsion and overlaid with 1 ml of sterile mineral oil. Both tests were incubated at 35 C. The rapid test was read at 4, 24, 48, and 72 h, and the Moeller test was read daily for

4 days and again at 7 days before discarding. In both tests, the development of a purple to violet color indicated a positive reaction as alkalization of the media occurred. Quality control of the media was monitored using a lysine- and ornithine-positive strain of *P. cepacia* and an arginine-positive strain of *P. aeruginosa*.

RESULTS

The results of the lysine and ornithine decarboxylase activity tests using the rapid medium and Moeller medium are shown in Table 1. All 56 strains of *P. maltophilia* were lysine positive and ornithine negative by both tests. In the rapid medium, 55 strains were positive in 4 h, and all 56 were positive within 24 h. In Moeller medium only 5 strains were positive within 24 h, and 21 required over 48 h of incubation for alkalization to occur. Although only five strains of *P. cepacia* were available for testing, there was complete agreement between the two tests for lysine decarboxylase. Again, the five strains were positive in the rapid test medium in 4 h but required at least 48 h incubation in Moeller medium. Early ornithine decarboxylase activity of three strains was detected by the rapid method, whereas two strains showed delayed activity by the Moeller method.

Tables 2 and 3 present the results of arginine dihydrolase tests using both methods. The lysine and ornithine activities of all organisms were negative in both tests. The rapid arginine dihydrolase test for apyocyanogenic *P. aeruginosa*, *P. putida*, and *P. fluorescens* agreed completely with the results obtained in Moeller medium (Table 2). All tests using the rapid method were completed within 24 h, whereas the Moeller method required at least 24 h before activity was evident. Eighty-five percent of the *P. pseudoalcaligenes* strains were arginine positive within 24 h using the rapid method, whereas only 20% of the strains were positive

after 48 h by the Moeller method. Indeed, three of the *P. pseudoalcaligenes* strains were only weakly positive at 7 days in Moeller medium. Eleven of 15 isolates of *P. stutzeri* (73%) were arginine positive within 24 h in the rapid medium, whereas none was positive in the Moeller medium. In contrast, all of the *P. stutzeri*-like cultures were arginine positive in both media, although 48 h of incubation was necessary to present a positive reaction in Moeller media. Seven of 39 strains of *Acinetobacter calcoaceticus* showed delayed (weak) dihydrolase activity in the rapid medium but failed to show activity in Moeller medium. Table 3 presents the arginine activity in infrequently encountered organisms. Both *P. mendocina* and *P. pseudomallei* agreed completely in the two tests, although results were obtained sooner in the rapid test medium. All the *P. alcaligenes* strains were positive in the rapid test in contrast to two delayed positive tests (7 days) in Moeller medium. Three peritrichous organisms conforming to the CDC group V-D, type 1, were arginine positive in the rapid test but negative in the Moeller test. Four strains of the yellow pigmented CDC group VE-1 were arginine positive within 24 h in the rapid medium, and two were delayed positive in Moeller medium.

A comparison of the rapid test and Moeller test to detect arginine dihydrolase is shown in Table 4. There was 91% agreement between the two tests; 157 of 439 organisms (36%) were positive and 241 (55%) were negative by both methods. Forty-one organisms (9%) were negative by the Moeller method but positive by the rapid method. At no time did the Moeller method detect a positive reaction that was negative by the rapid method. The 41 isolates that gave discrepant results in the two tests are listed in Table 5. The one *P. aeruginosa* culture was clearly false negative in Moeller medium. Since members of the *P. alcaligenes-pseudoalcali-*

TABLE 1. Results of lysine and ornithine decarboxylase tests using the rapid method and Moeller method^a

Organism	No. of strains tested	Method ^b	Lysine					Ornithine				
			No. positive				% Positive	No. positive				% Positive
			4 ^c	24	48	>48		4	24	48	>48	
<i>P. maltophilia</i>	56	R	55	1	— ^d	—	100	NEG ^e	NEG	NEG	NEG	—
		M	—	5	30	21	100	NEG	NEG	NEG	NEG	—
<i>P. cepacia</i>	5	R	5	—	—	—	100	2	1	—	—	60
		M	—	—	2	3	100	—	—	1	1	40

^a Arginine dihydrolase tests were negative by both methods.

^b R, Rapid method; M, Moeller method.

^c Incubation time (hours).

^d —, Indicates test completed or negative test.

^e NEG, Negative.

TABLE 2. Results of arginine dihydrolase tests using the rapid method and Moeller method^a

Organism	No. of strains tested	Method ^b	No. positive				% positive
			4 ^c	24	48	>48	
<i>P. aeruginosa</i>	79	R	76	3	— ^d	—	100
		M	—	63	12	3	99
<i>P. putida</i>	43	R	35	8	—	—	100
		M	—	17	19	7	100
<i>P. fluorescens</i>	11	R	8	3	—	—	100
		M	—	4	4	3	100
<i>P. pseudoalcaligenes</i>	20	R	4	13	—	—	85
		M	—	—	1	3	20
<i>P. stutzeri</i>	15	R	9	2	—	—	73
		M	—	—	—	—	0
<i>P. stutzeri</i> -like (CDC Vb-3)	11	R	5	4	2	—	100
		M	—	—	4	7	100
<i>A. calcoaceticus</i>	39	R	—	—	2	5	18
		M	—	—	—	—	0

^a Lysine and ornithine tests were negative by both methods.^b R, Rapid method; M, Moeller method.^c Incubation time (hours).^d —, Indicates completed or negative test.TABLE 3. Results of arginine dihydrolase tests of infrequently encountered organisms^a

Organism	No. of strains tested	Method ^b	No. positive			
			4 ^c	24	48	>48
<i>P. alcaligenes</i>	6	R	1	5	— ^d	—
		M	—	—	—	2
<i>P. mendocina</i>	3	R	3	—	—	—
		M	—	2	1	—
<i>P. pseudomallei</i>	3	R	2	1	—	—
		M	—	2	1	—
CDC V-D, type 1	3	R	—	2	1	—
		M	—	—	—	—
CDC V-E, type 1	4	R	—	4	—	—
		M	—	—	—	2

^a Lysine and ornithine tests were negative by both methods.^b R, Rapid method; M, Moeller method.^c Incubation time (hours).^d —, Indicates completed or negative test.

genes group, as well as the yellow-pigmented CDC VE-1 group, varied in ability to give a positive arginine test in Moeller medium, these results indicate that there was indeed a 37% false-negative rate in Moeller medium among these organisms. Data for decarboxylase reactions in Moeller medium of CDC VD, type 1, are not available. Thus, there is an apparent 4.1% false-positive rate of arginine reactions in the population of organisms tested.

Table 6 lists 141 organisms that were decarboxylase and dihydrolase negative by both

methods. These organisms are considered to lack decarboxylase and dihydrolase activity using methods currently available. Thus, the rapid method was specific as well as sensitive.

DISCUSSION

Rapid and accurate identification of microorganisms in the clinical laboratory is important. With the exception of tests for decarboxylase activity, rapid methods for the differentiation and identification of the nonfermentative,

gram-negative organisms are lacking. Use of thin-layer chromatography to detect arginine dihydrolase (13) and gas-liquid chromatography to detect lysine and ornithine decarboxylase (7) has been advocated. However, these methods often have limitations in their practicality although they may not be beyond the resources of some laboratories. Therefore, a simple procedure that can be implemented in any clinical microbiology laboratory is needed. This study clearly established the rapid test as an accurate and sensitive method to detect decarboxylase and dihydrolase activity among a wide variety of nonfermenting bacteria, as well as among the *Enterobacteriaceae* (1-3, 13).

The ease of performing the rapid test to detect decarboxylase and dihydrolase activity with organisms usually encountered makes it well suited for the clinical laboratory. Most test reactions were completed in 4 h, and some reactions took place as early as 1 to 2 h. Hourly time studies were not pursued and, thus, reactions between 4 and 24 h were not considered here.

Interpretation of test results in the rapid medium was not difficult since color change due to alkalization was rapid and intense. Slight, nonspecific alkalization of the test and base control occurred in certain instances, occasion-

ally among strains of *P. aeruginosa*, *P. maltophilia*, and *A. calcoaceticus*. Therefore, the reading of tests were terminated at 72 h since all tests, with a few exceptions, were completed within 48 h. Alkalization also had been noted when testing members of the *Enterobacteriaceae*, presumably as a result of metabolism of the peptone in the basal medium (1). In the present study this effect was minimized by the rapid decarboxylase or dihydrolase activity in a positive test when compared with the base control. Brooker and associates (1) noted the pK differential between bromocresol purple (6.3) used in the rapid medium and bromothymol blue (7.0) used as their indicator system. The choice of indicator, thus, could account for the slight, nonspecific change in the lower pH range with selected organisms in both the test and control tubes. The use of various indicators was not investigated in this evaluation.

There was 100% agreement between the two methods in tests for lysine decarboxylase. The rapid medium was superior to Moeller medium

TABLE 4. Comparison of methods for detecting arginine dihydrolase using 439 nonfermentative isolates

Rapid test	Moeller method		
	Arginine positive	Arginine negative	Total
Arginine positive	157 (36) ^a	41 (9)	198 (45)
Arginine negative	0	241 (55)	241 (55)
Total	157 (36)	282 (64)	439 (100)

^a Parenthetical values represent percentages.

TABLE 6. Organisms found to be negative for decarboxylase and dihydrolase activity by both Moeller method and rapid method

Organisms	No. tested
<i>P. acidovorans</i>	14
<i>P. diminuta-vesiculare</i>	7
<i>P. testosteroni</i>	7
<i>F. meningosepticum</i>	4
CDC II-b	11
CDC II-K	6
CDC V-A	4
CDC VE-2	8
<i>B. bronchiseptica</i>	3
<i>Achromobacter xylosoxidans</i>	15
<i>A. faecalis</i>	4
<i>A. odorans</i>	9
<i>A. denitrificans</i>	1
CDC IV-C	3
<i>A. lwoffii</i>	30
<i>Moraxella</i> spp.	15

TABLE 5. Distribution of 41 cultures that were arginine positive in the rapid test and arginine negative in the Moeller test

Organism	Rapid test		Remarks
	No. positive/no. tested	% False positive	
<i>P. aeruginosa</i>	1/79	1	Moeller false negative
<i>P. pseudoalcaligenes</i>	13/20	65	See text
<i>P. alcaligenes</i>	4/6	67	See text
<i>P. stutzeri</i>	11/15	73	Rapid false positive
<i>A. calcoaceticus</i>	7/39	18	Rapid false positive
CDC VD, type 1	3/3		See text
CDC VE-1	2/4	50	See text

in that all of 61 lysine-positive organisms tested were positive in the rapid medium in 24 h, whereas all but 5 organisms required at least 48 h of incubation to manifest a positive reaction in Moeller medium. Although few ornithine-positive organisms were tested, the result show that the rapid medium had a decided advantage (Table 1).

Discrepancies were noted between the two media in tests for arginase activity. In general, results were obtained more quickly in the rapid medium when testing both common and less common organisms. Many of the organisms that required more than 48 h to produce a positive reaction in Moeller medium indeed required 5 to 7 days to do so, an observation made by other investigators using Moeller medium for decarboxylase tests (7). Organisms of the *P. alcaligenes-pseudocalcaligenes* group are known to demonstrate arginine dihydrolase activity, although this activity is infrequently seen in Moeller medium (4, 12). Similar observations have been made among the yellow-pigmented organisms designated CDC V-E, type 1 (5, 11, 12). The positive arginine reactions detected with the rapid test medium show that this medium is more sensitive than Moeller and can present more uniform results when testing these organisms. The detection of arginine dihydrolase activity among the *P. alcaligenes-pseudocalcaligenes* group greatly assists to differentiate these organisms from other weakly saccharolytic or non-saccharolytic bacteria. It is interesting to note that, of the nonsaccharolytic bacteria tested, only *P. alcaligenes* demonstrated dihydrolase activity (Table 3).

The three isolates of the peritrichous organism conforming to the CDC designation V-D, type 1, presented a delayed positive arginine test in the rapid medium and a negative test in Moeller medium. Since decarboxylase reactions in Moeller medium are not described for these organisms (11, 12), it is difficult to assess whether the reactions shown in Tables 3 and 5 were false-positive arginine reactions in the rapid test. Therefore, with this consideration, in addition to that described above, it seems that the false-positive rapid arginine test was relegated to the 11 *P. stutzeri* and 7 *A. calcoaceticus* strains.

The production of arginine dihydrolase has been reported as a negative biochemical characteristic of *P. stutzeri* using Moeller medium (4, 6, 10). Weaver et al. (12) describe three organisms in the *P. stutzeri* group: Vb-1 (*P. stutzeri*), starch positive and arginine negative; Vb-3, starch positive and arginine positive; and Vb-2, starch negative and arginine positive. The

latter group, Vb-2, is suggestive of *P. mendocina* (10). Since all of the isolates of Vb-3 (Table 2) and *P. mendocina* (Table 3) were arginine positive in the rapid and Moeller tests, there is a suggestion that any organism which is arginine negative in Moeller medium is *P. stutzeri*. There is a further suggestion that any organism which is arginine positive in any medium other than Moeller is not *P. stutzeri*.

Moeller decarboxylase medium was developed principally for use in the bacteriology of the enteric organisms (9). The presence of glucose or glucose fermentation is not a prerequisite for decarboxylation or dihydrolation (9). The paucity of information concerning the origin of the adoption and adaptation of Moeller medium for use in studies of the nonfermentative bacteria may require a reassessment of its use as a standard. Since decarboxylation and dihydrolation reactions in nonfermenting organisms have not been studied as extensively as in the *Enterobacteriaceae* and since optimal conditions may vary for each enzyme, as well as organism, further testing of newly acquired strains will be necessary to determine the extent to which arginine dihydrolases are distributed among these organisms.

It seems apparent that detection of arginine dihydrolase activity among nonfermentative bacteria is based on (i) the population of organisms studied, (ii) the length of incubation of the tests, and (iii) the selection of the test to be performed. This latter contention is supported by the work of Williams and associates (13), who found that all 23 strains of *P. maltophilia* tested by thin-layer chromatography were arginine positive. It should be noted that *P. maltophilia* is considered to be arginine negative based on conventional (Moeller) tests.

The rapid method for lysine and ornithine decarboxylase has been shown to be extremely sensitive, as well as specific. The rapid method for arginine dihydrolase has also been shown to be far more sensitive than the Moeller test. The rapid medium has decided advantages over Moeller medium for the identification of nonfermenting organisms commonly encountered in the clinical laboratory. This medium provides quick test results and aids in the identification of nonfermenting bacteria, which often require extended periods of incubation by conventional methods.

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