Motility-Indole-Lysine Medium for Presumptive Identification of Enteric Pathogens of *Enterobacteriaceae*

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Detection of lysine decarboxylase activity is a useful supplement to reactions on triple sugar-iron (TSI) and urea agars in the initial examination of suspected pathogenic isolates from fecal cultures. Owing to the added value of motility and indole production in the differentiation of enteric pathogens, we prepared and evaluated a motility-indole-lysine (MIL) medium. The following 890 organisms were tested: 264 *Shigella*, 2 *Edwardsiella*, 182 *Salmonella enteritidis*, 235 *S. typhi*, 3 *Arizona*, 32 *Yersinia enterocolitica*, and 172 other members of the family *Enterobacteriaceae*. With few exceptions the MIL medium gave the same results as the standard motility, indole, and lysine decarboxylase (Moeller) test media. All discrepancies were with the indole reaction, which was weak in 2 of 67 strains of *Escherichia coli* and falsely negative in 6 of 32 strains of *Y. enterocolitica*. When both TSI agar and lysine-iron agar (LIA) slants are used in the evaluation isolates from fecal cultures, detection of H₂S is duplicated. Both LIA and MIL medium detect lysine decarboxylase and deaminase activity equally well. Because of its ability to detect motility and indole production, the MIL medium is more useful than LIA when used with TSI agar. The combination of TSI agar, MIL medium, and urea agar enables reliable initial recognition of enteric pathogens of the *Enterobacteriaceae*.

The lysine decarboxylase reaction is a useful test in the differentiation of members of the family *Enterobacteriaceae* (1, 2, 5, 6, 8, 10-14, 16). Although lysine-iron agar (LIA) was originally developed to aid recognition of *Arizona* cultures (9), LIA is widely used along with triple sugar-iron (TSI) agar in the selection of enteric pathogens from fecal cultures (6, 12, 13, 16). When both TSI agar and LIA slants are used, detection of H₂S is duplicated. Moreover, tests for motility and indole production are important in the recognition of *Edwardsiella*, *Shigella*, and *Yersinia enterocolitica* (5, 6, 8, 10, 12, 13, 22, 23). Therefore we prepared and evaluated a motility-indole-lysine (MIL) medium to be used with TSI agar and, optionally, urea agar, in the examination of colonies picked from primary isolation media. This MIL medium provides more useful information than LIA provides. When used with TSI agar and urea agar, the MIL medium enables the presumptive identification of enteric pathogens of the family *Enterobacteriaceae*. (This work was presented in part at the 75th annual meeting of

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MATERIALS AND METHODS

Media. All of the media for isolation and preliminary identification of bacteria were prepared from commercially available dehydrated stocks. Other differential media were prepared and tests were performed according to methods described by Edwards and Ewing (8). The Kovacs reagent used to test for indole was stored at 4 C in the dark (5). All tubes were loosely capped to permit gas exchange (21).

The MIL medium consisted of 9.0 g of decarboxylase medium base (Difco Laboratories, Inc., Detroit, Mich.), 5.0 g of peptone (Difco), 10.0 g of tryptone (Difco), 2.0 g of agar, 0.5 g of ferric ammonium citrate, 10.0 g of L-lysine monohydrochloride (Nutritional Biochemicals Corp., Cleveland, Ohio), and 1,000 ml of distilled water. The pH was adjusted to 6.6 ± 0.2. The MIL medium was heated to dissolve the agar, dispensed in 5-ml amounts into tubes (13 by 100 mm), and autoclaved at 121 C for 15 min.

Bacteria. A total of 718 strains of enteric pathogens were tested. These include 264 *Shigella*, 2 *Edwardsiella*, 182 *Salmonella enteritidis*, 235 *Salmonella typhi*, 3 *Arizona*, and 32 *Yersinia enterocolitica*. The Singapore Government Pathology Department provided 315 strains, including all *S. typhi* and *S. enteritidis* bioser Paratyphi A. These organisms were isolated from ill patients or carriers, lyophi-

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lized, and sent to us by air. The majority of strains of *S. typhi* were both Vi-phage typed and confirmed at the Center for Disease Control. The other organisms were either isolated in or confirmed by the Laboratory Division of the Colorado Department of Public Health. In addition 172 other strains of *Enterobacteriaceae* were identified in the Clinical Microbiology Laboratory at Colorado General Hospital during the examination of colonies picked from fecal cultures. These organisms included 67 *Escherichia coli*, 22 *Citrobacter freundii*, 46 *Klebsiella*, 12 *Enterobacter*, 3 *Serratia*, 11 *Proteus mirabilis*, 5 *P. morganii*, 3 *P. vulgaris*, 2 *P. rettgeri*, and 1 *Providencia*.

Taxonomy, nomenclature, and criteria for identification. The taxonomic system, nomenclature, and criteria for identification found in standard sources were followed (5, 8, 10, 22, 23).

Examination of isolates from fecal cultures. Fecal specimens were plated onto MacConkey and Hektoen enteric (18, 19) agars for primary isolation and inoculated into selenite-F broth for enrichment before subculture to secondary plates (6, 11, 12). After 18 to 24 h of incubation two or three of each type of colorless colony from MacConkey agar or blue-green colony, with or without black center, from Hektoen enteric agar were inoculated into TSI agar, motility, indole, and lysine decarboxylase (Moeller method) test media (8), MIL medium, and Christensen urea agar. Organisms referred for confirmation were tested on MIL medium and by standard methods (8, 10).

Reactions in MIL medium. The MIL medium was inoculated once by inserting a straight wire to the bottom of the tube. After 18 to 24 h of incubation at 25 C, 35 C, or both, motility and lysine decarboxylase and deaminase activity were read before testing for indole production. Diffuse turbidity or growth extending from the line of inoculation indicated motility. Nonmotile organisms grew only along the line of inoculation. Owing to fermentation

of glucose, all *Enterobacteriaceae* turned the bottom of the MIL tube yellow; those organisms that also formed cadaverine by lysine decarboxylase caused the entire tube to revert to purple (14). A bright yellow bottom with a narrow band of purple at the top of the tube indicated a negative test for lysine decarboxylase. Presence of lysine deaminase caused the top of the tube to turn deep red; the top remained purple in a negative test. To test for indole production 3 to 4 drops of Kovacs reagent were added to the medium. A red to pink reaction indicated the presence of indole and persistence of the bright yellow layer indicated a negative test.

RESULTS

In a preliminary evaluation of MIL different concentrations of L-lysine monohydrochloride from 0.5 to 2% were used. The color reactions were clearest and easiest to interpret at a concentration of 1% L-lysine or 2% DL-lysine monohydrochloride. Without added lysine the test organism *Proteus mirabilis* did not produce the deep red color attributed to the lysine deaminase reaction at the top of the tube of MIL medium.

The differentiation of members of the family *Enterobacteriaceae* by their reactions in MIL medium is summarized in Table 1. The results in MIL medium with *Enterobacteriaceae* other than *Shigella*, *Edwardsiella*, *Salmonella*, *Arizona*, and *Yersinia* are shown in Table 2. The proportion of strains yielding typical reactions is similar to published results from the Center for Disease Control (8, 10). Except for two of five strains of *P. morganii* all members of the tribe *Proteeae* gave a positive reaction for lysine deaminase. Two of 67 strains of *E. coli* gave faint

TABLE 1. Differentiation of *Enterobacteriaceae* with MIL medium^a

Motility ^b	Indole ^c	MIL tube		Probable organisms
		Top	Bottom	
+	+	Purple	Purple	<i>Escherichia</i> , <i>Edwardsiella</i>
+	—	Purple	Purple	<i>Enterobacter</i> , <i>Serratia</i> , <i>Salmonella</i> , <i>S. typhi</i> , <i>Arizona</i>
—	+	Purple	Purple	<i>Escherichia</i> , <i>Klebsiella</i>
—	—	Purple	Purple	<i>Klebsiella</i>
+	+	Purple	Yellow	<i>Escherichia</i> , <i>Citrobacter diversus</i> , <i>Proteus morganii</i> , <i>Yersinia enterocolitica</i> (25 C)
+	—	Purple	Yellow	<i>Citrobacter freundii</i> , <i>S. enteritidis</i> bioser Paratyphi A, <i>E. cloacae</i> , <i>Y. enterocolitica</i> (25 C)
—	+	Purple	Yellow	<i>Escherichia</i> , <i>Shigella flexneri</i>
—	—	Purple	Yellow	<i>S. sonnei</i> , <i>S. flexneri</i> , <i>Y. enterocolitica</i> (35 C)
+	+	Red	Yellow	<i>P. vulgaris</i> , <i>P. morganii</i> , <i>P. rettgeri</i> , <i>Providencia</i>
+	—	Red	Yellow	<i>P. mirabilis</i>

^a Symbols: +, positive; —, negative; purple top, negative lysine deaminase; red top, positive lysine deaminase; purple bottom, positive lysine decarboxylase; and yellow bottom, negative lysine decarboxylase.

^b Positive motility, diffuse growth outside line of inoculation.

^c Positive indole, pink to red reaction after addition of yellow Kovacs reagent.

indole reactions with MIL medium, but both were clearly positive by the tube-indole method (8). Results of motility and lysine decarboxylase reactions were identical by MIL and standard methods (8).

Reactions of the enteric pathogens of *Enterobacteriaceae* in MIL medium are shown in Table 3. Except for 6 of 32 strains of *Y. enterocolitica*, which gave falsely negative indole reactions in the MIL medium, all other results with 718 strains of potential pathogens were identical with MIL medium and standard methods (8).

Figure 1 shows the typical color reactions in MIL medium seen with enteric pathogens of the *Enterobacteriaceae*. In the United States *S. enteritidis* ser Typhimurium is the most often isolated *Salmonella* serotype and *S. sonnei* the most often isolated *Shigella* species (3, 4). *Edwardsiella* and *Arizona* are isolated infrequently in the United States (9, 17). Motility at 25 C and its absence at 35 C is an important characteristic of *Y. enterocolitica* (22, 23). An example of the added differential value of MIL medium in comparison with LIA is seen in Fig. 2. *S. sonnei* and *P.morganii* appear similar on

TABLE 2. Results in MIL medium with *Enterobacteriaceae* other than *Shigella*, *Edwardsiella*, *Salmonella*, *Arizona*, and *Yersinia*^a

Organism	No. of strains tested	Motility		Indole production		Lysine decarboxylase	
		Sign	% +	Sign	% +	Sign	% +
<i>E. coli</i>	67	+ or -	85	+	97	+ or -	76
<i>Citrobacter freundii</i>	22	+	100	-	100	-	100
<i>Klebsiella</i>	46	-	100	-	13	+	100
<i>Enterobacter cloacae</i>	2	+	100	-	100	-	100
<i>Enterobacter</i> spp.	10	+	100	-	100	+	100
<i>Serratia</i>	3	+	100	-	100	+	100
<i>Proteus</i>							
<i>mirabilis</i>	11	+	100	-	100	-	100
<i>morganii</i>	5	+	100	+	100	-	100
<i>vulgaris</i>	3	+	100	+	100	-	100
<i>rettgeri</i>	2	+	100	+	100	-	100
<i>Providencia</i>	1	+	100	+	100	-	100

^a +, Positive; and -, negative.

TABLE 3. Reactions of enteric pathogens of *Enterobacteriaceae* in MIL medium^a

Organism	No. of strains tested	Motility		Indole production		Lysine decarboxylase	
		Sign	% +	Sign	% +	Sign	% +
<i>Shigella</i>							
<i>dysenteriae</i>	2	-	100	-	100	-	100
<i>flexneri</i>	87	-	100	- or +	14	-	100
<i>sonnei</i>	175	-	100	-	100	-	100
<i>Edwardsiella</i>	2	+	100	+	100	+	100
<i>S. enteritidis</i>							
ser Typhimurium ^b	106	+	100	-	100	+	100
Other serotypes ^c	73	+	100	-	100	+	100
bioser Paratyphi A	3	+	100	-	100	-	100
<i>S. typhi</i>	235	+	100	-	100	+	100
<i>Arizona</i>	3	+	100	-	100	+	100
<i>Y. enterocolitica</i>	32						
25 C		+	100	- or +	15	-	100
35 C		-	100	- or +	12	-	100

^a +, Positive; and -, negative.

^b Includes 6 variety Copenhagen.

^c Includes 2 bioser Pullorum and the following serotypes: 20 Newport; 9 Infantis; 7 Oranienburg; 5 Bredeney; 5 Enteritidis; 5 Saintpaul; 4 Montevideo; 6 Paratyphi B; 3 Senftenberg; and one each of Bareilly, Derby, Heidelberg, Hvittingfoss, Javiana, and Minnesota.

MIL	MIL	MIL	MIL	MIL	MIL
+ - +	- - -	+++	+ - +	++ -	
<i>S. enter-</i>	<i>Shigella</i>	<i>Edward-</i>	<i>Arizona</i>	<i>Y. entero-</i>	Control
<i>itidis ser</i>	<i>sonnei</i>	<i>siella</i>	<i>hinshawii</i>	<i>colitica</i>	
Typhimurium		<i>tarda</i>		25 C

TSI	LIA	MIL	TSI	LIA	MIL
K/A-H ₂ S	K/A-H ₂ S	- - -	K/A-H ₂ S	K/A-H ₂ S	+ + -
<i>Shigella sonnei</i>			<i>Proteus morganii</i>

TSI	LIA	Urea	MIL	PA	LIA	MIL
K/A-H ₂ S	R/A-H ₂ S	-	+ + -	+	K/A-H ₂ S	- - -
<i>Providencia sp.</i>			<i>Shigella sonnei</i>			

FIGS. 1-3

TSI agar and LIA; the MIL medium clearly distinguishes between these organisms owing to the negative indole and motility reactions of *S. sonnei* and the positive indole and motility reactions of *P. morganii*. Figure 3 shows the deamination of lysine by *Providencia* species in both LIA and the MIL media as well as the added differential value of the MIL medium.

DISCUSSION

The importance of lysine decarboxylase as a differential character of members of the family *Enterobacteriaceae* is well recognized (1, 5, 6, 8, 10–13, 16). The value of lysine decarboxylase in the identification of *Salmonella* and *Shigella* has been emphasized (14, 16) and several methods for its detection have been published (1, 2, 8, 9, 14). LIA was originally proposed as an aid to the recognition of cultures of *Arizona* (9), but its use in the evaluation of colonies selected from primary isolation media is widely recommended (6, 12, 13, 16). Both LIA and TSI agar detect production of H_2S . However, neither LIA nor TSI agar provide information about motility or indole; these two tests are essential in the early separation of enteric pathogens from other *Enterobacteriaceae* (5, 6, 8, 10, 13, 22, 23).

To save time and media and thereby cost, many simplified approaches to the initial screening of fecal cultures have been proposed (6, 12, 15, 20). Owing to the success of a motility-indole-ornithine medium (7, 12), we devised a similar medium which incorporated lysine for the detection of lysine decarboxylase and deaminase activity and included the ability to detect motility and indole production. The only discrepancies with MIL medium when compared with standard methods (8) were in the detection of indole production. The MIL medium was about as sensitive in the detection of indole as published results with motility-indole-ornithine medium (7) and the spot-indole test (24). All of these combination or rapid tests are slightly less sensitive than the standard tube-indole test (8); however, their other advantages seem clear.

The results of this study show that the MIL

medium performs equally as well as LIA and the standard Moeller method in the detection of lysine decarboxylase. In contrast to LIA, the MIL medium provides results for motility and indole reactions, which are highly accurate. The use of MIL medium with TSI agar and urea agar enables the economic presumptive identification of isolates of *Shigella*, *Edwardsiella*, *Salmonella*, *Arizona*, and *Y. enterocolitica* obtained from fecal cultures.

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FIG. 1. Typical reactions of enteric pathogens of *Enterobacteriaceae* in motility-indole-lysine (MIL) medium. Symbols: M, motility; I, indole; L, lysine decarboxylase; +, positive; and –, negative.

FIG. 2. Reactions of *S. sonnei* and *P. morganii* in MIL and other media. Symbols: +, positive; –, negative; K, alkaline; and A, acid. The reactions in MIL medium clearly distinguish *S. sonnei* from *P. morganii*, whereas reactions on TSI agar and LIA are identical.

FIG. 3. Reactions of *Providencia* sp. and *Shigella sonnei* in MIL and other media. Symbols: +, positive; –, negative; K, alkaline; A, acid; R, deamination; PA, phenylalanine. Both LIA and the MIL medium show the deep red reaction from the deamination of lysine by *Providencia* sp. The MIL medium also shows the motility and indole reactions, which provide additional characters for the differentiation of *Providencia* sp. from *S. sonnei*.

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