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Use of the Minitek System for Characterizing Lactobacilli[1]

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Incubation of inoculated substrates of the Minitek system in anaerobic GasPak jars provided a method that produced results comparable to those of conventional tubed media for characterizing species of *Lactobacillus*. The use of sterile mineral oil to overlay inoculated substrate disks was responsible for erroneous results.

The Minitek (BBL, Cockeysville, Md.) miniaturized system for identifying bacteria provides clear-cut and accurate reactions for the characterization of *Enterobacteriaceae* (2, 4) and anaerobic bacteria (3, 8). It is flexible with respect to the substrates used. The method suggested for the *Enterobacteriaceae* requires that a number of the substrate disks, after being inoculated, be overlaid with sterile mineral oil to exclude air. Mineral oil is not used in the method for anaerobes. Rather, the inoculated substrates are incubated in GasPak (BBL) anaerobic systems. This study was undertaken to compare the two methods of using the Minitek system with conventional methods for characterizing *Lactobacillus* species.

Lactobacilli MRS broth (Difco Laboratories, Detroit, Mich.) prepared from individual ingredients without glucose and beef extract (7) was used as the basal (or identification) broth. The pH was adjusted so that it was 7.2 after the broth was autoclaved for 15 min at 121°C. Substrate disks and the other Minitek supplies were handled in accordance with directions from the manufacturer. However, amygdalin and melezitose were prepared in our laboratory according to procedures recommended by BBL (personal communication from BBL), which were as follows. A 2-g amount of the carbohydrate was dissolved in 20 ml of distilled water containing 0.1 g of anhydrous dibasic sodium phosphate and 0.1 ml of 8% aqueous phenol red. The solution was adjusted to pH 7.2 if necessary and filter sterilized with a 0.45-μm membrane filter (Millipore Corp., Bedford, Mass.). Sterile 0.25-inch (ca. 0.64-cm) filter paper disks, saturated by aseptically dipping them into the sterile carbohydrate solution, were placed in a sterile glass petri dish (covered) and dried in an oven at 80°C for 6 h. The dried disks were placed in sterile dry bottles and stored in a desiccator in a refrigerator until needed. They were placed in the wells of the Minitek plates with sterile forceps.

The basal broth was also used to prepare conventional tubed media following directions reported by Rogosa and Sharpe (6), except that bromocresol purple (0.02 g/liter) was used as the indicator in the media containing test carbohydrates. Esculin hydrolysis and production of ammonia from arginine were detected by the procedures described by Davis (1).

Cultures of lactobacilli were streaked onto MRS agar (MRS broth plus 1.5% agar) and incubated for 24 h at 37°C in a GasPak system. A portion of the growth was aseptically collected on a dry, sterile polyester-fiber swab (Fisher Scientific Co.) and transferred into 5 ml of sterile basal broth to an optical density approximating a McFarland 5 standard. This cell suspension was used to inoculate two sets of the Minitek plates (according to the manufacturer's directions) and the tubed media (0.05 ml/5 ml of broth). The inoculated tubed media were incubated for 48 h at 37°C. A tube of regular MRS broth was also inoculated in a similar manner and incubated at 15°C for 1 week. The inoculated substrate disks, except esculin, in one set of Minitek plates for each culture were each overlaid with 0.1 ml of sterile mineral oil (Fisher Scientific Co.), and the plates were incubated in a humidor (prepared by manufacturer's directions) for 48 h at 37°C (Minitek-oil). The remaining set of plates for each culture was not overlaid but was placed in a GasPak anaerobic system and incubated for 48 h at 37°C (Minitek-GP). After incubation, 0.05 ml of 0.25% aqueous phenol red was added to each carbohydrate and arginine disk to enhance the color of the reaction (3). Bromocresol purple was added to the appropriate tubed media in a similar manner. Only distinctive yellow color reactions were considered positive for

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TABLE 1. *Comparison of biochemical reactions produced by lactobacilli in Minitek and conventional test systems*

Isolate or culture	Identity system	Action on substrates																				Identity
		Growth at 15°C	Amygdalin	Arabinose	Arginine (NH_3)	Cellobiose	Esculin	Galactose	Glucose	Inositol	Lactose	Maltose	Mannitol	Mannose	Melezitose	Melibiose	Raffinose	Rhamnose	Sorbitol	Sucrose	Trehalose	Xylose	
1B1	Minitek-oil		−	−	−	+	+	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	
	Minitek-GP		+	−	−	+	+	−	+	−	+	+	+	−	−	−	−	−	−	+	+	−	*L. leichmanni*
	Tubed media	−	+	−	−	+	+	−	+	−	+	+	−	−	−	−	−	−	−	+	+	−	
	Bergey[a]	−	+	−	±	+	+	−	+	?	+	+	−	+	−	−	−	−	−	+	+	−	
1F1	Minitek-oil		−	−	−	−	+	−	−	−	−	−	−	+	−	−	−	−	−	−	+	−	
	Minitek-GP		+	−	−	+	+	+	+	−	+	+	−	+	−	−	−	−	−	+	+	−	*L. acidophilus*
	Tubed media	−	+	−	−	+	+	+	+	−	−	+	−	+	−	−	−	−	−	+	+	−	
	Bergey	−	+	−	−	+	+	+	+	?	+	+	−	+	−	±	±	−	−	+	+	−	
1A01	Minitek-oil		−	−	−	+	−	−	+	−	+	+	−	+	−	+	+	−	−	+	−	−	
	Minitek-GP		−	+	+	−	−	+	+	−	+	+	−	+	−	+	+	−	−	+	−	−	*L. fermentum*
	Tubed media	−	−	+	+	−	−	+	+	−	+	+	−	+	−	+	+	−	−	+	−	−	
	Bergey	−	−	±	+	−	−	+	+	?	+	+	−	±	−	+	+	−	−	±	±	±	
L. fermentum 36	Minitek-oil		−	−	+	−	−	+	+	−	+	+	+	+	−	+	+	−	−	−	−	+	
	Minitek-GP		+	−	+	−	−	+	+	−	+	+	−	+	−	+	+	−	−	−	−	+	*L. fermentum*
	Tubed media	−	−	−	+	−	−	+	+	−	+	+	−	+	−	+	+	−	−	−	−	+	
	Bergey	−	−	±	+	−	−	+	+	?	+	+	−	±	−	+	+	−	−	±	±	±	
L. acidophilus NCFM	Minitek-oil		+	+	−	+	+	+	+	+	+	+	−	+	+	+	+	−	−	+	+	−	
	Minitek-GP		+	−	−	+	+	+	+	−	+	+	−	+	−	+	+	−	−	+	+	−	*L. acidophilus*
	Tubed media	−	+	−	−	+	+	+	+	−	+	+	−	+	−	+	+	−	−	+	+	−	
	Bergey	−	+	−	−	+	+	+	+	?	+	+	−	+	−	±	±	−	−	+	+	−	

[a] 8th edition of *Bergey's Manual of Determinative Bacteriology*.

both indicators. The 48-h incubation gave fewer borderline reactions.

Early attempts using the Minitek system to identify lactobacilli isolated from rat and human feces were unsuccessful. Fermentation reactions observed for many of the isolates did not resemble those of known species of lactobacilli. When the Minitek system without mineral oil overlays was incubated in a GasPak anaerobic system, the resulting fermentation reactions permitted identification of the isolates as known *Lactobacillus* species. Both Minitek methods were compared with conventional tubed media for determining fermentation reactions (Table 1). Results obtained by the three methods for three isolates and two laboratory species of *Lactobacillus* are compared with the characteristics reported in the 8th edition of *Bergey's Manual of Determinative Bacteriology* (5). The results from the Minitek-GP system were in very close agreement with those from the conventional tubed media for all five cultures and coincided closely with fermentation patterns for known species of lactobacilli (5). However, results from the Minitek-oil system were quite different from those of the other two methods, particularly for the three isolates, and did not permit their identification with known species.

To obtain additional information on a comparison of the two Minitek systems to tubed media, they were evaluated by using 28 cultures of lactobacilli including strains of *L. acidophilus, L. fermentum, L. casei, L. bulgaricus, L. lactis, L. leichmanii,* and *L. viridescens*. Results obtained with the Minitek-GP method again agreed more closely with those from the tubed media than did those from the Minitek-oil method (Table 2). The results are presented for each substrate and Minitek method as the number positive in the tubed medium that were also positive in the Minitek system over the total number positive in the tubed media. The same was done for the negative reactions. The percentages of agreement for each substrate were determined by using the following formula:

$$\% \text{ agreement} = 100 \times \frac{\text{no. of reactions in Minitek agreeing with those of tubed media}}{\text{total number of tests for substrate}}$$

The Minitek-GP method exhibited an overall agreement of 93.6% with the conventional tubed media, whereas that for the Minitek-oil was only 84.6%. Of the 20 substrates, there was only one (raffinose) in which the results from

TABLE 2. *Comparison of Minitek system and conventional method for determining biochemical reactions of Lactobacillus species*

Substrate	GasPak			Oil		
	+/+ (tube)[a]	−/− (tube)[a]	% Agreeing with tubes[b]	+/+ (tube)[a]	−/− (tube)[a]	% Agreeing with tubes
Amygdalin	19/19	8/9	96.4	13/19	9/9	78.6
Arabinose	8/8	17/20	89.3	4/8	18/20	78.6
Arginine	5/8	20/20	89.3	5/8	20/20	89.3
Cellobiose	19/19	9/9	100.0	16/19	7/9	82.1
Esculin	19/20	7/8	92.9	19/20	7/8	92.9
Galactose	26/26	2/2	100.0	20/26	2/2	78.6
Glucose	28/28	0/0	100.0	24/28	0/0	85.7
Inositol	4/4	22/24	92.9	2/4	21/24	82.1
Lactose	25/25	2/3	96.4	22/25	3/3	89.3
Maltose	27/27	0/1	96.4	23/27	1/1	85.7
Mannitol	8/8	19/20	96.4	8/8	18/20	92.9
Mannose	23/23	2/5	89.3	22/23	2/5	85.7
Melezitose	4/4	18/24	78.6	4/4	17/24	75.0
Melibiose	14/15	12/13	92.9	9/15	13/13	78.6
Raffinose	13/13	12/15	89.3	12/13	14/15	92.9
Rhamnose	6/6	21/22	96.4	3/6	21/22	85.7
Sorbitol	12/13	14/15	92.3	8/13	15/15	82.1
Sucrose	23/23	5/5	100.0	20/23	5/5	89.3
Trehalose	19/19	9/9	100.0	17/19	9/9	92.9
Xylose	4/8	19/20	82.1	3/8	18/20	75.0
Total/avg.	306/316	218/244	93.6	254/316	220/244	84.6

[a] [Number + (or −) in tubed media that were also + (or −) in Minitek]/[number + (or −) in tubed media].

[b] % Agreement = (number of reactions in Minitek agreeing with those of tubed media/total number of tests for each substrate) × 100.

the Minitek-oil method agreed more closely than did those from the Minitek-GP system. The percent agreement (92.9%) for esculin hydrolysis was the same for both methods; this was probably because no oil overlay was required for this substrate.

Use of the Minitek system in a manner similar to that described for anaerobic bacteria provides a system for identifying species of *Lactobacillus* that produces results comparable to those obtained by using conventional tubed media. Use of the system as suggested (with mineral oil overlays) for the *Enterobacteriaceae* is not satisfactory. Apparently, the mineral oil contains some substance(s) that interferes with metabolism of the lactobacilli, thus causing erroneous results. Possibly, caution should be exercised in interpreting results obtained for other bacterial cultures with identification systems in which inoculated substrates are overlaid with mineral oil. It should be noted that two different lots of mineral oil were used in our studies, one of which was a new batch, and both gave comparable results.

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