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Evaluation of 36 Minitek Tests and a New Approach for Identification of Nonfermenters

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Thirty-six Minitek (BBL Microbiology Systems, Cockeysville, Md.) tests were evaluated with 625 Kansai Medical University stock strains of 22 species and one group of nonfermentative gram-negative rods able to grow on ordinary peptone media. Among the 36 tests, 15 were selected because a clear-cut reaction was shown by all 625 Kansai Medical University strains. Of these 15 tests, 12 were further selected for routine use because they were regarded as useful for the identification of nonfermenters. The 12 tests were arranged into the following four groups: (i) lysine-arginine-ornithine, (ii) urea-*ortho*-nitrophenyl-beta-D-galactopyranoside-dextrose aerobic, (iii) maltose-xylose-starch, and (iv) esculin-nitrate reduction-indole. A new profile system of four digits, the Minitek Y-Y (Yabuuchi and Yamanaka) system, consisting of 64 numbers which represent each single species and 11 numbers which give two to four species, is herein proposed. The system was designed primarily for a less expensive identification of gram-negative rods already confirmed in a butt of either triple sugar iron or Kligler iron agar for their lack of ability to ferment dextrose. Among the 539 clinical isolates obtained from 3 hospitals, 511 strains identifiable by classical methods were also identified by the Minitek Y-Y system.

Several kinds of simplified identification kits for nonfermenters are now commercially available. They are expected to be helpful tools for the routine identification of organisms by many clinical bacteriology laboratories. Besides the simplicity of such test systems, reliability, accuracy of identification, and low cost of materials are required for routine use.

A rapid and simplified method for the characterization and identification of clinical isolates, chiefly enterics, with dried paper disks has been investigated by several workers (9, 11, 12, 14) since 1949. Minitek (BBL Microbiology Systems, Cockeysville, Md.) is a miniaturized identification system consisting of paper disks impregnated with each of 35 substrates, inoculum broth, and a small plastic plate with 20 wells. The free selection of disks to be used, as documented by BBL, is convenient for the identification of a variety of organisms (1-8, 13). In 1978, BBL published a Minitek numerical identification system for nonfermenters. It utilized 15 disks supplemented with the oxidase test, growth ability on MacConkey agar medium, and indole production from tryptophan contained in the inoculum broth. BBL expressed the results of these tests as six-digit profile numbers and recommended application of the system for the oxidase-positive gram-negative isolates, either dextrose fermenting or nonfermenting. The 1978 profile numbers thus compiled, together with their confidence value and biotype validity, are for the final identification of 21 species and 13 groups of nonfermenters and 14 species of oxidase-positive fermenters. Since the identification of any organism by any such miniaturized system should depend on the profile number obtained by the system itself, compilation and publication of Minitek profile numbers for nonfermenters will contribute to simplifying the routine work in clinical bacteriology laboratories.

The BBL system for nonfermenters, however, has the following inconveniences: (i) preliminary selection of oxidase-positive isolates will lead to missing oxidase-negative nonfermenters; (ii) in certain species, the results of the oxidase test and growth on MacConkey agar vary considerably according to the product of different manufacturers; (iii) in certain species, acid is produced in the well of the Minitek anaerobic dextrose test in spite of the fact that the test strain never ferments dextrose in classical tube methods; (iv) the six-digit profile number is less easy to refer to, as compared with a 4-digit number; and (v) at the time when the present study was started, the maximum number of wells per plate was 12, and the use of two plates to make 15 tests per strain was thought to be uneconomical. To explore a more convenient and economical system for the identification of nonfermenters,

we evaluated all 36 Minitek tests by using 625 strains of 22 species and one group of nonfermenters.

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MATERIALS AND METHODS

Bacterial strains used. The 625 stock strains of 22 species and one group of nonfermentative gram-negative rods were obtained from the Kansai Medical University (KM) collection and are listed in Table 1. Culture collection accession numbers for the type or reference strain of each of the 22 species and one group of nonfermenters are listed in Table 2. Of the 625 KM test strains, 541 were of clinical origin; 59 were from soil, water, or plants, 7 were from animals, and 18 were from the hospital environment, such as from distilled water or chlorhexidine gluconate solution (Table 3). The 17 type strains (10) and 71 reference strains were characterized, and the remaining strains were identified before the present study according to the schema described previously (15).

TABLE 1. *Number of strains of nonfermenters tested*

Species or group	No. and source of strains[a]			
	KM collection	Clinical isolates		Total
		OH	OH, RCH, SN	
Pseudomonas aeruginosa	100	15	95	210
Pseudomonas fluorescens	15	1	2	18
Pseudomonas putida	27	2	27	56
Pseudomonas stutzeri	10	0	5	15
Pseudomonas cepacia	92	14	42	146
Pseudomonas mallei	4	0	0	4
Pseudomonas pseudomallei	6	0	0	6
Pseudomonas maltophilia	89	8	63	160
Pseudomonas paucimobilis	8	1	2	11
Pseudomonas putrefaciens	16	2	3	21
Pseudomonas acidovorans	15	0	1	16
Pseudomonas vesicularis	1	0	0	1
Pseudomonas diminuta	1	0	0	1
Achromobacter xylosoxidans	80	5	15	100
Alcaligenes faecalis	35	7	45	87
Agrobacterium radiobacter	10	0	2	12
Agrobacterium tumefaciens	1	0	0	1
Bordetella bronchiseptica	9	1	5	15
Flavobacterium meningosepticum	27	5	6	38
Flavobacterium odoratum	5	1	10	16
Flavobacterium sp. group IIb	13	6	8	27
Acinetobacter calcoaceticus	56	12	74	141
Acinetobacter lwoffi	5	1	24	30
Unidentified	0	8	20[b]	28
Total no. of strains tested	625	89	450	1163

[a] OH, Ohtemae Hospital, Osaka; RCH, Japan Red Cross Hospital, Osaka; SN, Osaka Saiseikai-Nakatsu Hospital.
[b] OH isolates, exclusively.

Minitek test procedure. Each test strain, grown on a heart infusion agar slant, was harvested and suspended in a Minitek enteric and nonfermenter broth to meet the McFarland turbidity standard no. 5. Homogeneous suspensions were obtained by repeated pipetting of the broth with a Minitek pipetter. Detailed procedures, according to the manufacturer's instructions, were followed. Four *Pseudomonas fluorescens* strains and one *Agrobacterium tumefaciens* strain from natural origin were incubated at 30°C. The remaining 620 KM strains were incubated at 37°C, except for those in the semisolid motility medium which were incubated at 25°C.

Selection and compilation of tests for the Y-Y profile system. Based on the results of 36 tests obtained with the 625 KM strains, 12 tests were selected (see box in Fig. 1) each of these tests gave a clear-cut reaction, either positive or negative, and was thought to be useful for the species identification of nonfermenters. The 12 tests were arranged into four groups to obtain a four-digit number for each strain tested (see box in Fig. 1).

Evaluation and routine use of the Y-Y profile system. To evaluate the Y-Y profile system, 89 isolates obtained in the Ohtemae Hospital during the period from February to October 1979 were identified by the system. Classical tube methods were simultaneously run for these 89 strains to confirm the identification. Since November 1979, the Y-Y profile system was used routinely in the bacteriology laboratory of three hospitals in Osaka (Ohtemae, Japan Red Cross, and Saiseikai-Nakatsu) for the identification of nonfermenters. Before the end of August 1980, 450 strains of nonfermenters, isolated in these three hospitals, were subjected to the Y-Y profile system and to classical methods.

RESULTS

Evaluation of each test. All 625 KM strains gave a clear-cut reaction, either positive or negative, to 15 of the 36 tests performed. These 15 tests, or substrates, were lysine, arginine, ornithine, urea, *ortho*-nitrophenyl-beta-D-galactopyranoside, citrate, nitrate reduction, indole, dextrose-anaerobic, maltose, xylose, starch (all recommended by BBL for the identification of nonfermenters), hydrogen sulfide, Voges-Proskauer, and esculin. Of the remaining 21 tests, unclear reactions were obtained with strains of nine species to glycerol, levulose and arabinose, with strains of eight species to mannitol, and with strains of 7 species to mannose (Table 4).

Of the 22 species and one group of nonfermenters, 8 species never gave an unclear reaction in any of the 36 tests. They were *Pseudomonas putrefaciens*, *Pseudomonas acidovorans*, *Pseu-*

TABLE 2. *Corresponding culture collection accession numbers and Y-Y profile number of nomenclatural type or reference strains of 22 species and one group of nonfermenters*

Species or group	Accession no. of[a]:				Status	Y-Y profile no.[c]
	ATCC	NCTC	CDC	KM		
Pseudomonas aeruginosa	10145	10332		2589	Type[b]	2120
Pseudomonas fluorescens	13525	10038		1076	Type[b]	2120
Pseudomonas putida	12633			888	Type[b]	2120
Pseudomonas stutzeri	17588			404	Type[b]	0132
Pseudomonas cepacia	25416			645	Type	5360
Pseudomonas mallei	15310			2067		2120
Pseudomonas pseudomallei	15682	4845		391		2142
Pseudomonas maltophilia	13637	10257		2591	Type	4046
Pseudomonas paucimobilis	29837	11030		2395	Type	0364
Pseudomonas putrefaciens			B6225	1194		0102
Pseudomonas acidovorans	15668			2083	Type	0002
Pseudomonas vesicularis	11426			2597	Type[b]	0144
Pseudomonas diminuta	11568	8545		2536	Type[b]	0000
Achromobacter xylosoxidans	27061	10807		543	Type	0022
Alcaligenes faecalis	8750			1056	Type[b]	0000
Agrobacterium radiobacter	19358	9042		1950	Type[b]	0766
Agrobacterium tumefaciens	7420			661		0764
Bordetella bronchiseptica	19395	452		1160	Type[b]	0400
Flavobacterium meningosepticum	13253	10016	14	2008	Type[b]	0145
Flavobacterium sp. group IIb		10795		2007		0357
Flavobacterium odoratum	4651			1357	Type	0400
Acinetobacter calcoaceticus	19606			1888		0160
Acinetobacter lwoffi	15309	5866		1951	Type[b]	0000

[a] ATCC, American Type Culture Collection, Maryland; NCTC, National Collection of Type Cultures, London; CDC, Center for Disease Control, Georgia; and KM, Kansai Medical University, Osaka.

[b] Type strain documented as such in the Approved Lists of Bacterial Names (10).

[c] Boldfaced digit indicates negative zinc dust in negative nitrite test. For further explanation, see Results.

domonas diminuta, Pseudomonas vesicularis, Alcaligenes faecalis, Flavobacterium meningosepticum, Flavobacterium odoratum, and *Acinetobacter lwoffi.* Unclear reactions were obtained with strains of *Agrobacterium radiobacter* in 16 tests, with strains of *Pseudomonas cepacia* in 12 tests, and with strains of *Pseudomonas aeruginosa* in 10 tests.

The results obtained from nine Minitek tests used in the Y-Y profile system were compared with those obtained by the classical culture method (Table 5). As summarized in Table 6, lysine, arginine, ornithine, xylose, starch, esculin, and indole gave greater than 90% agreement. The low agreement (77.6%) in dextrose was due to the character of *Pseudomonas maltophilia* and *Achromobacter xylosoxidans,* both of which are generally known as slow and weak oxidizers of dextrose. If the strains of these two species were excluded, dextrose agreement rose to 94.1% (bracketed data, Table 6). A fairly low agreement (85.6%) on maltose was seen with *Acinetobacter calcoaceticus.* Of 56 *A. calcoaceticus* strains, 44 were positive in the Minitek maltose medium but negative in the oxidation-fermentation maltose medium.

The results of nitrate reduction tests in the Minitek system were in 88.0% agreement with those in the casitone-yeast extract-nitrate broth (Table 6). As listed in Table 7, 12 nondenitrifying species showed 100% agreements, and 2 denitrifying species, *P. aeruginosa* and *A. xylosoxidans,* showed 84.0 and 63.8% agreement, respectively. Furthermore, a combination of negative nitrite-negative zinc dust in Minitek and negative nitrite-positive zinc dust in nitrate broth, or vice versa, was observed in a few strains of *P. aeruginosa, P. putrefaciens, A. radiobacter,* and *Flavobacterium* sp. group IIb.

Y-Y profile numbers and evaluation of the Y-Y profile system. Of the data from 22,500 tests (36 tests by 625 KM strains), data from 7,500 tests (12 tests by 625 KM strains) were extracted, arranged according to the Y-Y profile system (see box in Fig. 1), and calculated for four-digit Y-Y profile numbers. In recording

TABLE 3. *Distribution among the 12 species of nonfermenters of the 84 KM strains of nonclinical origin*

Species	Source of isolation		
	Soil, water, or plant	Animal	Hospital environment
Pseudomonas aeruginosa	2		
Pseudomonas fluorescens	4		
Pseudomonas putida	1		
Pseudomonas stutzeri	7		
Pseudomonas cepacia	5		15
Pseudomonas mallei		1	
Pseudomonas pseudomallei		2	
Pseudomonas maltophilia	30		2
Pseudomonas paucimobilis			1
Agrobacterium radiobacter	4		
Agrobacterium tumefaciens	1		
Bordetella bronchiseptica		4	
Total no. of strains	59	7	18

the results of the nitrate reduction tests, a combination of negative nitrite and negative zinc dust was understood as showing denitrification to have taken place; to differentiate denitrification from nitrite production, the last numeral of the profile number was boldfaced and the 66 numbers thus obtained for the 625 KM strains of 22 species and one group of nonfermenters are listed in numerical order in Table 8.

Each profile number of 81 of the 89 Ohtemae hospital isolates fell into one of the above mentioned 66 numbers, and the identification of these strains was in perfect agreement with classical tube methods. Thus, the Y-Y profile system was regarded as being useful for the routine identification of well-characterized species of nonfermenters frequently encountered in clinical specimens. Of 450 isolates from 3 hospitals, 407 gave profile numbers that were included in the 66 numbers. The remaining 23 strains were identified by classical methods, and nine numbers obtained for these 23 strains were confirmed to represent four species and one group of nonfermenters (footnote *b*, Table 9). Since the remaining 28 Ohtemae hospital isolates (Table 2) were not identified by the phenotypic characters obtained by classical methods, their Y-Y profile numbers were not included in Tables 8 and 9. A proposed list of Y-Y profile numbers and their frequency in each species of 1,135 (625 KM and 510 clinical) strains is shown in Table 9. Among 75 numbers, 64 represent single species, 8 represent two species, 2 represent three species, and 1 represents four species. To differentiate species, several additional tests were indicated for the profile numbers shared by two or more species.

DISCUSSION

Colonies of most species of nonfermenters are smaller than those of fermentative species after 20 to 24 h of incubation, and it is difficult at this time to distinguish each colonial morphology. In such cases, colonies should first be subcultured on appropriate solid medium to obtain enough growth for suspending inoculum broth and to avoid any improper fishing for two or more small colonies. If triple sugar iron or Kligler iron agar is used for primary subculture, the ability of the isolate to ferment dextrose can be determined the next day.

Certain strains of *P. cepacia* and *F. meningosepticum* were positive in anaerobic dextrose tests in the present study on the Minitek system. Strains of these species have often been said to be anaerobic-dextrose positive in other miniaturized kits, but on the contrary, they never ferment dextrose in oxidation-fermentation-dextrose medium. Although the Minitek anaerobic-dextrose tests for nonfermenters gave a clear-cut reaction (either positive or negative, as shown in Table 3), and BBL recommends that it be used in the identification system for nonfermenters, it was excluded from the Y-Y profile system because the positive reaction conflicts with the basic concept of a nonfermenter.

From these facts, we herein propose an identification scheme for nonfermenters, utilizing the Minitek Y-Y system, as illustrated in Fig. 1. In this scheme, final identification by the Y-Y profile number will usually be reached at 96 h after inoculating the test specimen on primary isolation media. The longer generation time and smaller colony size than those required for fermenters are the limitations for rapid identification of nonfermenters by culture methods. If all 12 tests of the Minitek Y-Y system could be read precisely after 24 h of incubation, the isolate could be identified on the third day, one day earlier.

As shown in Table 4, 19 sugar disks showed an unclear reaction by 14 species of nonfermenters after 48 h of incubation. In the color reaction of Minitek sugar disks, yellow indicates acid from sugar and red indicates alkali from peptone that was incorporated in the inoculum broth. There are some difficulties in the Minitek sys-

TABLE 4. *Distribution of strains among the 520 strains of 14 species and one group that gave unclear reactions to one or more substrates*[a]

Substrate	Species (no. of strains tested)															No. of strains tested that gave unclear reactions (%)	Total no. of species tested that gave unclear reactions
	Pseudomonas aeruginosa (100)	*P. fluorescens* (15)	*P. putida* (27)	*P. stutzeri* (10)	*P. cepacia* (92)	*P. mallei* (4)	*P. pseudomallei* (6)	*P. maltophilia* (89)	*P. paucimobilis* (8)	*Achromobacter xylosoxidans* (80)	*Agrobacterium radiobacter* (10)	*A. tumefaciens* (1)	*Bordetella bronchiseptica* (9)	*Flavobacterium* sp. group IIb (13)	*Acinetobacter calcoaceticus* (56)		
Glycerol	73		7	4	80		1		1	2	2			4		174 (33.5)	9
Levulose	65		11	10	34		1	12	2					3	12	150 (28.8)	9
Mannitol	64		1	2	38	1	2				4	1				113 (21.7)	8
Adonitol					71											71 (13.7)	1
Dulcitol					61					1	1	1				64 (12.3)	4
Sorbitol					56	1	2				4					63 (12.1)	4
Rhamnose	44						1				4					49 (9.4)	3
Inositol					37						4					41 (7.9)	2
Lactose	24				2				1		5					32 (6.2)	4
Mannose	1			1		1		9		11	2			1		26 (5.0)	7
Galactose	4				11					6	1					22 (4.2)	4
Arabinose	2	1	1	3	4		1		1	6			2			21 (4.0)	9
Trehalose	2				6	2		1	6		4					21 (4.0)	6
Phenylalanine				4				10			3					17 (3.3)	3
Cellobiose					2		1	3	2		2	1				11 (2.1)	6
Raffinose			4	3							1					8 (1.5)	3
Dextrose-aerobic										6				1		7 (1.3)	2
Sucrose									5		1					6 (1.2)	2
Malonate							1	1		4						6 (1.2)	3
Melibiose	1		1								3					5 (1.0)	3
Salicin											2					2 (0.4)	1

No. of tests performed per species	2100	315	567	210	1932	84	126	1869	168	1680	210	21	189	273	1176	10920	
No. of tests per species that gave unclear reactions	280	1	25	27	402	5	10	36	18	36	43	3	2	9	12	909	
% of tests per species that gave unclear reactions	13.3	0.3	4.4	12.9	20.8	6.0	7.9	1.9	10.7	2.1	20.5	14.3	1.1	3.3	1.0	8.3%	
No. of substrates on which reactions were unclear	10	1	6	7	12	4	8	6	7	7	16	3	1	4	1		

[a] None of the strains of eight other species listed in Table 1 gave unclear reactions.

tem in reading sugar reactions, as an intermediate coloration between positive and negative was observed in certain sugar disks inoculated with strains of nutritionally versatile species, such as *P. cepacia*. Such intermediate reaction is, unfortunately, rather close to the negative color. They are summarized in Table 4 as unclear reactions, and the tests were not included in the Y-Y profile system. If the uncertain reactions could be read with confidence as positive or negative, the reliability of the Minitek test would be further increased.

Of 6,250 test results obtained with the Minitek system, 5,771 (92.3%) were in agreement with those with classical methods (Table 5). However, the rate of agreement in each test varied from 77.6% (dextrose) to 100% (indole). When the Minitek Y-Y system is used for the identification of a nonfermenter, the Y-Y profile numbers should be referred to for obtaining the name of the organism in question. In the identification by any other system, either classical or miniaturized, the Y-Y profile numbers cannot be referred to because different test results to various extents were given.

As far as the 17 *Pseudomonas putida* and 2 *P. fluorescens* strains tested in this work were concerned, three profile numbers, 0120, 2100, and 2160, were not shared by these two species (Table 9). However, these numbers might be applicable to either *P. putida* or *P. fluorescens*, and the identification of such strains could be confirmed by testing their ability to oxidize trehalose and to hydrolyze gelatin.

The usefulness of the Y-Y profile system is that it is an effective and less expensive tool than other miniaturized systems for the routine identification of strains belonging to the well-characterized and named species of nonfermenters frequently encountered in clinical specimens and natural sources. However, as shown in Table 1, we had no clinical isolates of our own in five species, *Pseudomonas mallei*, *Pseudomonas pseudomallei*, *P. diminuta*, *P. vesicularis*, and *A. tumefaciens*. Because of their rare incidence, any isolate which gave a Y-Y profile number corresponding to one of these five species should be carefully examined by classical methods before its final identification. Although the BBL system for nonfermenters covers 14 oxidase-positive species of fermenters in four genera, *Vibrio*, *Aeromonas*, *Plesiomonas*, and *Pasteurella*, they are, at present, not considered in the Y-Y system.

To confirm the safety of the Minitek pipetter, the following experiment was performed. Sterile, melted MacConkey agar medium was distributed into the inside of a Minitek plate cover and

TABLE 5. *Comparison of nine test results of the Minitek and classical culture methods*

Substrate	Species or group	No. of strains used	No. of tests				Medium
			In agreement		Not in agreement		
			+[a] +[b]	−[a] −[b]	+[a] −[b]	−[a] +[b]	
Lysine	*Pseudomonas cepacia*	92	76			16	L-Lysine-Møller
	P. multophilia	89	73	3	6	7	
	Other 20 species plus one group	444		444			
		625	149	447	6	23	
Arginine	*Pseudomonas aeruginosa*	100	100				L-Arginine-Møller
	P. fluorescens	15	15				
	P. mallei	4	4				
	P. pseudomallei	6	6				
	P. putida	27	18	6	2	1	
	Other 17 species plus one group	473		473			
		625	143	479	2	1	
Ornithine	*Pseudomonas cepacia*	92	30	12	50		L-Ornithine-Møller
	P. putrefaciens	16	9			7	
	Other 21 species plus one group	517		517			
		625	39	529	50	7	
Dextrose	*Pseudomonas acidovorans*	15		15			OF- or PYP-glucose
	Alcaligenes faecalis	35		35			
	Bordetella bronchiseptica	9		9			
	Pseudomonas diminuta	1		1			
	Flavobacterium odoratum	5		5			
	Acinetobacter lwoffi	5		5			
	Pseudomonas aeruginosa	100	99	1			
	P. maltophilia	89	30	7	1	51	
	P. putrefaciens	16	2	10	1	3	
	P. stutzeri	10	6			4	
	Achromobacter xylosoxidans	80				80	
	Other 10 species plus one group	260	260				
		625	397	88	2	138	
Maltose	*Pseudomonas cepacia*	92	92				OF- or PYP-maltose
	Agrobacterium radiobacter	10	10				
	A. tumefaciens	1	1				
	Pseudomonas paucimobilis	8	8				
	P. mallei	4	4				
	P. vesicularis	1	1				
	P. maltophilia	89	79		2	8	
	P. stutzeri	10		1		9	
	P. pseudomallei	6	5			1	
	Acinetobacter calcoaceticus	56	11	1	44		
	Flavobacterium meningosepticum	27	8			19	
	Flavobacterium sp. group IIb	13	6			7	
	Other 11 species	308		308			
		625	225	310	46	44	

TABLE 5—*Continued*

Substrate	Species or group	No. of strains used	No. of tests				Medium
			In agreement		Not in agreement		
			+[a] +[b]	−[a] −[b]	+[a] −[b]	−[a] +[b]	
Xylose	*Pseudomonas putida*	27	27				OF or PYP-xylose
	P. paucimobilis	8	8				
	P. stutzeri	10	6	4			
	Achromobacter xylosoxidans	80	80				
	Agrobacterium radiobacter	10	10				
	Agrobacterium tumefaciens	1	1				
	Acinetobacter calcoaceticus	56	56				
	Pseudomonas aeruginosa	100	92	7	1		
	P. fluorescens	15	13			2	
	P. cepacia	92	89		3		
	P. pseudomallei	6	1	2	3		
	Flavobacterium meningosepticum	27		14		13	
	Other 10 species plus one group	193		193			
		625	383	220	7	15	
Starch	*Flavobacterium* sp. group IIb	13	13				HI agar with 0.2% soluble starch added
	Pseudomonas stutzeri	10	8			2	
	P. pseudomallei	6		4		2	
	P. paucimobilis	8		2		6	
	Other 19 species	588		588			
		625	21	594		10	
Esculin	*Pseudomonas maltophilia*	89	89				HI agar with 0.1% esculin and 0.05% ferric citrate added
	P. paucimobilis	8	8				
	P. vesicularis	1	1				
	Agrobacterium radiobacter	10	10				
	A. tumefaciens	1	1				
	Flavobacterium meningosepticum	27	27				
	Flavobacterium sp. group IIb	13	13				
	Pseudomonas cepacia	92		43		49	
	P. pseudomallei	6		2		4	
	Other 14 species	378		378			
		625	149	423		53	
Indole	*Flavobacterium meningosepticum*	27	27				1% tryptone broth supplemented with 0.5% L-tryptophan
	Flavobacterium sp. group IIb	13	13				
	Other 21 species	585		585			
		625	40	585			

[a] Minitek method.
[b] Classical method.
[c] Møller, Decarboxylase base, Møller (Difco Laboratories); OF, Oxidation—fermentation basal medium (Difco); PYP, Peptone—yeast extract—phenol red basal medium (Nissui); HI, heart infusion agar (Difco).

TABLE 6. *Summary of the compared results of 10 tests[a] by Minitek and conventional methods[b]*

Substrate	No. of tests: Agreed		Total (%)	No. of tests: Disagreed		Total (%)	No. of tests compared
	+[c] +[d]	−[c] −[d]		+[c] −[d]	−[c] +[d]		
Lysine	149	447	596 (95.7)	6	23	29 (4.3)	625
Arginine	143	479	622 (99.5)	2	1	3 (0.5)	625
Ornithine	39	529	568 (90.9)	50	7	57 (9.1)	625
Dextrose	397	88	485 (77.6)	2	138	140 (22.4)	625
	[367	81	448 (94.1)][e]	[1	7	8 (1.7)]	[476]
Maltose	225	310	535 (85.6)	46	44	90 (14.4)	625
Xylose	383	220	603 (96.5)	7	15	22 (3.5)	625
Starch	21	594	615 (98.4)	0	10	10 (1.6)	625
Esculin	149	423	572 (91.5)	0	53	53 (8.5)	625
Indole	40	585	625 (100)	0	0	0 (0)	625
Nitrate[f]			550 (88.0)			75 (12.0)	625
Total no. of tests			5771 (92.3)			479 (7.7)	6250
			[5734 (94.0)			347 (5.7)	6101]

[a] Data not available on urea and *ortho*-nitrophenyl-beta-D-galactopyranoside by conventional methods.
[b] Media used are indicated in Table 6.
[c] Minitek method.
[d] Classical method.
[e] Brackets contain data excluding 89 *P. maltophilia* strains and 80 *A. xylosoxidans* strains.
[f] For the detail of nitrate reduction test results, see Table 8.

TABLE 7. *Comparison of nitrate reduction test results of Minitek method and nitrate broth culture[a]*

Species or group (no. of strains used)	Tests	No. of tests in agreement: NO_2ZN	NO_2	NO_2Zn	No. of tests not in agreement (%): NO_2ZN	NO_2ZN	NO_2ZN	NO_2ZN	NO_2Zn	NO_2ZN
		−[b] −[c]	+[b] +[c]	−[b] +[c] −[c]	−[b] NT[c] −[c]	+[b] NT[b] −[c]	−[b] +[b] +[c] NT[c]	+[b] NT[b] −[c] +[c]	−[b] +[b] −[c]	−[b] −[b] −[c] +[c]
Pseudomonas cepacia	(92)		13	79						
P. maltophilia	(89)		36	53						
P. mallei	(4)		4							
P. putida	(27)			27						
P. paucimobilis	(8)			8						
P. vesicularis	(1)			1						
P. diminuta	(1)			1						
Agrobacterium tumefaciens	(1)			1						
Flavobacterium meningosepticum	(27)			27						
F. odoratum	(5)			5						
Acinetobacter calcoaceticus	(56)			56						
A. lwoffi	(5)			5						
Pseudomonas aeruginosa	(100)	81	2	1		10	1		1	4
P. fluorescens	(15)			6			8	1		
P. stutzeri	(10)	6	3		1					
P. pseudomallei	(6)	5				1				
P. putrefaciens	(16)		12	2				2		
P. acidovorans	(15)		13					2		
Achromobacter xylosoxidans	(80)	27	24		2	27				
Alcaligenes faecalis	(35)		3	31			1			
Bordetella bronchiseptica	(9)		6	1			2			
Agrobacterium radiobacter	(10)			5						5
Flavobacterium sp. group IIb	(13)			10						3
No. of tests	(625)	119	116	319	3	38	12	5	1	12
Total no. of strains (%)			554 (88.6)				71 (11.4)			

[a] +, Positive red coloration; −, negative red coloration; NT, not tested; Zn, zinc dust test for negative nitrite reaction.
[b] Minitek method.
[c] Nitrate broth culture.

TABLE 8. *Proposed list of Minitek Y-Y profile numbers and their frequency of occurrence among the 1,135 strains[a] of 22 species and one group of nonfermenters*

Y-Y profile no.	Species or group	No. of strains		Additional tests										
		Positive	Used	Triple sugar iron black butt	Motility	Oxidase	Flagellation	Deoxyribonuclease	Acylamidase	Colony			Oncogenicity for plants	Gelatin
										Appearance	Color	Margin		
0000	*Pseudomonas putrefaciens*	4	21	+	+	+								
	P. diminuta	1	1	−	+	+	1, Polar							
	Alcaligenes faecalis	71	87	−	+	+	Peritrichous							
	Acinetobacter lwoffi	30	30	−	−	−								
0002	*Pseudomonas putrefaciens*	3	21	+				+	−					
	P. acidovorans	16	16	−			>1, Polar	−	±					
	Alcaligenes faecalis	16	87	−			Peritrichous	−	±					
0012	*Pseudomonas stutzeri*	3	15											
0012[b]	*P. stutzeri*	1	15											
0015	*Flavobacterium* sp. group IIb	1	27											
0022	*Achromobacter xylosoxidans*	66	100											
0022	*Pseudomonas stutzeri*	1	15				1, Polar			Wrinkled				
	Achromobacter xylosoxidans	29	100				Peritrichous			Smooth				
0044	*Pseudomonas maltophilia*	1	160											
0046	*P. maltophilia*	3	160											
0102	*P. putrefaciens*	2	21											
0105	*Flavobacterium meningosepticum*	12	38											
0115	*Flavobacterium* sp. group IIb	9	27											
0117	*Flavobacterium* sp. group IIb	3	27											
0120	*Pseudomonas putida*	16	56		+	+								
	Acinetobacter calcoaceticus	17	142		−	−								

TABLE 8—*Continued*

Y-Y profile no.	Species or group	No. of strains		Additional tests										
		Positive	Used	Triple sugar iron black butt	Motility	Oxidase	Flagellation	Deoxyribonuclease	Acylamidase	Colony: Appearance	Colony: Color	Colony: Margin	Oncogenicity for plants	Gelatin
0122	*Achromobacter xylosoxidans*	4	100											
0122	*Pseudomonas stutzeri*	3	15				1, Polar			Wrinkled				
	Achromobacter xylosoxidans	1	100				Peritrichous			Smooth				
0132	*Pseudomonas stutzeri*	1	15											
0132	*P. stutzeri*	5	15											
0144	*P. maltophilia*	4	160			−	>1, Polar							
	P. vesicularis	1	1			+	1, Polar, short wavelength							
0145	*Flavobacterium meningosepticum*	5	38											
0154	*Flavobacterium* sp. group IIb	2	27											
0155	*Flavobacterium* sp. group IIb	7	27											
0157	*Flavobacterium* sp. group IIb	3	27											
0160	*Acinetobacter calcoaceticus*	116	142											
0162	*Pseudomonas cepacia*	2	148											
0172	*P. stutzeri*	1	15											
0204	*P. maltophilia*	2	160											
0206	*P. maltophilia*	2	160											
0244	*P. maltophilia*	3	160											
0305	*Flavobacterium meningosepticum*	7	38											
0345	*F. meningosepticum*	14	38											
0357	*Flavobacterium* sp. group IIb	2	27											
0360	*Pseudomonas cepacia*	6	148											

0364	*P. paucimobilis*	11	11								
0400	*Bordetella bronchiseptica*	4	15	+				Gray-white	Entire		
	Flavobacterium odoratum	16	16	−				Yellow	Spreading		
0402	*Bordetella bronchiseptica*	11	15								
0520	*Acinetobacter calcoaceticus*	3	142								
0560	*A. calcoaceticus*	6	142								
0666	*Agrobacterium radiobacter*	3	12								−
	A. tumefaciens	1	1								+
0764	*A. radiobacter*	8	12								
0766	*A. radiobacter*	3	12								
1002	*Pseudomonas putrefaciens*	11	21								
1102	*P. putrefaciens*	1	21								
1160	*P. cepacia*	8	148								
1360	*P. cepacia*	2	148								
2002	*P. aeruginosa*	3	211								
2100	*P. fluorescens*	2	18								
2102	*P. mallei*	4	4	−	−						
	P. aeruginosa	1	211	+	+						
2102	*P. aeruginosa*	6	211				+	Smooth			
	P. pseudomallei	1	6					Wrinkled			
2120	*P. fluorescens*	16	18				−				
	P. putida	39	56				−				
	P. aeruginosa	16	211				+				
2122	*P. aeruginosa*	32	211								
2122	*P. aeruginosa*	153	211								
2142	*P. pseudomallei*	1	6								
2160	*P. putida*	1	56								
2162	*P. pseudomallei*	1	6								
2162	*P. pseudomallei*	3	6								
4044	*P. maltophilia*	4	160								

TABLE 8—*Continued*

Y-Y profile no.	Species or group	No. of strains		Additional tests										
		Positive	Used	Triple sugar iron black butt	Motility	Oxidase	Flagellation	Deoxyribonuclease	Acylamidase	Colony			Oncogenicity for plants	Gelatin
										Appearance	Color	Margin		
4046	*P. maltophilia*	17	160											
4144	*P. maltophilia*	2	160											
4160	*P. cepacia*	1	148											
4162	*P. cepacia*	4	148											
4204	*P. maltophilia*	6	160											
4206	*P. maltophilia*	3	160											
4244	*P. maltophilia*	27	160											
4246	*P. maltophilia*	42	160											
4344	*P. maltophilia*	23	160											
4346	*P. maltophilia*	21	160											
4360	*P. cepacia*	4	148											
4362	*P. cepacia*	1	148											
5102	*P. cepacia*	1	148											
5140	*P. cepacia*	2	148											
5160	*P. cepacia*	77	148											
5162	*P. cepacia*	6	148											
5360	*P. cepacia*	23	148											
5362	*P. cepacia*	11	148											

[a] Total number of 625 KM stock cultures and 510 clinical isolates.
[b] See footnote c, Table 2.

TABLE 9. *Distribution of Y-Y profile numbers of 1,135 strains of nonfermentative gram-negative rods among 22 species and one group of nonfermenters*

Species or group (no. of strains tested)	Profile no.	No. of strains positive	% of strains positive
Pseudomonas aeruginosa (211)	2 1 2 **2**[a]	1 5 3	72.5
	2 1 2 2	3 2	15.2
	2 1 2 0	1 6	7.6
	2 1 0 **2**	6	2.8
	2 0 0 **2**	3	1.4
	2 1 0 2	1	0.5
P. fluorescens (18)	2 1 2 0	1 6	88.9
	2 1 0 0	2	11.1
P. putida (56)	2 1 2 0	3 9	69.6
	0 1 2 0	1 6	28.6
	2 1 6 0	1	1.8
P. stutzeri (15)	0 1 3 **2**	5	33.3
	0 0 1 2	3	20.0
	0 1 2 **2**	3	20.0
	0 0 1 **2**	1	6.7
	0 0 2 **2**	1	6.7
	0 1 3 2	1	6.7
	0 1 7 2[b]	1	6.7
P. cepacia (148)	5 1 6 0	7 7	52.0
	5 3 6 0	2 3	15.5
	5 3 6 2	1 1	7.4
	1 1 6 0	8	5.4
	0 3 6 0	6	4.1
	5 1 6 2	6	4.1
	4 1 6 2[b]	4	2.7
	4 3 6 0	4	2.7
	0 1 6 2	2	1.4
	1 3 6 0	2	1.4
	5 1 4 0[b]	2	1.4
	4 1 6 0	1	0.7
	4 3 6 2[b]	1	0.7
	5 1 0 2[b]	1	0.7
P. mallei (4)	2 1 0 2	4	
P. pseudomallei (6)	2 1 6 **2**	3	
	2 1 0 **2**	1	
	2 1 4 **2**	1	
	2 1 6 2	1	
P. maltophilia (160)	4 2 4 6	4 2	26.3
	4 2 4 4	2 7	16.9
	4 3 4 4	2 3	14.4
	4 3 4 6	2 1	13.1
	4 0 4 6	1 7	10.6
	4 2 0 4	6	3.8
	0 1 4 4	4	2.5
	4 0 4 4	4	2.5
	0 0 4 6	3	1.9
	0 2 4 4	3	1.9
	4 2 0 6	3	1.9
	0 2 0 4	2	1.3
	0 2 0 6	2	1.3
	4 1 4 4	2	1.3
	0 0 4 4	1	0.6
P. paucimobilis (11)	0 3 6 4	1 1	100
P. putrefaciens (21)	1 0 0 2	1 1	52.4
	0 0 0 0	4	19.1
	0 0 0 2	3	14.3
	0 1 0 2	2	9.5
	1 1 0 2	1	4.7
P. acidovorans (16)	0 0 0 2	1 6	100
P. vesicularis (1)	0 1 4 4	1	
P. diminuta (1)	0 0 0 0	1	
Achromobacter xylosoxidans (100)	0 0 2 2	6 6	66.0
	0 0 2 **2**	2 9	29.0
	0 1 2 2[b]	4	4.0
	0 1 2 **2**	1	1.0
Alcaligenes faecalis (87)	0 0 0 0	7 1	81.6
	0 0 0 2	1 6	19.4
Agrobacterium radiobacter (12)	0 7 6 4	8	66.7
	0 7 6 **6**	3	25.0
	0 6 6 **6**	1	8.3
A. tumefaciens (1)	0 6 6 **6**	1	
Bordetella bronchiseptica (15)	0 4 0 2	1 1	73.3
	0 4 0 0	4	26.7
Flavobacterium meningosepticum (38)	0 3 4 5	1 4	36.8
	0 1 0 5	1 2	31.6
	0 3 0 5	7	18.4
	0 1 4 5	5	13.2
F. odoratum (16)	0 4 0 0	1 6	100
Flavobacterium sp. group IIb (27)	0 1 1 5	9	33.3
	0 1 5 5	7	25.9
	0 1 1 **7**	3	11.1
	0 1 5 **7**	3	11.1
	0 1 5 4	2	7.4
	0 3 5 **7**	2	7.4
	0 0 1 5[b]	1	3.7
Acinetobacter calcoaceticus (141)	0 1 6 0	1 1 6	81.7
	0 1 2 0	1 7	12.0
	0 5 6 0[b]	6	4.2
	0 5 2 0[b]	3	2.1
A. lwoffi (30)	0 0 0 0	3 0	100

[a] See footnote *c*, Table 2.

[b] Profile number added after the identification of clinical isolates by classical methods. These nine numbers represent four species and one group of nonfermenters.

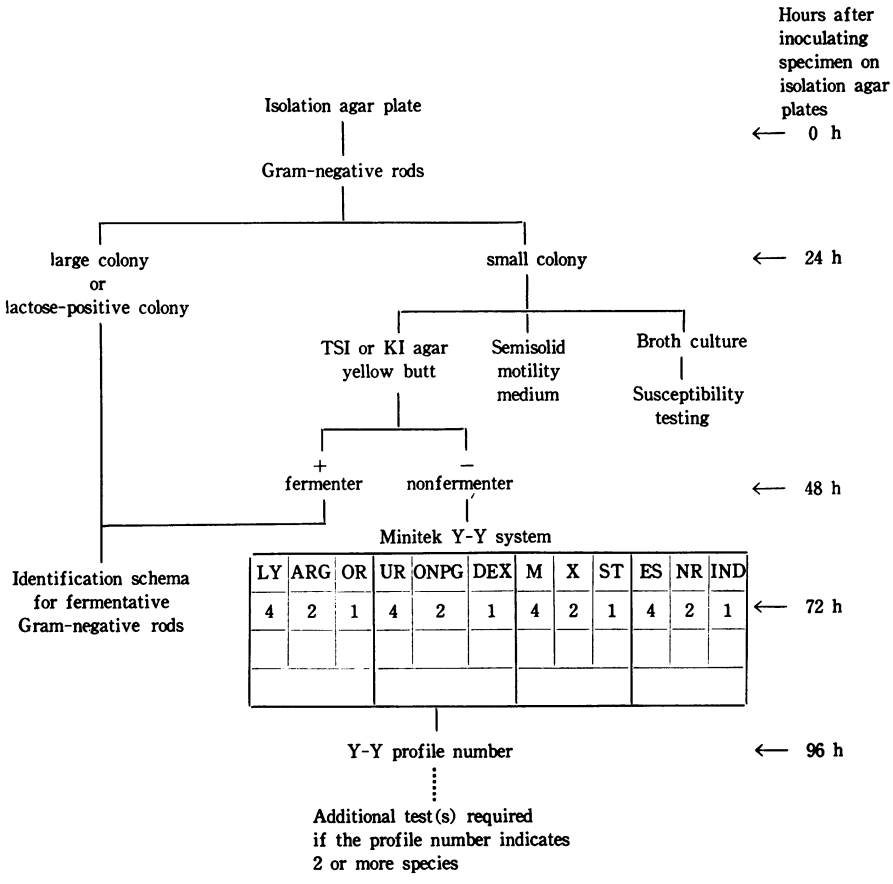

FIG. 1. *Identification schema for nonfermenting gram-negative rods by means of the Minitek Y-Y system. TSI, Triple sugar iron; KI, Kligler iron.*

solidified. Several bacterial suspensions (turbidity of a McFarland no. 5) were distributed into 20 wells of a Minitek plate with the pipetter, and closely covered with the above described plate cover with MacConkey agar. Each plate cover was then placed in a sterile petri dish and incubated at 37°C for 72 h. After 25 trials, five each for *Escherichia coli* and *Klebsiella pneumoniae* and 15 for nonfermenters, not a single colony appeared on the medium in the plate cover. The Minitek pipetter was thus regarded as not producing an aerosol during its proper manipulation.

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