APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Jan. 1992, p. 21–26
0099-2240/92/010021-06$02.00/0

Vol. 58, No. 1

Use of the API Rapid NFT System for Identifying Nonfermentative and Fermentative Marine Bacteria†

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Received 3 June 1991/Accepted 17 October 1991

Thirty-five American Type Culture Collection type strains of marine bacteria were used to evaluate the Rapid NFT system (API Analab Products, Plainview, N.Y.) for use in identifying heterotrophic marine bacteria. The 21 biochemical and assimilation tests on the Rapid NFT test strips were treated according to the manufacturer's protocol, which included use of AUX medium (provided with the Rapid NFT system) for preparing assimilation tests, and by substituting phenol red broth base (BBL Microbiology Systems, Cockeysville, Md.) with and without an oil overlay for the AUX medium. A seven-digit numerical profile was obtained for each NFT test strip from each of the three procedures and matched to its corresponding number in the Rapid NFT identification codebook. Also, all biochemical and assimilation test results were analyzed with SASTAXAN and SAS/GRAPH programs (SAS Institute, Inc., Cary, N.C.); similarity matrices were computed for all 35 strains. For comparison purposes, bacterial strains were grouped at a similarity level of 70%. The results indicated a low efficacy of identification for all three procedures. In addition, similarity matrix analysis showed more cohesive grouping based on results of phenol red broth base-treated strains than for the AUX medium provided by the manufacturer. However, none of the three treatments provided exclusive grouping of type strains at the genus level. Thus, the reliability of the data obtained from the NFT system and modifications thereof should be evaluated carefully when environmental isolates are characterized.

A detailed phenotypic characterization of bacterial strains, especially those from terrestrial and aquatic sources, is labor intensive. In many instances, the phenotypic traits are used in numerical taxonomic analyses to develop protocols for identifying particular types of bacteria (8, 10–14, 18, 19). Many of the biochemical tests used for determining phenotypic characteristics of bacteria from aquatic and terrestrial habitats are based on those used in clinical bacteriology (1, 3). Furthermore, efforts to expedite rapid characterization and identification of bacteria have yielded a variety of systems that allow simultaneous determination of numerous phenotypic characteristics. Although applicable to the majority of bacterial species routinely encountered in the clinical laboratory, multitest systems have also been used for characterizing environmental isolates (9, 13, 18). One such system, the API Rapid NFT profile index (API Analab Products, Plainview, N.Y.), was designed to identify gram-negative nonfermentative bacteria such as species of *Pseudomonas*, *Flavobacterium*, and related genera. In addition, the NFT system allows for the identification of some nonenteric fermentative bacteria, such as *Vibrio* and *Aeromonas* spp.

The Rapid NFT test strip contains test substrates freeze-dried in 20 individual cupules; the substrates are rehydrated upon the addition of a bacterial suspension. There are nine enzymatic assays: NO_3 reductase, tryptophanase, glucose fermentation, arginine dihydrolase, urease, esculin hydrolase, gelatinase, β-galactosidase, and cytochrome oxidase (which is performed independently). In addition, 12 carbohydrate assimilation tests (D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-D-glucosamine, maltose, D-gluconate, caprate, adipate, L-malate, citrate, and phenylacetate) are carried out on each test strip. Assimilation tests are carried out with inoculated cells suspended in the manufacturer's AUX medium (provided with the test strips), which contains ammonium sulfate, 0.15% agar, mineral base, amino acids, phosphate buffer, and distilled water.

During preliminary evaluation of the NFT system for identifying selected strains of marine bacteria (e.g., presumptive *Vibrio* spp.), we had difficulty in interpreting the results of the carbohydrate assimilation tests; a positive assimilation test is interpreted on the basis of the development of turbidity, indicating cell growth on a sole carbon source. However, the AUX medium used to inoculate assimilation tests is somewhat opaque, rendering unambiguous determination of results difficult. Therefore, we attempted to modify the system by substituting the AUX medium with a diluent containing phenol red (in the form of phenol red broth base) to allow for easier interpretation of results based on a pH-induced color change. The decision to use this type of modification was based on the observation that fermentative bacteria predominate in some regions of the oceans (5, 6, 15). The results reported herein summarize our findings of studies designed to evaluate the rapid NFT system (and modifications) for rapid identification and characterization of culturable heterotrophic marine bacteria.

MATERIALS AND METHODS

Bacterial strains. Thirty-five type strains of marine bacteria, representing 12 genera, used in this study were obtained from the American Type Culture Collection (Rockville, Md.) (ATCC strains). Four gram-positive marine species (two *Bacillus* spp. and two *Micrococcus* spp.) were among the type strains used in the study, even though the NFT system has no provision for identifying these seemingly ubiquitous species. Although not intended to be identified by the Rapid NFT system, these gram-positive bacteria were included as

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† Contribution no. 150 from the Center of Marine Biotechnology.

a means of evaluating the reliability of the NFT system by determining how these species grouped with other distantly related gram-negative bacteria after similarity matrix analysis.

Rapid NFT protocol and medium preparation. All marine strains were cultured and streaked onto marine agar 2216 (Difco, Detroit, MI). Three strains that did not grow on marine agar 2216 were grown instead on nutrient agar (Difco). Colonies from each marine isolate were suspended in sterile (autoclaved; 20 ppt) Instant Ocean (Aquarium Systems, Mentor, Ohio) that had been prefiltered through a 0.45-μm-pore-size membrane filter (Gelman Sciences, Ann Arbor, Mich.) to remove particulates (9). Strains grown on nutrient agar were suspended in 0.85% NaCl. The cell suspensions were diluted with the appropriate diluent to an A_{560} between 1.0 and 1.5, which corresponds to a McFarland barium sulfate standard of 0.5, according to the Rapid NFT protocol. Rapid NFT profile strips were inoculated according to the manufacturer's instructions with the exception that all strips were incubated at 27°C. All enzymatic and assimilation test results were recorded at 24 and 48 h, respectively. Oxidase tests were performed separately by transferring colonies from culture plates with a sterile platinum loop onto Whatman no. 3 filter paper moistened with 1% tetramethyl-*p*-phenylenediamine dihydrochloride (Sigma Chemical Co., St. Louis, Mo.). Oxidase-positive strains produced a color change to dark purple within 10 s (17).

PRBB modification. Phenol red broth base (PRBB) was prepared in 20-ppt Instant Ocean and dispensed in 5-ml aliquots in sterile culture tubes. Cell suspensions were prepared as described above, and test strips were inoculated in the manner used for the AUX suspension medium. Two sets of NFT strips were prepared from each strain, one without an oil overlay and one with a sterile mineral oil overlay (PRBBO) for the carbohydrate assimilation cupules. A PRBB color change from red to yellow (after 24 or 48 h) was interpreted as the ability of a strain to metabolize a specific carbon source, resulting in the formation of an acidic end product(s).

Controls were treated like samples, except that sterile 20-ppt Instant Ocean or sterile 0.85% NaCl was used as an inoculum instead of a bacterial suspension; controls were run simultaneously with experiments. The caprate assimilation test was positive for all PRBB and PRBBO controls and was therefore omitted from the similarity matrix analysis.

Data analysis. The results from each test strip for each treatment were recorded twice, after incubation for 24 and 48 h. For the purposes of data analyses, the 24- and 48-h readings were treated as independent results. Also, although there were 252 data sets obtained (i.e., 35 strains [plus 7 duplicates], three methods of preparing NFT strips, and two readings for each preparation), the results were analyzed and interpreted only for those seven genera (192 data sets) listed in the NFT identification codebook. The results obtained from the remaining five genera, although not listed in the NFT codebook, are presented in Tables 1 and 2 for comparison purposes.

The results were analyzed and interpreted in two ways: (i) according to the Rapid NFT protocol, a seven-digit number (designated as the numerical profile by API) was obtained for each data set and then compared with the numerical profiles in the NFT identification codebook and through the API computer identification system; and (ii) positive and negative results for each test strip were assigned a binary score of 0 and 1, respectively. The scores from all 252 data sets were used to generate similarity matrices by analysis with the SASTAXAN program (SAS Institute, Inc., Cary, N.C.) (7).

RESULTS AND DISCUSSION

In this study 26 ATCC type strains were used to evaluate the efficacy of the API Rapid NFT system for characterizing and identifying heterotrophic marine bacteria. In addition, we modified the Rapid NFT system protocol by incorporating a pH indicator (phenol red) in the carbohydrate assimilation tests to obtain a clearer interpretation of results. Numerical profiles obtained from the test strips prepared according to the manufacturer's protocol and the modifications reported herein were compared with those in the NFT codebook. The numerical profiles not listed in the codebook were submitted to API for further analysis. For identifications obtained from the numerical profiles, two levels of discrimination were considered for positive identification: genus level and species level.

Of the 26 type strains tested according to the manufacturer's protocol, all of which were reputed to be identifiable by the NFT codebook, only 5 were identified correctly to the species level and 2 were identified correctly to the genus level (Table 1). Duplicates of some type strains were tested as well; in the case of *Vibrio alginolyticus*, only one of three replicates yielded a correct identification. Even less reliable results were obtained from the Rapid NFT system prepared with PRBB and PRBBO. For example, only *Aeromonas hydrophila* and *Acinetobacter calcoaceticus* were identified correctly with the PRBB modification (Table 1). A large number of numerical profiles were obtained that did not match any in the NFT codebook. Subsequent computer analysis performed by API yielded more correct identifications (Table 1, computer match section). For the unmodified systems there were five more identifications to the genus level and three correct identifications to the species level. For PRBB and PRBBO modifications, three more strains were correctly identified: *Vibrio campbelli* to the genus level and *V. alginolyticus* and *Vibrio parahaemolyticus* to the species level. It is significant to note that the PRBB modifications correctly identified all replicates of *V. alginolyticus* (Table 1).

The total number of strains correctly identified for all three protocols was 17 (53.1%) of 32 strains (26 type strains plus duplicates) identifiable by the Rapid NFT system. Of these, 25% were correctly identified to the species level. In addition, 6 of the 32 strains were misidentified when the NFT codebook was used; 4 of these 6 were misidentified with the manufacturer's protocol, and 2 were misidentified with the PRBB and PRBBO modifications.

Approximately three-fourths (73.4%) of the 192 numerical profiles representing genera listed in the NFT codebook required computer assistance and were therefore submitted to API for further analysis (Table 1, computer match section; Table 2). These profiles either could not be identified, or were designated as unacceptable, doubtful, or low-discrimination profiles by API. Ironically, some of the doubtful profiles did list possibilities for identification at low confidence levels (typically below an accuracy level of 50%), which correctly identified some type strains (Table 2). For example, 4 of 32 strains tested with the AUX medium had numerical profiles designated as doubtful by API but provided correct identification of these type strains, albeit at 50% or lower confidence level. For the PRBB and PRBBO modifications, there were four and five strains, respectively, which were designated as doubtful, yet correct identifica-

TABLE 1. Results of identification of ATCC type strains with the API Rapid NFT system[a]

ATCC type strain	NFT codebook match						Computer match					
	AUX		PRBB		PRBBO		AUX		PRBB		PRBBO	
	24 h	48 h	24 h	48 h	24 h	48 h	24 h	48 h	24 h	48 h	24 h	48 h
Genera listed in NFT codebook												
7708 *Vibrio metschnikovii*	−	−	−	−	−	−	−	−	−	−	−	−
7708 *V. metschnikovii*[b]	−	−	−	−	−	−	−	−	−	−	−	−
19263 *Vibrio adaptatus*	mi	mi	mi	mi	mi	mi	■	■	■	■	■	■
19263 *V. adaptatus*[b]	mi	mi	−	mi	−	mi	■	■	−	■	−	■
25920 *Vibrio campbellii*	−	−	−	−	−	−	+	−	+	−	+	+
25920 *V. campbellii*[b]	−	−	−	−	−	−	−	−	+	+	+	+
14048 *Vibrio natriegens*	−	−	−	−	−	−	−	−	−	−	−	−
17749 *Vibrio alginolyticus*	−	−	−	−	−	−	−	−	+	+	+	+
17749 *V. alginolyticus*[b]	−	−	−	−	−	−	+	+	+	+	+	+
17749 *V. alginolyticus*[b]	+	+	−	−	−	−	■	■	+	+	+	+
27562 *Vibrio vulnificus*	−	−	−	−	−	−	+	+	−	−	−	−
17802 *Vibrio parahaemolyticus*	−	−	−	−	−	−	+	−	+	+	+	+
33509 *Vibrio ordalii*	−	−	−	−	−	−	−	mi	−	−	−	−
33564 *Vibrio hollisae*	+	+	−	−	−	−	■	■	−	−	−	−
33466 *Vibrio diazotrophicus*	−	−	−	−	−	−	+	+	−	−	−	−
33809 *Vibrio fluvialis*	−	−	−	−	−	−	+	−	−	−	−	−
19264 *Vibrio anguillarum*	−	−	−	−	−	−	−	−	−	−	−	−
35048 *Vibrio aestuarianus*	−	+	−	−	−	−	+	■	−	−	−	−
7744 *Vibrio fischeri*	+	+	−	−	−	−	■	■	−	−	−	−
25917 *Vibrio nereis*	−	−	mi	mi	mi	mi	−	−	■	■	■	■
10145 *Pseudomonas aeruginosa*	−	−	−	−	−	−	+	−	−	−	−	−
27123 *Pseudomonas duodoroffii*		−	−	−	−	−	−	−	−	−	−	−
13525 *Pseudomonas fluorescens*	+	+	−	−	−	−	■	■	−	−	−	−
33658 *Aeromonas salmonicida*	−	−	−	−	−	−	−	−	−	−	−	−
33658 *A. salmonicida*[b]	−	−	−	−	−	−	−	−	−	−	−	−
7965 *Aeromonas hydrophila*	+	+	+	+	+	+	■	■	■	■	■	■
19260 *Flavobacterium marinotypicum*	−	−	−	−	−	−	−	−	−	−	−	−
27951 *Flavobacterium lutescens*	mi	mi	−	−	−	−	■	■	−	−	−	−
11947 *Flavobacterium aquatile*	mi	mi	mi	−	mi	mi	■	■	■	−	■	■
8750 *Alcaligenes faecalis*	−	−	−	−	mi	mi	−	−	−	−	■	■
15918 *Achromobacter cholinophagum*	−	−	−	−	−	−	−	−	−	−	−	−
23055 *Acinetobacter calcoaceticus*	+	−	+	−	−	−	■	mi	■	−	−	−
Genera not listed in NFT codebook												
13880 *Serratia marcescens*	−	−	−	−	−	−	−	−	−	−	−	−
33670 *Serratia rubidaea*	−	−	−	−	−	−	−	−	−	−	−	−
8071 *Alteromonas putrefaciens*	−	−	−	−	−	−	−	−	−	−	−	−
14393 *Alteromonas haloplanktis*	−	−	mi	−	−	−	−	−	■	mi	−	−
8010 *Arthrobacter globiformis*	−	−	−	−	−	−	−	−	−	−	−	−
186 *Micrococcus roseus*	mi	−	−	−	−	−	■	−	−	−	−	−
4698 *Micrococcus luteus*	−	−	mi	mi	mi	−	−	−	■	■	■	mi
29841 *Bacillus marinus*	−	−	−	−	−	−	−	mi	−	−	−	−
29841 *B. marinus*[b]	−	−	−	−	−	−	−	mi	−	−	−	−
14581 *Bacillus megaterium*	−	−	−	−	−	−	mi	mi	−	−	−	−

[a] Observations were made at 24 and 48 h, with AUX, PRBB, or PRBBO for the assimilation medium. Numerical profiles either did not match (−) (Table 2) or matched (+) to the genus or species level of discrimination or matched but misidentified (mi) the type strains. Filled squares (■) represent results already accounted for in the NFT codebook match section. The correct/incorrect identification ratios were as follows for the NFT codebook match at 24 and 48 h, respectively: for AUX, 6/4 and 6/4; for PRBB, 2/3 and 1/3; for PRBBO, 1/4 and 1/5. The corresponding values for the computer match are as follows: for AUX, 8/0 and 3/2; for PRBB, 6/0 and 5/0; for PRBBO, 6/0 and 6/0.

[b] Duplicate.

tions were provided for these strains, although once again at a low confidence level (Table 2).

The low efficacy of identification with the Rapid NFT system in this study is in contrast to the higher efficacy of identification obtained with the Rapid NFT system in other studies (10, 13, 14). A plausible explanation for this discrepency centers on the fact that a large percentage of the strains tested previously were replicates or different strains of the same species (10, 13, 14). For example, Overman et al. (13) reported the Rapid NFT system to be 87% accurate in identifying members of the family *Vibrionaceae*. However, of the 60 strains tested, approximately one-half (27 strains) were *Aeromonas hydrophila* and 10 of the 60 were *Aeromonas sobria*; both of these species are listed frequently in the API Rapid NFT codebook.

Because of the low percentage of correct identifications that were obtained with the Rapid NFT system, the results were used to calculate similarity matrices. Similarity matrices, typically based on results of 60 to 120 phenotypic tests, have been used to group bacterial isolates from marine and aquatic environments (3, 4, 16, 19, 20). When used in concert with ATCC reference strains, similarity clusters have been

TABLE 2. Results of computer analysis of numerical profiles for which no matches existed in the API Rapid NFT codebook[a]

ATCC type strain	Unacceptable profiles						Doubtful profiles					
	AUX		PRBB		PRBBO		AUX		PRBB		PRBBO	
	24 h	48 h	24 h	48 h	24 h	48 h	24 h	48 h	24 h	48 h	24 h	48 h
Genera listed in NFT codebook												
7708 *Vibrio metschnikovii*	U	U	U	U	U	U	■	■	■	■	■	■
7708 *V. metschnikovii*	U	U	U	U	U	U	■	■	■	■	■	■
19263 *Vibrio adaptatus*	–	–	–	–	–	–	–	–	–	–	–	–
19263 *V. adaptatus*	–	–	–	–	–	–	–	–	D	–	L	–
25920 *Vibrio cambellii*	–	U	–	U	–	–	–	■	–	■	–	–
25920 *V. cambellii*	U	U	–	–	–	–	■	■	–	–	–	–
14048 *Vibrio natriegens*	–	U	–	–	–	–	G	■	D	D	D	D
17749 *Vibrio alginolyticus*	–	–	–	–	–	–	L	G	–	–	–	–
17749 *V. alginolyticus*	–	–	–	–	–	–	–	–	–	–	–	–
17749 *V. alginolyticus*	–	–	–	–	–	–	–	–	–	–	–	–
27562 *Vibrio vulnificus*	–	–	–	–	–	–	–	–	G	G	G	G
17802 *Vibrio parahaemolyticus*	–	–	–	–	–	–	–	D	–	–	–	–
33509 *Vibrio ordali*	–	–	–	U	–	U	D	–	D	■	D	■
33564 *Vibrio hollisae*	–	–	–	–	–	–	–	–	S	S	S	S
33466 *Vibrio diazotrophicus*	–	–	–	–	–	–	–	–	G	D	G	D
33809 *Vibrio fluvialis*	–	–	–	–	–	–	–	S	D	D	D	D
19264 *Vibrio anguillarum*	–	–	–	–	–	–	D	D	D	D	D	D
35048 *Vibrio aestuarianus*	–	–	–	–	–	–	–	–	D	D	D	D
7744 *Vibrio fischeri*	–	–	U	U	U	U	–	–	■	■	■	■
25917 *Vibrio nereis*	–	–	–	–	–	–	D	D	–	–	–	–
10145 *Pseudomonas aeruginosa*	–	U	–	U	–	U	–	■	D	■	D	■
27123 *Pseudomonas duodoroffii*	U	–	–	–	–	–	■	D	D	D	D	D
13525 *Pseudomonas fluorescens*	–	–	–	–	–	D	–	–	G	G	G	■
33658 *Aeromonas salmonicida*	–	–		U		U	D	D	D	■	D	■
33658 *A. salmonicida*	–	–	U	U	U	–	D	S	■	■	■	D
7965 *Aeromonas hydrophila*	–	–	–	–	–	–	–	–	–	–	–	–
19260 *Flavobacterium marinotypicum*	U	U	–	U	U	U	■	■	D	■	■	■
27951 *Flavobacterium lutescens*	–	–	–	–	–	–	–	–	D	D	D	D
11947 *Flavobacterium aquatile*	–	–	–	–	–	–	–	–	–	D	–	–
8750 *Alcaligenes faecalis*	U	–	–	–	–	–	■	D	D	D	–	–
15918 *Achromobacter cholinophagum*	–	–	–	U	–	U	D	D	D	■	D	■
23055 *Acinetobacter calcoaceticus*	–	–	–	U	–	U	–	–	–	■	S	■
Genera not listed in NFT codebook												
13880 *Serratia marcescens*	U	U	U	U	U	–	■	■	■	■	■	D
33670 *Serratia rubidaea*	–	–	–	U	–	U	D	D	D	■	D	■
8071 *Alteromonas putrefaciens*	–	–	–	–	–	–	D	D	D	D	D	D
14393 *Alteromonas haloplanktis*	U	U	–	–	–	–	■	■	–	–	D	D
8010 *Arthrobacter globiformis*	–	–	–	U	–	–	D	D	D	■	D	D
186 *Micrococcus roseus*	–	U	–	–	–	U	–	■	D	D	D	■
4698 *Micrococcus luteus*	–	–	–	–	–	–	D	D	–	–	–	–
29841 *Bacillus marinus*	U	–	–	U	–	U	■	–	D	■	D	■
29841 *B. marinus*	U	–	U	U	U	U	■	–	■	■	■	■
14581 *Bacillus megaterium*	–	–	U	U	U	U	–	–	■	■	■	■

[a] Unacceptable (U), doubtful (D), and low-discrimination (L) profiles are API designations which connote the following. Unacceptable indicates no matches in the API data base and that crucial tests are against the identification. Doubtful indicates that the isolate shares similarities with previously identified strains in the data base. These heretofore identified strains were listed by API as possible matches and typically had a frequency of correct identification less than 50%. Low-discrimination profiles offer possibilities for species identification between 50 and 70%. G and S indicate numerical profiles designated as doubtful but that identified the type strains at the genus or species level, respectively. Filled squares (■) and minus signs (–) represent numerical profiles already accounted for in the unacceptable category or in Table 1.

used to draw conclusions on the genus or class of an environmental isolate, based on its relative position in a group or cluster with other bacteria (8, 16, 19, 20). The results obtained for PRBB and PRBBO treatments of the Rapid NFT system were combined because only one type strain (*Bacillus marinus* ATCC 29841) differed in its placement among other groups of bacteria (Table 3). Results of all 252 numerical profiles obtained were analyzed by using SASTAXAN program and showed no exclusive grouping of the strains to the genus level with a similarity coefficient of 70% (Table 3). However, the results obtained from modifications of the Rapid NFT system do demonstrate obvious homology within the genus *Vibrio*. The majority of the *Vibrio* spp. grouped in three of four clusters for the modified API Rapid NFT system. One large group (group 2) contained predominantly *Vibrio* spp., and two small clusters (groups 1 and 3) contained only *Vibrio* spp. The other large group was heterogeneous, containing representatives from all 12 genera used in the study, a result not totally unexpected considering the small number of tests used to generate the similarity matrix. However, only three *Vibrio* spp. were included in this group and were related at the 50% similarity level (data

TABLE 3. Comparison of groupings of type strains by similarity matrix analysis[a] of phenotypic traits as determined with the API Rapid NFT system

API Rapid NFT system modified[b]			API Rapid NFT system		
Group[c] (n = 4)	Genera in group	No. of type strains in genus	Group (n = 8)	Genera in group	No. of type strains in genus
1	*Vibrio*	2	1	*Vibrio*	2
				Serratia	1
2	*Vibrio*	14		*Aeromonas*	1
	Aeromonas	3	2	*Vibrio*	5
	Serratia	1		*Serratia*	1
				Aeromonas	1
3	*Vibrio*	2	3	*Vibrio*	1
4	*Flavobacterium*	3	4	*Vibrio*	4
	Pseudomonas	3		*Alteromonas*	2
	Vibrio	3		*Flavobacterium*	2
	Alteromonas	2		*Bacillus*	2
	Micrococcus	2		*Micrococcus*	1
	Bacillus	2		*Acinetobacter*	1
	Acinetobacter	1		*Pseudomonas*	1
	Achromobacter	1			
	Alcaligenes	1			
	Arthrobacter	1			
			5	*Vibrio*	2
				Achromobacter	1
				Arthrobacter	1
				Bacillus	1
			6	*Flavobacterium*	1
			7	*Pseudomonas*	2
				Flavobacterium	1
			8	*Vibrio*	7
				Aeromonas	1

[a] Phenotypic test results were converted to binary scores and analyzed by using the SASTAXAN program.
[b] Modified by replacing AUX medium with PRBB and PRBBO (see text).
[c] Species were grouped at the 70% similarity level.

not presented) to the other remaining 17 *Vibrio* spp. Conversely, similarity clusters based on results from Rapid NFT systems prepared with the AUX medium were considerably more heterogeneous than those based on results with PRBB and PRBBO (Table 3). Eight distinct groups were obtained, and the *Vibrio* spp. were represented in all but two groups.

The combined results from the three treatments (AUX, PRBB, and PRBBO) demonstrated an overall lack of reliability of the Rapid NFT system for identifying marine bacteria (Table 4). Although the AUX medium yielded more correct identification of ATCC type strains than did the PRBB and PRBBO modifications of the Rapid NFT system (14 and 7 strains, respectively), the efficacy of identification for the manufacturer's protocol and for the modifications was very low: 43.8% for AUX and 21.9% for PRBB modifications.

Some plausible explanations for why the efficacy of identification is low for marine bacteria have been proposed by Austin (2). Many phenotypic tests designed for medical microbiology may not be applicable to the genetic and functional diversity of bacteria found in the aquatic environment (1, 2). Furthermore, the reproducibility of classical phenotypic tests may be low for marine bacteria, especially those containing plasmids whose expression may give inconsistent results for biochemical and morphological tests (1, 2).

The API Rapid NFT system currently accommodates only

TABLE 4. Efficacy of bacterial identification with the Rapid NFT profile index[a]

Matching of type strains with the API RAPID NFT data base	AUX	PRBB	PRBBO	Total
No. of correct identification of types strains	14	11	10	35
No. of correct identifications of type strains to genus	9	3	4	16
No. of matches which misidentified type strains	10	6	9	25
No. of doubtful profiles	14	22	19	55
No. of doubtful profiles providing correct identification of type strains	4	7	7	18
No. of unacceptable profiles	13	15	15	43

[a] The results are based on numerical profiles obtained from 26 strains, six duplicates, and readings at 24 and 48 h. The apparent discrepancy with the 252 total observations is due to the exclusion of the five genera not listed in the NFT identification codebook from calculations in the summary table.

13 genera of bacteria. Thus, the need for a larger data base that includes a wider array of genera more representative of bacteria indigenous to aquatic habitats is obvious. Until a larger, more reliable data base is available, results obtained from the NFT system should be evaluated carefully before they are used to identify bacterial isolates from the environment.

ACKNOWLEDGMENTS

This work was supported in part by the Center of Marine Biotechnology and by Office of Naval Research Grant N00014-86-K-0696.

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