6-1996

Comparison of Fatty Acid Methyl Ester Analysis with the Use of API 20E and NFT Strips for Identification of Aquatic Bacteria

Beverly J. Brown

Laura G. Leff
Kent State University - Kent Campus, lleff@kent.edu

Comparison of Fatty Acid Methyl Ester Analysis with the Use of API 20E and NFT Strips for Identification of Aquatic Bacteria.

B J Brown and L G Leff
Appl. Environ. Microbiol. **1996, 62(6):2183.**

APPLIED AND ENVIRONMENTAL MICROBIOLOGY, June 1996, p. 2183–2185 Vol. 62, No. 6
0099-2240/96/$04.00+0

Comparison of Fatty Acid Methyl Ester Analysis with the Use of API 20E and NFT Strips for Identification of Aquatic Bacteria

BEVERLY J. BROWN* AND LAURA G. LEFF

Department of Biological Sciences and the Water Resources Research Institute, Kent State University, Kent, Ohio 44242-0001

Received 21 December 1995/Accepted 21 March 1996

Aquatic bacteria grown on MacConkey agar and modified nutrient agar were identified by using API 20E and NFT strips and fatty acid methyl ester (FAME) analysis. Identifications agreed at the species level 35.7% of the time when API 20E strips and FAME analysis were used and in 4.3% of the cases when API NFT strips and FAME analysis were used. These techniques require further development before extended use in ecological studies.

Ecologists frequently use identification of organisms to learn more about a given community or ecosystem. Many organisms can be identified on the basis of morphological characters. However, this approach is not reliable for all groups of organisms, including bacteria which possess limited morphological differentiation (1, 2). Many techniques for bacterial identification rely on results of biochemical tests and assimilation assays (12). Such physiological tests have been performed by traditional microbiological methods or by using commercially available kits (e.g., the API rapid test strips, Enterotubes, Oxi-Ferm tubes, Minitek, or Micro-ID systems). Other techniques include fatty acid methyl ester (FAME) analysis. With the advent of molecular techniques, additional methods have been developed to facilitate identification of culturable bacteria such as the use of species-specific rRNA (1) or rRNA gene probes (10) and restriction endonuclease analysis of chromosomal DNA (12). Such approaches may be cost prohibitive, requiring substantial expenditure for equipment. However, identification of environmental bacteria is frequently desirable in the course of ecological studies.

The purpose of this study was to compare two commonly used methods for identifying environmental bacteria: API rapid test strips (bioMérieux Vitek, Inc., Hazelwood, Mo.) and FAME analysis (Microbial ID, Inc., Newark, Del.). Two types of API strips were used, depending on the medium used for bacterial isolation. The first was for nonfermenting, gram-negative bacteria (API NFT) which were isolated from modified nutrient agar (11) (Difco) plates, and the second was for members of the family *Enterobacteriaceae* and other gram-negative bacteria (API 20E) for isolates from MacConkey agar. API strips use a series of biochemical and assimilation tests; results are compared with a computerized database of results from known bacteria. In contrast, FAME analysis converts the phospholipids present in bacterial membranes into methyl esters. A methyl ester profile is determined by gas-liquid chromatography (4). Identification is made when the resulting profile is compared with profiles from known bacteria by using a similarity index.

Planktonic bacteria were collected in winter 1994 from five sites in the Cuyahoga River (Geauga, Portage, Summit, and Cuyahoga Counties, Ohio), a tributary of Lake Erie. Two types of media, modified nutrient agar and MacConkey agar, were inoculated with river water. Modified nutrient agar (11) (Difco) plates were incubated at room temperature for 3 days. MacConkey agar (BBL) plates were incubated at 37°C for 24 h. At the end of the incubation period, 47 colonies from modified nutrient agar and 80 colonies from MacConkey agar were randomly selected and isolated as pure cultures.

API NFT strip ratings are based on an estimate of how closely the unknown organism's profile matches profiles of taxa in the database and how similar the profile is to the most typical set of reactions for each taxon (6). Excellent, very good, good, and acceptable identifications must have percent of identification values equal to or greater than 99.9, 99.0, 90.0, and 80.0, respectively, with taxon similarity values equal to or greater than 0.75, 0.5, 0.25, and 0, respectively.

Isolates from MacConkey agar were identified with API 20E strips. API 20E ratings are based on three parameters, including the likelihood of a match between the unknown organism's profile and the computer profile, the relative value between the likelihood of the first and the likelihood of the second choices, and the number of tests against the first choice (5).

All MacConkey agar isolates with an identification confidence level of excellent or very good (16 isolates) were sent to Microbial ID, Inc., for FAME analysis. In addition, 20 additional isolates from MacConkey agar and 47 isolates from modified nutrient agar were randomly selected for FAME analysis. Identification confidence levels for FAME analysis were determined by using a similarity index. A similarity of 0.700 or higher with a separation between the first and second choices of 0.200 or more is considered an excellent match. If the similarity index is between 0.500 and 0.700 with a separation of 0.100 between the first and second choices, then the match is considered good. Similarity values between 0.300 and 0.500 could be an acceptable match but would indicate an atypical strain. Values lower than 0.300 suggest that the species is not included in the database.

All identifications from API strips were to the species level except where noted. Approximately 27.5% of the isolates identified with API 20E strips had an identification confidence level of excellent or very good (Fig. 1). None of the identifications from the API NFT strips had a confidence rating of excellent (Fig. 1). Six percent of isolates identified from API NFT strips had a confidence level of very good. Eighteen percent of isolates from modified nutrient agar were identified to the genus level only. API strips could not be used to identify 27.5% of

* Corresponding author. Phone: (330) 672-5659. Fax: (216) 672-3713. Electronic mail address: bbrown@kentvm.kent.edu.

TABLE 1. Identification confidence levels obtained by using FAME analysis

Confidence level	% of isolates	
	MacConkey agar (n = 36)	Modified nutrient agar (n = 47)
Excellent	27.8	34.0
Good	69.4	53.2
Acceptable	2.8	10.6
No match	0.0	2.1

isolates from MacConkey agar and 44.0% from nutrient agar (Fig. 1).

Isolates from MacConkey agar identified by using FAME analysis had an identification confidence level of excellent 27.8% of the time and a confidence level of good 69.4% of the time (Table 1). Isolates from modified nutrient agar identified by using FAME analysis had a confidence level of excellent 34.0% of the time and a confidence level of good 53.2% of the time (Table 1). One isolate had no match in the Microbial ID database. Identifications from API strips and FAME analysis for isolates from MacConkey agar agreed in 35.7% of the cases at the species level and 46.4% at the generic level. Species identifications determined with API 20E strips which were supported by FAME analysis were *Enterobacter agglomerans*, *Enterobacter cloacae*, *Escherichia coli*, and *Klebsiella pneumoniae*. A complete list of identifications for isolates from MacConkey agar is included in Table 2.

Identifications for isolates from modified nutrient agar determined to the species level by using API strips agreed with FAME analysis 4.3% of the time at the species level and 30.4% of the time at the genus level. The species confirmed with FAME analysis was *Pseudomonas chlororaphis*. Identification of isolates from modified nutrient agar given only to the genus level by using API NFT strips agreed 100% of the time with the genus determined by FAME analysis. Ten identifications were to the genus *Pseudomonas* and one was to the genus *Acinetobacter*. A complete list of identifications for isolates from modified nutrient agar is included in Table 3.

API strips and FAME analysis have been used in a variety of studies (8, 9, 12, 14, 16). Inferences based on bacterial identifications are useful only if identifications are accurate and if information regarding the ecology of the organisms is available. If the bacterial community of whole river water is being analyzed, the ability of these techniques to identify as many

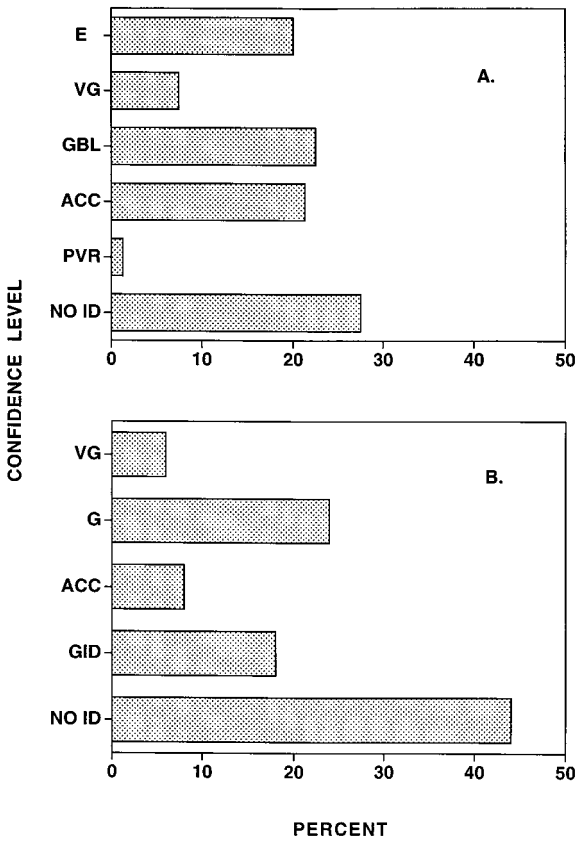

FIG. 1. (A) Identification confidence levels for 80 isolates from MacConkey agar obtained by using API 20E strips. (B) Identification confidence levels for 47 isolates from modified nutrient agar obtained by using API NFT strips. E, excellent; VG, very good; GBL, good likelihood but low selectivity; GID, identified to the genus level; ACC, acceptable; PVR, possibly very rare; NO ID, no identification possible.

TABLE 2. Comparison of species identification of isolates from MacConkey agar obtained by using API 20E strips and FAME analysis

Identification	Isolate(s) identified with[a]:	
	API 20E strips (n = 28)	FAME analysis (n = 36)
Acinetobacter baumanii		BM2C
Aeromonas caviae		BM2A, OP2C, RR3C, RR3B
Aeromonas hydrophila/veronii bv. *sobria*	RR3C	
Citrobacter freundii	KT1A, OP1A	
Enterobacter agglomerans	HR2A, HR2E, HR3F, KT1D, KT2D	HR2E, KT1A, OP1B, OP1C, BM1B, BM1E, RR2B, RR2E, RR2A
Enterobacter cloacae	KT1C, OP3E, BM1A, BM1E, RR3A	KT1C, OP3E, BM1A, BM2D, RR2D
Erwinia herbicola		KT2C
Escherichia coli	HR1E, OP3F, BM2F	HR1E, OP3F, BM2F
Hafnia alvei		HR1B, HR2A, HR3F, KT1D, KT2D, KT3D
Klebsiella oxytoca	OP1B, OP1C, BM1B, RR2B, RR3D	
Klebsiella pneumoniae	OP2A, BM3B, RR2E	OP2A, BM2B
Kluyvera sp.	KT1F	
Kluyvera ascorbata		KT1F
Kluyvera cryocrescens		OP1A
Rahnella aquatilis		HR1C
Vibrio fluvialis	BM2A	

[a] The first two characters of the isolate code refer to the site, the third refers to the replicate, and the fourth refers to the individual isolate.

TABLE 3. Comparison of species identification by isolate from modified nutrient agar obtained by using API NFT strips and FAME analysis

Identification	Isolate(s) identified with:	
	API NFT strips (n = 32)	FAME analysis (n = 46)
Acinetobacter lwoffii		I12
Acinetobacter johnsonii		K02, L49
Acinetobacter junii/johnsonii	I12, K02	
Aeromonas hydrophila	I79	
Aeromonas salmonicida subsp. *masoucida*/subsp. *achromogenes*	J83, L96	
Chrysomonas luteola	G92	
Comomonas testosteroni	M51	
Cytophaga johnsonae		I17, J48, L06, L16, N37
Enterobacter agglomerans		M57
Escherichia coli		K29
Hydrogenophaga pseudoflava		M51, M96
Janthinobacterium lividum		H11, H67, H30
Pseudomonas aureofaciens	G69, J76, J86, K01, N28	
Pseudomonas aeruginosa	I42	
Pseudomonas chlororaphis	I54, M60	G33, G36, G92, H61, H69, H84, I54, J12, K01, L82, L88, M68, N28
Pseudomonas fluorescens	G02, G75, H61, K49, L88, M68, M75	H70, I63, J83, J86, K09, K43
Pseudomonas marginalis		G02, G69, G75, I45, J76, K49, L96, M22
Pseudomonas putida		I42, I79, M75
Pseudomonas syringae pv. tomato		M60
Pseudomonas vesicularis	M96	
Sphingomonas paucimobilis	H11, H67, I17, J48, K43, L06, L16, L49, N37	

isolates as possible is highly desirable. Therefore, two qualities we looked for in our evaluation of API strips and FAME analysis were the ability to identify isolates and corroboration of identification between methods.

API strips were unable to identify a sizable number of isolates, whereas FAME analysis allowed identification in all but one case. The majority of identifications with API strips were not corroborated by FAME analysis. These findings are similar to those of Santos et al. (14), who found that the API 20E system could not accurately identify some strains of bacteria when identifications were compared with those made by standard biochemical tube and plate tests. Breschel and Singleton (7) encountered similar differences with API NFT strips and 35 American Type Culture Collection strains of marine bacteria. Both the inability to identify isolates and the inability to corroborate identifications may be attributable to computerized databases which lack extensive information on environmental bacteria.

API strips and FAME analysis were designed for analysis of clinical isolates and are effective tools in that regard (3, 13, 15). Use of API strips and FAME analysis for identification of aquatic freshwater bacteria is in a formative stage and should be used advisedly. As more environmental bacteria are collected and profiles are added to databases, discrepancies in identifications may be reconciled. Until identification of the nonculturable portion of bacterial assemblages is possible, improvements to the databases will enhance the value of API strips and FAME analysis as valuable ecological tools.

This research was supported by a grant (R823749-01-0) from the U.S. Environmental Protection Agency, Office of Exploratory Research, and the Department of Biological Sciences, Kent State University.

We thank the Kent State University Microbial Ecology Group for their comments on an early version of the manuscript.

REFERENCES

1. **Amann, R., W. Ludwig, and K.-H. Schleifer.** 1994. Identification of uncultured bacteria: a challenging task for molecular taxonomists. ASM News **60:**360–365.
2. **Amann, R. I., W. Ludwig, and K.-H. Schleifer.** 1995. Phylogenetic identification and in situ detection of individual microbial cells without cultivation. Microbiol. Rev. **59:**143–169.
3. **Appelbaum, P. C., and D. J. Leathers.** 1984. Evaluation of the Rapid NFT system for identification of gram-negative, nonfermenting rods. J. Clin. Microbiol. **20:**730–734.
4. **Bergan, T., and K. Sørheim.** 1984. Gas-liquid chromatography for the assay of fatty acid composition in gram-negative bacilli as an aid to classification. Methods Microbiol. **15:**345–362.
5. **bioMérieux Vitek, Inc.** 1992. 20E analytical profile index, 10th ed. bioMérieux Vitek, Inc., Hazelwood, Mo.
6. **bioMérieux Vitek, Inc.** 1992. Rapid NFT identification codebook. bioMérieux Vitek, Inc., Hazelwood, Mo.
7. **Breschel, T. S., and F. L. Singleton.** 1992. Use of the API Rapid NFT system for identifying nonfermentative and fermentative marine bacteria. Appl. Environ. Microbiol. **58:**21–26.
8. **Esteve, C., C. Amaro, E. G. Biosca, and E. Garay.** 1995. Biochemical and toxigenic properties of *Vibrio furnissii* isolated from a European eel farm. Aquaculture **132:**81–90.
9. **Haack, S. K., H. Garchow, D. A. Odelson, L. J. Forney, and M. J. Klug.** 1994. Accuracy, reproducibility, and interpretation of fatty acid methyl ester profiles of model bacterial communities. Appl. Environ. Microbiol. **60:**2483–2493.
10. **Leff, L. G., R. M. Kernan, J. V. McArthur, and L. J. Shimkets.** 1995. Identification of aquatic *Burkholderia* (*Pseudomonas*) *cepacia* by hybridization with species-specific rRNA gene probes. Appl. Environ. Microbiol. **61:**1634–1636.
11. **Leff, L. G., and J. L. Meyer.** 1991. Biological availability of dissolved organic carbon to bacteria along the Ogeechee River continuum. Limnol. Oceanogr. **36:**315–323.
12. **Logan, N. A.** 1994. Bacterial systematics. Blackwell Scientific Publications, London.
13. **Nord, C.-E., A. A. Lindberg, and A. Dahlbäck.** 1974. Evaluation of five test-kits—API, AuxoTab, Enterotube, PathoTec and R/B—for identification of Enterobacteriaceae. Med. Microbiol. Immunol. **159:**211–220.
14. **Santos, Y., J. L. Romalde, I. Bandin, B. Magarinos, S. Núñez, J. L. Barja, and A. E. Toranzo.** 1993. Usefulness of the API-20E system for the identification of bacterial fish pathogens. Aquaculture **116:**111–120.
15. **Søgaard, P., B. Gahrn-Hansen, Z. Hui-Ping, and W. Frederiksen.** 1986. An investigation of three commercial methods for rapid identification of non-enteric gram-negative rods. Acta Pathol. Microbiol. Immunol. Scand. Sect. B **94:**357–363.
16. **Tearle, P. V., and K. J. Richard.** 1987. Ecophysiological grouping of Antarctic environmental bacteria by API 20 NE and fatty acid fingerprints. J. Appl. Bacteriol. **63:**497–503.