JOURNAL OF CLINICAL MICROBIOLOGY, Oct. 1996, p. 2414–2420 Vol. 34, No. 10
0095-1137/96/$04.00+0
Copyright © 1996, American Society for Microbiology

Acinetobacter Species Identification by Using tRNA Spacer Fingerprinting

BORIS EHRENSTEIN,[1] ALEXANDRA T. BERNARDS,[2] LENIE DIJKSHOORN,[2] PETER GERNER-SMIDT,[3] KEVIN J. TOWNER,[4] PHILIPPE J. M. BOUVET,[5] FRANZ D. DASCHNER,[1] AND HAJO GRUNDMANN[1]*

Institut für Umweltmedizin und Krankenhaushygiene, Klinikum der Albert-Ludwigs-Universität, 79106 Freiburg, Germany[1]; Department of Medical Microbiology, Leiden University Hospital, 2300 RC Leiden, The Netherlands[2]; Department of Clinical Microbiology, Statens Seruminstitut, 2300 Copehagen S, Denmark[3]; Department of Microbiology and Public Health Laboratory Service Laboratory, University Hospital, Nottingham NG7 2UH, United Kingdom[4]; and Unité des Entérobactéries, Unité 389 Institut National de la Santé et de la Recherche Médicale, Institut Pasteur, 75724 Paris Cedex 15, France[5]

Received 17 January 1996/Returned for modification 6 March 1996/Accepted 16 July 1996

Identification of *Acinetobacter* spp. to the DNA group level by phenotypic techniques is problematic, and there is a need for an alternative identification method for routine use. The present study validated the suitability of a rapid identification technique based on tRNA spacer (tDNA) fingerprinting in comparison with that of a commercially available assay involving carbon source utilization tests (Biolog MicroStation System) for identifying the 21 DNA-DNA hybridization groups belonging to the genus. For this purpose, 128 strains identified previously by DNA-DNA hybridization were analyzed by both techniques. tDNA fingerprinting was highly reproducible and classified all strains into 17 groups. Six DNA groups belonging to the *A. calcoaceticus-A. baumannii* complex were grouped into two distinct clusters, indicating the high degree of genetic similarity within this complex. Strains of the more recently described DNA groups BJ13 to BJ16 were ambiguously grouped and displayed three pattern types. The software used with the commercial carbon source utilization method grouped the 128 strains into 12 clusters, explaining the less discriminatory power of this system. We conclude that tDNA fingerprinting offers a quick and reliable method for the routine differentiation of most *Acinetobacter* spp. at the subgenus level.

Members of the genus *Acinetobacter* are short, plump, nonmotile, gram-negative rods that are essentially nonmotile because of the absence of flagellae. They are nonfastidious, catalase-positive, oxidase-negative strict aerobes that grow on simple media containing a single carbon source. The genus has been known for many years to be genotypically heterogeneous (12, 17, 19). In the absence of objective criteria for subdivision of the genus only one genus and species, *Acinetobacter calcoaceticus*, was described in *Bergey's Manual of Systematic Bacteriology* (20). Many taxonomists have considered this classification insufficient given the considerable heterogeneity among members of the genus. Since 1986, DNA-DNA hybridization studies have resulted in the identification of 21 DNA groups (4, 5, 12, 13, 22, 24). Those studies were performed independently by different laboratories which has led to equivocal designations for some of the groups that have been described lately (Table 1). Unfortunately, there is no single biochemical test or set of tests that enables the accurate identification of strains at the DNA group level.

Strains of *Acinetobacter* have a wide distribution in nature. It has been estimated that acinetobacters may constitute 0.001% of the total heterotrophic population of soil and water (2). They have commonly been found in the hospital environment and are increasingly incriminated as one of the major pathogens of nosocomial concern. Numerous outbreaks of nosocomial infection have been reported, with most being caused by strains of DNA groups 2, 3, 5, 7, 8/9, or TU13. Long-lasting or repeated outbreaks are often caused by the recurrence of a single strain (9). Their importance in hospital epidemiology is partly explained by their ability to survive on inanimate and dry surfaces for prolonged periods of time (1, 15) and their success in acquiring resistance to almost all currently used antibiotics, including imipenem (16). There has been a recent dramatic increase in the incidence of these organisms (26), and it seems likely that they will be of increasing epidemiological importance in the future.

The current limited understanding of the ecology and distribution of *Acinetobacter* spp. within different habitats is caused by the lack of a rapid and practical method for identifying members of the genus to the species level. Carbon source utilization reactions and genotypic characterization by means of recent DNA-based methods, such as amplified rRNA gene (rDNA) restriction analysis and tRNA spacer (tDNA) fingerprinting, have been advocated as useful techniques for this purpose (25, 28). In the present study, we evaluated the suitability of tDNA fingerprinting and carbon source assimilation tests for identifying the 21 DNA groups using the 128 strains of *Acinetobacter* identified previously by DNA-DNA hybridization tests.

MATERIALS AND METHODS

Strains. A total of 128 *Acinetobacter* strains (Table 2) were studied, consisting mostly of hospital isolates originating from different parts of Scandinavia, France, Brazil, Belgium, and The Netherlands (kindly provided by the Statens Seruminstitut, Copenhagen, Denmark; Leiden University Hospital, Leiden, The Netherlands; the University Hospital, Nottingham, United Kingdom; and the Institut Pasteur, Paris, France). The 17 reference strains described previously for different DNA groups (4, 5) were included in the collection. All strains had been

* Corresponding author. Mailing address: Institut für Umweltmedizin und Krankenhaushygiene, Klinikum der Albert-Ludwigs-Universität, Hugstetter Str. 55, 79106 Freiburg, Germany. Phone: 49 761 270 5472. Fax: 49 761 270 5485.

TABLE 1. Comparison of the delineation of *Acinetobacter* genomic species by different laboratories

Species name	Genomic species no. according to:			
	Bouvet and Grimont (4) and Bouvet and Jeanjean (5)	Tjernberg and Ursing (24)	Nishimura et al. (22)	Gerner-Smidt and Tjernberg (13)
A. calcoaceticus	1	1	1	Not tested[a]
A. baumannii	2	2	1	2
NN	3	3	Not tested	3
NN	Ungrouped[c]	13	Not tested	13
NN	Not tested	Not tested	Not tested	Between 1 and 3
NN	Not tested	Not tested	Not tested	Close to TU13
A. haemolyticus	4	4	4	Not tested
A. junii	5	5	nt	Not tested
NN	6	6	4	Not tested
A. johnsonii	7	7	3	Not tested
A. lwoffii	8	8	2	Not tested
NN	9	8	Not tested	Not tested
NN	10	10	Ungrouped	Not tested
NN	11	11	Ungrouped	Not tested
A. radioresistens	(12)[d]	12	5	Not tested
NN	13	14	Not tested	Not tested
NN	14	Not tested	Not tested	Not tested
NN	15	Not tested	Not tested	Not tested
NN	16	Ungrouped	Not tested	Not tested
NN	17	Not tested	Not tested	Not tested
NN	Not tested	15	Not tested	Not tested

[a] Strains belonging to the group were not tested.
[b] NN, species not named.
[c] Strain(s) belonging to this group remained ungrouped.
[d] Parentheses indicate unpublished data.

extensively characterized previously by diverse methods, including DNA-DNA hybridization (4, 5, 10, 12, 13, 22, 24).

tDNA fingerprinting. The strains were cultured overnight on Mueller-Hinton blood agar (Unipath, Wesel, Germany) at 30°C. A 1-μl loopful of colony growth was suspended in 100 μl of H_2O, boiled for 10 min, and centrifuged briefly in a microcentrifuge. One microliter of crude cell lysate provided sufficient target DNA for PCR amplification. Each PCR was carried out in 25-μl volumes containing 1× PCR buffer and 0.1 mM (each) deoxynucleoside triphosphate (Pharmacia, Freiburg, Germany), 1 μM (each) primer, and 0.6 U of *Taq* polymerase (Pharmacia) overlaid with mineral oil. After initial denaturation for 2 min at 94°C, 45 cycles of 40 s at 94°C, 40 s at 50°C, and 2 min at 72°C followed. Final extension was for 3 min at 72°C. The oligonucleotide primers used for tDNA outward spacer amplification were consensus sequences derived from the tDNA 3′ and 5′ ends, as described by Welsh and McClelland (27). The primers and their sequences were as follows: T3A, 5′-GGG GGT TCG AAT TCC CGC CGG CCC CA-3′, and T5B, 5′-AAT GCT CTA CCA ACT GAA CT-3′. Both primers were 5′ end-labelled with fluorescein isothiocyanate during custom synthesis (Pharmacia).

The amplification products were analyzed with an automated laser fluorescence (A.L.F.) sequencer (Pharmacia). The DNA fragments were separated by electrophoresis through 5% acrylamide–7 M urea gels in 0.6× TBE (Tris-borate-EDTA) buffer. Sample preparation and running conditions were as described previously (18). The fragment patterns were expressed as fluorescence densitograms by the automated sequencer. Inconsistencies within and between gels were normalized with Fragment Manager Software (Pharmacia) by comparing internal standards and size markers. Identical patterns were perceived by visual comparison of the fluorescence densitograms, and the patterns were reevaluated by automated cluster analysis after import of digitized fluorescence data directly into the GelCompar software (Applied Maths, Kortrijk, Belgium). The analysis was carried out by using similarity matrices calculated from the Pearson product-moment correlation coefficient. Clusters were recognized by using the unweighted pair group method with arithmetic averages.

Carbon source assimilation tests. The carbon source assimilation test used in the present study was based on the Biolog MicroStation system (Biolog Inc., Hayward, Calif.) (23). Oxidation of 95 different carbon sources was assessed in 96-well GN microplates supplied by the manufacturer. Briefly, colonies of overnight cultures were grown on tryptic soy agar (Unipath), harvested with sterile swabs, and suspended in sterile saline. The suspensions were turbidimetrically adjusted to approximately 4.5×10^8 cells per ml, and 150 μl was added to each of the wells. Oxidation of the substrates was shown by a change of color from colorless to purple. The results were recorded by visual inspection, and the 95-test color patterns resulted in a 32-digit "bionumber" for each isolate. Because the commercial identification database did not include references for some of the recently described DNA groups (groups BJ13 to BJ17) (3), a cluster analysis of the oxidation patterns was performed with Biolog software, release 3.01A, to establish the potency of the system for identifying all DNA groups. The program uses a modified unweighted pair group method with arithmetic averages. Distance values, representing the number of tests with discrepant results between two strains, are calculated for all pairs of strains. A distance level of 14 is used by the software to separate clusters.

RESULTS AND DISCUSSION

tDNA fingerprinting uses primers designed to amplify the spacer regions of tDNA clusters (27). Amplification profiles are likely to differ at the species or genus level and would thus be a measure for genetic relatedness at this level of classification. In order to improve the resolution of the amplification products during electrophoresis, fluorescein-labelled primers and an automated DNA sequencer were used in the study. tDNA fingerprinting of 128 *Acinetobacter* strains belonging to all 21 previously described DNA groups produced 17 patterns, coded A to Q (Table 2), that were clearly distinguishable by visual comparison of the fluorescence densitograms. Six tDNA fingerprinting pattern types, represented by 66 strains, matched single DNA groups. All 31 strains belonging to the *A. calcoaceticus-A. baumannii* complex clustered into two distinct tDNA fingerprinting pattern types, with one cluster containing the unnamed DNA groups 1, 3, and "between 1 and 3" (13), while the other included DNA groups 2 (A. baumannii), TU13, and "close to TU13" (13). Figure 1 provides the 12 patterns displayed by the strains of the four major *A. calcoaceticus-A. baumannii* complex DNA groups. Only two strains each of DNA groups 6, TU15, and BJ17 were obtainable for investigation. Each of these strains displayed a unique pattern. Strains of the more recently described DNA groups BJ13 to BJ16 displayed only three distinctive pattern types, but the DNA group designations of these strains did not correlate with the tDNA pattern types (Table 2 and Fig. 2).

TABLE 2. Classification of 128 strains of *Acinetobacter* spp. by tDNA fingerprinting and phenotypic clustering with the Biolog MicroStation system

Strain	Source	DNA group[a]	tDNA fingerprint pattern type	Phenotypic cluster (Biolog)
ATCC 23055^T	K. Towner	1	A	a
ATCC 17902	P. Gerner-Smidt	1	A	b
42[b]	P. Gerner-Smidt	1	A	b
59[b]	P. Gerner-Smidt	1	A	b
RUH 582 (6[c])	P. Gerner-Smidt	1	A	b
RUH 584 (3[c])	P. Gerner-Smidt	1	A	b
RUH 944 (5[c])	P. Gerner-Smidt	1	A	b
10095[d]	P. Gerner-Smidt	Between 1 and 3	A	b
10169[d]	P. Gerner-Smidt	Between 1 and 3	A	b
ATCC 19606^T	K. Towner	2	B	b
9771[d]	P. Gerner-Smidt	2	B	b
10073[d]	P. Gerner-Smidt	2	B	b
10074[d]	P. Gerner-Smidt	2	B	b
10508[d]	P. Gerner-Smidt	2	B	b
ATCC 19004	K. Towner	3	A	c
9907[d]	P. Gerner-Smidt	3	A	b
10078[d]	P. Gerner-Smidt	3	A	b
10084[d]	P. Gerner-Smidt	3	A	b
10088[d]	P. Gerner-Smidt	3	A	b
10089[d]	P. Gerner-Smidt	3	A	b
10790[d]	P. Gerner-Smidt	3	A	b
12174a[d]	P. Gerner-Smidt	3	A	b
12398[d]	P. Gerner-Smidt	3	A	b
ATCC 17906^T	K. Towner	4	C	d
RUH 415 (57[c])	L. Dijkshoorn	4	C	d
RUH 44 (61[c])	L. Dijkshoorn	4	C	d
RUH 406 (59[c])	L. Dijkshoorn	4	C	d
LMD 70.9[c]	L. Dijkshoorn	4	C	d
61[b]	L. Dijkshoorn	4	C	e
197[b]	L. Dijkshoorn	4	C	d
ATCC 17908^T	K. Towner	5	D	a
22[c]	P. Gerner-Smidt	5	D	e
23[c]	P. Gerner-Smidt	5	D	e
27[c]	P. Gerner-Smidt	5	D	e
53a[c]	P. Gerner-Smidt	5	D	e
74a[c]	P. Gerner-Smidt	5	D	e
74b[c]	P. Gerner-Smidt	5	D	e
80[c]	P. Gerner-Smidt	5	D	e
96[c]	P. Gerner-Smidt	5	D	e
113:3[c]	P. Gerner-Smidt	5	D	e
117[c]	P. Gerner-Smidt	5	D	f
124[c]	P. Gerner-Smidt	5	D	e
127[c]	P. Gerner-Smidt	5	D	e
138[c]	P. Gerner-Smidt	5	D	e
140[c]	P. Gerner-Smidt	5	D	e
155a[c]	P. Gerner-Smidt	5	D	e
177[c]	P. Gerner-Smidt	5	D	e
178[c]	P. Gerner-Smidt	5	D	e
189[c]	P. Gerner-Smidt	5	D	e
RUH 204 (64[c])	P. Gerner-Smidt	5	D	e
RUH 383 (65[c])	P. Gerner-Smidt	5	D	e
ATCC 17979	K. Towner	6	E	d
RUH 286	L. Dijkshoorn	6	F	d
ATCC 17909^T	K. Towner	7	G	a
68[b]	L. Dijkshoorn	7	G	a
92[b]	L. Dijkshoorn	7	G	a
97[b]	L. Dijkshoorn	7	G	a
112[b]	L. Dijkshoorn	7	G	a
134[b]	L. Dijkshoorn	7	G	a

Continued on following page

TABLE 2—*Continued*

Strain	Source	DNA group[a]	tDNA fingerprint pattern type	Phenotypic cluster (Biolog)
137[b]	L. Dijkshoorn	7	G	a
153[b]	L. Dijkshoorn	7	G	a
NCTC 5866[T]	K. Towner	8/9	H	a
ATCC 9957	K. Towner	8/9	H	a
ATCC 17910	P. Gerner-Smidt	8/9	H	a
ATCC 17968	P. Gerner-Smidt	8/9	H	a
ATCC 17987	P. Gerner-Smidt	8/9	H	a
82[e]	P. Gerner-Smidt	8/9	H	a
201[e]	P. Gerner-Smidt	8/9	H	a
256[e]	P. Gerner-Smidt	8/9	H	a
283[e]	P. Gerner-Smidt	8/9	H	a
284[e]	P. Gerner-Smidt	8/9	H	a
286[e]	P. Gerner-Smidt	8/9	H	a
65109-84[e]	P. Gerner-Smidt	8/9	H	a
86981-84[e]	P. Gerner-Smidt	8/9	H	a
44[b]	P. Gerner-Smidt	8/9	H	a
122[b]	P. Gerner-Smidt	8/9	H	a
135[b]	P. Gerner-Smidt	8/9	H	a
145[b]	P. Gerner-Smidt	8/9	H	a
202[b]	P. Gerner-Smidt	8/9	H	a
1101[b]	P. Gerner-Smidt	8/9	H	a
RUH 45 (79[c])	P. Gerner-Smidt	8/9	H	a
RUH 74 (82[c])	P. Gerner-Smidt	8/9	H	a
RUH 549 (86[c])	P. Gerner-Smidt	8/9	H	a
RUH 551 (78[c])	P. Gerner-Smidt	8/9	H	a
RUH 709 (88[c])	P. Gerner-Smidt	8/9	H	a
RUH 1104 (84[c])	P. Gerner-Smidt	8/9	H	a
U100[e]	P. Gerner-Smidt	8/9	H	a
ATCC 17924	K. Towner	10	I	g
113:2[b]	L. Dijkshoorn	10	I	g
198[b]	L. Dijkshoorn	10	I	g
ATCC 11171	K. Towner	11	J	e
51[b]	L. Dijkshoorn	11	J	g
73[b]	L. Dijkshoorn	11	J	g
174[b]	L. Dijkshoorn	11	J	g
SEIP 12.81	K. Towner	12	K	h
RUH 2225	L. Dijkshoorn	12	K	a
RUH 2863	L. Dijkshoorn	12	K	a
RUH 3517	L. Dijkshoorn	12	K	a
ATCC 17905	K. Towner	BJ13/TU14	C	i
134[f]	L. Dijkshoorn	BJ13/TU14	C	j
376[f]	L. Dijkshoorn	BJ13/TU14	C	j
496[f]	L. Dijkshoorn	BJ13/TU14	C	j
552[f]	L. Dijkshoorn	BJ13/TU14	L	k
943[f]	L. Dijkshoorn	BJ13/TU14	C	j
1001[f]	L. Dijkshoorn	BJ13/TU14	L	j
1158[f]	L. Dijkshoorn	BJ13/TU14	L	j
1191[f]	L. Dijkshoorn	BJ13/TU14	L	j
382[f]	K. Towner	BJ14	L	l
513[f]	L. Dijkshoorn	BJ14	C	b
743[f]	L. Dijkshoorn	BJ14	L	b
79[f]	K. Towner	BJ15	M	e
81[f]	L. Dijkshoorn	BJ15	C	i
78[f] (CIP 70.18)	K. Towner	BJ16	M	i
673[f]	L. Dijkshoorn	BJ16	M	i
1011[f]	L. Dijkshoorn	BJ16	M	i
1211[f]	L. Dijkshoorn	BJ16	M	i
942[f]	K. Towner	BJ17	N	e
641[f]	L. Dijkshoorn	BJ17	O	i

Continued on following page

TABLE 2—*Continued*

Strain	Source	DNA group[a]	tDNA fingerprint pattern type	Phenotypic cluster (Biolog)
4419[d]	P. Gerner-Smidt	TU13	B	b
9836[d]	P. Gerner-Smidt	TU13	B	b
9894[d]	P. Gerner-Smidt	TU13	B	b
10716[d]	P. Gerner-Smidt	TU13	B	b
10717[d]	P. Gerner-Smidt	TU13	B	b
12112[d]	P. Gerner-Smidt	TU13	B	b
5804[d]	P. Gerner-Smidt	Close to TU13	B	b
10090[d]	P. Gerner-Smidt	Close to TU13	B	b
118[b]	P. Gerner-Smidt	TU15	P	a
151a[b]	P. Gerner-Smidt	TU15	Q	a

[a] DNA group designation of strains by DNA-DNA hybridization (see Table 1 for species names). DNA groups 8 and 9 are considered a single entity (12, 24); TU13 to TU15, DNA groups 13 to 15, respectively, described by Tjernberg and Ursing (24); BJ13 to BJ17, DNA groups 13 to 17, respectively, described by Bouvet and Jeanjean (5); DNA groups BJ13 and TU14 are regarded as a single entity (24). The DNA groups originally named "between 1 and 3" and "close to TU13" have not yet received an individual number (13).

[b] Strain originally characterized by Tjernberg and Ursing (24).

[c] Strain originally characterized by Dijkshoorn et al. (10).

[d] Strain originally characterized by Gerner-Smidt and Tjernberg (13).

[e] Strain originally characterized by Gerner-Smidt et al. (14).

[f] Strain originally characterized by Bouvet and Jeanjean (5).

The fluorescence density data obtained from the DNA sequencer were also used for direct similarity analysis with a computer-based algorithm and strain alignment by cluster analysis. All strains clustered into the same groups distinguished previously by visual analysis (Fig. 2). Discernible DNA groups differed at the 75% similarity level.

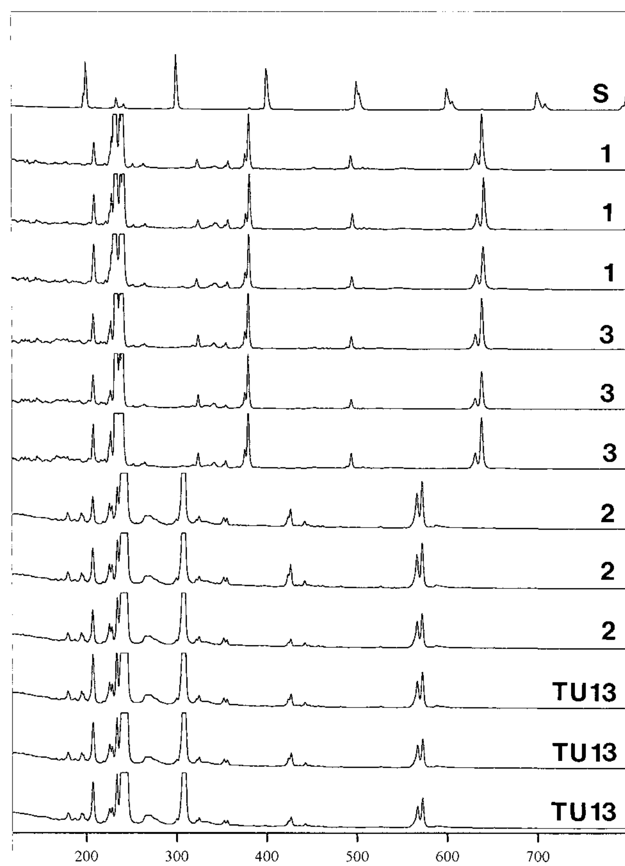

FIG. 1. Fluorescence densitogram of the tDNA fingerprint patterns representative of DNA groups of the *A. calcoaceticus-A. baumannii* complex (groups 1, 2, 3, and TU13). Row S, a 100-bp ladder was used for base pair calibration, with the number of base pairs indicated at the bottom of the densitogram.

The strains were also investigated with a commercially available identification system that uses 95 oxidation reactions; however, the system does not include an assessment of the temperature dependence for replication of the various *Acinetobacter* DNA groups. Identification with the database supplied by the manufacturer correctly classified 57 of the 128 strains to the DNA group level. Of the remaining 71 strains, 15 were identified as "*Acinetobacter* spp." (i.e., to the genus level only), 41 were incorrectly classified at the DNA group level, 5 were identified wrongly at the genus level, and no identification at all was obtained for 10 strains. Cluster analysis with these carbon source oxidation patterns grouped the strains into 12 entities, coded a to l (Table 2). With the exception of two strains, the strains of the *A. calcoaceticus-A. baumannii* complex were grouped in one cluster (cluster b). All strains of the genomic species 8/9, TU15, the type strain of DNA group 1, one strain of DNA group 5, six strains of DNA group 7, and three strains of DNA group 12 were combined in one cluster (cluster a). The two strains of DNA group 6 were grouped together with six strains of DNA group 4 (cluster d). The strains of DNA group 10, three strains of DNA group 11, and two strains of DNA group 7 were also in one cluster (cluster g). The majority of the strains of DNA groups 5 and BJ13/TU14 were in separate clusters (clusters e and j, respectively). Cluster i comprised all strains of DNA group BJ16 and one strain each of DNA groups BJ13/TU14, BJ15, and BJ17. Five strains remained unclustered (clusters c, f, h, k, and l).

The accepted standard for subdividing *Acinetobacter* spp. classifies strains by virtue of DNA heteroduplex hybridization kinetics (8). Correct identification of some species may also be achieved by ribotyping (11, 13), but both of these techniques are labor-intensive and are not suited for rapid screening purposes. Amplification of rDNA and subsequent restriction analysis provides an alternative approach for the identification of most DNA groups (25), but it requires the digestion of amplified DNA by several restriction endonucleases, which is an additional time-consuming step in the classification process. Direct amplification of intervening sequences in the tRNA-coding sequences (tDNA fingerprinting) provides a discriminatory amplification profile that does not require any further

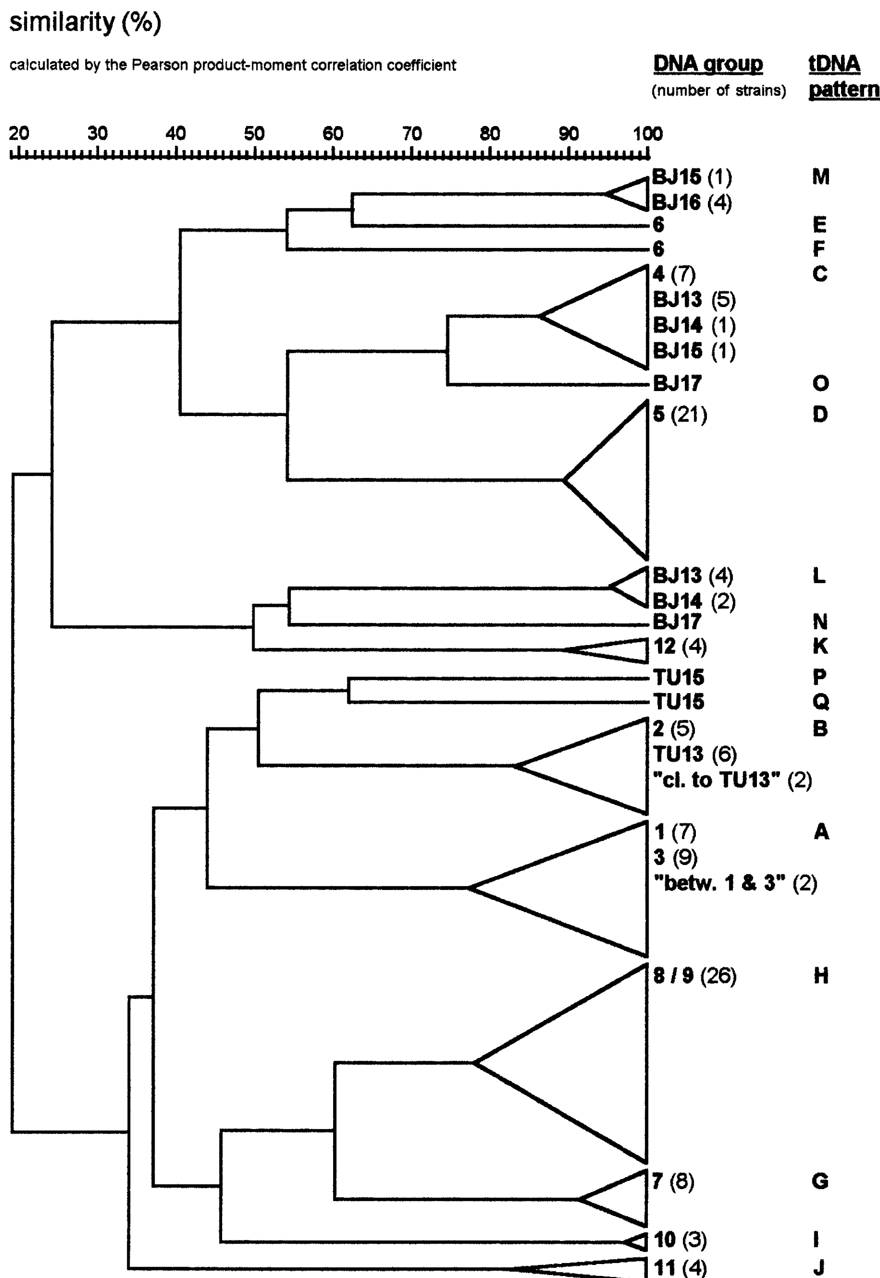

FIG. 2. Dendrogram derived from cluster analysis of tDNA fingerprint patterns for 128 *Acinetobacter* strains of 21 DNA groups by the unweighted pair group method with arithmetic averages. The vertex of the triangle indicates the average similarity value of the cluster.

processing of the PCR products. This approach has already been advocated for the identification of *Acinetobacter* spp. at the DNA group level for isolates mainly of environmental origin (28), but it has never been evaluated against a large panel of strains identified previously by DNA-DNA hybridization.

Although tDNA fingerprinting could not discriminate a number of strains at the DNA group level, it allowed the classification of most strains at a different taxonomic level. The lack of discrimination between DNA groups 1, 3, and "between 1 and 3" and between DNA groups 2, TU13, and "close to TU13" may be explained by the high degree of overall sequence homology. Even by DNA-DNA hybridization (24) these groups were distinguished only at a similarity level of 70%, which by convention represents the cutoff value for the definition of new DNA groups. Ribotyping also linked these clusters at the same level of similarity (13), and in a recent phenotypic study that used the electrophoretic polymorphisms of three enzymes (6), strains of DNA group 2 (*A. baumannii*) and DNA group TU13 could not be discriminated. It may thus be argued that the six DNA groups of the *A. calcoaceticus*-*A. baumannii* complex should be regarded as only two taxonomic entities at this level of classification. DNA groups 8 and 9, which have also been regarded as a single entity (24), could not be separated in the present study. Only two isolates constitute each of DNA group 6, Tjernberg and Ursing's (24) DNA group

15, and Bouvet and Jeanjean's (5) DNA group 17. We investigated these three pairs, and they displayed similarity values of only 58, 62, and 23%, respectively, possibly reflecting considerable divergence of the tRNA gene clusters. These strains and those belonging to DNA groups BJ13 to BJ16 are isolated infrequently from clinical specimens, and as a result of the limited number of strains identified so far (5), these groups are still vaguely defined by typing approaches other than DNA-DNA hybridization. Our results reflect the uncertainties still pertaining to these newly described groups. Further investigations will be required to determine the validity of taxa 6, BJ13 to BJ17, and TU15 if more strains could be assigned to these DNA groups.

Analysis of tDNA fingerprinting data can be accomplished easily by visual comparison (Fig. 1), but this becomes difficult when a large panel of unclassified strains is being investigated. Digital similarity analysis of the patterns generated by high-resolution gel electrophoresis on an automated sequencer may support the screening of such strain collections by clustering similar amplification profiles. It must, however, be emphasized that the degree of similarity expressed in the dendrograms cannot be regarded as absolute, since subtle variations in the fluorescence data contribute to the results calculated by the computer. This flaw may be overcome by future improvements in the standardization of the PCR reagents and combining a more accurate pattern alignment between different gels with the use of advanced decision algorithms (7).

Classification by oxidation profiles coherently grouped a considerable number of strains that belonged to individual DNA groups into distinct clusters. However, one to three strains of every DNA group aberrantly clustered with members of other DNA groups or remained unclustered. Overall, the results obtained with the oxidation profiles were in agreement with those of a previous study (3), except that DNA groups 7, 8/9, and 12 appeared to be linked to a single cluster in the current investigation. Adjustment of the commercially available database may improve the identification of some DNA groups. Nevertheless, grouping by tDNA fingerprinting showed a better discrimination of DNA groups and more consistency of patterns within DNA groups than grouping by carbon source oxidation. These findings are consistent with those of other studies showing that the DNA groups of *Acinetobacter* spp. do not fully correlate with the phenotypic classification (14, 21).

In conclusion, the combined use of tDNA fingerprinting with an automated sequencer offers a substantial time gain, a high degree of reproducibility, and accurate resolution compared with conventional methods. The ease and simplicity of the technique make this approach appealing for use in the differentiation of clinical isolates, but these advantages must be weighed against the cost of the equipment.

ACKNOWLEDGMENT

We gratefully acknowledge the contribution of all strains marked with a superscript *b* in Table 2 by Ingela Tjernberg, Department of Medical Microbiology, University of Lund, Malmö, Sweden.

REFERENCES

1. **Allan, K. D., and H. T. Green.** 1987. Hospital outbreak of multiresistant *Acinetobacter anitratus*. An airborne mode of spread? J. Hosp. Infect. **7:**169–175.
2. **Baumann, P.** 1968. Isolation of *Acinetobacter* from soil and water. J. Bacteriol. **96:**39–42.
3. **Bernards, A. T., L. Dijkshoorn, J. Van der Toorn, B. R. Bochner, and C. P. A. van Boven.** 1995. Phenotypic characterization of Acinetobacter strains of 13 DNA-DNA hybridisation groups by means of the Biolog system. J. Med. Microbiol. **42:**113–119.
4. **Bouvet, P. J. M., and P. A. D. Grimont.** 1986. Taxonomy of the genus *Acinetobacter* with the recognition of *Acinetobacter baumannii* sp. nov., *Acinetobacter haemolyticus* sp. nov., *Acinetobacter johnsonii* sp. nov., and *Acinetobacter junii* sp. nov. and emended description of *Acinetobacter calcoaceticus* and *Acinetobacter lwoffii*. Int. J. Syst. Bacteriol. **36:**228–240.
5. **Bouvet, P. J. M., and S. Jeanjean.** 1989. Delineation of new proteolytic genomic species in the genus *Acinetobacter*. Res. Microbiol. **140:**291–299.
6. **Bouvet, P. J. M., and S. Jeanjean.** 1995. Differentiation of *Acinetobacter calcoaceticus* sensu stricto from related *Acinetobacter* species by electrophoretic polymorphism of malate dehydrogenase, glutamate dehydrogenase and catalase. Res. Microbiol. **146:**773–785.
7. **Carlson, C. A., J. N. Keller, K. K. McAdoo, D. Y. Wang, B. Higgins, C. W. Baily, J. G. Thorne, B. J. Payne, M. Skala, and A. W. Hahn.** 1995. *Escherichia coli* O157:H7 restriction pattern recognition by artificial neural network. J. Clin. Microbiol. **33:**2894–2898.
8. **Crosa, J. H., D. J. Brenner, and S. Falkow.** 1973. Use of single-strand-specific nuclease for analysis of bacterial and plasmid deoxyribonucleic acid homo- and heteroduplexes. J. Bacteriol. **115:**904–911.
9. **Crowe, M., K. J. Towner, and H. Humphreys.** 1995. Clinical and epidemiological features of an outbreak of *Acinetobacter* in an intensive care unit. J. Med. Microbiol. **43:**55–62.
10. **Dijkshoorn, L., I. Tjernberg, B. Pot, M. F. Michel, J. Ursing, and K. Kersters.** 1990. Numerical analysis of cell envelope protein profiles of *Acinetobacter* strains classified by DNA-DNA hybridisation. Syst. Appl. Microbiol. **13:**338–344.
11. **Gerner-Smidt, P.** 1992. Ribotyping of the *Acinetobacter calcoaceticus-Acinetobacter baumannii* complex. J. Clin. Microbiol. **30:**2680–2685.
12. **Gerner-Smidt, P.** 1994. *Acinetobacter*. Epidemiological and taxonomic aspects. AMPIS **102**(Suppl. 47)**:**5–41.
13. **Gerner-Smidt, P., and I. Tjernberg.** 1993. Acinetobacter in Denmark. II. Molecular studies of the *Acinetobacter calcoaceticus-Acinetobacter baumannii* complex. AMPIS **101:**826–832.
14. **Gerner-Smidt, P., I. Tjernberg, and J. Ursing.** 1991. Reliability of phenotypic tests for identification of *Acinetobacter* species. J. Clin. Microbiol. **29:**277–282.
15. **Getchell-White, S. J., L. G. Donowitz, and D. H. M. Gröschel.** 1989. The inanimate environment of an intensive care unit as a potential source of nosocomial bacteria: evidence for long survival of *Acinetobacter calcoaceticus*. Infect. Control. Hosp. Epidemiol. **10:**402–407.
16. **Go, E. S., C. Urban, J. Burns, B. Kreiswirth, W. Eisner, N. Mariano, K. Mosinka-Snipas, and J. J. Rahal.** 1994. Clinical and molecular epidemiology of *Acinetobacter* infections sensitive only to polymyxin B and sulbactam. Lancet **344:**1329–1332.
17. **Grimont, P. A. D., and P. J. M. Bouvet.** 1991. Taxonomy of *Acinetobacter*, p. 25–36. *In* K. J. Towner, E. Bergogne-Bèrèzin, and C. A. Fewson (ed.), The biology of *Acinetobacter*. Plenum Press, New York.
18. **Grundmann, H., C. Schneider, H. V. Tichy, R. Simon, I. Klare, D. Hartung, and F. D. Daschner.** 1995. Automated laser fluorescence analysis of randomly amplified polymorphic DNA: a rapid method for investigating nosocomial transmission of *Acinetobacter baumannii*. J. Med. Microbiol. **43:**446–451.
19. **Johnson, J. L., R. S. Anderson, and E. J. Ordal.** 1970. Nucleic acid homologies among oxidase-negative *Moraxella* species. J. Bacteriol. **101:**568–573.
20. **Juni, E.** 1984. Genus III. Acinetobacter Brisou et Prévôt 1954, p. 303–307. *In* N. R. Krieg and J. G. Hold (ed.), Bergey's manual of systematic bacteriology, vol. 1. The Williams & Wilkins Co., Baltimore.
21. **Kämpfer, P., I. Tjernberg, and J. Ursing.** 1993. Numerical classification and identification of *Acinetobacter* genomic species. J. Appl. Bacteriol. **75:**259–268.
22. **Nishimura, Y., T. Ino, and H. Hzuka.** 1988. *Acinetobacter radioresistens* sp. nov. isolated from cotton and soil. Int. J. Syst. Bacteriol. **38:**209–211.
23. **Stager, C. E., and J. R. Davis.** 1992. Automated systems for identification of microorganisms. Clin. Microbiol. Rev. **5:**302–327.
24. **Tjernberg, I., and J. Ursing.** 1989. Clinical strains of *Acinetobacter* classified by DNA-DNA hybridisation. AMPIS **97:**595–605.
25. **Vaneechoutte, M., L. Dijkshoorn, I. Tjernberg, A. Elaichouni, P. de Vos, G. Claeys, and G. Verschraegen.** 1995. Identification of *Acinetobacter* genomic species by amplified ribosomal DNA restriction analysis. J. Clin. Microbiol. **33:**11–15.
26. **Washington, J. A., and the International Collaborative Blood Culture Study Group.** 1992. An international multicenter study of blood culture practices. Eur. J. Clin. Microbiol. Infect. Dis. **11:**1115–1128.
27. **Welsh, J., and M. McClelland.** 1991. Genomic fingerprints produced by PCR with consensus tRNA primers. Nucleic Acids Res. **19:**861–866.
28. **Wiedmann-Al-Ahmad, M., H. V. Tichy, and G. Schön.** 1994. Characterization of *Acinetobacter* type strains and isolates obtained from wastewater treatment plants by PCR-fingerprinting. Appl. Environ. Microbiol. **60:**4066–4071.