CHARACTERISATION AND TYPING OF MICRO-ORGANISMS

Phenotypic characterisation of *Acinetobacter* strains of 13 DNA-DNA hybridisation groups by means of the Biolog system

ALEXANDRA T. BERNARDS, L. DIJKSHOORN, J. VAN DER TOORN*, B. R. BOCHNER† and C. P. A. VAN BOVEN

Department of Medical Microbiology, Laboratory of Clinical Bacteriology, University Hospital Leiden, PO Box 9600, 2300 RC Leiden, * Kluyver Laboratory for Biotechnology, Julianalaan 67, University of Technology, Delft, The Netherlands and † Biolog Inc., 3938 Trust Way, Hayward, CA 94545, USA

Summary. A collection of 129 *Acinetobacter* strains belonging to DNA groups (genomic species) 1–14 (1–7 and 10–12 *sensu* Bouvet and Grimont; 8 and 13–14 *sensu* Tjernberg and Ursing) were investigated for their ability to oxidise 95 carbon sources in the Biolog system. The strain groupings obtained by cluster analysis with the Biolog software were compared with the results of DNA-DNA hybridisation studies. Strains of DNA groups 1 (*A. calcoaceticus*), 2 (*A. baumannii*), 3 and 13 were linked in one cluster, as were DNA groups 4 (*A. haemolyticus*) and 6, DNA groups 10 and 11, and DNA groups 8 (*A. lwoffii*) and 12 (*A. radioresistens*). Strains of DNA group 5 (*A. junii*) were grouped in a single cluster with one strain of DNA group 4. Strains of DNA groups 7 (*A. johnsonii*) and 14 formed separate clusters. With the exception of the linkage of DNA groups 8 and 12, these results correlated with classification of reference strains of the DNA groups by DNA-DNA hybridisation, but six strains of four different DNA groups were not allocated to the clusters of their respective DNA groups. In the case of DNA groups 4, 5, 6, 7, 10, 11 and 14, at least one carbon source oxidation test could be used to differentiate them from the other DNA groups.

Introduction

Micro-organisms of the genus *Acinetobacter* are widespread in nature and have been isolated from environmental and human sources. Many outbreaks of infection with multiresistant strains have occurred¹ and rapid identification methods are needed to identify these strains. Although the taxonomy of *Acinetobacter* has been studied extensively,^{2–5} there is as yet no uniform agreement. Bouvet and Grimont⁴ identified 12 genomic species (DNA groups) by DNA-DNA hybridisation. Tjernberg and Ursing⁵ described three additional DNA groups, named 13–15, and the type strain of another species, designated by Nishimura *et al.*⁶ as *A. radioresistens*, was found by Tjernberg and Ursing⁵ to belong to DNA group 12. Concurrently, Bouvet and Jeanjean⁷ delineated five additional DNA groups (named 13–17) among proteolytic strains, and group 13 of this study apparently corresponds to DNA group 14 of Tjernberg and Ursing.⁵

Carbon source utilisation tests, supplemented with several physiological tests, are unable to discriminate all of the various *Acinetobacter* DNA groups.^{4,7–11} A possible alternative is offered by the Biolog system, which is based on metabolic fingerprinting by determination of carbon source utilisation profiles. Oxidation of substrates, rather than assimilation, leads to reduction of the colourless indicator tetrazolium dye, thereby forming a purple colour. Several recent studies have used the Biolog system to identify various genera and species,^{12–14} but most *Acinetobacter* strains studied previously¹⁴ were of unknown genomic group, making conclusions on identification to species level difficult.

The aim of the present study was to characterise and group a collection of *Acinetobacter* strains of known genomic species by the Biolog system. The grouping of the strains following cluster analysis by the Biolog system was compared with classification based on DNA-DNA hybridisation^{4,5} to evaluate the Biolog system as a possible rapid alternative method for identifying *Acinetobacter* isolates to the genomic species (DNA group) level.

Materials and methods

Bacterial strains

In total, 129 *Acinetobacter* strains allocated to DNA groups 1–7 and 10–12 (*sensu* Bouvet and Grimont), and to DNA groups 8 and 13–14 (*sensu* Tjernberg and Ursing) by DNA-DNA hybridisation were investigated. Of these, 120 strains, including field isolates from The Netherlands and Sweden and strains from culture collections, have been described previously.^{5,15} Six additional strains, RUH nos. 3212 and 3323 of DNA group 2, nos. 2784 and 3289 of group 3, no. 3198 of group 5 and no. 3206 of group 14 were isolated in Dutch hospitals between 1988 and 1989. These six strains were identified to their DNA groups by DNA-DNA hybridisation with a filter method.¹⁶ Two strains, UHL no. 286 of DNA group 6 and no. 287 of group 11, originated from the collection of Gerner-Smidt and were kindly provided by Dr I. Tjernberg. Strain LMD 90.19 of DNA group 7 was obtained from the LMD Collection, Delft, and has been described recently by Bonting *et al.*¹⁷ Type and reference strains used in this study are shown in table I.

The Biolog system

The Biolog system (Biolog Inc., Hayward, CA, USA) was used according to the manufacturer's instructions with a few alterations. Briefly, strains stored at -70°C were subcultured on to sheep blood

agar (Oxoid CM271 with sheep blood 5% v/v) and incubated at 30°C . Fresh overnight cultures on Trypticase Soy Agar (BBL; 11043) were used for the tests. Cell suspensions were prepared in pre-warmed saline with a wire loop. Mucoid colonies were harvested with a moistened cotton swab. Inocula were set to the appropriate density, i.e., within the limits of the Biolog GN turbidity standards, with an ATB Densitometer model 1550 (bioMérieux, Benelux B.V., Den Bosch, The Netherlands). The Biolog plates were incubated at 30°C and read automatically, by means of the Biolog Microstation plate reader, and visually after incubation for 4 h and 24 h. Cluster analysis of the oxidation profiles was performed with Biolog's software release 3.01A. The program uses a modified UPGMA method (unweighted pair-group method with arithmetic averages)¹⁸ which is explained in detail in the Biolog reference manual. Briefly, "distance values", representing in essence the number of tests with discrepant results between two strains, are calculated for all pairs of strains. The structure of the final dendrogram is based on these values, with a distance level of 14 used as a cutting level for separating clusters. This is shown in the dendrogram as a blank line between clusters. If the distance between strains adjacent to these "cut points" is less than 14, they are joined by a dashed line in the dendrogram at their actual distance value.

Results

Duration of incubation and reproducibility

Cluster analysis of the profiles obtained after incubation for 4 h resulted in a clustering pattern that did not correspond to the recognised DNA groups (data not shown). At 24 h, there was weak colour development in some wells that gave rise to doubtful interpretations on visual reading of the microplates. The 24-h readings by the plate reader were considered to give the most consistent and objective results, and these were used for further analysis.

The 129 strains were tested in 16 runs. One strain of DNA group 14 was included in every run for testing reproducibility. Numerical analysis of all strains linked the replicates of this strain in one cluster with the other strains of DNA group 14 (data not shown).

Cluster analysis

Cluster analysis of the oxidation profiles was performed with Biolog's software release 3.01A. Nine clusters (I–IX) were distinguished in the dendrogram (figure). Cluster I contained all strains of DNA groups 1 (*A. calcoaceticus*), 2 (*A. baumannii*), 3 and 13, except for the type strain (ATCC 23055) and one other strain of DNA group 1 (LMD 22.17). No subclusters corresponding to DNA groups 1, 2, 3 and 13 were distinguished within this cluster. Cluster II contained

Table I. Strains of *Acinetobacter* used in the study

Genomic species	Number of strains	Type and reference strains*
1 (<i>A. calcoaceticus</i>)	8	ATCC 23055 ^T , LMD 22.17
2 (<i>A. baumannii</i>)	36	CCUG 19096 ^T (= ATCC 19606), LMD 82.54, NCTC 7844, ATCC 17904
3	15	ATCC 19004, LMD 79.41
4 (<i>A. haemolyticus</i>)	7	ATCC 17906 ^T , LMD 70.9, Gilardi 2890
5 (<i>A. junii</i>)	6	ATCC 17908 ^T
6	2	ATCC 17979
7 (<i>A. johnsonii</i>)	9	ATCC 17909 ^T , LMD 90.19
8 (<i>A. lwoffii</i>)	13	NCTC 5866 ^T
10	3	ATCC 17924
11	7	CIP 63.46 (= ATCC 11171), LMD 81.109
12 (<i>A. radioresistens</i>)	7	FOR-1 ^T (= IAM 13186), SEIP 12.81
13	11	ATCC 17903
14	5	ATCC 17905

^T, denotes type strain.

*ATCC, American Type Culture Collection, Rockville, MD, USA; LMD Collection, Kluyver Laboratory of Biotechnology, Delft, The Netherlands; CCUG, Culture Collection, University of Göteborg, Göteborg, Sweden; NCTC, National Collection of Type Cultures, London; CIP, Collection de l'Institut Pasteur, Paris, France; IAM, Institute of Applied Microbiology, University of Tokyo, Tokyo, Japan; SEIP, Service des Entérobactéries de l'Institut Pasteur, Paris, France.

Figure. Dendrogram of numerical analysis of oxidation profiles of 129 *Acinetobacter* strains, as calculated by Biolog software release 3.01A. Vertical axis shows strains arranged by their DNA group, with type (T) and reference strains (table I) indicated at their appropriate locations. Clusters designated by Roman numerals are based on a "distance level" of 14, where a "distance level" essentially represents the number of tests with discrepant results between two strains (see text), as indicated by the scale at the top of the figure.

Table II. Differential characteristics for 13 DNA-DNA hybridisation groups of *Acinetobacter* based on 34 organic substrates tested in the Biolog system

Substrate	Percentage of strains giving positive reactions in each DNA group (number of strains tested) and cluster group												
	1	2	3	13	14	4	6	5	10	11	7	8	12
	(8) I	(36) I	(15) I	(11) I	(5) II	(7) III	(2) III	(6) IV	(3) V	(7) V	(9) VI	(13) VII	(7) VII
L-Arabinose	75	100	100	100	100	86	100	0	33	0	6	8	0
L-Fucose	25	0	0	0	0	7	0	0	100	0	0	0	0
D-Galactose	63	100	93	100	100	86	100	0	67	0	0	8	0
α -D-Glucose	88	100	100	100	100	86	100	0	0	0	0	8	0
D-Mannose	75	100	100	100	100	86	50	0	67	14	0	0	0
Citric acid	75	100	63	91	100	71	100	0	100	14	0	0	0
Formic acid	75	63	77	100	100	100	75	17	0	14	0	8	0
α -Hydroxybutyric acid	50	100	83	82	100	0	0	83	100	14	94	0	57
β -Hydroxybutyric acid	100	100	100	100	100	100	50	75	100	100	78	0	57
α -Ketobutyric acid	75	100	93	100	90	57	100	100	100	100	94	0	43
α -Ketoglutaric acid	37	43	30	9	80	86	100	58	100	100	39	0	79
α -Ketovaleric acid	88	100	100	100	40	57	100	0	0	0	33	0	43
D,L-Lactic acid	100	100	100	100	80	0	0	100	100	100	100	62	86
Malonic acid	100	100	100	27	60	0	0	0	17	29	22	0	86
Propionic acid	69	100	93	91	100	93	75	100	100	100	89	0	14
Quinic acid	100	100	87	100	100	100	100	0	67	86	56	0	0
D-Saccharic acid	25	86	100	0	0	0	0	0	0	0	11	0	0
Sebacic acid	75	94	100	100	40	21	0	0	100	100	22	73	86
Bromo succinic acid	100	100	100	100	100	100	100	100	100	100	100	19	0
Glucuronamide	69	100	100	82	90	86	100	0	33	0	0	8	0
D-Alanine	88	100	100	100	30	86	100	17	100	100	100	65	0
L-Asparagine	100	100	100	100	100	100	100	100	100	71	100	0	57
L-Aspartic acid	100	100	80	100	10	29	100	17	83	71	89	0	29
L-Histidine	100	100	100	100	100	86	100	67	100	100	0	0	0
Hydroxy L-proline	12	100	7	55	0	14	0	0	0	0	0	0	0
L-Leucine	75	100	87	100	40	86	100	33	0	0	6	0	29
L-Phenylalanine	100	94	80	100	100	0	0	0	0	0	0	0	43
L-Serine	75	42	27	27	20	86	100	33	67	25	0	0	0
L-Pyroglytamic acid	100	100	100	100	100	0	0	0	100	93	100	0	57
D,L-Carnitine	0	86	73	91	90	0	0	0	100	93	11	0	29
γ -Amino butyric acid	100	100	100	100	0	100	0	67	100	86	67	31	86
Urocanic acid	100	90	73	82	40	0	50	8	0	0	11	0	0
Putrescine	100	100	100	91	0	0	0	0	0	0	0	0	71
2-Amino ethanol	25	0	27	0	100	0	0	0	100	79	67	0	0

all five strains of DNA group 14. Cluster III comprised six of the seven strains of DNA group 4 (*A. haemolyticus*) and the two strains of DNA group 6. Cluster IV contained the remaining DNA group 4 strain and the six strains of DNA group 5 (*A. junii*). However, this DNA group 4 strain was linked to a DNA group 4 strain in cluster III at a higher similarity level than to the strains of DNA group 5. Cluster V comprised the three DNA group 10 strains and six of the eight DNA group 11 strains. Two small subclusters were distinguished within this cluster, one with five strains of DNA group 11, and the other with the three DNA group 10 strains. One strain of DNA group 11 was equally distant from these two subclusters. Cluster VI contained eight of the nine strains of DNA group 7 (*A. johnsonii*). Cluster VII contained all strains of DNA group 8 (*A. lwoffii*) and five strains of DNA group 12 (*A. radioresistens*). Two subclusters were distinguished, one with all DNA group 8 strains and the type strain of DNA group 12, and the other with four strains of DNA group 12. Cluster VIII comprised two strains of DNA group 1 (*A. calcoaceticus*), both of which, including the type strain of DNA group 1, were

isolated by Beyerinck early this century. Cluster IX contained two strains of DNA group 12. One strain of DNA group 7 and one strain of DNA group 11 remained unclustered.

Reactions of the 13 DNA groups with 95 carbon sources

Positive oxidation reactions according to the automatic reader for the 13 DNA groups with the 95 carbon sources were scored after incubation for 24 h. Tests were considered useful for discriminating DNA groups if there was a 100 % difference in the occurrence of positive reactions for strains from at least two groups. Thirty-four tests met this criterion (table II). Of the remaining 61 tests, two were positive for all strains and 16 were negative for all strains (table III). With 33 tests, positive reactions were obtained for between 0 and 71 % of strains, depending on the DNA group, while eight tests were positive with 8–100 % of strains. Two tests were negative for three and two strains respectively (table III).

Tests with the best capacity for discriminating

Table III. Tests not considered to discriminate between DNA groups*Positive for all strains*

Tween 40, Tween 80

*Negative for all strains*Glycogen, *N*-acetyl-D-galactosamine, *N*-acetyl-D-glucosamine, adonitol, D-arabitol, lactulose, maltose, D-psicose, D-raffinose, L-rhamnose, D-sorbitol, D-galactonic acid lactone, phenyl ethylamine, glycerol, D,L- α -glycerol phosphate, glucose-1-phosphate*Positive reactions for 0–71% of strains tested in each DNA group* α -Cyclodextrin, dextrin, cellobiose, *i*-erythritol, D-fructose, gentobiose, *m*-inositol, α -D-lactose, D-mannitol, D-melibiose, β -methyl-D-glucoside, sucrose, D-trehalose, turanose, xylitol, D-galacturonic acid, D-gluconic acid, D-glucosaminic acid, D-glucuronic acid, γ -hydroxybutyric acid, *p*-hydroxyphenylacetic acid, itaconic acid, alaninamide, L-alanyl glycine, glycyl-L-aspartic acid, glycyl-L-glutamic acid, L-ornithine, D-serine, L-threonine, inosine, uridine, thymidine, glucose-6-phosphate*Positive reactions for 8–100% of strains tested in each DNA group*Mono-methyl succinate, *cis*-aconitic acid, succinic acid, succinimic acid, L-alanine, L-glutamic acid, L-proline, 2,3-butanediol*Negative for a few strains*

Methylpyruvate*, acetic acid†

*Negative for two strains of DNA group 4 and one strain of DNA group 13.

†Negative for one strain of DNA group 1 and one strain of DNA group 12.

Table IV. Summary of test substrates used to discriminate DNA groups 1, 2, 3 and 13

Substrate	Percentage of strains giving positive reactions in each DNA group (number of strains tested)			
	1 (8)	2 (36)	3 (15)	13 (11)
Malonic acid	100	100	100	27
D-Saccharic acid	25	86	100	0
Hydroxy L-proline	12	100	7	55
D,L-Carnitine	0	86	73	91

Table V. Summary of test substrates used to discriminate DNA groups 8 and 12

Substrate	Percentage of strains giving positive reactions in each DNA group (number of strains tested)	
	8 (13)	12 (7)
α -Ketoglutaric acid	0	79
Malonic acid	0	86
L-Glutamic acid	8	100
Putrescine	0	71

between DNA groups 1, 2, 3 and 13 are summarised in table IV. The only test substrate that differentiated DNA group 3 from group 13 clearly was D-saccharic acid. DNA group 14 (cluster II; figure) linked closest to DNA groups 1, 2, 3 and 13 (cluster I), but could be

distinguished from these groups by between two and four tests (table II).

Between DNA groups 4 and 6 (cluster III; figure), oxidation of γ -amino butyric acid made a clear distinction (table II). Oxidation tests discriminating DNA groups 4 and 5 (cluster IV; figure) were D,L-lactic acid and quinic acid, but seven additional tests (L-arabinose, D-galactose, α -D-glucose, D-mannose, formic acid, α -hydroxybutyric acid and glucuronamide) showed 83–86% differences in the percentage of strains giving positive reactions, thereby adding to the distinction of these two groups (table II). DNA groups 10 and 11, linked in cluster V (figure) were separated by the L-fucose test. The citric acid and α -hydroxybutyric acid tests added to the discrimination of these groups as, in both cases, all DNA group 10 strains and only one (14%) of seven strains of DNA group 11 were positive (table II).

No tests distinguished DNA group 8 from DNA group 12 clearly. For α -ketoglutaric acid, malonic acid, L-glutamic acid and putrescine, the differences in the percentage of strains giving positive reactions were 79%, 86%, 92% and 71%, respectively (table V). Two strains of DNA group 12 were linked separately (cluster IX; figure). Cluster analysis was repeated on all strains excluding the two strains in cluster IX, but no improvement in separating DNA groups 8 and 12 was found (data not shown).

Identification to genus and DNA group level by Biolog software release 3.01A

Although grouping based on Biolog's "breathprints" was the primary purpose of the study, the data were also used to evaluate the then current database release (3.01A) of Biolog's software for its ability to identify the strains. Automated readings after incubation for 4 h showed correct identification to the genus level for 10% of strains, while no identification was given for 89% of strains. After incubation for 24 h, 109 (84.5%) of the 129 strains were identified as belonging to the genus *Acinetobacter* (table VI), and 58 (45%) of these strains were identified to the correct DNA group. Of the remaining 20 strains, one (0.8%) was wrongly identified to the genus *Psychrobacter* while no identification was reached for the remaining 19 strains (14.7%). Visual readings after incubation for 24 h identified 80.7% of the strains to the genus level and 45% to the correct DNA group, while no identification was reached for 14.7% of strains. Six strains (4.7%) were wrongly identified to other genera.

Discussion

Identification of oxidative micro-organisms by their catabolic enzymes was first described by den Dooren de Jong¹⁹ who, while studying the nutritional versatility of soil and water bacteria with over 200 organic compounds, distinguished separate groups among the

Table VI. Identification of 129 *Acinetobacter* strains by Biolog software release 3.01A

Level of identification	Number of strains of each DNA group identified to the level shown (number of strains tested)												
	1 (8)	2 (36)	3 (15)	4 (7)	5 (6)	6 (2)	7 (9)	8 (13)	10 (3)	11 (7)	12 (7)	13 (11)	14 (5)
Correctly identified to:													
genus and DNA group	1	14	0	4	2	1	6	6	2	5	2	10	5
genus only	6	20	15	1	1	1	1	5	0	0	0	1	0
Misidentified to other genus	0	0	0	0	1	0	0	0	0	0	0	0	0
No identification	1	2	0	2	2	0	2	2	1	2	5	0	0

aerobic pseudomonads that were useful for taxonomic purposes. Baumann *et al.*² used the same principle to characterise strains of the genus *Acinetobacter*. Following the identification of at least 17 DNA groups within the genus *Acinetobacter* by DNA-DNA hybridisation,^{4,5,7} utilisation of carbon sources has been studied as a tool for the possible phenotypic identification of the different groups.^{4,7,9-11} Although several DNA groups could be distinguished, this approach was unsatisfactory for discriminating the DNA groups that were closely related genetically.

The present study demonstrated that the Biolog system resulted in the clustering of DNA group 1 (*A. calcoaceticus*) with groups 2 (*A. baumannii*), 3 and 13 (cluster I); of DNA group 4 (*A. haemolyticus*) with group 6 (cluster III); of DNA group 10 with group 11 (cluster V); and in the identification of separate clusters for DNA groups 7 (cluster VI) and DNA group 14 (cluster II). These results were in overall agreement with previous DNA-DNA relatedness studies,^{4,5} except for the close linking of DNA groups 8 and 12 which has not been shown previously by DNA homology studies.

The discrimination of DNA groups 1, 2, 3 and 13 has been achieved by ribotyping,²⁰ but this method is beyond the skills of most routine laboratories. The separation of these DNA groups on the basis of utilisation of organic compounds does not seem possible at the moment.^{4,9,10} Growth at 44°C has been shown to distinguish DNA group 2 from DNA groups 1 and 3,^{4,9} and D-asparagine assimilation differentiates DNA group 1 from DNA groups 2 and 3.¹⁰ The present paper adds D-saccharic acid oxidation to the characteristics distinguishing DNA group 3 from 13. The two aberrant strains of DNA group 1 (*A. calcoaceticus*) in the present study (the type strain ATCC 23055 and strain LMD 22.17; cluster VIII, figure) were described in 1911 by Beyerinck. Both of these strains showed atypical results in the study of Baumann *et al.*,² and it has been suggested that cultivation of the strains under laboratory conditions for many years might have caused the acquisition of deviant traits.

None of the tests used in the study of Gerner-Smidt *et al.*⁹ separated DNA group 4 completely from DNA

group 6. The best distinguishing trait was found to be growth on 4-aminobutyrate (DNA group 4, 95%; DNA group 6, 0%). In the present study, oxidation of γ -amino butyric acid (= 4-aminobutyrate) separated the groups clearly, although only a limited number of strains of DNA group 4 were tested.

According to Bouvet and Grimont,⁴ DNA groups 10 and 11 can be separated by testing for glucose acidification. This finding was confirmed by Gerner-Smidt *et al.*⁹ and Kämpfer *et al.*,¹⁰ who also found growth at 37°C to be a distinguishing test. In the present study, oxidation of L-fucose was found to be a discriminating test. However, none of the strains used by Kämpfer *et al.*¹⁰ used this compound for growth. This discrepancy may be explained by the fact that the Biolog system tests for oxidation rather than growth.

DNA homology studies have not shown a close relationship between DNA groups 8 and 12. In the studies of Bouvet and Grimont⁴ and Gerner-Smidt *et al.*,⁹ groups 8 and 12 were easily separated phenotypically. However, Kämpfer *et al.*¹⁰ found the two groups to be related more closely to each other, and to the one strain of DNA group 15, than to any of the other DNA groups. The only discriminating test in their study, L-leucine assimilation, was positive for all DNA group 12 strains, whereas in the present study only 29% of DNA group 12 strains oxidised this substrate and no clearly discriminating tests were found for DNA groups 8 and 12. At best, four tests (α -ketoglutaric acid, malonic acid, L-glutamic acid and putrescine) showed differences in the percentage of strains from the two groups giving positive reactions of 71%–92%. The results obtained with L-phenylalanine, malonate, L-leucine (table II) and 2,3-butanediol (not shown) differed considerably from those of Bouvet and Grimont⁴ in that no discriminating potential was found in the present study. The type strain of DNA group 12 appeared to be closer phenotypically to the strains of DNA group 8 than to the strains of DNA group 12. Gerner-Smidt *et al.*⁹ also found that this strain behaved atypically.

In the present study, 109 (84.5%) strains were identified correctly by Biolog to the genus level, which is in accordance with the 82.8% finding of Knight *et al.*¹⁴ Of the 51 strains identified to an

incorrect DNA group, 42 belonged to DNA groups 1, 2, 3 or 13. Therefore, given the close relationship of these DNA groups, we support the suggestion of Tjernberg and Ursing⁵ and Gerner-Smidt *et al.*⁹ that these DNA groups should be linked in a "complex" to help microbiologists in their daily practice to identify strains unambiguously.

Phenotypic identification of *Acinetobacter* to the genomic species level is currently unsatisfactory, and the various systems described^{8,10} are not applied widely. Commercial systems are, in principle, within reach of any laboratory worldwide, and might, there-

fore, serve as a reference system. Construction of data bases founded on well-defined strains is of paramount importance for correct identification by these systems. The clustering pattern obtained in the present study indicates that incorporation of the data into the Biolog software will improve identification of *Acinetobacter* species by the system. The results also suggest that the Biolog system may be helpful in grouping strains as part of taxonomic investigations.

We thank Ms Tanny J. K. van der Reijden for technical assistance.

References

- Bergogne-Bérézin E, Joly-Guillou ML, Vieu JF. Epidemiology of nosocomial infections due to *Acinetobacter calcoaceticus*. *J Hosp Infect* 1987; **10**: 105-113.
- Baumann P, Doudoroff M, Stanier RY. A study of the *Moraxella* Group; II. oxidative-negative species (genus *Acinetobacter*). *J Bacteriol* 1968; **95**: 1520-1541.
- Henriksen SD. *Moraxella*, *Acinetobacter*, and the *Mimiae*. *Bacteriol Rev* 1973; **37**: 522-561.
- Bouvet PJM, Grimont PAD. 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 descriptions of *Acinetobacter calcoaceticus* and *Acinetobacter lwoffii*. *Int J Syst Bacteriol* 1986; **36**: 228-240.
- Tjernberg I, Ursing J. Clinical strains of *Acinetobacter* classified by DNA-DNA hybridization. *APMIS* 1989; **97**: 595-605.
- Nishimura Y, Ino Y, Iizuka H. *Acinetobacter radioresistens* sp. nov. isolated from cotton and soil. *Int J Syst Bacteriol* 1988; **38**: 209-211.
- Bouvet PJM, Jeanjean S. Delineation of new proteolytic genomic species in the genus *Acinetobacter*. *Res Microbiol* 1989; **140**: 291-299.
- Bouvet PJM, Grimont PAD. Identification and biotyping of clinical isolates of *Acinetobacter*. *Ann Inst Past/Microbiol* 1987; **138**: 569-578.
- Gerner-Smidt P, Tjernberg I, Ursing J. Reliability of phenotypic tests for identification of *Acinetobacter* species. *J Clin Microbiol* 1991; **29**: 277-282.
- Kämpfer P, Tjernberg I, Ursing J. Numerical classification and identification of *Acinetobacter* genomic species. *J Appl Bacteriol* 1993; **75**: 259-268.
- Soddell JA, Beacham AM, Seviour RJ. Phenotypic identification of non-clinical isolates of *Acinetobacter* species. *J Appl Bacteriol* 1993; **74**: 210-214.
- Miller JM, Rhoden DL. Preliminary evaluation of Biolog, a carbon source utilization method for bacterial identification. *J Clin Microbiol* 1991; **29**: 1143-1147.
- Klingler JM, Stowe RP, Obenhuber DC, Groves TO, Mishra SK, Pierson DL. Evaluation of the Biolog automated microbial identification system. *Appl Environ Microbiol* 1992; **58**: 2089-2092.
- Knight GC, McDonnell SA, Seviour RJ, Soddell JA. Identification of *Acinetobacter* isolates using the Biolog identification system. *Lett Appl Microbiol* 1993; **16**: 261-264.
- Dijkshoorn L, Tjernberg I, Pot B, Michel MF, Ursing J, Kersters K. Numerical analysis of cell envelope protein profiles of *Acinetobacter* strains classified by DNA-DNA hybridization. *Syst Appl Microbiol* 1990; **13**: 338-344.
- Tjernberg I, Lindh E, Ursing J. A quantitative bacterial dot method for DNA-DNA hybridization and its correlation to the hydroxyapatite method. *Curr Microbiol* 1989; **18**: 77-81.
- Bonting CFC, Willemsen BMF, Akkermans-van Vliet W, Bouvet PJM, Kortstee GJJ, Zehnder AJB. Additional characteristics of the polyphosphate-accumulating *Acinetobacter* strain 210A and its identification as *Acinetobacter johnsonii*. *FEMS Microbiol Ecol* 1992; **102**: 57-64.
- Sneath PHA. Computer taxonomy. In: Norris JR, Ribbons DW (eds) *Methods in microbiology*, vol 7A. London, Academic Press. 1972: 29-98.
- den Dooren de Jong LE. Bijdrage tot de kennis van het mineralisatieproces. (Thesis). Nijgh & van Ditmar's uitgevers Mij. Rotterdam 1926.
- Gerner-Smidt P. Ribotyping of the *Acinetobacter calcoaceticus-Acinetobacter baumannii* complex. *J Clin Microbiol* 1992; **30**: 2680-2685.