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# The rapid identification of *Acinetobacter* species using Fourier transform infrared spectroscopy

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## ABSTRACT

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**Aims:** Fourier transform infrared (FT-IR) was used to analyse a selection of *Acinetobacter* isolates in order to determine if this approach could discriminate readily between the known genomic species of this genus and environmental isolates from activated sludge.

**Methods and Results:** FT-IR spectroscopy is a rapid whole-organism fingerprinting method, typically taking only 10 s per sample, and generates 'holistic' biochemical profiles (or 'fingerprints') from biological materials. The cluster analysis produced by FT-IR was compared with previous polyphasic taxonomic studies on these isolates and with 16S–23S rDNA intergenic spacer region (ISR) fingerprinting presented in this paper. FT-IR and 16S–23S rDNA ISR analyses together indicate that some of the *Acinetobacter* genomic species are particularly heterogeneous and poorly defined, making characterization of the unknown environmental isolates with the genomic species difficult.

**Conclusions:** Whilst the characterization of the isolates from activated sludge revealed by FT-IR and 16S–23S rDNA ISR were not directly comparable, the dendrogram produced from FT-IR data did correlate well with the outcomes of the other polyphasic taxonomic work (E.L. Carr, P. Kämpfer, B.K.C. Patel, V. Gürtler and R.J. Seviour, 2003, *International Journal of Systematic and Evolutionary Microbiology* **53**, 953–963).

**Significance and Impact of the Study:** We believe it would be advantageous to pursue this approach further and establish a comprehensive database of taxonomically well-defined *Acinetobacter* species to aid the identification of unknown strains. In this instance, FT-IR may provide the rapid identification method eagerly sought for the routine identification of *Acinetobacter* isolates from a wide range of environmental sources.

**Keywords:** 16S rDNA sequencing, *Acinetobacter*, Fourier transform infrared spectroscopy.

## INTRODUCTION

The important role that many of the genomic species of *Acinetobacter* play in nosocomial infections, together with their ability to develop resistance to many of the commonly used antibiotics (Towner 1997) makes rapid, reliable epidemiological typing of these organisms a necessity. Typing not only allows differentiation between epidemic and sporadic strains, but can also provide valuable epidemiological information about the geographical spread and pathogenicity of particular strains of interest (Dijkshoorn 1996). This information may prove invaluable in developing strategies to prevent the spread of infections caused by *Acinetobacter* species. For this reason, a number of phenotypic and genotypic techniques have been applied to characterize and type clinical isolates of *Acinetobacter* species, with a view to identifying them reliably at the subspecies level. Unfortunately, a single technique is not universally accepted or applied in clinical laboratories for the rapid identification of strains, because the majority of the techniques produce different classifications. For example, Vila *et al.* (1994) used

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four typing methods to characterize two strains of *Acinetobacter baumannii* and strains of *Acinetobacter* isolated from an infectious outbreak. All the strains were previously demonstrated to have the same ribotype. However, the four typing methods indicated the strains to be quite different to each other. Thus a polyphasic taxonomic approach (Vandamme *et al.* 1996) should be adopted where a combination of typing methods (including at least one molecular method), are used before indistinguishable isolates are designated as the same strain (Dijkshoorn 1996). Some molecular techniques used for typing *Acinetobacter* strains are claimed to provide reproducible and discriminatory results (e.g. ribotyping, pulsed-field gel electrophoresis and amplified fragment length polymorphisms). However, these methods are laborious, slow, expensive and often require considerable technical expertise. Therefore, they are deemed unattractive for routine application in busy clinical laboratories. Consequently, it would be very advantageous to have a rapid typing method, which provides the reliable identification of both clinical and environmental strains of *Acinetobacter*. It is considered that this would greatly assist in the understanding of the ecology of members of this genus.

Whole-organism fingerprinting (Magee 1993; Goodacre *et al.* 1998) by physico-chemical spectroscopic methods such as pyrolysis mass spectrometry (PyMS; Goodacre and Kell 1996), Fourier transform infrared spectroscopy (FT-IR; Helm *et al.* 1991; Naumann *et al.* 1991a,b) and Raman spectroscopy (Maquelin *et al.* 2002) seem to represent attractive alternative identification methods, because they do not demonstrate the problems associated with genotypic typing methods. That is to say, they are rapid, require minimal sample preparation, are relatively inexpensive and can be readily automated to handle large sample numbers (Goodacre *et al.* 1998). Moreover, Stackebrandt *et al.* (2002) especially highlighted these techniques as developments of particular interest in prokaryotic systematics.

PyMS and FT-IR spectroscopy both measure the phenotype of an organism and provide a 'snap-shot' of its expressed genotype (Timmins *et al.* 1998a). Unlike PyMS which predominantly measures the bond strengths of molecules, FT-IR spectroscopy measures vibrations of functional chemical groups and highly polar bonds, therefore, the 'fingerprints' generated by this method consist of the vibrational features of all the cell components (e.g. DNA, RNA, proteins and lipids; Naumann *et al.* 1994). This method enables the generation of distinct chemical fingerprints of micro-organisms, without their destruction, and appears to have sufficient resolving power to distinguish

**Table 1** Details of the known genomic species used in this study

| Abbreviation used in study | Species name | Origin/Culture Collection numbers |
|---|---|---|
| BG1 | *Acinetobacter calcoaceticus* | ATCC 23055T/CIP 81·08T |
| BG2 | *Acinetobacter baumannii* | ATCC 19606T/CIP 70·34T |
| BG3 | *Acinetobacter* sp. 3 | ATCC 19004/CIP 70·29 |
| BG4 | *Acinetobacter haemolyticus* | ATCC 17906T/CIP 64·3T |
| BG5 | *Acinetobacter junii* | ATCC 17908T/CIP 64·5T |
| BG6 | *Acinetobacter* sp. 6 | ATCC 17979/CIP A165 |
| BG7 | *Acinetobacter johnsonii* | ATCC 17909T/CIP64·6T |
| BG8 | *Acinetobacter lwoffii* | ATCC 17925T/CIP 64·10T |
| BG9 | *Acinetobacter* sp. 9 | ATCC 9957/CIP 70·31 |
| BG10 | *Acinetobacter* sp. 10 | ATCC 17924/CIP 70·12 |
| BG11 | *Acinetobacter* sp. 11 | ATCC 11171/CIP 63·46 |
| BG12 | *Acinetobacter radioresistens* | SEIP 12·81 |
| BJ13 | *Acinetobacter* sp. 13 | ATCC 17905/CIP 64·2 |
| BJ14 | *Acinetobacter* sp. 14 | K.Irino 105/85 |
| BJ15 | *Acinetobacter* sp. 15 | M.M. Adam Ac606 180 : 40va |
| BJ16 | *Acinetobacter* sp. 16 | ATCC 17988/CIP 70·18 |
| BJ17 | *Acinetobacter* sp. 17 | SEIP Ac87·314 |
| TU13(ATTC) | *Acinetobacter* sp. 13 | ATCC 17903 |
| TU13 (165) | *Acinetobacter* sp. 13 | Lund University |
| TU14 | *Acinetobacter* sp. 14 | ATCC 151a1 |
| TU15 | *Acinetobacter* sp. 15 | ATCC 71 |
| CTTU13 | 'Close to TU13' | Statens Serum Institut |
| B/W1+3 | 'Between BG1 and BG3' | Statens Serum Institut |

ATCC, American Type Culture Collection (Maryland, USA); CIP, Collection de l'Institut Pasteur (Paris); SEIP, Collection du Service des Enterobacteries, Institut Pasteur (Paris).

microbes to strain level (Helm *et al.* 1991; Naumann *et al.* 1991b; Sandt *et al.* 2003). Indeed, it has sufficient resolution power to distinguish between single-gene knock-out mutants in yeast (Oliver *et al.* 1998).

By contrast to FT-IR, PyMS has been shown to suffer from lack of reproducibility because of instrument drift problems (Shute *et al.* 1988; Goodacre and Kell 1996). Coupled with the findings that PyMS and FT-IR give very similar microbial classifications (Timmins *et al.* 1998a,b), we believe that FT-IR is a particularly robust and reproducible whole-organism fingerprinting method. The aim of the present study was to apply FT-IR spectroscopy to the characterization of a selection of *Acinetobacter* isolates in order to determine if this approach could discriminate readily between the known genomic species of this genus and environmental isolates from activated sludge. The results from the FT-IR analysis were compared with those obtained using the molecular techniques of 16S–23S rDNA intergenic spacer region (ISR) fingerprinting.

## MATERIALS AND METHODS

### Cultivation of *Acinetobacter* isolates for FT-IR spectroscopy

The strains for the FT-IR analysis were selected in order to represent all the known genomic species of *Acinetobacter* with the exception of the recently described species *A. schindleri* and *A. ursingii* (Nemec *et al.* 2001; Table 1). In addition, 43 isolates from activated sludge were included in this study, including the seven newly described species (Carr *et al.* 2003) detailed in Table 2. The selected isolates were previously analysed by PyMS (Carr *et al.* 2001a).

All strains were cultured on R2A medium (Reasoner and Geldreich 1985) and incubated at 30°C for 24 h. Isolates were subcultured three times in triplicate before FT-IR analysis was performed, in order to minimize possible phenotypic variation.

### FT-IR analysis of *Acinetobacter* isolates

Bacterial cells of each isolate were harvested from agar plates by washing them with 400 μl physiological saline (0·9% NaCl) and were gently mixed to produce an emulsion, which was stored on ice until required. A 10 × 10-cm aluminium plate was rinsed with acetone and dried at 50°C for 10 min. FT-IR analysis was initially performed on the plate without samples to provide a reference reading for each well. The plate was loaded onto the motorized stage of a reflectance thin-layer chromatography accessory (Glauninger *et al.* 1990; Bouffard *et al.* 1994; Goodacre *et al.* 1996; Winson *et al.* 1997), attached to a Bruker IFS28 FT-IR spectrometer (Bruker Spectrospin Ltd, Conventry, UK). This was equipped with a mercury–cadmium–telluride (MCT) detector, which was cooled with liquid $N_2$. A 10-μl aliquot of each sample was evenly applied in triplicate onto the 100-well plate (so called machine replicates). The plate was then

**Table 2** The activated sludge isolates of *Acinetobacter* investigated in this study along with their Biolog identifications

| *Acinetobacter* strain name | Biolog identification | Culture collection number |
|---|---|---|
| 7B02 | UN | DSMZ 14966/CIP 107466 |
| AB2010 | BG5 | NA |
| AB2104 | BG5 | NA |
| 2B07 | BG7 | NA |
| 6A07 | BG7 | NA |
| M10/15 | BG5 | NA |
| C5 | UN | DSMZ 14963/CIP 107473 |
| 4B02 | UN | DSMZ 14964/CIP 107468 |
| 5N13 | UN | NA |
| 3A02 | UN | NA |
| 6A05 | UN | NA |
| 12A02 | UN | NA |
| 21B02 | UN | NA |
| 17A04 | UN | DSMZ 14968/CIP 107470 |
| A7 | UN | DSMZ 14959/CIP 107476 |
| 1SRO6 | BG7 | NA |
| C1 | UN | NA |
| 9B03 | BG7 | NA |
| 26N03 | UN | NA |
| 5B02 | BG9 | DSMZ 14965/CIP 107467 |
| 7N16 | UN | DSMZ 14971/CIP 107465 |
| 5N03 | BG2 | NA |
| AB3316 | BG5 | NA |
| AB1030 | BG5 | NA |
| 6N01 | BG7 | NA |
| 1A08 | BG7 | NA |
| 11N04 | BG7 | NA |
| 9A01 | UN | DSMZ 14967/CIP 107464 |
| 11A04 | BG7 | NA |
| 22B02 | BG12 | NA |
| 26B02 | BG12 | NA |
| 2N01 | BG12 | DSMZ 14969/CIP 107471 |
| 6N03 | BG7 | NA |
| A23 | UN | DSMZ 14960/CIP 107475 |
| D11 | UN | NA |
| 25A01 | BG7 | NA |
| AB1141 | BG8/9 | NA |
| AB1110 | BG7 | DSMZ 14962/CIP 107472 |
| 19A01 | BG7 | NA |
| AB1160 | BG5 | NA |
| 4N13 | UN | DSMZ 14670/CIP 107469 |
| B2 | UN | DSMZ 14961/CIP 107474 |
| J22/15 | BG10 | NA |

UN, unidentified; NA, not applicable; DSMZ, Deutsche Sammlung von Mikroorganismen und Zellkulturen; CIP, Collection de l'Institut Pasteur, Paris.

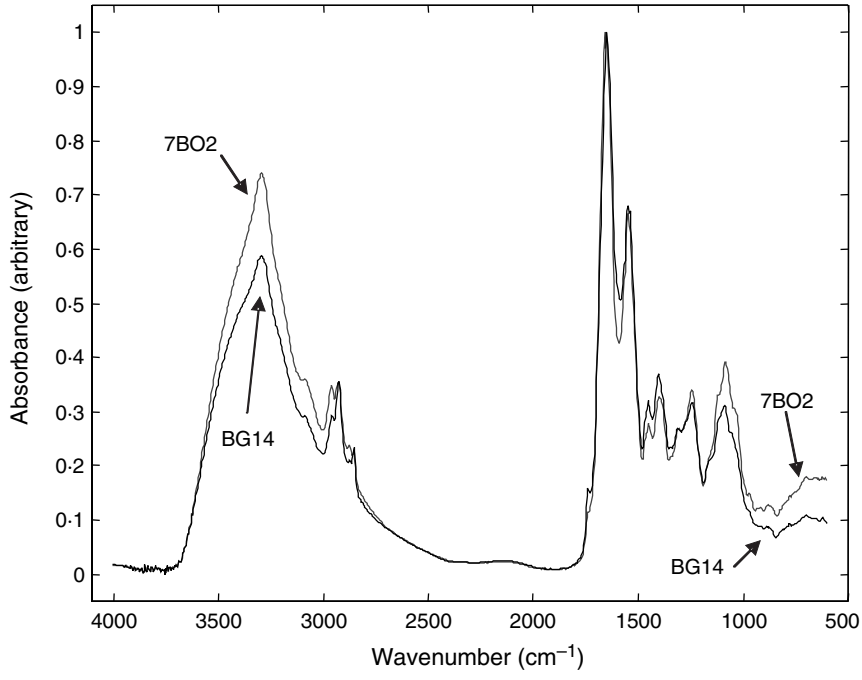


**Fig. 1** Typical FT-IR spectra of both the environmental (7B02) and known genomic species (BG14) of *Acinetobacter*

dried at 50°C for 30 min before FT-IR analysis was performed. Spectra were collected over the wavelength range of 4000 to 600 cm$^{-1}$ under the control of an IBM-compatible computer (Dell Computer Corporation, Bracknell, UK) programmed with Opus 2.1 running under IBM OS/2 Warp, which was provided by the manufacturers. Spectra were acquired at a rate of 20 s$^{-1}$, with a resolution of 4 cm$^{-1}$, so that each sample represents a spectrum containing 882 data points. To improve the signal-to-noise ratio, 256 spectra were co-added and averaged. The spectra are displayed in terms of absorbance (see Fig. 1 for typical spectra), which was calculated from the reflectance–absorbance spectra using OPUS software (Bruker Spectrospin Ltd).

The ASCII data were imported into Matlab ver. 5 (The MathWorks, Inc., Natick, MA, USA). To minimize problems arising from baseline shifts Matlab was used to correct for $CO_2$ vibrations (the $CO_2$ peaks at 2403–2272 cm$^{-1}$ and 683–656 cm$^{-1}$ were removed, and filled with a trend) and the data were normalized such that the smallest recorded absorbance was set to 0 and the highest was set to 1 for each spectrum. Principal components analysis (PCA; Jolliffe 1986) was performed on the spectra to reduce the dimensionality of the multivariate data prior to discriminant function analysis (DFA). DFA discriminates between groups on the basis of the retained principal components with *a priori* knowledge of which spectra were replicates (MacFie *et al.* 1978; Windig *et al.* 1983) and minimizes 'within group' variance and maximizes 'between group' variance. As this was based on the machine replicates this does not bias the analysis in any way. Finally, hierarchical cluster analysis (HCA) was used to construct a dendrogram from the *a priori* group centres in DFA space using Euclidean distances as described by Goodacre *et al.* (1998) and the dendrogram was produced using average linkage clustering algorithm (Manly 1994).

## RESULTS

All samples gave reproducible characteristic biological infrared absorption spectra (see Fig. 1 for examples). However, the spectra and all the others collected have complex and broad contours with little qualitative difference between them, although on closer inspection quantitative differences can be observed. Such spectra, essentially un-interpretable by the naked eye, demonstrate the need to use multivariate statistical methods for the analyses of these data.

DFA was performed on these spectra and the resultant plot of discriminant function (DF) 1 vs DF 2 is given in Fig. 2. The relationship between the known genomic species strains is observed more readily when only the *a priori* group centres in the DFA space are plotted (Fig. 2b) and some clustering is observed. For example, the strains representing the genomic species TU13, TU14 and TU15 of Tjernberg and Ursing (1989) cluster together, as do strains BJ14 and BJ17 of Bouvet and Jeanjean (1989), however, the strains of BJ15 and BJ16 cluster elsewhere. The strains of the 12 genomic species described by Bouvet and Grimont (1986) are recovered in several separate clusters in the DFA plot. Thus whilst the strains of BG2,

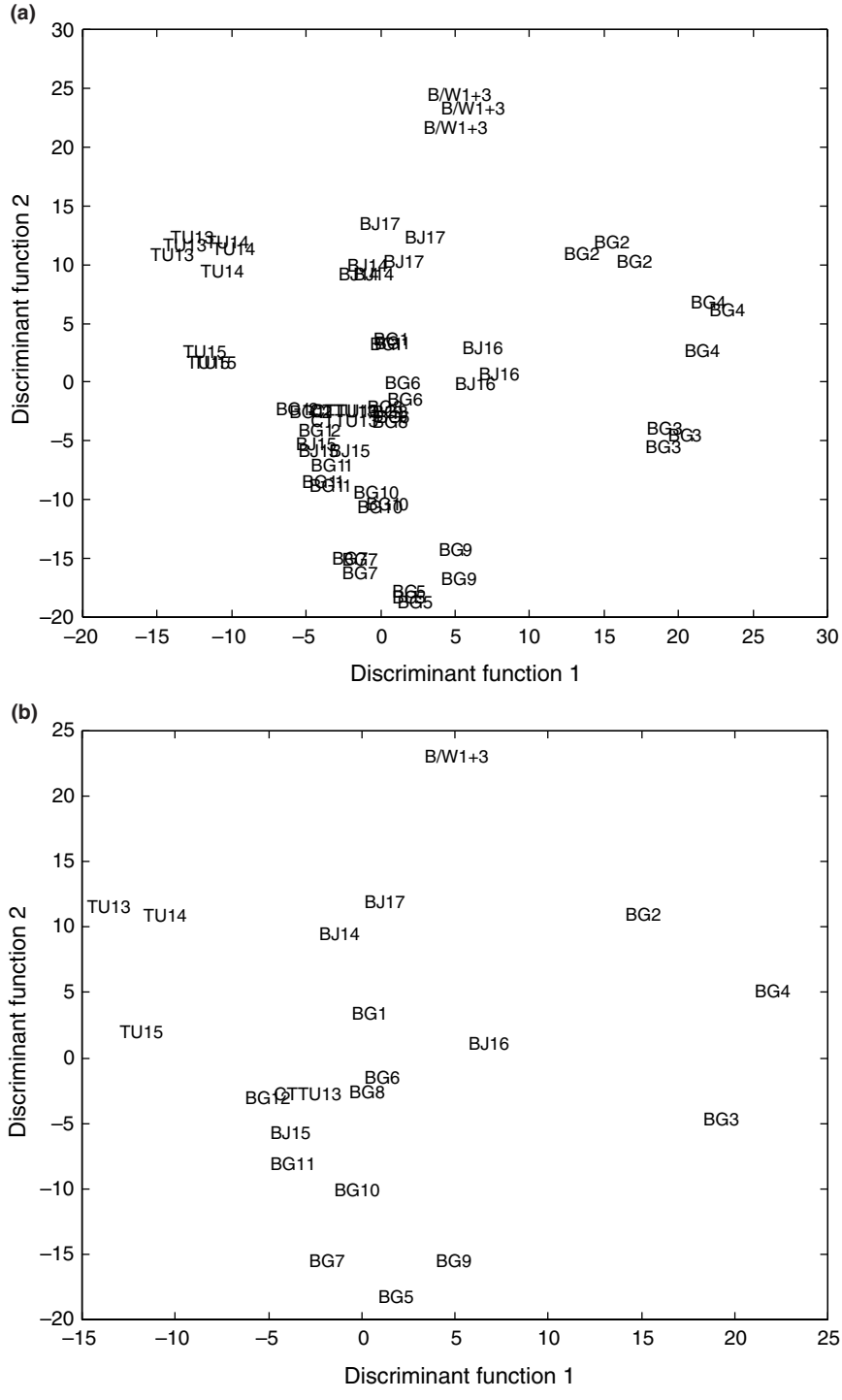


**Fig. 2** Discriminant function analysis plot based on FT-IR data of the known genomic species of *Acinetobacter*. (a) The three machine replicates are plotted. (b) The group centres of the machine replicates are plotted

BG3 and BG4 are recovered together, as are BG5, BG7 and BG9, strains of the other BG genomic species (BG1, BG6, BG8, BG10, BG11 and BG12) are dispersed through the DFA cluster space.

Fourty-three activated sludge isolates of *Acinetobacter* were selected for analysis by FT-IR. The vast majority of the environmental isolates and genomic species clustered in one large group with several outliers (Fig. 3). The relationships between the strains are perhaps better illustrated using HCA analysis of the FT-IR data (Fig. 4). In general, the majority of the activated sludge isolates failed to cluster closely with strains of the known genomic species after

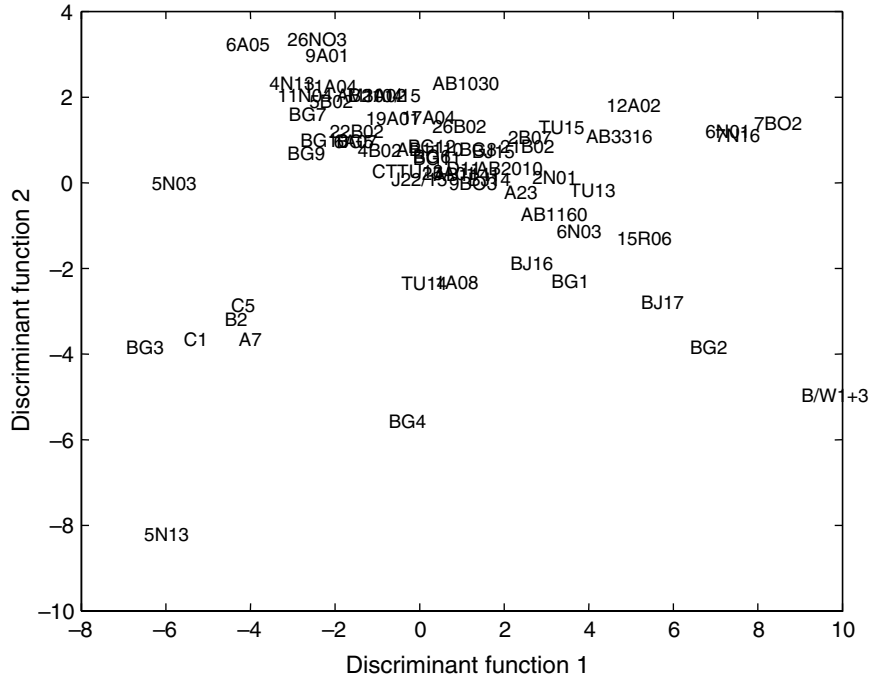


**Fig. 3** Discriminant function analysis plot based on FT-IR data of both environmental isolates and known genomic species of *Acinetobacter*

FT-IR analysis. However, some exceptions were observed. For example, strains BG5 and 22B02, BG7 and 11N04, and BG11 and AB1110 all cluster very closely together and BG3 clusters with the four environmental isolates B2, A7, C5 and C1.

## DISCUSSION

### Analysis of the known genomic species of *Acinetobacter* by FT-IR spectroscopy

These findings from FT-IR analysis do not wholly agree with those suggested from earlier DNA–DNA hybridization data (Bouvet and Grimont 1986; Bouvet and Jeanjean 1989; Tjernberg and Ursing 1989). For example, FT-IR failed to group BG8 and BG9 together, whilst DNA–DNA hybridization illustrated the two samples to be taxonomically very similar (Bouvet and Grimont 1986; Tjernberg and Ursing 1989). The FT-IR analysis indicates that the 12 genomic species described by Bouvet and Jeanjean (1989) to be heterogenous because they are recovered throughout the DFA space. However, separation of the two genomic BG 8 and BG9 by FT-IR was not surprising, because they can also be distinguished from each other when characterized by other techniques (Wiedmann-Al-Ahmad *et al.* 1994; Carr *et al.* 2001a,b), hence, reinforcing the accepted problems using DNA–DNA hybridization as a method for determining speciation in bacteria (Stackebrandt *et al.* 2002). Likewise, strains of the genomic species BG1, BG2, BG3, TU13, CTTU13 and B/W1+3 belonging to the *Acb* complex (Gerner-Smidt *et al.* 1991) did not cluster together by FT-IR analysis, by contrast 16S–23S rDNA fingerprinting (Carr *et al.* 2001a) groups all these species closely together, with the exception of TU13. However, both methods grouped TU13 with TU14 (Fig. 2b).

Characterization of the known genomic species of *Acinetobacter* by PyMS indicated that BG1 and BG2 had very similar fingerprints. However, a direct comparison between the PyMS and FT-IR data is complicated by the omission of some members of the *Acb* complex in the PyMS analysis (Carr *et al.* 2001a). The phenotypic similarity of the organisms belonging to this complex is well documented (e.g. Gerner-Smidt *et al.* 1991) and causes great difficulties in the typing of this complex by clinical laboratories. FT-IR and PyMS analysis (Carr *et al.* 2001a) grouped BJ14 and BJ17 together. However, strains of the other proteolytic genomic species (BJ15 and BJ16) of Bouvet and Jeanjean (1989) were recovered separately. The 16S–23S rDNA ISR fingerprinting (Carr *et al.* 2001a) and 16S rDNA sequencing (Ibrahim *et al.* 1997) grouped BJ15, BJ16 and BJ17 together, but not BJ14. Strains BG10 and BG11 were recovered in the same cluster as BJ15 by FT-IR analysis, this may be expected from DNA–DNA hybridization data, which indicates that BG11 is more similar to BJ15. These observations are perhaps not surprising when considering the findings from the genomic fingerprinting methods (Wiedmann-Al-Ahmad *et al.* 1994; Vaneechoutte *et al.* 1995; Janssen *et al.* 1997).

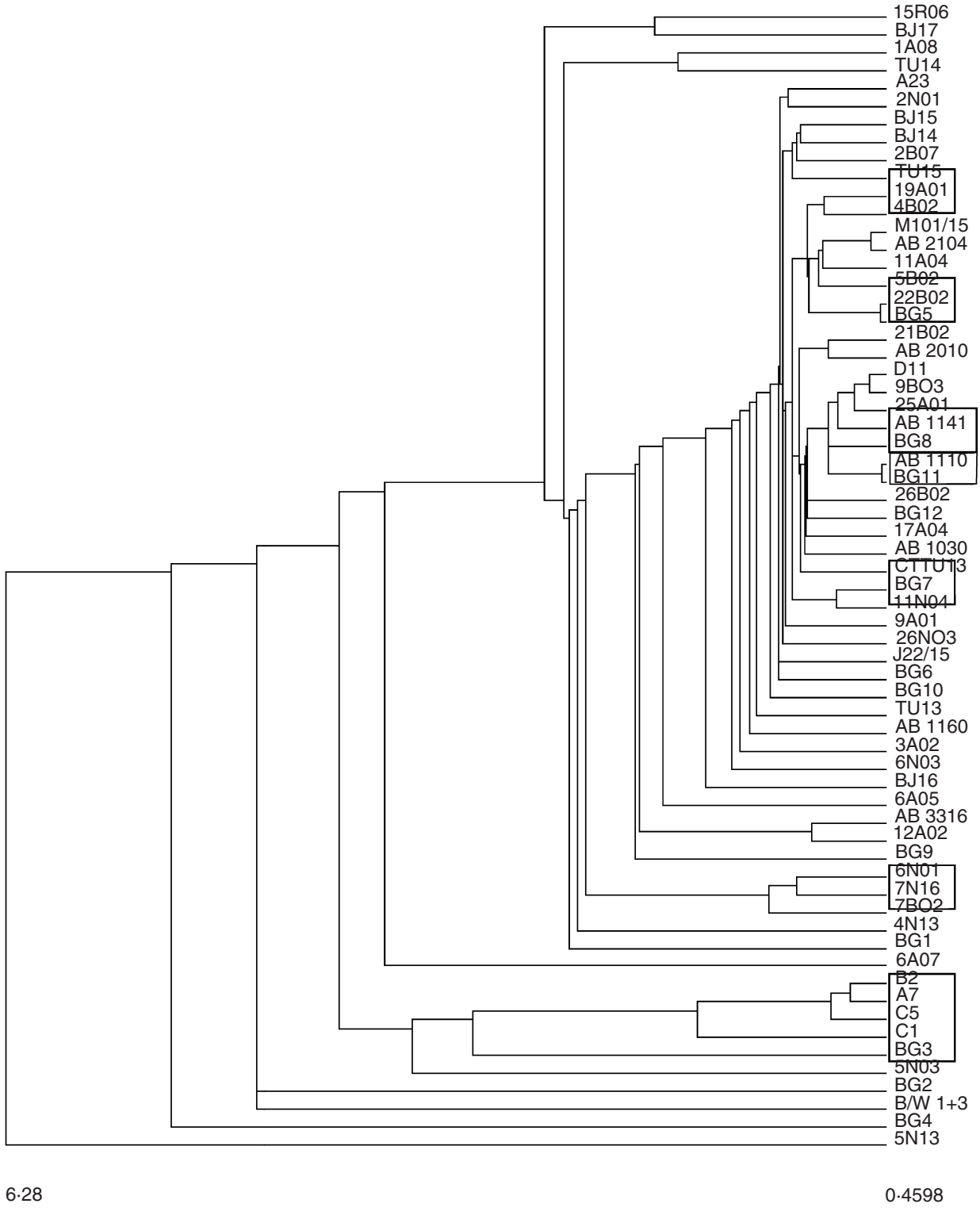


**Fig. 4** Dendrogram based on FT-IR data showing the relationships between the environmental and known genomic species of *Acinetobacter* generated by hierarchical cluster analysis

**Table 3** Comparison of various techniques to identify a sub-set of *Acinetobacter* strains

| | Characterization technique | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | PyMS* | | | | | | |
| Reference | 16S–23S rRNA ISR | Run A | Run B | Run C | FT-IR | RAPD-PCR† | DNA–DNA‡ | 16S rRNA‡ |
| BG3 | A | A | | | E | B2 | A2 | |
| BG8 | A | D | | | **A** | B2 | | |
| BG10 | A | | | | | B1 | A2 | **C1** |
| BG11 | A | B | | | **A** | A1 | B2 | **C1** |
| A23 | A | | | | | A1 | B4 | **C1** |
| D11 | A | | | | **A** | A2 | | |
| BG5 | B1 | B | | | B | D | | |
| 25A01 | B1 | G | | | A | | | |
| BJ13 | B1 | B | | | | B2 | | **D2** |
| BJ15 | B1 | E | | | C | A2 | | **D2** |
| BJ16 | B1 | C | | | | B1 | B4 | **D2** |
| 6N01 | B1 | F | | | **D** | | | |
| TU15 | B1 | | | | C | B2 | A3 | C2 |
| 4N13 | B1 | | C | | | | B3 | **D2** |
| 7N16 | B1 | | **D** | | **D** | | | **D2** |
| 7B02 | B1 | | **D** | | **D** | | | **D2** |
| BJ17 | B2 | | | | | | B4 | D2 |
| TU13 | B2 | | | | | A2 | **A3** | C2 |
| TU14 | B2 | | | | | D | **A3** | |
| BG12 | B2 | D | | | A | B2 | | |
| AB2104 | C | | B2 | | B | | | |
| 2B07 | C | G | | | C | | | |
| 11A04 | C | C | | | B | | | |
| 9B03 | C | F | | | A | | | |
| AB1110 | D | F | | | A | | B1 | C1 |
| A23 | D | E | | | B | | A1 | |
| AB1141 | E | | | **F** | **A** | | | |
| 22B02 | E | | | E | B | | | |
| 17A04 | E | | | **F** | **A** | | B4 | C2 |
| BG7 | F | A | | | | B2 | | |
| 5B02 | F | | | B1 | B | | B1 | C2 |
| C5 | G | | | | **E** | **C2** | **A1** | **C1** |
| B2 | G | | | | **E** | **C1** | **A1** | **C1** |
| A7 | G | | | | **E** | **C1** | **A1** | **C1** |
| C1 | G | | | | **E** | **C2** | | |
| BG9 | | B | | | | B2 | | B |
| BJ14 | | | | | C | B2 | | D2 |
| 4B02 | | | | | B | | B4 | D1 |

Characters in bold indicate a correlation of clustered strains between the individual technique and 16S–23S rRNA ISR.
*Run A, B and C refers to three samples run during the acquisition of PyMS data; Carr *et al.* (2001a).
†Carr *et al.* (2001b).
‡Carr *et al.* (2003).

## Characterization of activated sludge isolates of *Acinetobacter* by FT-IR spectroscopy

The activated sludge isolates for the study were selected on the basis of the PyMS screening and 16S–23S rDNA ISR fingerprinting (Carr *et al.* 2001a). The clustering pattern observed in the DFA resembles that observed in the ball and stick diagrams from the PyMS analysis of these activated sludge isolates previously reported (Carr *et al.* 2001a), in which the vast majority of the strains clustered in one large group, but with several outliers. The majority of strains could not be confidently assigned

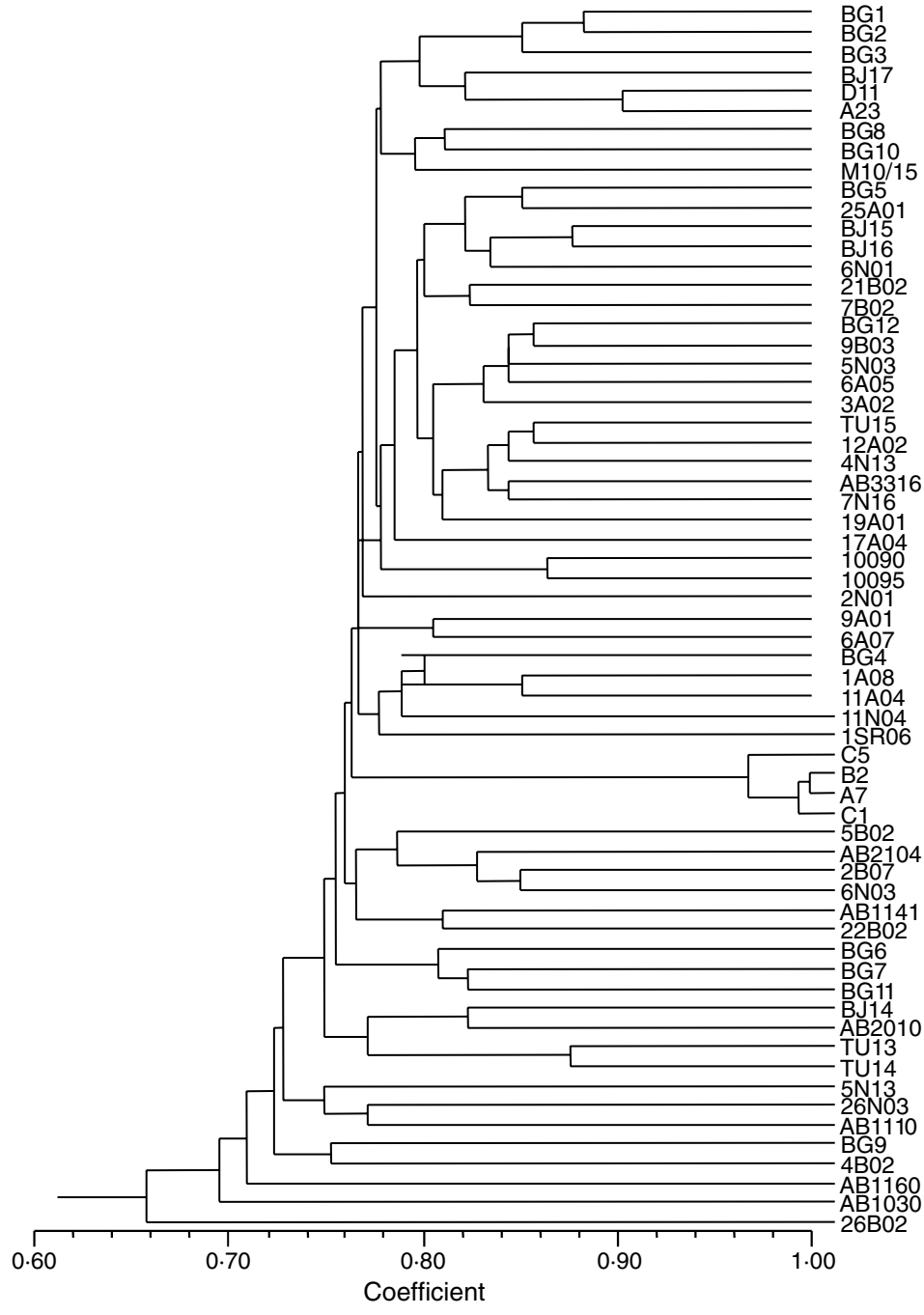


**Fig. 5** Dendrogram of the environmental and known genomic species of *Acinetobacter* based on data from fingerprinting of the 16S–23S rRNA ISR generated using the UPGMA algorithm

to a particular DNA group based on the DFA, because of the lack of clustering with the known genomic species. However, in general, the FT-IR data appear to support the findings based on the polyphasic taxonomic approach adopted by Carr *et al.* (2003), to characterize these strains further. Isolates A7, C5 and B2 are all strains of *A. baylyi*, as proposed by the polyphasic study and they form a very distinct group on the basis of the FT-IR data. This taxonomic relationship is supported by the data from 16S–23S rDNA ISR fingerprinting, 16S rDNA sequence data and DNA–DNA hybridization data (Table 3; Carr *et al.* 2001a, 2003). The FT-IR analysis clusters BG3 with the

three strains of *A. baylyi*, this may imply that BG3 belongs to the species *A. baylyi*, however, results from the 16S–23S ISR, PyMS, RAPD-PCR and DNA–DNA hybridization (Carr *et al.* 2001a,b; Carr *et al.* 2003) fail to support this observation. Strains designated 7B02 and 7N16 occur in the same FT-IR cluster this supports the polyphasic taxonomic study of Carr *et al.* (2003), in which both strains were assigned to the new genomic species, *A. tjernbergiae*.

Strains 9A01, 17A04, 4N13 and 4B02 have also been placed in four newly described genomic species (Carr *et al.* 2003) and, FT-IR agrees with these findings. Moreover, in the present study FT-IR also groups new activated sludge isolates with these genomic species (e.g. 19AO1 and 4BO2), suggesting that there may be additional members for further characterization. Interestingly, strains AB1110 and 2N01 which were shown to belong to the same genomic species (*A. towneri*) on the basis of DNA–DNA hybridization and 16S rDNA sequencing (Carr *et al.* 2003) but were not grouped together by FT-IR analysis. In fact, strain AB1110 clusters very closely with BG11. Strain 5B02 was considered to belong to *A. johnsonii* from DNA–DNA hybridization data (Carr *et al.* 2003), the FT-IR analysis clusters this isolate with the type strain of *A. johnsonnii* (ATCC 17909T).

Table 2 gives the Biolog identifications of these environmental isolates. There was very little agreement observed between the Biolog designations and the position of the environmental isolates in the dendrogram produced from the FT-IR data (Fig. 4). Numerical taxonomic analysis of the 16S–23S rDNA ISR fingerprints of the same set of isolates analysed by FT-IR are presented in Fig. 5, allowing a direct comparison of inter-strain relationships revealed with the two methods and Table 3 shows comparisons between various techniques used to characterize a subgroup of test strains. With the exception of the distinct cluster containing *A. baylyi* C5, B2 and A7 with strain C1 (whose identity is unknown; Figs 4 and 5, Table 3), the relationships from 16S–23S rDNA ISR fingerprinting failed to agree with any of the previous polyphasic taxonomic conclusions (Carr *et al.* 2003) or the other studies characterizing the *Acinetobacter* (Table 3; Carr *et al.* 2001a,b, 2003).

### Concluding remarks

The clustering illustrated by the FT-IR analysis showed limited correlation with DNA–DNA hybridization, in terms of the interspecies relationships between the known genomic species. In the majority of examples there is limited correlation between the various techniques used to identify a subset of *Acinetobacter* (Table 3). It is generally accepted that FT-IR is a very powerful method for discriminating bacteria at the species to subspecies levels (Naumann *et al.* 1991a; Schmitt and Flemming 1998; Maquelin *et al.* 2002). Therefore, it is possible that these discrepancies come from an inadequacy in the *definitions* of these genomic species of *Acinetobacter*, which are poorly represented by multiple strains. Indeed, some genomic species are clearly very heterogeneous and possibly comprised of multiple species. In particular the BG genomic species are diverse and from both FT-IR analyses (Fig. 2b) and 16S–23S rDNA ISR fingerprinting (Fig. 5) emerge as widely dispersed. It is likely that these attempts at finding rapid identification methods for members of this genus will be improved when multiple strains of each carefully defined genomic species were included in the analyses.

When FT-IR was applied to the selected activated sludge isolates, the data obtained from some species correlated well with the outcomes of earlier polyphasic taxonomic work with these strains (Carr *et al.* 2003). It is considered important to pursue this technique further as a rapid typing tool for bacteria, as suggested by Stackebrandt *et al.* (2002). It would be advantageous to establish a *comprehensive* database of known distinct *Acinetobacter* species to aid in the identification of unknown strains, similar to that established and evaluated successfully for coryneform bacteria (Oberreuter *et al.* 2002) in which it would be advantageous to include a greater number of strains for each species. In this instance FT-IR may provide the rapid identification method eagerly sought for the routine identification of *Acinetobacter* isolates from a wide range of habitats.

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