Acinetobacter parvus sp. nov., a small-colony-forming species isolated from human clinical specimens

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The taxonomic status of seven glucose-non-acidifying, non-proteolytic Acinetobacter strains characterized by forming small colonies on agar media was studied. With one exception, all strains were from human specimens. They could be distinguished from all described Acinetobacter (genomic) species by their ability to grow on ethanol and acetate as sole sources of carbon but not on 22 other substrates tested including DL-lactate or DL-4-aminobutyrate. DNA–DNA hybridization studies, 16S rRNA gene sequence analysis, amplified rDNA restriction analysis and DNA polymorphism analysis by AFLP showed that these strains represent a hitherto unknown species of the genus Acinetobacter, for which the name Acinetobacter parvus (type strain LMG 21765T = LUH 4616T = NIPH 384T = CCM 7030T) is proposed.

The genus Acinetobacter comprises non-motile, strictly aerobic, oxidase-negative, Gram-negative bacteria that grow well on simple media. Twenty-four (genomic) species are currently recognized within the genus (Bouvet & Grimont, 1986; Tjernberg & Ursing, 1989; Bouvet & Jeanjean, 1989; Gerner-Smidt & Tjernberg, 1993; Vaneechoutte et al., 1999; Nemec et al., 2001) and strains of these species usually form colonies of 1·0–2·0 mm in diameter after 24 h incubation under optimum growth conditions (Bouvet & Grimont, 1986; Nemec et al., 2001). In a taxonomic study of Acinetobacter clinical isolates (Nemec et al., 2000), two strains were found which formed notably small colonies on routine agar media and could not be identified as any known (genomic) species. These strains were glucose-non-acidifying, non-proteolytic, did not utilize any of the 14 carbon sources of the identification scheme of Bouvet & Grimont (1987) and had highly similar amplified rDNA restriction analysis (ARDRA) profiles. Later, five strains similar to the two strains were found among archive strains in our collections. The aim of the present study was to define the taxonomic status of these strains by a polyphasic analysis.

The seven strains used in this study are listed in Table 1. All had the properties of the genus Acinetobacter (Juni, 1984), i.e. they were Gram-negative, strictly aerobic, oxidase-negative, non-motile coccobacilli, and were positive in the transformation assay of Juni (1972). The methods for genotypic characterization included ARDRA, AFLP fingerprinting and comparative 16S rDNA sequence analysis. Phenotypic characterization was done essentially according to Bouvet & Grimont (1987) and Gerner-Smidt et al. (1991), with some modifications. Details of the methods and their interpretative criteria have been given by Dijkshoorn et al. (1998) and Nemec et al. (2000, 2001). The assimilation tests were performed in tubes containing the fluid medium of Cruze et al. (1979) supplemented with 0·1 % (w/v) carbon source. Results were read after 2, 6 and 10 days incubation at 30 °C.

High-molecular-mass DNA for determination of the G+C

Abbreviation: ARDRA, amplified rDNA restriction analysis.

The EMBL accession numbers for the 16S rRNA gene sequences of Acinetobacter parvus LMG 21765T and LMG 21766 are AJ293691 and AJ293690, respectively.

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content and for DNA–DNA hybridization was prepared from cells grown aerobically on Tryptone Soya Agar (TSA; Oxoid) at 28 °C by the method of Wilson (1987), with minor modifications. Strains *Acinetobacter haemolyticus* LMG 996T, *Acinetobacter baumannii* LMG 1041T, *Acinetobacter calcoaceticus* LMG 1046T and ‘*Acinetobacter venetianus*’ LMG 19082, which produced large amounts of exopolysaccharides, were subjected to a mild alkaline hydrolysis step before cell lysis, as described by Willems *et al.* (1998). The G + C content of the DNA was determined by HPLC according to the method of Mesbah *et al.* (1989). Non-methylated phage λ DNA (Sigma) was used as the calibration reference. DNA–DNA hybridizations were performed using a modification of the microplate method described by Ezaki *et al.* (1989) and Goris *et al.* (1998). Hybridizations were performed at 37 °C in a hybridization solution [2 × SSC, 5 × Denhardt’s solution, 50 % (v/v) formamide, 2-5 % (w/v) dextran sulfate, low-molecular-mass denatured salmon sperm DNA to a final concentration of 100 µg ml⁻¹, 1-25 µg biotinylated probe DNA ml⁻¹]. The DNA–DNA relatedness percentages presented are means based on at least two hybridization experiments. Reciprocal reactions (e.g. AxB and BxA) were performed and the variation between them was within the limit of this method (Goris *et al.*, 1998).

Colonies of all strains grown on TSA or Nutrient Agar (NA; Oxoid) were circular, convex, smooth and slightly opaque with entire margins. These colonies were notably smaller than those of the other described *Acinetobacter* species (Fig. 1). On NA, the colonies were 0-1-0-4 mm and 0-3-0-9 mm in diameter after 24 and 48 h incubation at 30 °C, respectively, while on TSA, the colonies were 0-3-0-7 mm and 1-0-1-4 mm in diameter after 24 and 48 h of incubation at 30 °C, respectively. The use of other agar media including chocolate and blood agar did not significantly affect colony size as compared with TSA.

Table 1. Strains of *Acinetobacter parvus* used in this study

<table>
<thead>
<tr>
<th>Strain designation</th>
<th>Other strain designation</th>
<th>Reference/received from</th>
<th>Specimen</th>
<th>Location and year of isolation</th>
</tr>
</thead>
<tbody>
<tr>
<td>LUH 3067</td>
<td>–</td>
<td>A. T. Bernardis</td>
<td>Forehead (inpatient)</td>
<td>Enschede, NL, 1995</td>
</tr>
<tr>
<td>RUH 2714</td>
<td>–</td>
<td>–</td>
<td>Blood (inpatient)</td>
<td>Rotterdam, NL, 1986</td>
</tr>
<tr>
<td>RUH 7036</td>
<td>V0102891</td>
<td>J. Wagenaar</td>
<td>Eye (man)</td>
<td>Rotterdam, NL, 1988</td>
</tr>
<tr>
<td>LUH 7036</td>
<td>–</td>
<td>–</td>
<td>Ear (dog)</td>
<td>Leiderdorp, NL, 2001</td>
</tr>
</tbody>
</table>

All strains grew in Brain–Heart Infusion (Difco) broth at temperatures ranging from 25 to 35 °C but not at 41 °C. All but one strain (LMG 21766) grew at 37 °C, although the growth of LUH 3067 and RUH 2008 was reduced at this temperature as compared to growth at 30 °C. All strains utilized ethanol and acetate as sole sources of carbon and energy and their growth on these two substrates was clear after 2 days incubation. All strains were negative in the following tests: acid production from D-glucose, haemolysis of sheep blood, gelatinase production, and the utilization of DL-lactate, DL-4-aminobutyrate, trans-aconitate, citrate (Simmons), glutarate, L-aspartate, azelate, β-alanine, L-histidine, D-malate, malonate, histamine, L-phenylalanine, phenylacetate, levulinate, citraconate, 4-hydroxybenzoate, L-tartrate, L-ornithine, L-leucine, L-arabinose and 2,3-butanediol.

The result of the comparative analysis of AFLP patterns of...
the seven strains and type and reference strains of all described Acinetobacter (genomic) species is shown in Fig. 2. The seven strains grouped at 63 %, which is well above the 50 % level seen in previous studies for the delineation of Acinetobacter species (Nemec et al., 2001). They were clearly separated from the other Acinetobacter (genomic) species (each species represented by one strain) at 34 %. The 16S rDNA sequences of strains LMG 21765 T and LMG 21766 (EMBL accession nos AJ293691 and AJ293690, respectively) showed 99-8 % similarity. The similarity values between these sequences and those of the other 24 (genomic) species of the genus Acinetobacter (EMBL accession nos Z93434–Z93454, AJ275038, AJ278311 and AJ295007) were in the range of 95-9–98-1 %, which corresponds to the interspecies similarity values of the genus Acinetobacter (Ibrahim et al., 1997; Nemec et al., 2001).

Structural homogeneity of 16S rDNA was confirmed by ARDRA. All strains had identical or almost identical restriction patterns: CfoI 1 (LMG 21765 T, LUH 3067, LMG 21766 and RUH 2714) or CfoI 1 + 5 (RUH 2008, LUH 4619 and LUH 7036), Alul 2, Mbol 1, RsaI 2 and MspI 3.

DNA–DNA relatedness was determined between LMG 21765 T, LMG 21766 and the type strains of the nomenclature species that had shown highest similarity (> 96-5 %) of 16S rDNA sequences with the two strains (Table 2). The level of DNA–DNA binding between LMG 21765 T and LMG 21766 was 82 %. DNA–DNA binding values between these strains and the type strains of Acinetobacter junii, A. haemolyticus, A. baumannii, Acinetobacter johnsonii, A. calcoaceticus and the reference strain of 'A. venetianus' were not higher than 35 %. The DNA G + C content of LMG 21765 T and LMG 21766 was 41·8 and 41·5 %, respectively.

On the basis of phenotypic and genotypic characteristics, it is proposed that the seven small-colony-forming strains represent a hitherto unknown species of the genus Acinetobacter, for which the name Acinetobacter parvus is proposed.

A. parvus can be differentiated from other Acinetobacter (genomic) species by its negative results in biochemical tests suggested by Bouvet & Grimont (1987), in particular by the inability to oxidize D-glucose, to hydrolyse gelatin and to utilize D-lactate, Dl-4-aminobutyrate, citrate (Simmons), azelate, β-alanine and l-histidine (Bouvet & Grimont, 1987; Bouvet & Jeanjean, 1989; Gerner-Smidt et al., 1991; Vaneechoutte et al., 1999; Nemec et al., 2001). The acetate utilization test which is positive in A. parvus is necessary to differentiate prototrophic A. parvus strains from auxotrophic strains of other Acinetobacter (genomic) species. Notably, its typical colony size is an important feature to recognize A. parvus amidst colonies of other species and genera, and to differentiate it from biochemically inactive strains of other Acinetobacter (genomic) species.

ARDRA allowed for differentiation of A. parvus from all described (genomic) species of Acinetobacter, except A. junii and proteolytic genomic species 17 (Dijkshoorn et al., 1998; Vaneechoutte et al., 1999; Nemec et al., 2001). Four A. parvus strains had the same ARDRA combination pattern (CfoI 1, Alul 2, Mbol 1, Rsai 2, MspI 3) as the latter two (genomic) species. However, A. parvus strains can easily be distinguished from genomic species 17 and A. junii strains sharing this ARDRA profile by their small colonies and the inability to lyse sheep erythrocytes.

The A. parvus strains were isolated from human and animal non-sterile body sites, except for RUH 2008, which originated from the blood of a human. Isolation of this strain was
followed by other isolates with similar characteristics from intravenous catheters, which indicates that the strain was involved in a catheter-related blood-stream infection. Strain LUH 7036 was isolated from the ear of a dog with refractory otitis media.

During this study, an additional strain (LUH 4826) that was phenotypically indistinguishable from the A. parvus strains was isolated from a human clinical specimen. However, LUH 4826 had AlaI and Rsal ARDRA patterns different from those of the A. parvus strains and AFLP fingerprinting showed no significant similarity between this strain and any of the described Acinetobacter (genomic) species including A. parvus (not shown). Therefore, LUH 4826 may represent an as-yet-undescribed species of the genus Acinetobacter that is phenotypically similar to A. parvus. This finding demonstrates that, as is the case with most Acinetobacter (genomic) species, definitive species identification requires the use of genotypic methods.

Description of Acinetobacter parvus sp. nov.

Acinetobacter parvus (par’vus. L. masc. adj. parvus small, referring to the fact that its colonies on agar media are significantly smaller than those of the other known Acinetobacter species).

Characteristics correspond to those of the genus (Juni, 1984). The description is based on the characterization of seven strains of different origin. Colonies on TSA after 24 h incubation at 30 °C are approximately 0·3–0·7 mm in diameter, circular, convex, smooth and slightly opaque with entire margins. Growth occurs at 35 °C but not at 41 °C. Growth at 37 °C usually occurs but may be reduced. Good growth on ethanol and acetate as sole sources of carbon and energy. Negative results in the following tests: acid production from D-glucose, haemolysis of sheep blood, proteolytic genomic species in the genus Acinetobacter (genomic) species, definitive species identification requires the use of genotypic methods.

Acknowledgements

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Note added in proof

Since the study was completed, seven additional strains with typical A. parvus colonies have been studied in our laboratories. All of them were isolated from human clinical specimens (blood, ear pus, vaginal swab) and showed AFLP fingerprints, ARDRA profiles and biochemical properties typical of A. parvus. The only exception was the ability of three of these strains to grow on L-ornithine. Since this article was accepted for publication, seven new species of Acinetobacter have been described (Carr et al., 2003). Comparison of published 16S rDNA sequences and phenotypic characteristics did not show the identity of A. parvus with any of these species.

References


Acinetobacter parvus sp. nov.


