Acinetobacter beijerinckii sp. nov. and *Acinetobacter gyllenbergii* sp. nov., haemolytic organisms isolated from humans

Alexandr Nemec,¹ Martin Musílek,¹ Martina Maixnerová,¹ Thierry De Baere,² Tanny J. K. van der Reijden,³ Mario Vaneechoutte² and Lenie Dijkshoorn³

Correspondence
Alexandr Nemec
anemec@szu.cz

¹Centre of Epidemiology and Microbiology, National Institute of Public Health, Šrobárova 48, 100 42 Prague 10, Czech Republic

²Department of Clinical Chemistry, Microbiology and Immunology, University Hospital, Blok A, B-9000 Ghent, Belgium

³Department of Infectious Diseases, Leiden University Medical Centre C5-P, PO Box 9600, 2300 RC Leiden, The Netherlands

The taxonomic status of 24 haemolytic, non-glucose acidifying *Acinetobacter* strains that did not belong to any previously described species was investigated by means of a polyphasic approach. Using AFLP fingerprinting, amplified rDNA restriction analysis and phenotypic characterization, the strains were classified into two phenetically coherent groups (comprising 15 and 9 strains) that were distinct from each other and from all known *Acinetobacter* species. Confirmation that these groups formed two separate lineages within the genus *Acinetobacter* was obtained from comparative analysis of partial sequences of the gene encoding the β -subunit of RNA polymerase in all strains and also from 16S rRNA gene sequence analysis of representative strains. Previously published DNA–DNA reassociation data for some of the strains used also supported the species rank for both groups, for which the names *Acinetobacter beijerinckii* sp. nov. and *Acinetobacter gyllenbergii* sp. nov. are proposed. The strains of *A. beijerinckii* sp. nov. originated from human and animal specimens and from various environmental sources, whereas those of *A. gyllenbergii* sp. nov. were isolated exclusively from human clinical specimens. The phenotypic characteristics most useful for the differentiation of these species from other *Acinetobacter* species that comprise haemolytic strains were the inability of *A. beijerinckii* sp. nov. to grow on L-arginine and the ability of *A. gyllenbergii* sp. nov. to grow on azelate. The type strain of *A. beijerinckii* sp. nov. is NIPH 838^T (=LUH 4759^T=CCUG 51249^T=CCM 7266^T=58a^T) and the type strain of *A. gyllenbergii* sp. nov. is NIPH 2150^T (=RUH 422^T=CCUG 51248^T=CCM 7267^T=1271^T).

The genus *Acinetobacter* currently comprises 32 genomic species, of which the names of 17 have been validly published (Dijkshoorn *et al.*, 2007). Some *Acinetobacter* strains are able both to lyse mammalian erythrocytes and to

liquefy gelatin. These features have been associated with particular species and therefore are taxonomically relevant. *Acinetobacter haemolyticus*, '*Acinetobacter venetianus*' and genomic species 6, 13BJ (=14TU), 14BJ, 15BJ, 16 and 17 comprise strains that are typically both proteolytic and haemolytic (Bouvet & Grimont, 1986; Bouvet & Jeanjean, 1989; Gerner-Smidt *et al.*, 1991; Vaneechoutte *et al.*, 1999). In contrast, strains of the other known genomic species are usually neither proteolytic nor haemolytic (Bouvet & Grimont, 1986; Tjernberg & Ursing, 1989; Nemec *et al.*, 2001, 2003; Carr *et al.*, 2003); *Acinetobacter junii* is an exception, as a significant proportion of its strains are haemolytic but non-proteolytic (Gerner-Smidt *et al.*, 1991).

Abbreviations: ARDRA, amplified rDNA restriction analysis.

The GenBank/EMBL/DDBJ accession numbers for the *rhoB* partial gene sequences determined in this study are EU477105–EU477158 and those for the 16S rRNA gene sequences of strains NIPH 838^T, LUH 6214, NIPH 2150^T, LUH 1740 and LUH 1737 are AJ626712, AJ303013, AJ293694, AJ293693 and AJ293692, respectively.

Detailed data on the origins of the *A. beijerinckii* sp. nov. and *A. gyllenbergii* sp. nov. strains and a dendrogram derived from a cluster analysis of AFLP fingerprints are available as supplementary material with the online version of this paper.

Recently, we identified two phenotypically and genotypically distinct groups of *Acinetobacter* strains that did not belong to any of the described species. All these strains produced strong haemolysis on agar media supplemented with sheep erythrocytes. Some of them have already been subjected to DNA–DNA hybridization by others and were shown not to belong to any of the species known at that time (Bouvet & Grimont, 1986; Tjernberg & Ursing, 1989; Bouvet & Jeanjean, 1989). In this study, we present the results of phenotypic characterization, AFLP fingerprinting and comparative analysis of the RNA polymerase β -subunit (*rpoB*) and 16S rRNA gene sequences of the organisms of the two groups, and discuss these results in the light of previously published DNA–DNA reassociation data. We conclude that both groups are genetically coherent and distinct from each other and from all hitherto known genomic species and propose the names *Acinetobacter beijeirinkii* sp. nov. and *Acinetobacter gyllenbergii* sp. nov. for these groups.

The 24 strains of *A. beijeirinkii* sp. nov. ($n=15$) and *A. gyllenbergii* sp. nov. ($n=9$) investigated in this study are listed in Supplementary Table S1 (available in IJSEM Online). All of the strains studied had the properties of the genus *Acinetobacter* (Baumann *et al.*, 1968), i.e. they were Gram-negative, strictly aerobic, oxidase-negative, non-motile coccobacilli that were positive in the transformation assay of Juni (1972). Additionally, 29 strains representing all described *Acinetobacter* genomic species, which are known to contain haemolytic and/or proteolytic strains, were selected from previous studies for comparative taxonomic analysis (Supplementary Fig. S1, available in IJSEM Online). Finally, the type or reference strains of the remaining non-haemolytic species of the genus were included in the genotypic analyses (Figs 1 and 2), except *Acinetobacter grimontii*, which has recently been shown to be a later synonym of *A. junii* (Vanechoutte *et al.*, 2008).

AFLP analysis was used as the main genome fingerprinting method to assess the overall genotypic similarity of strains at species level (Nemec *et al.*, 2001). The result of the comparative analysis of the AFLP patterns of the strains of *A. beijeirinkii* sp. nov. and *A. gyllenbergii* sp. nov. and of those representing known *Acinetobacter* species is shown in Supplementary Fig. S1. The strains of *A. beijeirinkii* sp. nov. and *A. gyllenbergii* sp. nov. grouped into two clusters at similarity levels of 53 and 72 %, respectively, i.e. above the 50 % level that was determined in previous studies as the threshold for the delineation of *Acinetobacter* species (Nemec *et al.*, 2001, 2003). The two clusters were separate from each other and from all other strains at similarity cut-off levels ≤ 37 %. These results indicate both the genomic coherence and the distinctness of each of the two novel species.

Comparative sequence analysis of the *rpoB* gene was used to confirm the intraspecies relatedness of strains of *A. beijeirinkii* sp. nov. and *A. gyllenbergii* sp. nov. and their separation from each other and from all hitherto described *Acinetobacter* species. The method of La Scola *et al.* (2006)

was followed with minor modifications. Two sets of primers were used to amplify two variable regions of the *rpoB* gene: zone 1, spanning nucleotide positions 2916–3267 [primers Ac696F (5'-TAYCGYAAAGAYTTGAAA-GAAG-3') and Ac1093R (5'-CMACACCYTTGTTMCC-RTGA-3')], and zone 2, spanning nucleotide positions 3263–3773 [primers Ac1055F (5'-GTGATAARATGGCBG-GTCGT-3') and Ac1598R (5'-CGBGCRTGCATYTTGTC-RT-3')] (La Scola *et al.*, 2006). PCR amplification was performed in 20 μ l containing 10 μ l *Taq* PCR Master Mix (Qiagen), 0.2 μ M each primer and 1.5 μ l DNA suspension obtained by alkaline lysis as described by Nemec *et al.* (2000), with the following cycling conditions: 94 °C for 2 min; 32 cycles of 30 s at 94 °C, 30 s at 55 °C and 2 min at 72 °C; and 5 min at 72 °C. Sequencing reactions were carried out with a BigDye Terminator cycle sequencing kit (Applied Biosystems) using the aforementioned primers. Sequencing products were analysed using an ABI Prism 3100 genetic analyzer (Applied Biosystems) and edited using the program LASERGENE (DNASTAR). Comparative sequence analysis was performed using the BioNumerics 5.0 software package (Applied Maths). First, a similarity matrix based on pairwise alignment of sequences was calculated using the UPGMA clustering algorithm; this was followed by multiple sequence alignment and global cluster analysis using the Jukes–Cantor algorithm (to calculate evolutionary distances; Jukes & Cantor, 1969) and the neighbour-joining clustering method. The calculations were carried out for concatenated zones 1 and 2 using nucleotide positions 2917–3267 for zone 1 and positions 3322–3723 for zone 2. The position numbers correspond to those of the *rpoB* encoding sequence of *Acinetobacter baumannii* (La Scola *et al.*, 2006).

Partial sequences for *rpoB* were determined for all of the strains of *A. beijeirinkii* sp. nov. and *A. gyllenbergii* sp. nov. as well as for the type or reference strains representing all known *Acinetobacter* genomic species. Twenty-two strains used by La Scola *et al.* (2006) were resequenced in the present study: the results were identical to those of La Scola *et al.* (2006). The only discrepancies, found for the *rpoB* sequences of genomic species 13TU, CIP 70.18 and genomic species 16, CIP 64.2, could be explained by the confusion between the designations of these two strains in the Collection of the Pasteur Institute (CIP; Paris, France) from which the strains had been obtained by La Scola *et al.* (2006) (this error has been corrected by the CIP). The results of the cluster analysis of concatenated *rpoB* zones 1 and 2 are shown in Fig. 1. The strains of *A. beijeirinkii* and *A. gyllenbergii* spp. nov. grouped into two respective clusters with bootstrap values supporting the monophyly of each of these clusters. The intraspecies similarity values (expressed as the percentages of identical nucleotides in corresponding positions in two aligned sequences) for the strains of *A. beijeirinkii* sp. nov. and *A. gyllenbergii* sp. nov. ranged from 97.9 to 100 % and 98.8 to 100 %, respectively, whereas the interspecies similarity values between the strains of *A. beijeirinkii* and *A. gyllenbergii* spp. nov. and

Fig. 1. Neighbour-joining tree, based on two concatenated variable zones of *rpoB* of *Acinetobacter beijeirackii* sp. nov., *Acinetobacter gyllenbergii* sp. nov. and representatives of known species of the genus *Acinetobacter*. Numbers at branch points are bootstrap percentages (based on 1000 resamplings) >80%. GenBank accession numbers are given in parentheses. Bar, 2% sequence divergence.

other members of the genus *Acinetobacter* were 78.2–91.6 and 78.6–93.9%, respectively.

The 16S rRNA gene sequence analysis was performed as described previously (Nemec *et al.*, 2001) and included comparative analysis of the almost-complete 16S rRNA gene sequences as well as amplified 16S rDNA restriction analysis (ARDRA), which is based on restriction enzyme digestion of the PCR-amplified 16S rRNA gene with restriction endonucleases. Almost-complete 16S rRNA gene sequences were obtained for *A. beijeirackii* sp. nov. strains NIPH 838^T and LUH 6214 and for *A. gyllenbergii* sp. nov. strains NIPH 2150^T, LUH 1737 and LUH 1740. The

sequences clustered with those of other members of the genus *Acinetobacter* (Fig. 2). The sequences of the two *A. beijeirackii* sp. nov. strains were identical, as were those of the three *A. gyllenbergii* sp. nov. strains. The similarity between the sequences of *A. beijeirackii* sp. nov. and those of other members of the genus *Acinetobacter* ranged from 95.0 (*Acinetobacter townneri*) to 99.3% (genomic species 16). The similarity values between the 16S rRNA gene sequences of *A. gyllenbergii* sp. nov. and those of other members of the genus ranged from 94.6 (*A. townneri*) to 99.1% (genomic species 16). The sequence similarity between *A. beijeirackii* sp. nov. and *A. gyllenbergii* sp. nov. was 98.6%. To estimate the intraspecies sequence

homogeneity of the 16S rRNA gene, ARDRA was performed for all strains of both species. On the basis of the ARDRA numbering system of Dijkshoorn *et al.* (1998), all *A. beijerinckii* sp. nov. strains had the restriction pattern *Cfo*I 1, *Alu*I 4, *Mbo*I 2, *Rsa*I 2, *Msp*I 2 and *Bfa*I 14, whereas the *A. gyllenbergii* sp. nov. strains shared the pattern *Cfo*I 1, *Alu*I 4, *Mbo*I 1, *Rsa*I 4 and *Msp*I 3.

Tjernberg & Ursing (1989). *A. gyllenbergii* sp. nov. strain LUH 1737 (=80) was shown to be less than 34 % similar to the type or reference strains of *Acinetobacter calcoaceticus*, *A. haemolyticus*, *A. junii*, *Acinetobacter lwoffii*, *Acinetobacter johnsonii* and genomic species 6, 9, 10, 11 and 12 (Bouvet & Grimont, 1986) and to be only 43–55 % similar to the proteolytic genomic species 13BJ, 14BJ, 15BJ, 16 and 17, with ΔT_m ranging from 6.5 to 9.5 °C (Bouvet & Jeanjean, 1989). Similarly, three other *A. gyllenbergii* sp. nov. strains [NIPH 2150^T (=1271^T), LUH 1740 (=930), LUH 1741 (=944)] were found to be 28–55 % similar to genomic species 13BJ, 14BJ, 15BJ, 16 and 17, with ΔT_m ranging from 6.5 to 12 °C (Bouvet & Jeanjean, 1989). These archive data indicate that both *A. beijerinckii* and *A. gyllenbergii* spp. nov. are distinct from 18 genomic species described by Bouvet & Grimont (1986), Tjernberg & Ursing (1989) and Bouvet & Jeanjean (1989). As the results of both AFLP and comparative sequence analysis of housekeeping genes (16S rRNA gene, *rpoB*) did not indicate a close relationship between the novel species and species described by Nemec *et al.* (2001, 2003) and Carr *et al.* (2003), additional DNA–DNA hybridization studies were not carried out.

The results of phenotypic tests are given in the species descriptions. The tests used were those described by Nemec *et al.* (2000), with some modifications and additions. The cultivation temperature was 30 °C and tests were evaluated after 2 days incubation, unless indicated otherwise. Aerobic acid production from glucose was tested using Hugh and Leifson's agar (OF basal medium; Merck) supplemented with 1% (w/v) D-glucose. The gelatinase test was performed on tryptone soya agar (Oxoid) containing 4% (w/v) gelatin, with Frazier's reagent (15%, w/v, HgCl₂ in 20%, v/v, HCl) being used to visualize zones of gelatin hydrolysis. Haemolysis was tested on Columbia agar plates supplemented with 5% sheep or horse blood (bioMérieux). Growth tests at 25, 30, 35, 37 and 41 °C were performed in 5 ml aliquots of brain-heart infusion broth (Oxoid) dispensed into tubes (16 mm inner diameter). Each tube was inoculated with a drop (~30 µl) of cell suspension of standardized turbidity (~10⁸ c.f.u. ml⁻¹) prepared in saline from an overnight agar culture and incubated in a thermostatically controlled water bath. Utilization of citrate was tested on Simmons' citrate agar (Oxoid) and was recorded after 2, 4 and 6 days incubation. Tests for the assimilation of the other 32 carbon sources were performed using the basal mineral medium of Cruze *et al.* (1979) supplemented with 0.1% (w/v) carbon source. The basal medium consisted of the following (l⁻¹): 10.0 g KH₂PO₄, 5.0 g Na₂HPO₄, 2.0 g (NH₄)₂SO₄, 0.2 g MgSO₄·7H₂O, 0.001 g CaCl₂·2H₂O and 0.001 g FeSO₄·7H₂O (pH 7.0). The supplemented medium was dispensed into tubes (12 mm inner diameter) in 3 ml volumes and was inoculated as in the growth-temperature tests. Growth on carbon sources was evaluated after 2, 4, 6 and 10 days by means of visual comparison between inoculated tubes containing carbon sources and control tubes containing only inoculated basal medium. *A. baumannii* NIPH 146 was used as a positive control for all assimilation tests except those for histamine and tryptamine (positive control: *Acinetobacter* genomic species 11 LMG 988) and those for D-glucose and D-gluconate (positive control: *Acinetobacter baylyi* CCM 7195^T). All phenotypic tests were performed three times in different sessions and were repeated when inconsistent results were obtained.

All of the strains of *A. beijerinckii* sp. nov. and *A. gyllenbergii* sp. nov. produced large zones of haemolysis after 24 h on agar media supplemented with sheep or horse erythrocytes; haemolysis was greater at a cultivation temperature of 37 °C. Notably, *A. gyllenbergii* sp. nov. strain NIPH 2353 (=CCM 1976) was used by Lehmann (1973) in a study on the haemolytic properties of eight *Acinetobacter* sp. strains. His results suggested that an extracellular cytolytic protein was responsible for the haemolysis. In addition, the strains could be classified into two groups on the basis of the data from detailed biochemical characterization of their haemolytic properties. Whereas the strains of the first group showed direct haemolysis, with no detectable increase in lysis after cooling, the strains of the second group exhibited hot-

cold haemolysis. The two types of haemolytic activities were also associated with immunological differences. As five of the strains used by Lehmann were available at the Czech Collection of Microorganisms (Brno, Czech Republic), we attempted to identify these strains at the species level. The three strains (CCM 1989, CCM 2358^T and CCM 2359) with hot-cold haemolysis were allocated to *A. haemolyticus*, whereas the two strains showing direct haemolysis were identified as *A. gyllenbergii* sp. nov. (CCM 1976) and genomic species 13BJ (CCM 2357=ATCC 17905). These results suggest that the differences in haemolytic activity have taxonomic relevance.

A. beijerinckii sp. nov. and *A. gyllenbergii* sp. nov. could be phenotypically differentiated from other non-haemolytic *Acinetobacter* genomic species by the ability to lyse sheep erythrocytes or, in the case of *A. gyllenbergii* sp. nov. by gelatinase activity, and on the basis of the results of some other biochemical tests described by Bouvet & Grimont (1987) (e.g. tests for acid production from glucose and utilization of *trans*-aconitate, glutarate, L-aspartate, β-alanine, L-histidine and L-phenylalanine). Table 1 shows phenotypic characteristics useful for the differentiation of *A. beijerinckii* sp. nov. and *A. gyllenbergii* sp. nov. from each other and from all of the species that include haemolytic strains. The 10 tests shown were selected to provide reliable discrimination in terms of reproducibility and ease of interpretation of results. For *A. beijerinckii* sp. nov. the number of discriminative tests ranged from nine (genomic species 14BJ) to two (*A. haemolyticus*, '*A. venetianus*') whereas for *A. gyllenbergii* sp. nov. this number ranged from six (*A. beijerinckii* sp. nov., genomic species 6) to two (genomic species 14BJ, genomic species 16). The most useful characteristics for the differentiation of these species from the other haemolytic *Acinetobacter* species were the inability of *A. beijerinckii* sp. nov. to grow on L-arginine and the ability of *A. gyllenbergii* sp. nov. to grow on azelate. While the former feature was observed only in strains of *A. beijerinckii* sp. nov., the latter was confined to *A. gyllenbergii* sp. nov. and genomic species 14BJ. Both of the novel species could be identified genotypically using *rpoB* sequence analysis or AFLP library fingerprinting. Finally, strains of *A. beijerinckii* sp. nov. and *A. gyllenbergii* sp. nov. had ARDRA profiles (see above) that are unique among the species studied to date (Dijkshoorn *et al.*, 1998; Nemec *et al.*, 2001, 2003).

Description of *Acinetobacter beijerinckii* sp. nov.

Acinetobacter beijerinckii [bei.je.rin'cki.i. N.L. masc. gen. n. *beijerinckii* of Beijerinck, named in honour of Martinus Willem Beijerinck (1851–1931), the Dutch microbiologist and botanist].

The description is based on the characterization of 15 strains (Supplementary Table S1 in IJSEM Online). Phenotypic characteristics correspond to those of the genus (Baumann *et al.*, 1968), i.e. cells are Gram-negative, strictly aerobic, oxidase-negative, catalase-positive, non-motile coccobacilli, capable of growth in mineral media

Table 1. Phenotypic characteristics useful for distinguishing *Acinetobacter beijerinckii* sp. nov. and *Acinetobacter gyllenbergii* sp. nov. from each other and from haemolytic strains of other species

Taxa: 1, *A. beijerinckii* sp. nov.; 2, *A. gyllenbergii* sp. nov.; 3, *A. haemolyticus*; 4, *A. junii*; 5, genomic species 6; 6, genomic species 13BJ/14TU; 7, genomic species 14BJ; 8, genomic species 15BJ; 9, genomic species 16; 10, genomic species 17; 11, '*A. venetianus*'; 12, *Acinetobacter tjernbergiae*. The results shown were obtained in the present study. Strains of previously described genomic species were selected from the studies of Bouvet & Grimont (1987), Tjernberg & Ursing (1989), Janssen *et al.* (1997), Vaneechoutte *et al.* (1999) and Carr *et al.* (2003). All of them are indicated in Supplementary Fig. S1 (in IJSEM Online). The type strain of *A. tjernbergiae* was included as it revealed haemolysis when tested in our laboratory. Numbers of strains are indicated in parentheses. +, All strains positive within 4 days incubation; −, all strains negative within 10 days incubation; v, strain-dependent results or weak, delayed or non-reproducible reactions.

Characteristic	1 (n=15)	2 (n=9)	3 (n=5)	4 (n=4)	5 (n=2)	6 (n=6)	7 (n=3)	8 (n=2)	9 (n=3)	10 (n=2)	11 (n=2)	12 (n=1)
Acid from D-glucose	−	−	v	−	+	+	+	−	−	−	−	−
Utilization of:												
DL-Lactate	−	+	−	+	−	+	+	+	+	+	−	−
Azelate	−	+	−	−	−	−	+	−	−	−	−	−
β-Alanine	−	+	−	−	−	v	+	−	v	+	−	−
Malonate	+	v	−	−	−	v	+	v	v	v	+	−
L-Arginine	−	+	+	+	+	+	+	+	+	+	+	+
Ethanol	+	v	v	v	+	−	−	−	−	−	+	+
Benzoate	−	+	−	+	−	v	+	+	+	+	+	+
Adipate	−	+	−	−	−	−	+	−	−	−	−	−
Putrescine	−	−	−	−	−	−	+	−	−	+	−	−

with acetate as the sole carbon source and ammonia as the sole source of nitrogen but incapable of dissimilative denitrification. Positive in the transformation assay of Juni (1972). Colonies on tryptone soya agar after 24 h incubation at 30 °C are approximately 1.0–2.0 mm in diameter, circular, convex, smooth and slightly opaque with entire margins. Growth occurs at temperatures ranging from 25 to 37 °C, but not at 41 °C. Sheep and horse erythrocytes are lysed in 24 h at 30–37 °C. Acid is not produced from D-glucose; gelatin is hydrolysed by some (13 %) strains. Acetate, ethanol, citrate (Simmons'), 4-aminobutyrate, L-aspartate, L-histidine, D-malate and malonate are utilized as sole sources of carbon, with growth becoming visible after 2 days incubation. No growth occurs on DL-lactate, *trans*-aconitate, glutarate, azelate, β-alanine, histamine, L-phenylalanine, phenylacetate, levulinate, citraconate, 4-hydroxybenzoate, L-tartrate, L-ornithine, L-arginine, D-glucose, D-gluconate, D-ribose, L-arabinose, 2,3-butanediol, benzoate, adipate, gentisate, tryptamine, trigonelline, tricarballoylate or putrescine within 10 days. L-Leucine is utilized by most (93 %) strains.

The strains studied were isolated from human (sputum, peritoneal dialysis fluid, gall, throat swab, perineal swab, faeces) and equine (airways) clinical specimens, from a hospital environment and staff (skin), and soil and water (Supplementary Table S1 in IJSEM Online). The type strain, NIPH 838^T (=LUH 4759^T=CCUG 51249^T=CCM 7266^T=58a^T), was isolated in 1980 from the wound of a patient at the hospital in Malmö, Sweden (Tjernberg & Ursing, 1989). The type strain grows weakly on L-leucine and does not hydrolyse gelatin after 2 days incubation.

Description of *Acinetobacter gyllenbergii* sp. nov.

Acinetobacter gyllenbergii (gyl.len.ber'gi.i. N.L. masc. gen. n. *gyllenbergii* of Gyllenberg, named in honour of Helge G. Gyllenberg, the Finnish bacteriologist and taxonomist).

The description is based on the characterization of 9 strains (Supplementary Table S1 in IJSEM Online). Phenotypic characteristics correspond to those of the genus (Baumann *et al.*, 1968), i.e. cells are Gram-negative, strictly aerobic, oxidase-negative, catalase-positive, non-motile coccobacilli, capable of growth in mineral media with acetate as the sole carbon source and ammonia as the sole source of nitrogen but incapable of dissimilative denitrification. Positive in the transformation assay of Juni (1972). Colonies on tryptone soya agar after 24 h incubation at 30 °C are approximately 1.0–2.0 mm in diameter, circular, convex, smooth and slightly opaque with entire margins. Growth occurs at temperatures ranging from 25 to 37 °C, but not at 41 °C; weak growth of some strains occurs at 37 °C. Sheep and horse erythrocytes are lysed in 24 h at 30–37 °C. Acid is not produced from D-glucose; gelatin is hydrolysed by all strains. Acetate, citrate (Simmons'), DL-lactate, azelate, β-alanine, L-histidine, D-malate, phenylacetate, L-arginine, L-leucine, benzoate and adipate are utilized as sole sources of carbon, with growth becoming visible after 2 days incubation. No growth occurs on *trans*-aconitate, L-aspartate, histamine, levulinate, citraconate, L-tartrate, D-glucose, D-gluconate, D-ribose, L-arabinose, 2,3-butanediol, tryptamine, trigonelline, tricarballoylate or putrescine within 10 days. Malonate is utilized by 78 % of the strains, L-phenylalanine by 89 %, 4-hydroxybenzoate by 89 %, ethanol by 22 %, L-ornithine by 56 % and

gentisate by 11%. For most strains, growth on 4-aminobutyrate or glutarate is not reproducible or is delayed (7–10 days).

The strains studied were isolated exclusively from human clinical specimens (blood, urine, sputum, vaginal swab, throat swab) (Supplementary Table S1 in IJSEM Online). The type strain, NIPH 2150^T (=RUH 422^T=CCUG 51248^T=CCM 7267^T=1271^T), was isolated in 1978 from the urine of a patient in Leiden University Hospital, The Netherlands (Bouvet & Jeanjean, 1989). The type strain grows on malonate, L-phenylalanine, L-ornithine, gentisate and quinate, but does not utilize ethanol or L-glutarate.

Acknowledgements

We thank all those who generously provided strains (listed in Supplementary Table S1). A.N. was supported by grant no. MSM 0021620812 from the Czech Ministry of Education, Youth and Sports. T. De B. is indebted to the Fund for Scientific Research Flanders (FWO) for a position as postdoctoral fellow. Part of this study was presented at the 14th European Congress of Clinical Microbiology and Infectious Diseases (Prague, Czech Republic) in 2004 (abstract P1855) and at the 6th International Symposium on the Biology of *Acinetobacter* (Dublin, Ireland) in 2004 (abstract D2).

References

- Baumann, P., Doudoroff, M. & Stanier, R. Y. (1968). A study of the *Moraxella* group. II. Oxidative-negative species (genus *Acinetobacter*). *J Bacteriol* **95**, 1520–1541.
- Bouvet, P. J. M. & Grimont, P. A. D. (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 descriptions of *Acinetobacter calcoaceticus* and *Acinetobacter lwoffii*. *Int J Syst Bacteriol* **36**, 228–240.
- Bouvet, P. J. M. & Grimont, P. A. D. (1987). Identification and biotyping of clinical isolates of *Acinetobacter*. *Ann Inst Pasteur Microbiol* **138**, 569–578.
- Bouvet, P. J. M. & Jeanjean, S. (1989). Delineation of new proteolytic genomic species in the genus *Acinetobacter*. *Res Microbiol* **140**, 291–299.
- Carr, E. L., Kämpfer, P., Patel, B. K. C., Gürtler, V. & Seviour, R. J. (2003). Seven novel species of *Acinetobacter* isolated from activated sludge. *Int J Syst Evol Microbiol* **53**, 953–963.
- Cruze, J. A., Singer, J. T. & Finnerty, W. R. (1979). Conditions for quantitative transformation in *Acinetobacter calcoaceticus*. *Curr Microbiol* **3**, 129–132.
- Dijkshoorn, L., van Harselaar, B., Tjernberg, I., Bouvet, P. J. M. & Vanechoutte, M. (1998). Evaluation of amplified ribosomal DNA restriction analysis for identification of *Acinetobacter* genomic species. *Syst Appl Microbiol* **21**, 33–39.
- Dijkshoorn, L., Nemec, A. & Seifert, H. (2007). An increasing threat in the hospital: multidrug-resistant *Acinetobacter baumannii*. *Nat Rev Microbiol* **5**, 939–951.
- Gerner-Smidt, P., Tjernberg, I. & Ursing, J. (1991). Reliability of phenotypic tests for identification of *Acinetobacter* species. *J Clin Microbiol* **29**, 277–282.
- Janssen, P., Maquelin, K., Coopman, R., Tjernberg, I., Bouvet, P., Kersters, K. & Dijkshoorn, L. (1997). Discrimination of *Acinetobacter* genomic species by AFLP fingerprinting. *Int J Syst Bacteriol* **47**, 1179–1187.
- Jukes, T. H. & Cantor, C. R. (1969). Evolution of protein molecules. In *Mammalian Protein Metabolism*, vol. 3, pp. 21–132. Edited by H. N. Munro. New York: Academic Press.
- Juni, E. (1972). Interspecies transformation of *Acinetobacter*: genetic evidence for a ubiquitous genus. *J Bacteriol* **112**, 917–931.
- La Scola, B., Gundi, V. A., Khamis, A. & Raoult, D. (2006). Sequencing of the *rpoB* gene and flanking spacers for molecular identification of *Acinetobacter* species. *J Clin Microbiol* **44**, 827–832.
- Lehmann, V. (1973). Haemolytic activity of various strains of *Acinetobacter*. *Acta Pathol Microbiol Scand [B] Microbiol Immunol* **81**, 427–432.
- Nemec, A., Dijkshoorn, L. & Ježek, P. (2000). Recognition of two novel phenons of the genus *Acinetobacter* among non-glucose-acidifying isolates from human specimens. *J Clin Microbiol* **38**, 3937–3941.
- Nemec, A., De Baere, T., Tjernberg, I., Vanechoutte, M., van der Reijden, T. J. K. & Dijkshoorn, L. (2001). *Acinetobacter ursingii* sp. nov. and *Acinetobacter schindleri* sp. nov., isolated from human clinical specimens. *Int J Syst Evol Microbiol* **51**, 1891–1899.
- Nemec, A., Dijkshoorn, L., Cleenwerck, I., De Baere, T., Janssens, D., Van Der Reijden, T. J., Ježek, P. & Vanechoutte, M. (2003). *Acinetobacter parvus* sp. nov., a small-colony-forming species isolated from human clinical specimens. *Int J Syst Evol Microbiol* **53**, 1563–1567.
- Seifert, H., Dijkshoorn, L., Gerner-Smidt, P., Pelzer, N., Tjernberg, I. & Vanechoutte, M. (1997). Distribution of *Acinetobacter* species on human skin: comparison of phenotypic and genotypic identification methods. *J Clin Microbiol* **35**, 2819–2825.
- Tjernberg, I. & Ursing, J. (1989). Clinical strains of *Acinetobacter* classified by DNA-DNA hybridization. *APMIS* **97**, 595–605.
- Vanechoutte, M., Tjernberg, I., Baldi, F., Pepi, M., Fani, R., Sullivan, E. R., van der Toorn, J. & Dijkshoorn, L. (1999). Oil-degrading *Acinetobacter* strain RAG-1 and strains described as '*Acinetobacter venetianus* sp. nov.' belong to the same genomic species. *Res Microbiol* **150**, 69–73.
- Vanechoutte, M., De Baere, T., Nemec, A., Musilek, M., van der Reijden, T. J. K. & Dijkshoorn, L. (2008). Reclassification of *Acinetobacter grimontii* Carr *et al.* 2003 as a later synonym of *Acinetobacter junii* Bouvet and Grimont 1986. *Int J Syst Evol Microbiol* **58**, 937–940.