Acinetobacter bereziniae sp. nov. and *Acinetobacter guillouiae* sp. nov., to accommodate *Acinetobacter* genomic species 10 and 11, respectively

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Acinetobacter genospecies (genomic species) 10 and 11 were described by Bouvet and Grimont in 1986 on the basis of DNA–DNA reassociation studies and comprehensive phenotypic analysis. In the present study, the names *Acinetobacter bereziniae* sp. nov. and *Acinetobacter guillouiae* sp. nov., respectively, are proposed for these genomic species based on the congruence of results of polyphasic analysis of 33 strains (16 and 17 strains of genomic species 10 and 11, respectively). All strains were investigated by selective restriction fragment amplification (i.e. AFLP) analysis *rpoB* sequence analysis, amplified rDNA restriction analysis and tDNA intergenic length polymorphism analysis, and their nutritional and physiological properties were determined. Subsets of the strains were studied by 16S rRNA gene sequence analysis and matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) MS or had been classified previously by DNA–DNA reassociation. Results indicate that *A. bereziniae* and *A. guillouiae* represent two phenetically and phylogenetically distinct groups within the genus *Acinetobacter*. Based on the comparative analysis of housekeeping genes (16S rRNA and *rpoB* genes), these species together represent a monophyletic branch within the genus. Despite their overall phenotypic similarity, the ability to oxidize D-glucose and to grow at 38 °C can be used in the presumptive differentiation of these two species from each other: with the exception of three strains that were positive for only one test, *A. bereziniae* strains were positive for both tests, whereas *A. guillouiae* strains were negative in these tests. The strains of *A. bereziniae* originated mainly from human clinical specimens, whereas *A. guillouiae* strains were isolated from different environmental sources in addition to human specimens. The type strain of *A. bereziniae* sp. nov. is LMG 1003^T (=CIP 70.12^T =ATCC 17924^T) and that of *A. guillouiae* sp. nov. is LMG 988^T (=CIP 63.46^T =ATCC 11171^T =CCUG 2491^T).

Abbreviations: ARDRA, amplified rDNA restriction analysis; MALDI-TOF MS, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry; tDNA-PCR, tDNA-intergenic length polymorphism analysis.

The GenBank/EMBL/DDBJ accession numbers for the partial *rpoB* gene sequences determined in this study are FJ754429–FJ754463 and those for the 16S rRNA gene sequences of strains LUH 2634, LUH 9667, LUH 7013 and A23 are FM177773–FM177776, respectively.

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

The genus *Acinetobacter* currently encompasses 19 species with validly published names and a number of tentative species with provisional designations (Dijkshoorn *et al.*, 2007; Vaneechoutte & De Baere, 2007; Vaneechoutte *et al.*, 2009; Nemec *et al.*, 2009). *Acinetobacter* genospecies 10 and 11 (here referred to as genomic species 10 and 11) were delineated in 1986 on the basis of DNA–DNA reassociation studies and comprehensive phenotypic analysis (Bouvet & Grimont, 1986). As the number of strains in each group was small (four and three strains, respectively), the authors intentionally used provisional designations for these groups and did not propose formal species names. Later, other authors reported the isolation of strains belonging to genomic species 10 or 11 from various human, animal and environmental sources, which suggests that these genomic species are widespread biological entities (Tjernberg & Ursing, 1989; Berlau *et al.*, 1999; Chu *et al.*, 1999; Nemec *et al.*, 2000; Dijkshoorn *et al.*, 2005). In this study, results of a polyphasic taxonomic analysis of strains of genomic species 10 and 11 are presented. Both our results and archive data published by others indicate that these genomic species represent two phenetically and phylogenetically distinct groups, which together represent a monophyletic branch within the genus. It is concluded that these groups represent two novel species for which the names *Acinetobacter bereziniae* sp. nov. and *Acinetobacter guillouiae* sp. nov., respectively, are proposed.

The 16 strains of genomic species 10 (*A. bereziniae*) and 17 strains of genomic species 11 (*A. guillouiae*) investigated in this study are listed in Table 1. Detailed information on the origin and properties of these strains is shown in Supplementary Table S1 (available in IJSEM Online).

Selective restriction fragment amplification (i.e. AFLP) analysis, a high-resolution DNA fingerprinting method, was used to assess overall genomic similarity of strains (Nemec *et al.*, 2001; Dijkshoorn & Nemec, 2007). Fingerprints of the strains were compared to a database of reference strains of all described named and unnamed *Acinetobacter* species. Results of comparative analysis of AFLP fingerprints of strains of *A. bereziniae* and *A. guillouiae* and of those representing all known *Acinetobacter* species are shown in Supplementary Fig. S1. The strains of *A. bereziniae* and *A. guillouiae* created two clusters at levels of 51 and 59%, respectively, which are above the 50% level that has been established as the threshold for the delineation of *Acinetobacter* species (Nemec *et al.*, 2001). The two clusters were separate from each other and from all other strains at similarity levels of $\leq 33\%$. These data indicate both genomic coherence and distinctness of each of the two species.

Comparative sequence analysis of the RNA polymerase β -subunit (*rpoB*) gene was performed as described previously (Nemec *et al.*, 2009). Similarity calculations and cluster analysis were carried out for two concatenated zones encompassing nucleotide positions 2917–3267 and 3322–3723. The *rpoB*-based dendrogram for strains of

A. bereziniae and *A. guillouiae* and for representative strains of other species of the genus is shown in Fig. 1. Intraspecies similarity values (expressed as percentages of identical nucleotides in corresponding positions in two aligned sequences) for strains of *A. bereziniae* and *A. guillouiae* were 98.9–100 and 97.6–100%, respectively, whereas the similarity between these two species was 91.5–92.7%. The interspecies similarity values of strains of *A. bereziniae* and *A. guillouiae* with other members of the genus (except for *Acinetobacter baylyi* CCM 7195^T and C5; see below) were 78.5–86.6 and 77.6–87.5%, respectively. When *A. baylyi* CCM 7195^T and C5 were excluded from cluster analysis, strains of the two novel species formed two respective clusters each supported by a bootstrap value of 100%; these clusters were linked to form a broader cluster, which was also supported by a bootstrap value of 100%. These results demonstrate both the relatedness and distinctiveness of *A. bereziniae* and *A. guillouiae* and suggest that these species are monophyletic taxa that together represent a broader clade within the genus.

The *rpoB* results obtained for the five *A. baylyi* strains deserve more detailed comment. Three of these strains (ADP1, A7 and 93A2) formed a relatively homogeneous cluster (intracluster similarity values of 99.3–99.8%) that was markedly separate from other species of the genus (Fig. 1), with similarity values between the three *A. baylyi* strains and strains representing the other *Acinetobacter* species ranging from 78.5 to 83.9%. In contrast, the two remaining *A. baylyi* strains (C5 and CCM 7195^T) clustered with the *A. guillouiae* strains and were only 82.2–88.1% similar to the other *A. baylyi* strains. However, although the *rpoB* similarity between CCM 7195^T and the *A. guillouiae* strains (97.6–99.7%) was in the intraspecies range found for *A. guillouiae*, the similarity for *A. baylyi* C5 and the *A. guillouiae* strains was only 93.1–94.2%. Comparative analysis of *rpoB* sequences using nucleotide positions 2915–3775 revealed that the sequence of *A. baylyi* C5 consisted of two segments. Whereas the *rpoB* region of C5 between positions 2915 and 3134 corresponded to the *rpoB* orthologues of *A. baylyi* ADP1, A7 and 93A2 (similarity 99.1–100%), the region between positions 3140 and 3775 corresponded to those of the *A. guillouiae* strains (similarity 98.5–99.7%). These data suggest that the *rpoB* sequences of *A. baylyi* strains ADP1, A7 and 93A2 represent the authentic *A. baylyi* *rpoB* orthologues, whereas the two other *A. baylyi* strains (C5 and CCM 7195^T) carry sequences that may have resulted from intragenic recombination events following the acquisition of *rpoB* sequences from *A. guillouiae*. This hypothesis is consistent with the fact that *A. baylyi* is known to be transformable by DNA from other *Acinetobacter* species (Juni, 1972; Vaneechoutte *et al.*, 2006) and that both *A. baylyi* and *A. guillouiae* can be found in the same environment (Carr *et al.*, 2003).

16S rRNA gene sequence analysis was carried out as described previously (Nemec *et al.*, 2001; Vaneechoutte & De Baere 2007). It included comparative analysis of 16S rRNA gene sequences and amplified 16S rDNA restriction

Table 1. Strains of *Acinetobacter bereziniae* sp. nov. and *Acinetobacter guillouiae* sp. nov.

Culture collections: ATCC, American Type Culture Collection, Manassas, USA; CCM, Czech Collection of Microorganisms, Brno, Czech Republic; CCUG, Culture Collection, University of Göteborg, Sweden; LMG, Bacteria Collection, Laboratorium voor Microbiologie Gent, Gent, Belgium; CIP, Collection de l'Institut Pasteur, Institut Pasteur, Paris, France. ANC and NIPH are strain designations used by A. Nemec and LUH and RUH are strain designations used by L. Dijkshoorn. CZ, Czech Republic; NL, Netherlands; in, inpatient; out, outpatient.

Strain designation	Specimen	Location and year of isolation	Donor	Reference
<i>Acinetobacter bereziniae</i> sp. nov.				
LMG 1003 ^T (=NIPH 521 ^T =ATCC 17924 ^T =CIP 70.12 ^T =69 ^{T*})	Wound (human)	Before 1960		Bouvet & Grimont (1986)
CCUG 28268 (=113:2*)	Wound (human)	Malmö, Sweden, 1980	I. Tjernberg	Tjernberg & Ursing (1989)
RUH 2222 (=198*)	Urine (human)	Malmö, Sweden, 1980	I. Tjernberg	Tjernberg & Ursing (1989)
NIPH 870	Urine (human, in)	Č. Budějovice, CZ, 1997	M. Horníková	Nemec <i>et al.</i> (2000)
NIPH 1741	Sputum (human, in)	Plzeň, CZ, 2001	T. Bergerová	
LUH 2634	Sink (hospital environment)	Enschede, NL, 1995		Nemec <i>et al.</i> (2000)
LUH 6973	Faeces (human, out)	Leiden, NL, 2000		
LUH 9667 (=8630†)	Eye (rabbit)	Dublin, Ireland	S. Fanning	Nemec <i>et al.</i> (2000)
NIPH 3	Hospital environment	Prague, 1991	J. Vráňková	
NIPH 1050	Urine (human, in)	Sedlčany, CZ, 1998	P. Ježek	Nemec <i>et al.</i> (2000)
NIPH 1054	Faeces (human, out)	Příbram, CZ, 1998	P. Ježek	
LUH 5624 (=R1-82†)	Sewage	Denmark, 1997	L. Guardabassi	Nemec <i>et al.</i> (2000)
LUH 5852	Sputum (human, in)	Leiden, NL, 1999		
LUH 7438 (=118FFC†)	Blood (human)	Coimbra, Portugal, 1998	G. da Silva	Nemec <i>et al.</i> (2000)
LUH 7832 (=V0112893†)	Wound (seal)	Utrecht, NL, 2001	J. Wagenaar	
LUH 8524 (=130380-2†)	Clinical specimen (human)	Heerlen, NL, 2003	Wagenvoort	
<i>Acinetobacter guillouiae</i> sp. nov.				
LMG 988 ^T (=NIPH 522 ^T =ATCC 11171 ^T =CIP 63.46 ^T =73 ^{T*})	Sewage	Before 1951		Bouvet & Grimont (1986)
RUH 2234 (=174*)	Contact lens	Malmö, Sweden, 1980	I. Tjernberg	Tjernberg & Ursing (1989)
RUH 2236 (=51*)	Urine (human)	Malmö, Sweden, 1980	I. Tjernberg	Tjernberg & Ursing (1989)
NIPH 682	Blood (human, in)	Č. Budějovice, CZ, 1997	O. Hausner	Nemec <i>et al.</i> (2000)
RUH 2860 (=58b*)	Wound (human)	Malmö, Sweden, 1980	I. Tjernberg	Tjernberg & Ursing (1989)
LUH 287 (=225*)	Sputum (human)	Odense, Denmark, 1988–9	P. Gerner-Smidt	Gerner-Smidt <i>et al.</i> (1991)
RUH 1050	Not known	Not known	J. van der Toorn	Janssen <i>et al.</i> (1997)
A23* (=CIP 107475)	Activated sludge	Albury, Australia	E. Carr	Carr <i>et al.</i> (2003)
LUH 5606 (=F4AUG7†)	Freshwater	Fish farm, Denmark, 1997	L. Guardabassi	Guardabassi <i>et al.</i> (2000)
LUH 5653	Blood (human, in)	Leiden, NL, 1999		Nemec <i>et al.</i> (2000)
LUH 7830 (=V0112205†)	Eye (cat)	Utrecht, NL, 2001	J. Wagenaar	
LUH 4560 (=A130d†)	Soil (barley field)	West Cornwall, UK, 1993–4	H. Seifert	Nemec <i>et al.</i> (2000)
LUH 6980	Faeces (human, out)	Leiden, NL, 2000		
NIPH 991	Ear swab (human, out)	Sedlčany, CZ, 1998	P. Ježek	Nemec <i>et al.</i> (2000)
ANC 3626	Soil (beech forest)	CZ, 2007	L. Křížová	
LUH 7013	Faeces (human, out)	Leiden, NL, 2000		
CCM 4725	Raw milk	North Moravia, CZ		

*Strain designation used in the reference publication.

†Strain designation used by the donor.

analysis (ARDRA), which is based on restriction endonuclease digestion of the amplified 16S rRNA gene (Dijkshoorn *et al.*, 1998). In the present study, 16S rRNA

gene sequences were obtained for *A. bereziniae* LUH 2634 and LUH 9667 and for *A. guillouiae* A23 and LUH 7013. The dendrogram of cluster analysis of these sequences and

Fig. 1. Rooted neighbour-joining tree based on two concatenated zones of *rpoB* of strains of *A. bereziniae* sp. nov. and *A. guillouiae* sp. nov. and representatives of all known species of the genus *Acinetobacter*. Cluster analysis was carried out using BioNumerics 5.1 (Applied Maths) with the *rpoB* sequence of *Pseudomonas aeruginosa* PAO1 as the outgroup. Bootstrap values $\geq 80\%$ (based on 1000 resamplings) are shown. GenBank accession numbers are given in parentheses. Bar, 5% sequence divergence.

the sequences of the type strains of *A. bereziniae*, *A. guillouiae* and other members of the genus *Acinetobacter* is shown in Fig. 2. The intraspecies sequence similarities of strains of *A. bereziniae* and *A. guillouiae* were 99.8–100 and 99.6–100%, respectively. The interspecies sequence similarities between the strains of *A. bereziniae* and *A. guillouiae* were in the range 99.1–99.6%, whereas the interspecies similarity between these strains and the type strains of other members of the genus *Acinetobacter* varied from 94.6% (*Acinetobacter townneri*) to 98.0% (*Acinetobacter johnsonii*). These data indicate a close re-

latedness between *A. bereziniae* and *A. guillouiae*, as well as their distinctness from other species of the genus. The sequences of *A. bereziniae* and *A. guillouiae* formed two respective clusters in the 16S rRNA gene-based dendrogram (Fig. 2), although they were supported only moderately by bootstrap values (86 and 69%, respectively). The relative homogeneity of the 16S rRNA gene sequences of the two species was also confirmed by ARDRA. Based on the ARDRA numbering system (Dijkshoorn *et al.*, 1998), all *A. bereziniae* and *A. guillouiae* strains shared the patterns *CfoI* 4, *AluI* 2, *MboI* 1, *RsaI* 2 and *MspI* 3 and only differed in

Fig. 2. Rooted 16S rRNA gene sequence-based tree showing the relationships between strains of *A. bereziniae* sp. nov. and *A. guillouiae* sp. nov., other members of the genus *Acinetobacter* and representatives of closely related genera. The cluster analysis, performed using GENEBASE (Applied Maths), was based on the neighbour-joining method using *Alkanindiges illinoisensis* GTI MVAB Hex1^T, *Psychrobacter immobilis* ATCC 43116^T and *Moraxella lacunata* ATCC 17967^T as the outgroup, an open gap penalty of 100% and a unit gap cost of 20%. The region used corresponds to *Escherichia coli* positions 99–1470. Bootstrap percentages ($\geq 50\%$) after 100 resamplings are shown. GenBank/EMBL accession numbers are given in parentheses. Bar, 1% sequence divergence.

the *BsmI* pattern: strains of *A. bereziniae* yielded *BsmI* pattern 1, whereas those of *A. guillouiae* had *BsmI* pattern 2.

DNA–DNA reassociation studies to delineate genomic species 10 and 11 have been performed previously in the studies of Bouvet & Grimont (1986) and Tjernberg & Ursing (1989). In the former study, four and three strains of genomic species 10 and 11, respectively, were investigated by DNA–DNA reassociation. The four strains of genomic species 10, including *A. bereziniae* LMG 1003^T (=69^T), were 65–68% similar to each other, with ΔT_m ranging from 0.8 to 1.2 °C, whereas the three strains of genomic species 11, including *A. guillouiae* LMG 988^T (=73^T), were 71–74% similar to one another. In contrast, genomic species 10 and 11 were only 21–30% similar to each other and the DNA–DNA reassociation values between these species and the strains of the other 10 genomic species were in the range 3–21%. Similar results have been obtained by Tjernberg & Ursing (1989). In their study, three strains, including *A. bereziniae* LMG 1003^T (=ATCC 17924^T), RUH 2222 (=198) and CCUG 28268 (=113:2), were allocated to genomic species 10, whereas five strains, including LMG 988^T (=CIP 63.46^T), RUH 2234 (=174), RUH 2236 (=51) and RUH 2860 (=58b), were classified as genomic species 11. These archive data further support the distinctiveness of the genomes of

A. bereziniae and *A. guillouiae* from each other and from the other (genomic) species described by Bouvet & Grimont (1986) and Tjernberg & Ursing (1989).

tDNA–intergenic length polymorphism analysis (tDNA–PCR) was carried out using one fluorescent primer to allow visualization of the amplified intergenic tRNA–spacer regions using ABI 310 capillary electrophoresis apparatus as described previously (Vanechoutte *et al.*, 1998; Baele *et al.*, 2000, 2001). Electrophoretograms were compared with BaseHopper, a software program developed at Ghent University (Baele *et al.*, 2001), available upon request from the authors. Both species yielded more than 50 amplified tRNA–intergenic spacer fragments, ranging between 50 and 477 bp in length. Most fragments were observed for both species, indicating their relatedness, but several fragments enabled differentiation between them: spacers with lengths of 139.6 ± 0.3 (mean $\pm$ SD), 166.3 ± 0.2 , 181.6 ± 0.2 , 220.7 ± 0.1 , 228.6 ± 0.2 and 238.5 ± 0.3 bp were observed exclusively for *A. bereziniae* and present in almost all strains of this species, whereas spacers with lengths of 109.6 ± 0.4 , 173.6 ± 0.3 , 221.7 ± 0.1 , 229.7 ± 0.1 , 235.6 ± 0.5 and 477.7 ± 1.2 bp were present exclusively in almost all *A. guillouiae* strains.

Results of phenotypic tests are given in Table 2 and in the species descriptions. All tests have been described in detail

by Nemec *et al.* (2009). Strains of *A. bereziniae* and *A. guillouiae* were unable to lyse sheep erythrocytes in the blood agar test or produce gelatinase, but they were able to grow on a number of carbon sources. Table 2 shows phenotypic characters that are useful for the differentiation of these species from the *Acinetobacter* species that include non-haemolytic and/or non-proteolytic strains. A unique property of both *A. bereziniae* and *A. guillouiae* compared to all known *Acinetobacter* species was the ability to grow on histamine, although only 63–65 % of strains of these species were positive in this test. The two species showed an overall phenotypic similarity to each other, leaving only a few features that could be used for their presumptive differentiation from each other. With three exceptions,

the *A. bereziniae* strains oxidized D-glucose and grew in brain-heart infusion (BHI) agar at 38 °C, whereas *A. guillouiae* strains were negative in these tests. The exceptions included two *A. bereziniae* strains (NIPH 3 and LUH 9667) that were negative in the D-glucose oxidation test and one *A. guillouiae* strain (NIPH 682) that could grow at 38 °C. In addition, 82 % of the *A. guillouiae* strains grew on tryptamine, whereas none of the *A. bereziniae* strains utilized this carbon source. However, growth of *A. guillouiae* strains on tryptamine was usually slow and visible only after 4 or 6 days of incubation.

Matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) MS measurements were carried out using

Table 2. Differential phenotypic properties of *A. bereziniae* sp. nov., *A. guillouiae* sp. nov. and other species comprising non-haemolytic or proteolytic strains

Species: 1, *A. bereziniae* sp. nov. (16 strains); 2, *A. guillouiae* sp. nov. (17); 3, *A. baumannii* (8); 4, *A. calcoaceticus* (3); 5, genomic species 3 (7); 6, genomic species 13TU (9); 7, *A. junii* (4); 8, *A. johnsonii* (8); 9, *A. lwoffii* (8); 10, *A. radioresistens* (6); 11, *A. ursingii* (15); 12, *A. schindleri* (13); 13, *A. parvus* (10); 14, *A. baylyi* (5); 15, *A. towneri* (2); 16, *A. bouvetii* (1); 17, *A. gernerii* (1); 18, *A. tandoii* (1). All results were obtained in the present study. Type or reference strains ($n=103$) of previously described genomic species were selected from the studies of Bouvet & Grimont (1986), Tjernberg & Ursing (1989), Janssen *et al.* (1997), Carr *et al.* (2003), Nemec *et al.* (2001, 2003) and Vaneechoutte *et al.* (2009). +, All strains positive within 4 days of incubation; –, all strains negative after 10 days of incubation; v+, 85–99 % of strains positive within 6 days of incubation; v, 16–84 % of strains positive within 6 days of incubation; v–, 1–15 % of strains positive within 6 days of incubation; ND, not determined. For *A. bereziniae* and *A. guillouiae*, percentages of positive reactions are indicated for strain-dependent reactions.

Characteristic	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Growth at 44 °C	–	–	+	–	–	v	–	–	–	–	–	–	–	–	–	–	–	–
Growth at 38 °C	+	6	+	+	+	+	+	v	v+	+	v	+	ND	+	+	ND	–	+
Acid from D-glucose	88	–	+	+	+	+	–	–	v–	–	–	–	–	+	–	–	+	–
Utilization of:																		
4-Aminobutyrate	+	88	+	+	+	+	v	v	v+	+	–	–	–	+	–	–	+	+
trans-Aconitate	38	12	+	+	+	v	–	–	–	–	–	–	–	+	–	–	–	+
Citrate (Simmons')	+	+	+	+	+	+	v	v+	v–	–	v+	v	–	+	–	+	+	+
Glutarate	+	+	+	+	v+	+	–	–	–	+	v+	v+	–	v	–	+	+	–
L-Aspartate	+	+	+	+	+	+	v	v	–	–	v+	–	–	+	–	–	+	+
Azelate	63	+	+	+	+	+	–	–	+	+	+	v	–	+	–	–	+	–
β-Alanine	+	94	+	+	+	+	–	–	–	–	–	–	–	–	–	–	+	–
L-Histidine	94	94	+	+	+	+	v	–	–	–	–	–	–	–	–	+	–	+
Malonate	–	18	+	+	+	v	–	v	–	+	–	–	–	+	–	–	–	+
Histamine	63	65	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
L-Phenylalanine	–	–	+	+	v	+	–	–	–	v	–	–	–	–	–	–	+	+
Phenylacetate	25	65	+	+	v	+	–	–	v+	+	–	–	–	–	–	–	+	+
4-Hydroxybenzoate	88	88	+	+	+	v+	–	v–	–	–	v+	v	–	+	–	–	+	+
L-Ornithine	–	–	v	+	v+	v+	–	v–	–	–	–	–	v–	–	–	–	–	+
L-Arginine	–	–	+	+	+	+	+	v	–	–	–	–	–	+	–	–	–	+
L-Leucine	–	–	+	+	+	+	v	–	–	+	–	–	–	–	–	–	–	+
L-Arabinose	–	–	+	v	+	+	–	–	–	–	–	–	–	–	–	–	–	–
2,3-Butanediol	+	+	+	+	v	v+	–	v	–	+	–	–	–	+	v	–	+	+
D-Glucose	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–	–
Trigonelline	+	59	v+	v	v	v	–	–	–	–	–	–	–	+	–	–	–	–
Tricarballoylate	38	12	+	+	+	v+	–	–	–	–	–	v	–	v	–	–	–	+
Putrescine	–	–	+	+	+	v+	–	–	–	–	–	–	–	–	–	–	–	+
Adipate	63	+	+	+	+	+	–	–	v+	v	+	ND	–	+	–	–	+	–

an Ultraflex III instrument (Bruker Daltonik) operated in linear positive mode. External calibration of the mass spectra was performed using *Escherichia coli* DH5 α standard peaks. Preparation of samples included sterilization in 75 % ethanol and protein extraction with an acetonitrile/formic acid/water (50:35:15) mixture. Aliquots of protein extracts (0.3 μ l per well) were deposited on the MALDI target and overlaid with 0.3 μ l saturated solution of an α -cyano-4-hydroxycinnamic acid in acetonitrile/water/trifluoroacetic acid (50:47.5:2.5) mixture (Williams *et al.*, 2003). Each extract was spotted onto three MALDI target wells. Five consecutive spectrum accumulations including 1000 laser shots aimed using a lattice raster were acquired from each of the wells. Obtained spectra were processed using BioTyper software (version 1.1; Bruker Daltonik). Signals present in at least 70 % of the spectrum accumulations were taken into account for statistical analysis. The MALDI-TOF MS-based dendrogram was generated using the correlation distance measure with the mean linkage algorithm. MALDI-TOF MS analysis of 11 strains of each of *A. bereziniae* and *A. guillouiae* showed specific signals for all representatives of the particular species, namely peaks 7156, 7407 and 7796 (*m/z*) for *A. bereziniae* and 3258, 3690, 6513, 6978, 7378 and 7813 (*m/z*) for *A. guillouiae* (see Supplementary Fig. S2). Supplementary Fig. S2 also shows the results of cluster analysis, which demonstrate a clear separation of the respective mass spectra of *A. bereziniae* and *A. guillouiae*.

Description of *Acinetobacter bereziniae* sp. nov.

Acinetobacter bereziniae (be.re.zi.ni'ae. N.L. fem. gen. n. *berezinae* of Bérézin, named after Eugénie Bergogne-Bérézin, French medical microbiologist).

Phenotypic characteristics correspond to those of the genus (Baumann *et al.*, 1968), i.e. Gram-stain-negative, strictly aerobic, oxidase-negative, catalase-positive, non-motile coccobacillus, capable of growing in mineral media with acetate as sole carbon source and ammonia as sole source of nitrogen, and incapable of dissimilative denitrification. Positive in the transformation assay of Juni (1972). The species description is based on characterization of 16 strains. Colonies on tryptic soy agar (TSA; Oxoid) after 24 h incubation at 30 °C are 1.0–2.0 mm in diameter, circular, convex, smooth and slightly opaque with entire margins. Growth occurs in BHI (Oxoid) at 25–38 °C, but not at 41 °C. Acid is produced from D-glucose by the majority of strains (88 % of the strains studied), gelatin is not hydrolysed and haemolysis is not observed on agar media supplemented with sheep erythrocytes. Acetate, ethanol, DL-lactate, 4-aminobutyrate, citrate (Simmons'), glutarate, L-aspartate, β -alanine, 2,3-butanediol, trigonelline, benzoate and L-glutamate are utilized as sole sources of carbon with growth visible within 4 days (generally 2 days) of incubation. No growth on malonate, L-phenylalanine, laevulinate, citraconate, L-tartrate, L-ornithine, L-arginine, L-leucine, D-glucose, D-gluconate, D-ribose, L-arabinose, gentisate, tryptamine or putrescine occurs within 10 days.

Some strains utilize *trans*-aconitate (38 % of the strains studied), azelate (63 %), L-histidine (94 %), D-malate (88 %), histamine (63 %), phenylacetate (25 %), 4-hydroxybenzoate (88 %), adipate (63 %) and tricarballoylate (38 %) within 6 days.

The type strain is LMG 1003^T (=CIP 70.12^T =ATCC 17924^T), isolated from a human wound before 1960. It was used as the reference strain of genospecies 10 by Bouvet & Grimont (1986). This strain produces acid from D-glucose and grows on azelate, L-histidine, D-malate, histamine, 4-hydroxybenzoate and adipate, but does not utilize *trans*-aconitate, phenylacetate or tricarballoylate. Strain LMG 1003^T produces two stable colony types.

Description of *Acinetobacter guillouiae* sp. nov.

Acinetobacter guillouiae (guil.lou'i.æ. N.L. fem. gen. n. *guillouiae* of Guillou, named after Marie-Laure Joly-Guillou, French medical microbiologist).

Phenotypic characteristics correspond to those of the genus (Baumann *et al.*, 1968), i.e. Gram-stain-negative, strictly aerobic, oxidase-negative, catalase-positive, non-motile coccobacillus, capable of growing in mineral media with acetate as sole carbon source and ammonia as sole source of nitrogen, and incapable of dissimilative denitrification. Positive in the transformation assay of Juni (1972). The species description is based on characterization of 17 strains. Colonies on TSA after 24 h incubation at 30 °C are 1.0–2.0 mm in diameter, circular, convex, smooth and slightly opaque with entire margins. Growth occurs in BHI at 25–35 °C; no growth usually occurs at 38 °C. Acid is not produced from D-glucose, gelatin is not hydrolysed and haemolysis is not observed on agar media supplemented with sheep erythrocytes. Acetate, ethanol, DL-lactate, azelate, citrate (Simmons'), glutarate, L-aspartate, 2,3-butanediol, adipate and L-glutamate are utilized as sole sources of carbon with growth visible within 4 days (generally 2 days) of incubation. No growth on L-phenylalanine, laevulinate, citraconate, L-tartrate, L-ornithine, L-arginine, L-leucine, D-glucose, D-gluconate, D-ribose, L-arabinose or putrescine occurs within 10 days. Some strains utilize *trans*-aconitate (12 % of the strains studied), 4-aminobutyrate (88 %), β -alanine (94 %), L-histidine (94 %), D-malate (94 %), histamine (65 %), phenylacetate (65 %), 4-hydroxybenzoate (88 %), malonate (18 %), gentisate (18 %), trigonelline (59 %), tryptamine (82 %), benzoate (88 %) and tricarballoylate (12 %) within 6 days.

The type strain is LMG 988^T (=CIP 63.46^T =ATCC 11171^T =CCUG 2491^T), isolated from sewage containing gas-work effluent before 1951. It was used as the reference strain of genospecies 11 by Bouvet & Grimont (1986). This strain grows on β -alanine, L-histidine, D-malate, histamine, phenylacetate, 4-hydroxybenzoate, tryptamine and benzoate, but does not grow at 38 °C or utilize *trans*-aconitate, malonate, gentisate, trigonelline or tricarballoylate. Growth of the type strain on 4-aminobutyrate is not reproducible.

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