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Acinetobacter equi sp. nov. isolated from horse faeces

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The GenBank accession numbers for the partial 16S rRNA, *rpoB* and *gyrB* gene sequences of strain 114^T (=DSM 27228^T=CCUG 65204^T) are KC494698, KC494699 and KP690075, respectively. The whole genome sequence of strain 114^T is available at GenBank under accession number CP012808 (Bioproject PRJNA296861).

SUMMARY

We studied the taxonomic position of five strains isolated from horse faeces which shared identical 16S rRNA gene sequences. The cells of all isolates are Gram-negative staining, obligate aerobic and individuals have a rod shaped appearance. The strains show highest 16S rRNA gene sequence similarities to *A. lwoffii* (98.3%), *A. haemolyticus* (98.0%), *A. johnsonii* (97.9%), and *A. brisouii* (97.9%). Whole genome sequencing of strain 114^T and phylogeny reconstruction based on a core set of 1,061 *Acinetobacter* genes indicated *A. bouvetii* CIP 107468^T as the closest relative among the *Acinetobacter* species for which whole genome sequences are available. The genomic G+C content of strain 114^T is 34.9 mol%, which is lower than any other value reported for *Acinetobacter*. The predominant polyamine is 1,3-diaminopropane, which is typical for the genus *Acinetobacter*. The most abundant fatty acids are $C_{16:1}$ ω7c and/or iso-$C_{15:0}$ 2-OH (36%) and $C_{16:0}$ (28%). The proportion of $C_{18:1}$ ω9c (7%) is distinctively low compared to most *Acinetobacter* species. Major ubiquinone of strain 114^T is Q-9. Microscopic studies revealed the presence of pili and the absence of flagella. The capability of all five strains to utilize L-arabinose and gentisate as well as their lack of growth at temperatures of 41 °C and above provide sufficient criteria to distinguish them from all *Acinetobacter* species with validly published names. Strain 114^T (=DSM 27228^T=CCUG 65204^T) is considered as type strain of a novel species within the genus *Acinetobacter*, for which the name *Acinetobacter equi* sp. nov. is proposed.

49 The genus *Acinetobacter* is highly diverse (Touchon *et al.*, 2014). It comprises species
50 ubiquitous in soil and water as well as species appearing as opportunistic pathogens in the
51 hospital setting but with poorly defined natural reservoirs such as *A. baumannii*, *A. pittii* and
52 *A. nosocomialis*. At present there are around 40 species with standing in nomenclature
53 (http://www.bacterio.net/-allnamesac.html), several genomic species and a number of species
54 with not yet validated names. Notably, a recent ecological study from Lebanon reported as
55 many as 30 unique isolates which represent potential new species (Rafei *et al.*, 2015). This
56 indicates that the genus *Acinetobacter* is more species-rich than hitherto acknowledged.
57
58 In search of natural reservoirs of the nosocomial pathogen *Acinetobacter baumannii*, we
59 collected faeces from a horse at Minsleben, Germany (zip code D-38855, Harz district,
60 Saxony-Anhalt, coordinates: 51° 52′ 0.84″ N, 10° 49′ 43.68″ E) in October, 2012. Fresh
61 faeces samples were collected with Amies agar gel medium transport swabs (108C, Copan,
62 Italy), stored over night at room temperature and plated on CHROMagar™ Acinetobacter
63 (CHROMagar, France) the next day. After incubation at 37 °C for 24 hours, *Acinetobacter*-
64 like red colonies grew to a size of 1 - 2 mm. Single colonies were transferred into 50 µl of
65 sterile water and boiled for 10 minutes. After centrifugation of the samples, 2 µl of the
66 supernatant was taken as template to test by PCR for the $bla_{\text{OXA-51-like}}$ carbapenemase gene
67 intrinsic to *A. baumannii* (Turton *et al.*, 2006). As the result was negative, partial sequencing
68 of the *rpoB* gene from strain 114^{T} was conducted using primers Ac696F and Ac1598R as
69 described previously (Nemec *et al.*, 2009). The resulting sequence of 861 bp in length
70 (GenBank accession no. KC494699) served as query in a subsequent BLAST search against
71 NCBI nucleotide collection and non-redundant protein database, respectively. The search
72 revealed the *rpoB* gene of *A. johnsonii* as the highest scoring hit, showing however only 88%
73 sequence identity to the query sequence and 93% sequence identity for the corresponding

amino acid sequence. Sequence identity values for *A. lwoffii* (87% and 92%), *A. haemolyticus* (83% and 90%) and *A. brisouii* (83% and 88%) are in a similar range.

Next, spectroscopic DNA-DNA hybridization of strain 114^T against type strains *A. johnsonii* DSM 6963^T, *A. lwoffii* DSM 2403^T, *A. haemolyticus* DSM 6962^T, and *A. brisouii* DSM 18516^T was performed by the Identification service and Dr. Cathrin Spröer, DSMZ, Braunschweig, Germany. Cells were disrupted by using a Constant Systems TS 0.75 KW (IUL Instruments, Germany) and the DNA in the crude lysate was purified by chromatography on hydroxyapatite (Cashion *et al.*, 1977). Spectrophotometric determination of DNA-DNA hybridization was performed as described (De Ley *et al.*, 1970; Huss *et al.*, 1983). Two determinations each at 66°C in 2 x SSC yielded average DNA-DNA similarity of strain 114^T with *A. johnsonii* DSM 6963^T of 11,75 (±1.75%), 16.25% (± 4.15%) with *A. lwoffii* DSM 2403^T, 27.45 (±1.85%) with *A. haemolyticus* DSM 6962^T, and 35.15% (±0.95%) with *A. brisouii* DSM 18516^T (dDDH values 21.4, 22.0, 21.4, and 22.1%, respectively). This suggests an isolated position of strain 114^T within the genus *Acinetobacter*. To corroborate this assumption we further characterized this strain in a polyphasic approach.

The Identification service of the German Collection of Microorgamisms and Cell Cultures (DSMZ, Braunschweig, Germany; Dr. Cathrin Spröer) was commissioned to perform a 16S rRNA gene sequence analysis of strain 114^T. The resulting partial 16S rRNA gene sequence encompassed 1523 nucleotides (GenBank accession no. KC494698). We then performed a structure guided alignment of this sequence to 16S rRNA genes from representatives across the genus *Acinetobacter*. This analysis revealed highest similarities with *A. lwoffii* (98.3%), *A. haemolyticus* (98.0%), *A. johnsonii* (97.9%), and *A. brisouii* (97.9%). The neighbour-joining tree based on these sequences (Fig. 1) lent support to our initial notion that strain 114^T takes a distinct position within the genus, however the branch support for strain 114^T was low

(bootstrap value 32%). The distinct position of strain 114^T was further substantiated by Bayesian consensus tree searches based on *rpoB* and *gyrB* nucleotide sequence alignments, respectively (Suppl. Figures S1 and S2, Supplementary Information S1, and Table S1).

Next, we determined the whole genome sequence of strain 114^T. To this end, chromosomal DNA from an overnight culture was prepared using a QIAGEN Genomic-tip 500/G according to the manufacturer's instructions, and GATC Biotech (Konstanz, Germany) was commissioned to perform genome sequencing (PacBio RS II technology). This attempt yielded the complete genome of 3,054,269 base pairs (assembly program HGAP3, coverage 250-fold). The data are available at GenBank under accession no. CP012808 (Bioproject PRJNA296861).

To decisively determine the phylogenetic placement of strain 114^T we pursued a phylogenomic approach integrating the analysis of 1,061 *Acinetobacter* core genes (see Supplementary Information S1 and Table S2). A maximum likelihood tree reconstruction considering 59 strains from the genus *Acinetobacter* revealed that strain 114^T is only distantly related to any so far described and sequenced species with *A. bouvetii* CIP 107468^T being the closest relative (Fig. 2).

In line with its isolated position in the *Acinetobacter* phylogeny, also the G + C content of the 114^T genome is with 34.9 mol% the lowest among all hitherto analysed members of this genus (G + C contents ranging from 36.6 to 54.7 mol% (Alvarez-Perez *et al.*, 2013; Choi *et al.*, 2013; Kim *et al.*, 2014; Li *et al.*, 2014; Smet *et al.*, 2014)).

Using the GGDC 2.0 web server we next calculated genome-based pairwise digital DNA-DNA hybridization values for strain 114^T and all available genomes from *Acinetobacter* type strains (http://ggdc.dsmz.de/distcalc2.php, (Meier-Kolthoff *et al.*, 2013)). The DDH estimates were all in a very narrow range from 20.7% (*A. harbinensis*) to 22.4% (*A. guillouiae*) (GGDC 2.0, formula 2). Collectively, DNA-DNA relatedness of strain 114^T with all sequenced type

strains can be estimated to be significantly below the threshold value of 70% recommended for delineation of a species (Stackebrandt & Goebel, 1994; Wayne *et al.*, 1987). To obtain a more refined picture, we assessed the genome-wide pairwise sequence similarity between strain 114^T and its closest relative according to core genome-based phylogeny, *A. bouvetii* CIP 107468^T (see Fig. 2); (Average nucleotide identity (ANI), http://enve-omics.ce.gatech.edu/ani/). The obtained value of 80.8% is substantially below the value of 95% that is considered as the maximal intraspecific sequence divergence (Rodriguez-R & Konstantinidis, 2014).

Cell morphology of strain 114^T as depicted in Supplementary Fig. S3 was examined by transmission electron microscopy (TEM; negative staining and ultrathin sectioning) and scanning electron microscopy (SEM) (for details of the preparation protocols see Supplementary Information S2). Bacteria appeared rod-shaped with a mean size of 1.5 x 0.9 µm (Suppl. Fig. S3 A and B). TEM further revealed the presence of pili especially if bacteria were grown or transferred in liquid media before fixation for electron microscopy (Suppl. Fig. S3 C and D). While some of the bacteria showed pili all around their surface others displayed pili only at one pole or did not show any pili at all. In line with the notion that all members of the genus *Acinetobacter* lack flagella, we could not observe any flagella by TEM and SEM in strain 114^T (Suppl. Fig. S3). Accordingly, no swimming motility could be noticed for strain 114^T (data not shown).

For production of biomass used for chemotaxonomic analyses we harvested cells grown in PYE broth (0.3% peptone from casein, 0.3% yeast extract, pH 7.2). Cells subjected to polyamine analysis were harvested at the late exponential growth phase as recommended by Busse & Auling (Busse & Auling, 1988). Cells subjected to the integrated procedure for extraction of quinones and polar lipids (Altenburger *et al.*, 1996; Tindall, 1990a; b) were

149 harvested from the stationary growth phase. For polyamine anaylsis HPLC conditions were
150 applied as described by Busse *et al*. (Busse *et al.*, 1997). The equipment used for HPLC
151 analysis was described by Stolz *et al*. (Stolz *et al.*, 2007).
152 The predominant polyamine of strain 114^T was 1,3-diaminopropane [100.2 µmol (g dry
153 weight)$^{-1}$] and minor polyamines were spermidine [2.6 µmol (g dry weight)$^{-1}$]), spermine [2.2
154 µmol (g dry weight)$^{-1}$], putrescine [1.3 µmol (g dry weight)$^{-1}$]) and cadaverine [0.3 µmol (g
155 dry weight)$^{-1}$]). Our results integrate with the previous observation that 1,3-diaminopropane is
156 the major polyamine in *Acinetobacter* (Auling *et al.*, 1991; Busse & Auling, 1988; Hamana &
157 Matsuzaki, 1992; Kämpfer *et al.*, 1992). Major ubiquinone of strain 114^T was Q-9 (84%),
158 further quinones were Q-8 (13%) and Q-10 (3%).
159 Major polar lipids of strain 114^T were diphosphatidyl glycerol, phosphatidyl glycerol,
160 phosphatidyl ethanolamine, phosphatidyl serine, an unidentified aminophospholipid (APL)
161 and an unidentified phospholipid (PL). Additionally, 11 unidentified lipids without amino-,
162 phosphate- or sugar residues were detected (Supplementary Fig. S4). This polar lipid profile
163 was almost identical to that of *Acinetobacter baumannii* ATCC 19606^T with the sole
164 difference that the profile of the latter showed absence of few minor lipids (results not
165 shown).
166
167 Fatty acids were extracted and analysed by the Identification service of the DSMZ,
168 Braunschweig, Germany, according to the standard protocol of the Microbial Identification
169 System (MIDI Microbial ID Inc.) by using the TSB40 method. To this end strain 114^T was
170 cultivated on trypticase soy broth agar for 24 h at 37°C. The major fatty acids determined for
171 strain 114^T were $C_{16:1}$ ω7c and/or iso-$C_{15:0}$ 2-OH (36.2%; summed feature 3), $C_{16:0}$ (28.2%),
172 $C_{18:1}$ ω9c (7.0%), and $C_{12:0}$ (6.5%) (Supplementary Table S3). Importantly, the portion of
173 $C_{18:1}$ ω9c (7.0%) is considerably lower than in most species with portions ranging from 17-

45% (Anandham *et al.*, 2010; Kämpfer *et al.*, 1992; Lee & Lee, 2010; Lee *et al.*, 2009; Malhotra *et al.*, 2012; Vaz-Moreira *et al.*, 2011; Yoon *et al.*, 2007). In the closest relative, *A. bouvetii*, the proportion of $C_{18:1}$ ω9c had been determined at a relatively low level of 13.5-13.8% (Abbas *et al.*, 2014; Lee & Lee, 2010) and in the distantly related species *A. apis*, *A. nectaris* and *A. boissieri* it was even lower (1-6%) (Kim *et al.*, 2014).

Recently, the *Galleria mellonella* infection model has been established to characterize the virulence potential of *Acinetobacter* spp. (Peleg *et al.*, 2009). We found that infection doses of strain 114^T ten times higher than that used for the virulent reference strain *A. baumannii* ATCC 17978 (1 - 2 x 10^6 CFU of strain 114^T compared to 2 - 3 x 10^5 CFU of ATCC 17978) caused only very limited lethality. Also, melanisation of the larvae, being indicative of a stress response, was very weak (see Supplementary Fig. S5).

In the year 2015, in an attempt to isolate additional representatives of the supposedly new species, we collected horse faeces in the Wernigerode region. While we were unsuccessful in isolating related bacteria from horse samples in Minsleben where strain 114^T was collected, we isolated related bacteria from faeces of four different horses in the neighbouring village of Silstedt (51° 51′ 41″ N, 10° 50′ 55″ E) using CHROMagar™ Acinetobacter as above. The partial 16S rRNA sequencing of strains Sil3.1, Sil4.1, Sil7.1 and Sil10.2 revealed no difference to strain 114^T and only one of the strains (Sil7.1) showed a distinct *rpoB* sequence with 99.5% identity to strain 114^T (KT599435). A RAPD-PCR using primer A (5'-CTGGCGGCTTG-3'; (Ziemke *et al.*, 1997)) was performed and confirmed the distinctness of each of the isolates (Supplementary Fig. S6).

198 All five strains were oxidase negative, catalase-positive and grew well in 2xYT broth (16 g/l
199 tryptone, 10 g/l yeast extract, 5 g/l NaCl) as well as in brain-heart-infusion broth at 37 °C
200 under aerobic conditions but not at 41°C. They grew well on CHROMagar™ Acinetobacter,
201 on bile-chrysoidine-glycerol agar (GCG agar; Ziesché *et al.*, 1985; Heipha Diagnostika) and
202 on MacConkey agar after incubation for 24 hours at 27°C and at 37°C.
203 The API 20NE (Biomérieux, France) test system showed nitrate reduction under aerobic
204 conditions in all five strains (two repetitions each), which is rare among members of the genus
205 (Bouvet & Grimont, 1986), and assimilation of caprate (weak), adipate, malate and citrate
206 after incubation at 30 °C for 48 hours. All other API 20NE reactions were negative.
207 We have further characterized all five strains phenotypically using the Biolog GEN III
208 MicroPlate (protocol A, 22 h at 33°C) to identify the *Acinetobacter* species with most similar
209 metabolic capabilities. With all five strains tested 2-5 times each, the highest similarity was
210 either to *Acinetobacter variabilis* ("*Acinetobacter* genomospecies 15TU" according to the
211 OMNILOG database), to *Acinetobacter ursingii*, or to *Acinetobacter guillouiae*.
212 Reproducibly, all strains showed respiration on L-alanine, L-glutamic acid, methylpyruvate,
213 L-malic acid, bromosuccinic acid, tween 40, and acetic acid. Utilization of these compounds
214 in liquid mineral medium was confirmed following established protocols (Nemec *et al.*,
215 2009).
216 The metabolic capabilities of the five strains were further tested following protocols
217 established for *Acinetobacter* (Nemec *et al.*, 2009) and compared to the data provided for all
218 species with validly published names recently (Krizova *et al.*, 2015). Table 1 summarizes the
219 phenotypes of the proposed species *A. equi* and of the three most similar species according to
220 Biolog analyses, the most closely related species according to core genome-based phylogeny,
221 *A. bouvetii*, as well as the species with less than 6 differentiating traits (Krizova *et al.*, 2015).
222 The capability of all five strains to assimilate L-arabinose and gentisate in combination with

the inability to grow at 41°C and above are sufficient to differentiate them from all species with validly published names (Krizova *et al.*, 2015) as well as from the recently described *A. populi* sp. nov. (Li *et al.*, 2015), and at least four differentiating traits were identified for all species (Table 1).

Collectively, on the basis of these results it is obvious that strain 114^T is the representative of a novel species within the genus *Acinetobacter* for which the name *Acinetobacter equi* sp. nov. is proposed.

Description of *Acinetobacter equi* sp. nov.

Acinetobacter equi (e′qui. L. gen. n. *equi* of the horse).

Cells are rods, 0.8 µm wide and 1 - 1.5 µm long and stain Gram-negative. Aerobic, oxidase negative, catalase positive. Cells carry no flagella. Pigments are not produced. Colonies appear red on CHROMagar™ Acinetobacter and grow up to 1 - 2 mm in diameter within 24 h at 37 °C. Growth occurs on MacConkey agar and on bile-chrysoidine-glycerol agar after incubation at both 27 °C and 37 °C. Non-haemolytic on sheep blood agar, no gelatinase activity. The API 20NE test system shows nitrate reduction, variable arginine dihydrolase activity, utilization of L-arabinose, caprate (weak), adipate, malate and citrate. Acid production from D-glucose is variable. The following compounds are utilized on mineral medium: acetate, adipate, L-alanine, L-arabinose, bromosuccinic acid, citrate, gentisate, L-glutamate, L-histidine, 4-hydroxybenzoate, DL-lactate, L-malate, methylpyruvate, phenylacetate, tween 40. The following compounds are not utilized on mineral medium: trans-aconitate, β-alanine, 4-aminobutyrate, L-arginine, L-aspartate, azelate, benzoate, 2,3-butanediol, D-glucose, glutarate, L-leucine, D-malate, malonate, L-ornithine, L-phenlyalanine, propionic acid, putrescine, L-tartrate, trigonelline, tryptamine. Utilization of

ethanol, tricarballylate, and L-pyroglutamic acid is variable. The type strain of the species has a DNA G + C content of 34.9 mol%. Major fatty acids of the type strain are $C_{16:1}$ ω7c and/or iso-$C_{15:0}$ 2-OH, $C_{16:0}$, $C_{18:1}$ ω9c, and $C_{12:0}$. Major ubiquinone is Q-9. Major polyamine is 1,3-diaminopropane. Major polar lipids are diphosphatidyl glycerol, phosphatidyl glycerol, phosphatidyl ethanolamine, phosphatidyl serine, an unidentified aminophospholipid and an unidentified phospholipid. The type strain, 114^T (=DSM 27228^T=CCUG 65204^T), was isolated from faeces of a horse collected in Minsleben (Germany).

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TABLES

Table 1: Phenotypic features of *Acinetobacter equi* sp. nov. and of selected *Acinetobacter* species

Feature	*A. equi* (5)	*A. bouvetii* (1)	*A. lwoffii* (16)	*A. junii* (14)	*A. nosocomialis* (20)	*A. schindleri* (22)	*A. guillouiae* (17)	*A. ursingii* (29)	*A. variabilis* (16)
Growth at 41°C	-	-	6	93	+	+	-	-	+
Growth at 37°C	+	D	+	+	+	+	D	+	+
Acid from D-Glucose	20W (-)	-	19	-	+	-	-	-	13
Hemolysis (sheep blood)	-	-	-	50	-	-	-	-	-
Gelatinase	-	-	-	-	-	-	-	-	-
Utilization of *trans*-Aconitate	-	-	6	-	60	-	12	-	6
Adipate	+	-	81	-	95	41	+	+	69
β-Alanine	-	-	-	-	85	-	94	-	-
4-Aminobutyrate	-	-	88	86	+	-	88	-	19
L-Arabinose	+	-	-	-	+	-	-	-	19
L-Arginine	-	-	-	93	+	-	-	-	19
L-Aspartate	-	-	-	21	+	-	+	97W	-
Azelate	-	-	+	-	95	64	+	+	81
Benzoate	-	+	88	79	90	91	88	52	88
2,3-Butanediol	-	-	6	-	90	32	+	-	81
Citrate (Simmons)	+	+	13	79	+	59	+	+	25
Ethanol	80 (-)	+	+	93	+	95	+	+	+
Gentisate	+	-	-	-	10	41	18	-	-
L-Glutamate	+	+	6	+	+	-	+	+	25
Glutarate	-	+	-	-	95	95	+	97	19
L-Histidine	+	+	-	93	+	-	94	-	-
4-Hydroxybenzoate	+	-	-	-	80	64	88	97	-
DL-Lactate	+	+	89	93	+	+	+	+	6
L-Leucine	-	-	-	14	95	-	-	-	-
D-Malate	-	-	19W	79	+	95W	94	+W	13
Malonate	-	-	6	-	20	-	18	-	-
L-Ornithine	-	-	-	-	95	-	-	-	-
Phenylacetate	+	-	69	-	85	-	65	-	75
L-Phenylalanine	-	-	-	-	85	-	-	-	38
Putrescine	-	-	-	-	95	-	-	-	-
L-Tartrate	-	-	-	-	-	18	-	-	-
Tricarballylate	80 (-)	-	6	-	95	45	12	-	-
Trigonelline	-	-	-	-	20	-	59	-	-
Tryptamine	-	-	-	-	-	-	82	-	-

Table 1. Phenotypic features of *Acinetobacter equi* sp. nov. and of selected *Acinetobacter* species. *Acinetobacter* species selected were: *A. bouvetii*, the closest relative according to core genome-based phylogeny; species with validly published names with less than 6 distinguishing features based on the data by (Krizova *et al.*, 2015); the species with most similar metabolic profiles according to Biolog analyses (*A. variabilis*, *A. ursingii* and *A. guillouiae*); data for all species except *A. equi* from (Krizova *et al.*, 2015); data for *A. equi* from triplicates. Utilization of compounds was tested in mineral medium as described (Nemec *et al.*, 2009). Biolog data were not included. Acidification from D-glucose and gelatinase activity were tested with API 20 NE; temperature growth tests were performed in brain-heart-infusion broth. Number of strains considered given in parenthesis after species name. +, all strains positive; -, all strains negative; numbers give the percentage of positives in case of variability; (-) indicates the trait of strain 114^T in case of variability; W, (mostly) weak positive reaction, and D, (mostly) doubtful or irreproducible reaction (Krizova *et al.*, 2015). Grey shading indicates features with discriminatory power relative to *A. equi* (all strains positive compared to a negative result for all strains of *A. equi* or *vice versa*). Type strains *A. johnsonii* DSM 6963^T, *A. lwoffii* DSM 2403^T, *A. haemolyticus* DSM 6962^T, *A. brisouii* DSM 18516^T, and *A. baumannii* ATCC 19606^T were used for quality control and results compared to data provided by (Krizova *et al.*, 2015).

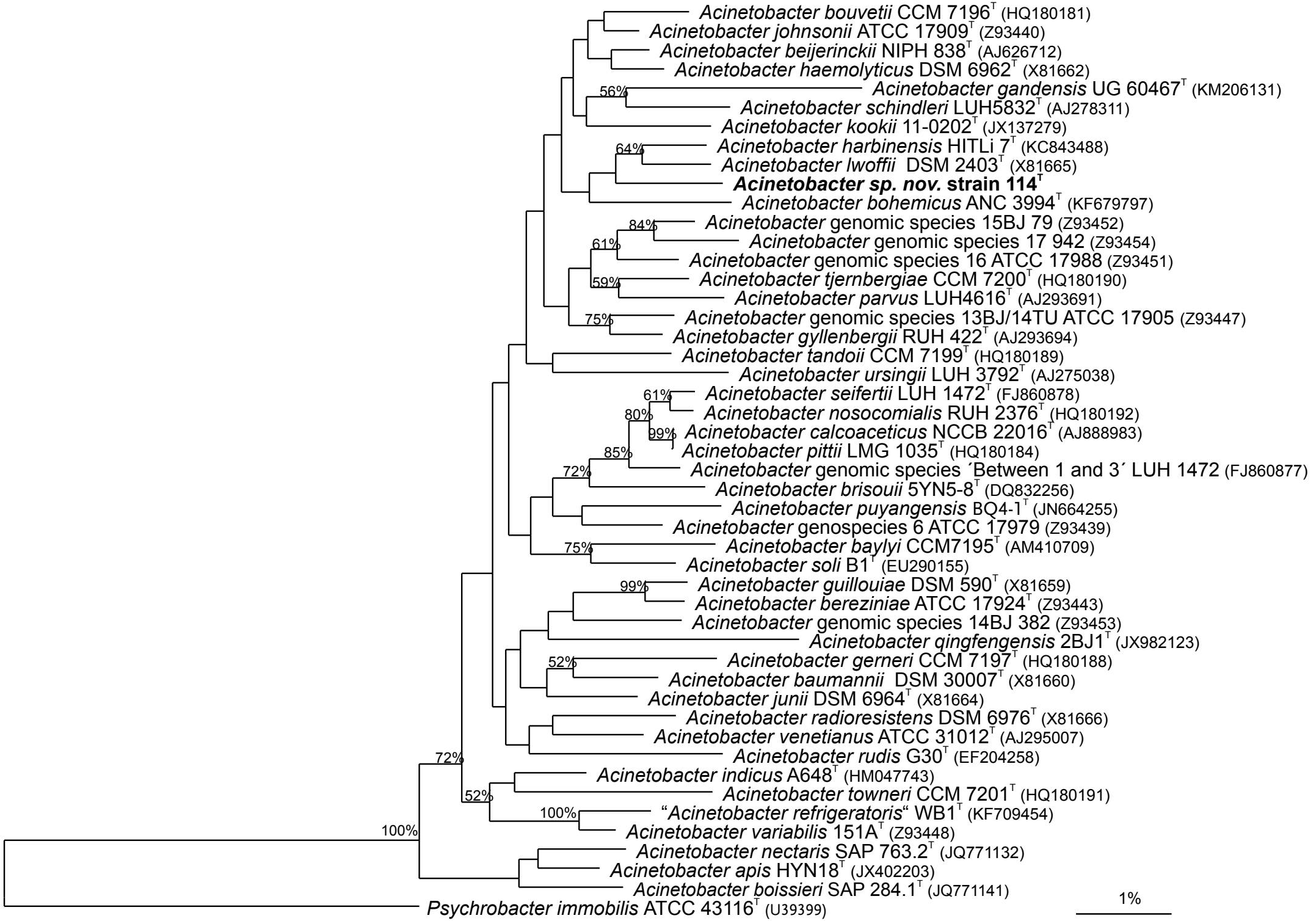
LEGENDS TO FIGURES

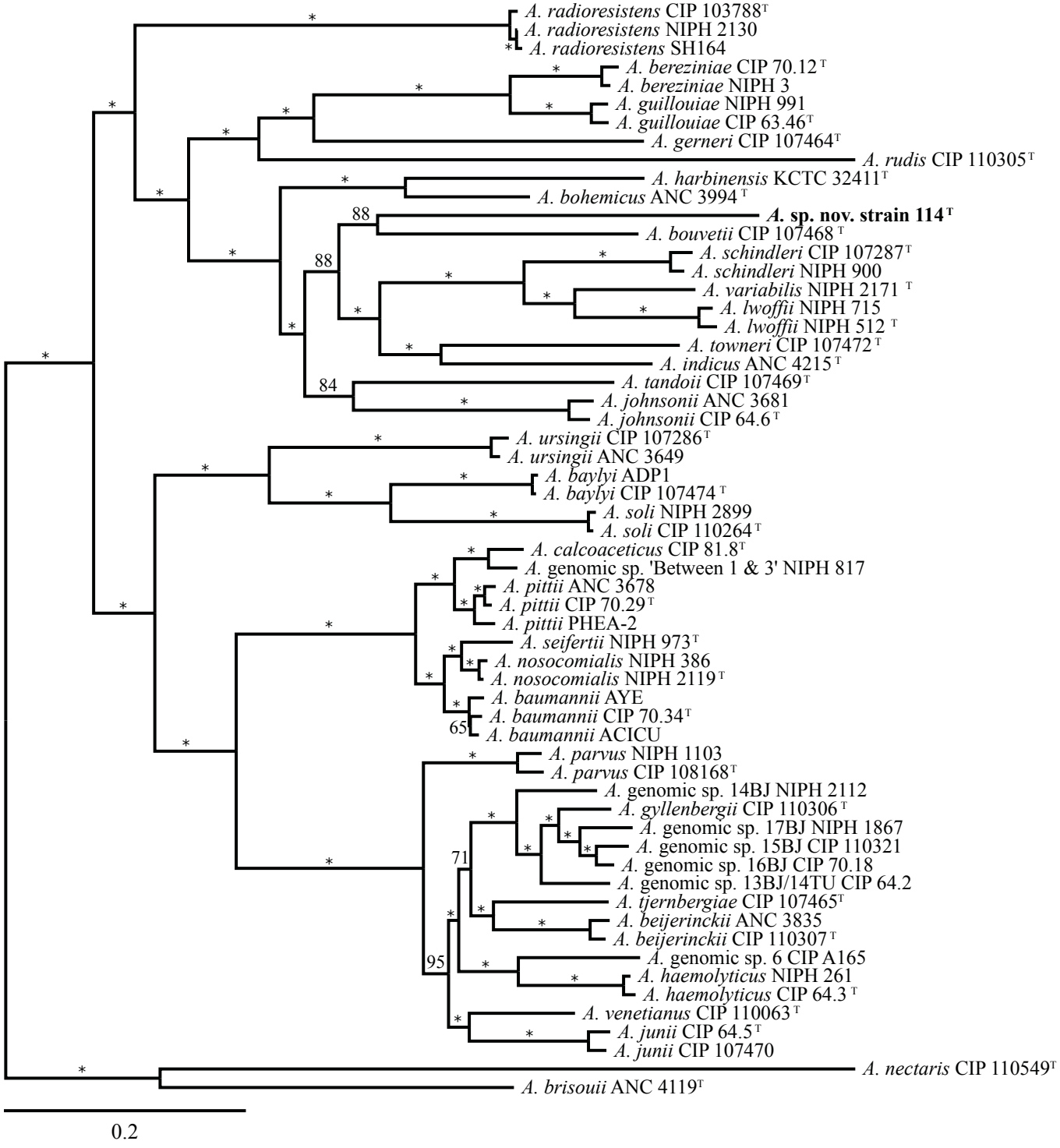
Fig. 1. Neighbour-joining phylogenetic reconstruction from nearly complete 16S rRNA gene sequences (region 165 - 1438 according to *E. coli* nomenclature) using the ARB package (Pruesse *et al.*, 2007) and the correction of Jukes & Cantor (1969). Scale bar indicates 1 nucleotide substitution per 100 nucleotides. Bootstrap values above 50% (1000 resamplings) are indicated. *Psychrobacter immobilis* ATCC 43116^T (U39399) was used as an outgroup.

Fig. 2. Phylogenetic tree for the genus *Acinetobacter*. Branch support values are given as branch labels and represent ML bootstrap values. "*" denotes 100% bootstrap support. Branch lengths are given in expected substitutions per site (see Supplementary Information S1 for details).

FOOTNOTES

The GenBank accession numbers for the partial 16S rRNA, *rpoB* and *gyrB* gene sequences of strain 114^T (=DSM 27228^T=CCUG 65204^T) are KC494698, KC494699 and KP690075, respectively. The whole genome sequence of strain 114^T is available at GenBank under accession number CP012808 (Bioproject PRJNA296861). Strain 114^T was deposited at the DSMZ strain collection (DSM 27228^T) and at the Culture Collection of the University of Göteborg (CCUG 65204^T). Supplementary material is available in IJSEM Online.


Acinetobacter bouvetii CCM 7196^{T} (HQ180181)
Acinetobacter johnsonii ATCC 17909^{T} (Z93440)
Acinetobacter beijerinckii NIPH 838^{T} (AJ626712)
Acinetobacter haemolyticus DSM 6962^{T} (X81662)
Acinetobacter gandensis UG 60467^{T} (KM206131)
56%
Acinetobacter schindleri LUH5832^{T} (AJ278311)
Acinetobacter kookii 11-0202^{T} (JX137279)
Acinetobacter harbinensis HITLi 7^{T} (KC843488)
64%
Acinetobacter lwoffii DSM 2403^{T} (X81665)
***Acinetobacter sp. nov.* strain 114^{T}**
Acinetobacter bohemicus ANC 3994^{T} (KF679797)
Acinetobacter genomic species 15BJ 79 (Z93452)
84%
Acinetobacter genomic species 17 942 (Z93454)
61%
Acinetobacter genomic species 16 ATCC 17988 (Z93451)
Acinetobacter tjernbergiae CCM 7200^{T} (HQ180190)
59%
Acinetobacter parvus LUH4616^{T} (AJ293691)
Acinetobacter genomic species 13BJ/14TU ATCC 17905 (Z93447)
75%
Acinetobacter gyllenbergii RUH 422^{T} (AJ293694)
Acinetobacter tandoii CCM 7199^{T} (HQ180189)
Acinetobacter ursingii LUH 3792^{T} (AJ275038)
Acinetobacter seifertii LUH 1472^{T} (FJ860878)
61%
Acinetobacter nosocomialis RUH 2376^{T} (HQ180192)
80%
Acinetobacter calcoaceticus NCCB 22016^{T} (AJ888983)
99%
Acinetobacter pittii LMG 1035^{T} (HQ180184)
85%
Acinetobacter genomic species ´Between 1 and 3´ LUH 1472 (FJ860877)
72%
Acinetobacter brisouii 5YN5-8^{T} (DQ832256)
Acinetobacter puyangensis BQ4-1^{T} (JN664255)
Acinetobacter genospecies 6 ATCC 17979 (Z93439)
Acinetobacter baylyi CCM7195^{T} (AM410709)
75%
Acinetobacter soli B1^{T} (EU290155)
Acinetobacter guillouiae DSM 590^{T} (X81659)
99%
Acinetobacter bereziniae ATCC 17924^{T} (Z93443)
Acinetobacter genomic species 14BJ 382 (Z93453)
Acinetobacter qingfengensis 2BJ1^{T} (JX982123)
Acinetobacter gerneri CCM 7197^{T} (HQ180188)
52%
Acinetobacter baumannii DSM 30007^{T} (X81660)
Acinetobacter junii DSM 6964^{T} (X81664)
Acinetobacter radioresistens DSM 6976^{T} (X81666)
Acinetobacter venetianus ATCC 31012^{T} (AJ295007)
Acinetobacter rudis G30^{T} (EF204258)
72%
Acinetobacter indicus A648^{T} (HM047743)
Acinetobacter towneri CCM 7201^{T} (HQ180191)
52%
100%
“*Acinetobacter refrigeratoris*“ WB1^{T} (KF709454)
Acinetobacter variabilis 151A^{T} (Z93448)
100%
Acinetobacter nectaris SAP 763.2^{T} (JQ771132)
Acinetobacter apis HYN18^{T} (JX402203)
Acinetobacter boissieri SAP 284.1^{T} (JQ771141)
Psychrobacter immobilis ATCC 43116^{T} (U39399)
1%


A. radioresistens CIP 103788T
A. radioresistens NIPH 2130
A. radioresistens SH164
A. bereziniae CIP 70.12T
A. bereziniae NIPH 3
A. guillouiae NIPH 991
A. guillouiae CIP 63.46T
A. gerneri CIP 107464T
A. rudis CIP 110305T
A. harbinensis KCTC 32411T
A. bohemicus ANC 3994T
A. sp. nov. strain 114T
A. bouvetii CIP 107468T
A. schindleri CIP 107287T
A. schindleri NIPH 900
A. variabilis NIPH 2171T
A. lwoffii NIPH 715
A. lwoffii NIPH 512T
A. towneri CIP 107472T
A. indicus ANC 4215T
A. tandoii CIP 107469T
A. johnsonii ANC 3681
A. johnsonii CIP 64.6T
A. ursingii CIP 107286T
A. ursingii ANC 3649
A. baylyi ADP1
A. baylyi CIP 107474T
A. soli NIPH 2899
A. soli CIP 110264T
A. calcoaceticus CIP 81.8T
A. genomic sp. 'Between 1 & 3' NIPH 817
A. pittii ANC 3678
A. pittii CIP 70.29T
A. pittii PHEA-2
A. seifertii NIPH 973T
A. nosocomialis NIPH 386
A. nosocomialis NIPH 2119T
A. baumannii AYE
A. baumannii CIP 70.34T
A. baumannii ACICU
A. parvus NIPH 1103
A. parvus CIP 108168T
A. genomic sp. 14BJ NIPH 2112
A. gyllenbergii CIP 110306T
A. genomic sp. 17BJ NIPH 1867
A. genomic sp. 15BJ CIP 110321
A. genomic sp. 16BJ CIP 70.18
A. genomic sp. 13BJ/14TU CIP 64.2
A. tjernbergiae CIP 107465T
A. beijerinckii ANC 3835
A. beijerinckii CIP 110307T
A. genomic sp. 6 CIP A165
A. haemolyticus NIPH 261
A. haemolyticus CIP 64.3T
A. venetianus CIP 110063T
A. junii CIP 64.5T
A. junii CIP 107470
A. nectaris CIP 110549T
A. brisouii ANC 4119T
88
88
84
65
71
95
0.2

***Acinetobacter equi* sp. nov. isolated from horse faeces**
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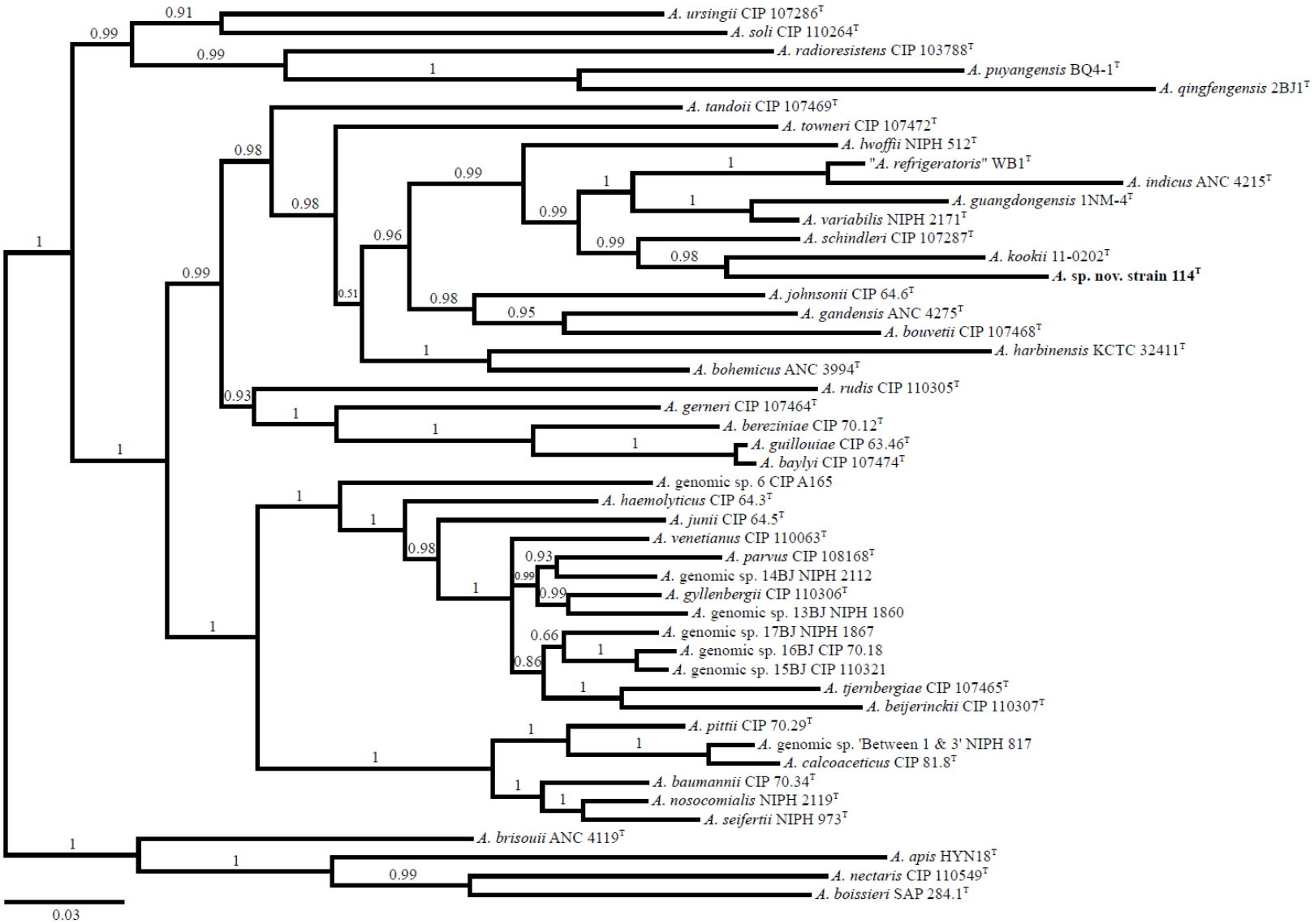
Supplementary Fig. S1



Supplementary Figure S1. Bayesian consensus tree based on the *rpoB* nucleotide sequence alignment. Branch support values are given as branch labels and represent Bayesian posterior probabilities. Branch lengths are given in expected substitutions per site (see Supplementary Information S1 for details and sequence identifiers).

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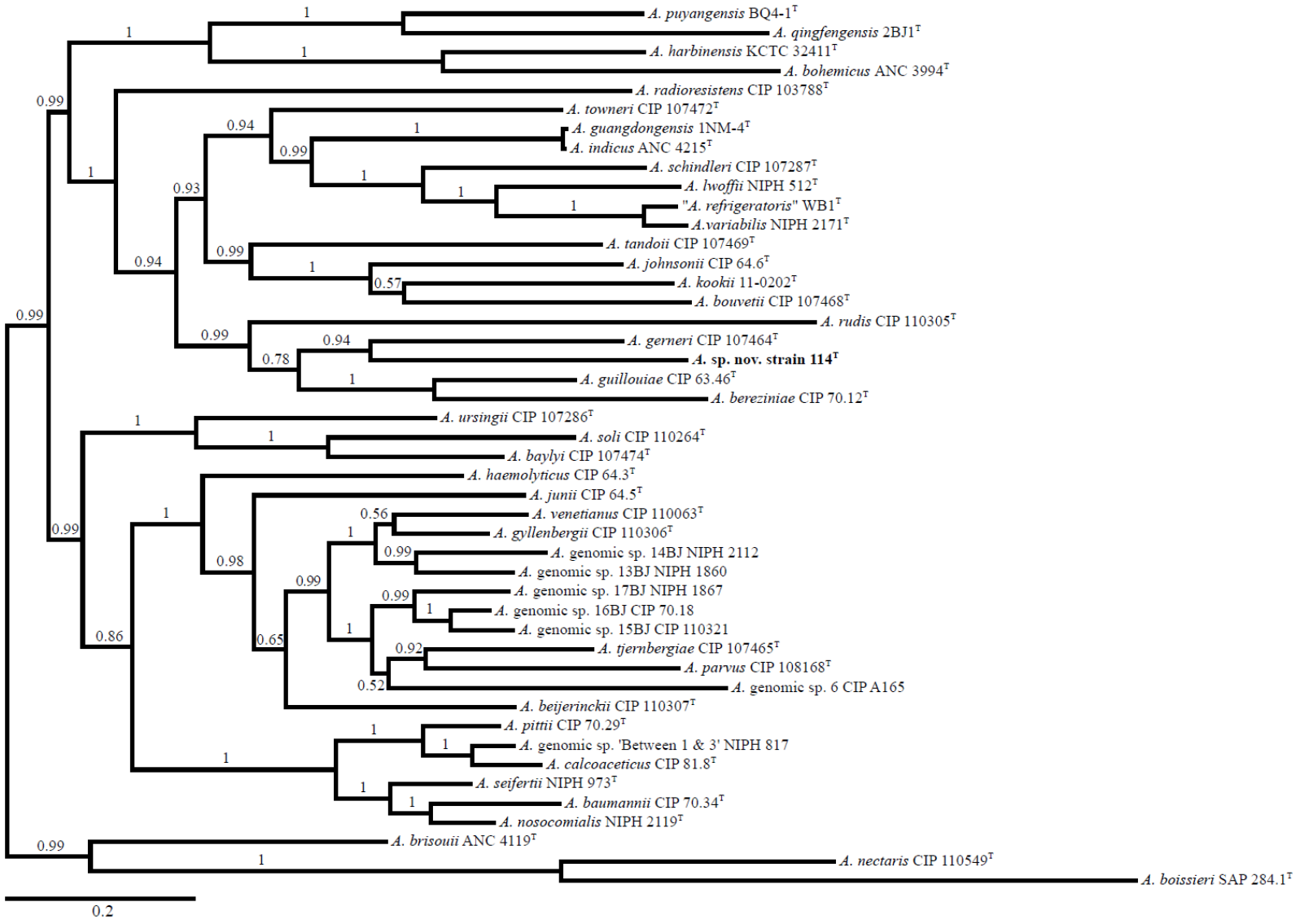
Supplementary Fig. S2



Supplementary Figure S2. Bayesian consensus tree based on the *gyrB* nucleotide sequence alignment. Branch support values are given as branch labels and represent Bayesian posterior probabilities. Branch lengths are given in expected substitutions per site (see Supplementary Information S1 for details and sequence identifiers).

***Acinetobacter equi* sp. nov. isolated from horse faeces**

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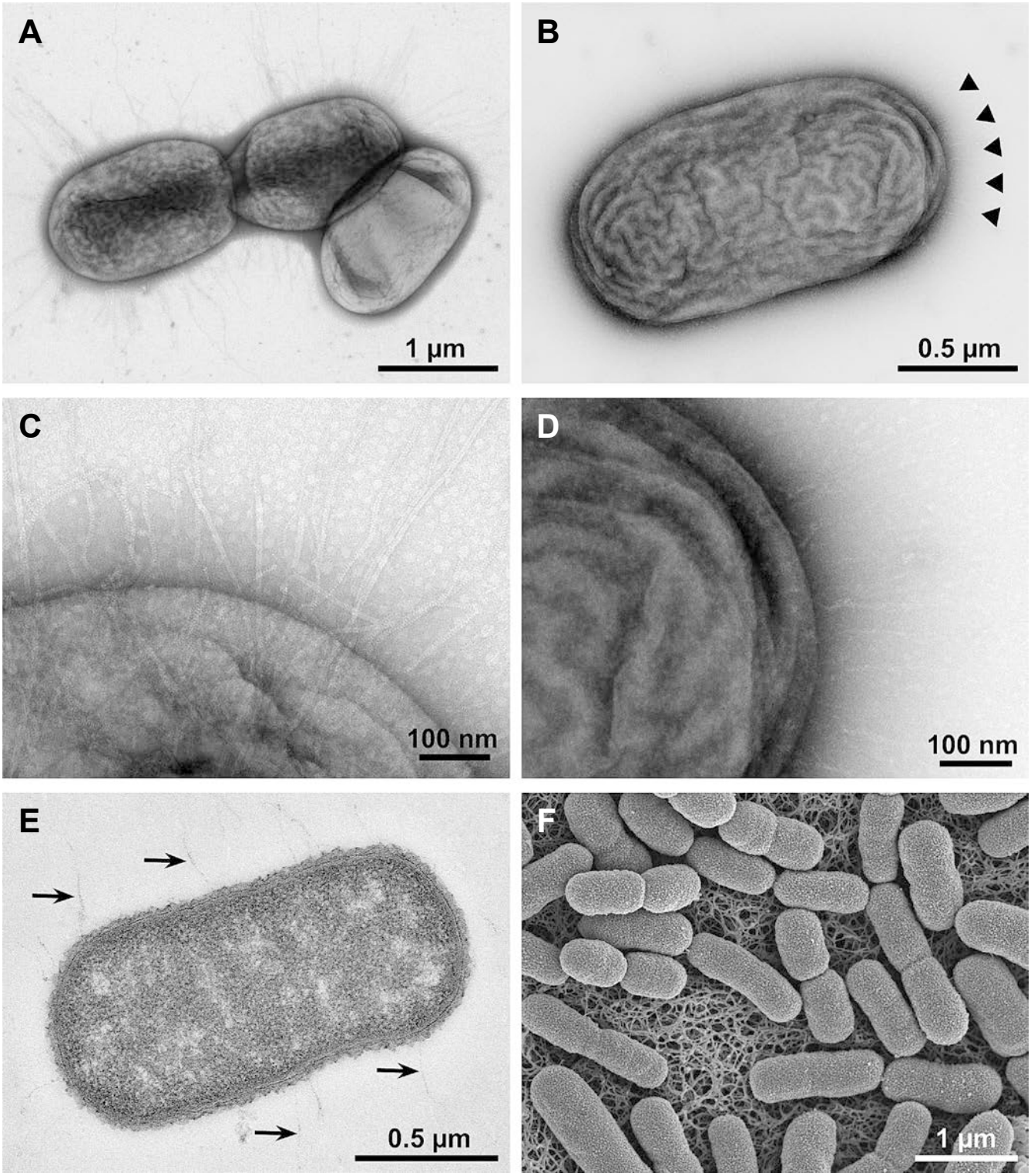
Supplementary Fig. S3



Supplementary Figure S3. Electron microscopy of strain 114^{T}. (A)-(D) Negative staining transmission electron microscopy after cultivation on nutrient agar. (A) A group of three bacteria of which two possess many pili all around their surface and one no or few pili at all. (B) A single bacterium which shows thin pili (arrow heads) only at one cell pole. (C, D) Higher magnification of the various pili from bacteria shown in (A) or (B), respectively. (E) Ultrathin section transmission electron microscopy of a single bacterium (longitudinal section) from a liquid LB culture which reveals the typical cell wall structure of Gram-negative bacteria (i.e. outer and inner membrane) and some pili (arrows). The cytoplasm reveals no remarkable structural features. (F) Scanning electron microscopy (SEM) of bacteria at the bottom of a colony on LB agar (which is visible as a web-like background). Note that the bacteria appear smaller in size than in (A)-(E) which is probably due to extensive shrinking of the sample during preparation for SEM.

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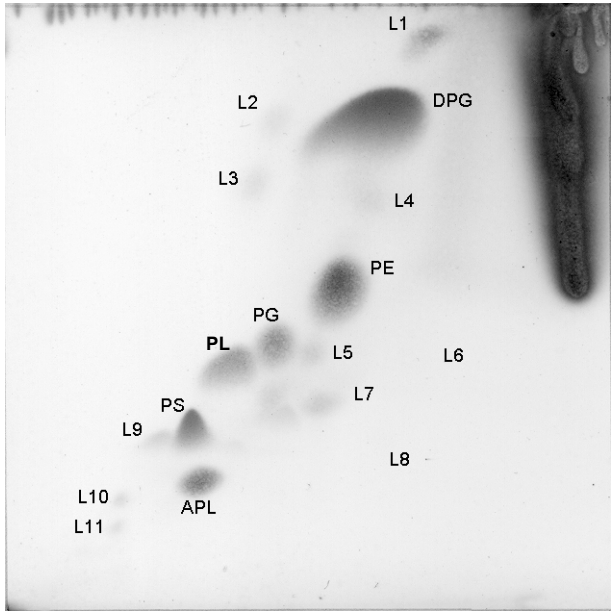
Supplementary Fig. S4



Polar lipids profile of _Acinetobacter_ strain 114^T after separation by two-dimensional thin layer chromatography and detection using molybdatophosphoric acid. DPG, diphosphatidylglycerol; PG, phosphatidylglycerol; PE, phosphatidylethanolamine; PS, phosphatidylserine; APL, unidentified aminophospholipid; PL, unidentified phospholipid; L1-11, unidentified polar lipids not detectable with any of the spray reagents specific for lipids containing a phosphate group, an amino group or a sugar moiety.

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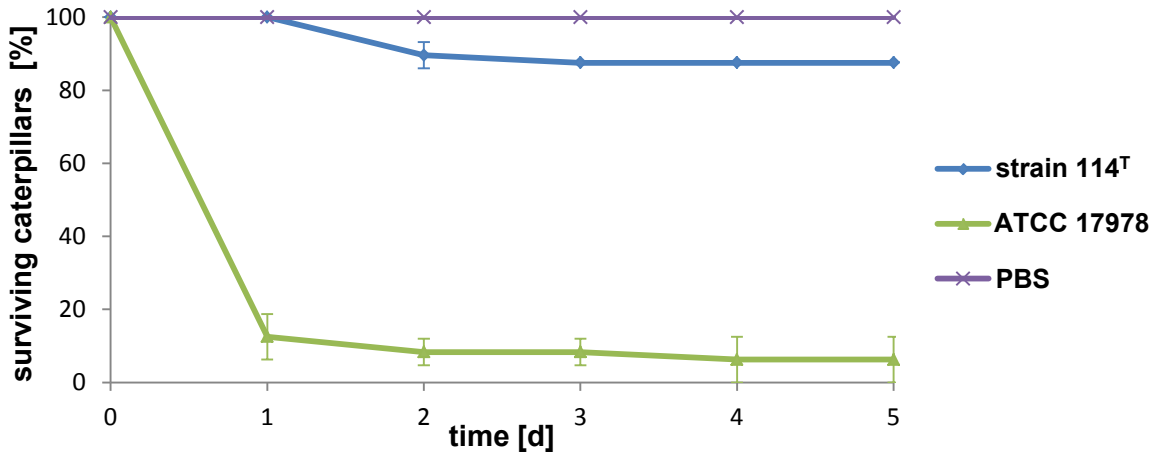
Supplementary Fig. S5

(b)



(b)

Low virulence potential of strain 114^T compared to _Acinetobacter baumannii_ ATCC 17978 in the _Galleria mellonella_ infection model. _Galleria_ larvae were infected as recently described (Skiebe et al., Int. J. Med. Microbiol. 302 (2012) 117-128) with ~2x10^6 bacteria of strain 114^T or ~2x10^5 bacteria of _A. baumannii_ ATCC 17978 or mock infected with PBS and the larvae incubated in Petri dishes for 5 days at 37°C. (a) Survival of larvae over time. (b) Photographs of the larvae taken 24 hours after infection.

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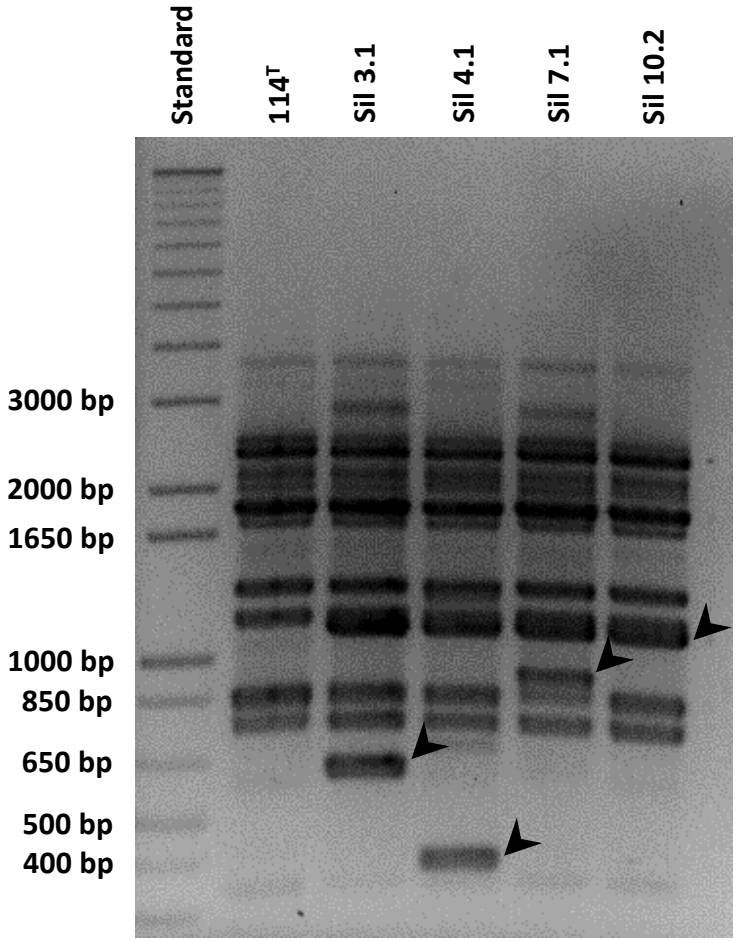
Supplementary Fig. S6



Comparative genomic fingerprint analysis by RAPD-PCR using primer A (5'-CTGGCGGCTTG-3'; (Ziemke _et al._, 1997)). PCR conditions were as follows using AccuPrime _Pfx_ polymerase (Life Technologies): Initial denaturation for 2 min at 95°C followed by 30 cycles with denaturation for 15 s at 95°C, annealing for 30 s at 46°C and extension for 1 min 30 s at 68°C, followed by a final extension for 7 min. 1% agarose gel stained with ethidium bromide. Arrowheads indicate distinctive bands in comparison to _Acinetobacter_ strain 114T.

Acinetobacter equi sp. nov. isolated from horse faeces

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Supplementary Information S1

Phylogeny of the genus *Acinetobacter* based on the core gene set of 59 *Acinetobacter* strains.

1 Summary

We have determined the genome sequence of *A.* sp. nov. strain 114^T isolated from horse faeces and annotated the protein-coding genes within the genome. To determine the phylogenetic placement of this new taxon we have pursued a phylogenomic approach integrating the analysis of 1,061 *Acinetobacter* core genes. A maximum likelihood tree reconstruction considering 59 taxa from the genus *Acinetobacter* reveals that *A.* sp. nov. strain 114^T is only distantly related to any so far described species. The patristic distance to its closest relative, *A. bouvetii* CIP 107468^T, exceeds with 0.51 substitutions per site by far the hitherto observed genetic diversity between strains of the same *Acinetobacter* species. This strongly suggests that *A.* sp. nov. strain 114^T is the first representative of a newly detected species within the genus *Acinetobacter*.

2 Material and Methods

2.1. Coding Sequences and Gene Prediction

Bacterial coding sequences of 58 *Acinetobacter* strains where retrieved from GenBank (http://www.ncbi.nlm.nih.gov/genome/browse/representative/) (Table S2). Protein coding genes in the genome of *A.* sp. nov. strain 114^T were predicted with Prokka (Seemann, 2014). Additionally, we used partial coding sequences of further 11 *Acinetobacter* strains for a gene tree analysis of the RNA polymerase beta subunit (*rpoB*) and the DNA gyrase beta subunit (*gyrB*) (Table S1).

2.2 Ortholog Search

We used OrthoMCL (Li et al., 2003) to identify initial sets of orthologs from five *Acinetobacter* type strains spanning the *Acinetobacter* clade. As primer taxa we used *A. radioresistence* CIP 103788^T, *A. baylyi* CIP 107474^T, *A. baumannii* CIP 70.34^T, *A. calcoaceticus* CIP 81.8^T, and *A. nosocomialis* NIPH 2119^T. Only orthologous groups harboring sequences from all five primer taxa were further processed. For each group we aligned the corresponding protein sequences with MAFFT-LINSI (Katoh et al., 2008) and converted the alignment subsequently into a profile hidden Markov model (pHMM) using hmmbuild from the HMMER3 package (http://hmmer.janelia.org). These pHMMs served then as input for a HaMStR ortholog search (Ebersberger et al., 2009) in 59 *Acinetobacter* strains. We then retained only those genes for the phylogeny reconstruction that were represented by an ortholog in all 59 taxa.

2.3 Phylogeny reconstruction

Amino acid sequence alignments of the selected orthologous groups were computed with MAFFT-LINSI. The resulting alignments were concatenated into a supermatrix alignment of 379,559 amino acids in length. ProtTest 3.2 (Darriba et al., 2011) identified the LG model (Le et al., 2008) in combination with modeling invariant sites (+I), rate heterogeneity across sites (+G) and estimating amino acid frequencies from the data (+F) as the best-fitting model to the data. Maximum Likelihood (ML) tree reconstruction on the supermatrix was then conducted with RAxML 8.1.9 (Stamatakis, 2014) using the PROTGAMMAILGF model for amino acid sequence evolution. For statistical support 100 nonparametric bootstrap replicates were computed. The resulting tree was rooted using the mid point rooting method.

The sets of *rpoB* and *gyrB* orthologs of known *Acinetobacter* type strains were completed by the partial sequences and aligned with MAFFT-LINSI. Subsequently, for each protein sequence alignment a separate codon alignment of the extracted nucleotide sequences was built with PAL2NAL (Suyama et al., 2006). Bayesian consensus trees were reconstructed with PhyloBayes 3.3f using the GTR model (Lartillot et al., 2009) for nucleotide sequence evolution. For each alignment of *rpoB* and *gyrB* nucleotide sequences two independent runs were performed. The two runs were checked for convergence with *bpcomp* (maxdiff < 0.03 and meandiff < 0.002) discarding the first 2000 trees as burn-in and then taking every 10th tree into account for a majority rule consensus tree.

3 Results

Ortholog search with HaMStR identified 1,061 genes with orthologs present in all of the 59 analyzed *Acinetobacter* strains. Subsequent to aligning the sequences in the individual orthologous groups and alignment concatenation we used the resulting supermatrix for maximum likelihood (ML) tree reconstruction (Fig. 2). Please note that we used multiple strains of *Acinetobacter* species, if available, to outline the placement of *Acinetobacter* strains according to their species assignment and to delineate between intra- and interspecies separation. We conclude that the stable placement of strain 114^T distinguishes it form other *Acinetobacter* species in the genus. *A.* sp. nov. strain 114^T is clustered with *A. bouvetii* CIP 107468^T as a sister clade to the *A. schindleri-indicus* subclade. Mid point rooting revealed the clade of *A. nectaris* CIP 110549^T and *A. brisouii* ANC 4119^T as the earliest branching subclade of the genus *Acinetobacter* which resembles the placement of the root applied by Touchon et al. (2014).

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Table S1

	rpoB			gyrB		
Strain	**Nucleotide NCBI accession no.**	**Protein ID**	**Locus Tag**	**Nucleotide NCBI accession no.**	**Protein ID**	**Locus Tag**
A. apis HYN18^T	JX863071.1	-	-	-	-	-
A. baumannii CIP 70.34^T	NZ_KB849992.1	WP_000331899.1	F911_RS22805	NZ_KB849981.1	WP_000093729.1	F911_RS05765
A. baylyi CIP 107474^T	NZ_KB849623.1	WP_004718475.1	F952_RS01550	NZ_KB849630.1	WP_004930060.1	F952_RS15460
A. beijerinckii CIP 110307^T	NZ_KB849765.1	WP_005050903.1	F933_RS10540	NZ_KB849765.1	WP_005060443.1	F933_RS08865
A. bereziniae CIP 70.12^T	NZ_KB849756.1	WP_004826241.1	F938_RS22045	NZ_KB849756.1	WP_005034820.1	F938_RS20580
A. bohemicus ANC 3994^T	KJ124834.1	-	-	KJ147467.1	-	-
A. boissieri SAP 284.1^T	JQ771155.1	-	-	KF493699.1	-	-
A. bouvetii CIP 107468^T	NZ_KB849725.1	WP_005006986.1	F941_RS01395	NZ_KB849725.1	WP_005006472.1	F941_RS00080
A. brisouii ANC 4119^T	NZ_KB849592.1	WP_004902489.1	F954_RS09275	NZ_KB849592.1	WP_004902971.1	F954_RS10705
A. calcoaceticus CIP 81.8^T	NZ_KB849779.1	WP_003654504.1	F936_RS04235	NZ_KB849779.1	WP_003656037.1	F936_RS04940
A. gandensis ANC 4275^T	KJ569689.1	-	-	-	-	-
A. gerneri CIP 107464^T	NZ_KB849545.1	WP_004868035.1	F960_RS17160	NZ_KB849554.1	WP_004871613.1	F960_RS20900
A. guangdongensis 1NM-4^T	KJ701021.1	-	-	KJ716796.1	-	-
A. guillouiae CIP 63.46^T	NZ_KB849282.1	WP_004718475.1	F981_RS03520	NZ_KB849282.1	WP_004718929.1	F981_RS04795
A. gyllenbergii CIP 110306^T	NZ_ATGG01000025.1	WP_016660496.1	F957_RS05260	NZ_ATGG01000001.1	WP_005277650.1	F957_RS20235
A. haemolyticus CIP 64.3^T	NZ_KB849803.1	WP_004637643.1	F927_RS09310	NZ_KB849808.1	WP_004637310.1	F927_RS13675
A. harbinensis KCTC 32411^T	KF803234.1	-	-	KF803235.1	-	-
A. indicus ANC 4215^T	NZ_KE340342.1	WP_016659679.1	F956_RS11990	NZ_KE340347.1	WP_016659765.1	F956_RS12775
A. johnsonii CIP 64.6^T	NZ_KB849234.1	WP_004692766.1	F986_RS05225	NZ_KB849237.1	WP_004697421.1	F986_RS16700
A. junii CIP 64.5^T	NZ_KB849653.1	WP_004958566.1	F948_00111	NZ_KB849655.1	WP_004950013.1	F948_RS14610
A. kookii 11-0202^T	JX844152.1	-	-	JX844154.1	-	-
A. lwoffii NIPH 512^T	KB851227.1	ENW25623.1	F925_00976	KB851227.1	ENW25371.1	F925_00710
A. nectaris CIP 110549^T	NZ_KI530734.1	WP_023273527.1	P256_RS09800	NZ_KI530738.1	WP_023274092.1	P256_RS12640
A. nosocomialis NIPH 2119^T	NZ_KB849239.1	WP_004710883.1	F984_RS16875	NZ_KB849239.1	WP_002051923.1	F984_RS18360
A. parvus CIP 108168^T	NZ_KB849210.1	WP_004680736.1	F988_RS01620	NZ_KB849215.1	WP_004683893.1	F988_RS14080
A. pittii CIP 70.29^T	NZ_KB849797.1	WP_002114857.1	F928_RS20405	NZ_KB849785.1	WP_002115497.1	F928_RS04265

A. puyangensis BQ4-1^{T}	JX499272.1	-	-	JQ411219.1	-	-
A. radioresistens CIP 103788^{T}	NZ_KB849747.1	WP_005023022.1	F939_RS01550	NZ_KB849748.1	WP_005023820.1	F939_RS03925
"A. refrigeratoris" WB1^{T}	KJ701022.1	-	-	KJ716797.1	-	-
A. rudis CIP 110305^{T}	NZ_KE340355.1	WP_016657952.1	F945_RS17735	NZ_KE340348.1	WP_016654493.1	F945_RS00205
A. qingfengensis 2BJ1^{T}	KC631629.1	-	-	KC686827.1	-	-
A. schindleri CIP 107287^{T}	NZ_KB849574.1	WP_004890577.1	F955_RS02105	NZ_KB849587.1	WP_004814017.1	F955_RS15785
A. seifertii NIPH 973^{T}	KB851199.1	ENU43909.1	F985_01401	KB851195.1	ENU45289.1	F985_00004
A. soli CIP 110264^{T}	NZ_KB849634.1	WP_004932374.1	F951_00127	NZ_KB849635.1	WP_004933512.1	F951_RS02295
A. sp. nov. strain 114^{T}	KC494699.2	-	-	KP690075.1	-	-
A. tandoii CIP 107469^{T}	AQFM01000044.1	EOR04360.1	I593_03440	AQFM01000049.1	EOR02142.1	I593_03976
A. tjernbergiae CIP 107465^{T}	AYEV01000042.1	ESK53759.1	F990_03202	AYEV01000021.1	ESK55130.1	F990_02168
A. towneri CIP 107472^{T}	NZ_KB849691.1	WP_004975783.1	F947_RS11390	NZ_KB849679.1	WP_004969755.1	F947_RS00385
A. ursingii CIP 107286^{T}	NZ_KB849710.1	WP_004985068.1	F944_RS00040	NZ_KB849711.1	WP_004986198.1	F944_RS02370
A. variabilis NIPH 2171^{T}	KB850111.1	ENX11474.1	F897_00318	KB850112.1	ENX10903.1	F897_00582
A. venetianus CIP 110063^{T}	EU477136.2	-	-	JX523707.1	-	-
Genomic sp. 6 CIP A165	KB849200.1	ENU31799.1	F991_00284	APOK01000036.1	ENU29484.1	F991_02715
Genomic sp. 13BJ/14TU CIP 64.2	KB850121.1	ENX17345.1	F895_01290	KB850125.1	ENX11271.1	F895_03793
Genomic sp. 14BJ NIPH 1847	KB850107.1	ENX10404.1	F898_00129	KB850108.1	ENX08282.1	F898_01182
Genomic sp. 15BJ CIP 110321	AQFL01000028.1	EOR03170.1	F896_03831	AQFL01000001.1	EOR10741.1	F896_00015
Genomic sp. 16BJ CIP 70.18	APRN01000028.1	ENX62181.1	F902_00375	APRN01000034.1	ENX59490.1	F902_01230
Genomic sp. 17BJ NIPH 1867	APRO01000007.1	ENX54084.1	F901_01373	APRO01000007.1	ENX53787.1	F901_01059
Genomic sp. 'Between 1 & 3' NIPH 817	KB849420.1	ENV01566.1	F968_03526	KB849420.1	ENV01174.1	F968_03831

Table S1: *Acinetobacter* strains used for phylogenetic tree reconstruction. Database entries are given as NCBI accession numbers, Protein IDs and Locus tags that specify the used sequences for *rpoB* and *gyrB*.

Table S2

Strain	Genome NCBI accession no.
A. calcoaceticus CIP 81.8^T	APQI00000000.1
A. calcoaceticus PHEA-2	NC_016603.1
A. baumannii CIP 70.34^T	APRG00000000.1
A. baumannii ACICU	NC_010611.1
A. baumannii AYE	NC_010410.1
A. baylyi CIP 107474^T	APPT00000000.1
A. baylyi ADP1	NC_005966.1
A. beijerinckii CIP 110307^T	APQL00000000.1
A. beijerinckii ANC 3835	APQK00000000.1
A. bereziniae CIP 70.12^T	APQG00000000.1
A. bereziniae NIPH 3	APPK00000000.1
A. bohemicus ANC 3994^T	APOH00000000.1
A. bouvetii CIP 107468^T	APQD00000000.1
A. brisouii ANC 4119^T	APPR00000000.1
A. gerneri CIP 107464^T	APPN00000000.1
A. guillouiae CIP 63.46^T	APOS00000000.1
A. guillouiae NIPH 991	APPJ00000000.1
A. gyllenbergii CIP 110306^T	ATGG00000000.1
A. haemolyticus CIP 64.3^T	APQQ00000000.1
A. haemolyticus NIPH 261	APQR00000000.1
A. harbinensis KCTC 32411^T	NZ_JXBK00000000.1
A. indicus ANC 4215^T	ATGH00000000.1
A. johnsonii CIP 64.6^T	APON00000000.1
A. johnsonii ANC 3681	APPZ00000000.1
A. junii CIP 64.5^T	APPX00000000.1
A. junii CIP 107470	APPS00000000.1
A. lwoffii NIPH 512^T	AYHO00000000.1
A. lwoffii NIPH 715	APOT00000000.1
A. nectaris CIP 110549^T	AYER00000000.1
A. nosocomialis NIPH 2119^T	APOP00000000.1
A. nosocomialis NIPH 386	APPP00000000.1
A. parvus CIP 108168^T	APOM00000000.1
A. parvus NIPH 1103	APOL00000000.1
A. pittii CIP 70.29^T	APQP00000000.1
A. pittii ANC 3678	APQN00000000.1
A. radioresistens CIP 103788^T	APQF00000000.1
A. radioresistens NIPH 2130	APQE00000000.1
A. radioresistens SH164	ACPO00000000.1
A. rudis CIP 110305^T	ATGI00000000.1
A. schindleri CIP 107287^T	APPQ00000000.1
A. schindleri NIPH 900	APPI00000000.1
A. seifertii NIPH 973 ^{T}	APOO00000000.1
A. soli CIP 110264^T	APPU00000000.1
A. soli NIPH 2899	APPV00000000.1
A. tandoii CIP 107469^T	AQFM00000000.1
A. tjernbergiae CIP 107465^T	AYEV00000000.1

A. towneri CIP 107472^{T}	APPY00000000.1
A. ursingii CIP 107286^{T}	APQA00000000.1
A. ursingii ANC 3649	APQC00000000.1
A. variabilis NIPH 2171 ^{T}	APRS00000000.1
A. venetianus CIP 110063^{T}	APPO00000000.1
Genomic sp. 6 CIP A165	APOK00000000.1
Genomic sp. 13BJ/14TU CIP 64.2	APRT00000000.1
Genomic sp. 14BJ NIPH 1847	APRR00000000.1
Genomic sp. 15BJ CIP 110321	AQFL00000000.1
Genomic sp. 16BJ CIP 70.18	APRN00000000.1
Genomic sp. 17BJ NIPH 1867	APRO00000000.1
Genomic sp. 'Between 1 & 3' NIPH 817	APPF00000000.1

Table S2: *Acinetobacter* strains used for phylogenetic tree reconstruction. Database entries are given as NCBI accession numbers und specify the used genomes.

Supplementary Table S3. Fatty acid composition of strain 114^T and selected *Acinetobacter* strains.

Fatty acids	***A. equi*** **114^T**	***A. bouvetii*** **KCTC 12414^T** **(Lee & Lee, 2010)**	***A. lwoffii*** **KCTC 12407^T** **(Lee & Lee, 2010)**	***A. junii*** **LMG998^T** **(Malhotra *et al.*, 2012)**	***A. schindleri*** ***KCTC* 12409^T** **(Lee & Lee, 2010)**	***A. guillouiae*** **LMG 988^T** **(Vaz-Moreira *et al.*, 2011)**	***A. ursingii*** **KCTC 12410^T** **(Lee & Lee, 2010)**	***A. variabilis*** **KCTC12687** **(Lee *et al.*, 2009)**
$C_{10:0}$	-	1.7	-	2.5	1.0	-	-	4.5
$C_{12:0}$	6.5	5.2	6.6	3.8	10.2	5.3	6.8	-
$C_{12:0}$ 2-OH	2.0	-	-	7.6	-	1.7	1.7	-
$C_{12:0}$ 3-OH	4.0	5.0	5.4	8.9	7.0	5.5	4.0	2.8
$C_{14:0}$	2.8	1.4	1.8	1.3	1.5	-	-	-
$C_{15:0}$	1.1	-	-	-	-	-	-	-
$C_{16:0}$	28.2	26.9	17.4	16.3	17.8	15.3	19.9	20.9
$C_{17:1}$ ω8c	2.8	-	-	1.0	-	-	-	-
$C_{17:0}$	2.6	-	-	1.7	-	-	-	-
$C_{18:1}$ ω9c	7.0	13.8	18.5	28.1	18.4	47.2	45.5	28.1
$C_{18:1}$ ω7c	2.5	3.4	3.1	-	2.7	2.9	-	2.7
$C_{18:0}$	1.6	1.3	1.3	2.3	-	2.0	-	3.1
Summed feature 2 ($C_{12:0}$ aldehyde, iso-$C_{16:1}$ I and/or $C_{14:0}$ 3-OH)	1.8	-	1.0	0.4	2.1	-	4.7	3
Summed feature 3 ($C_{16:1}$ ω7c and/or iso-$C_{15:0}$ 2-OH)	36.2	40.5	37.5	23.5	38.1	17.4	14.7	30.9

Source of data as indicated in headline; -, trace amounts below 0.5% or not detected. Selection of strains in dependence on selection for Table 1.

References:

Lee, H. J. & Lee, S. S. (2010). Acinetobacter kyonggiensis sp. nov., a beta-glucosidase-producing bacterium, isolated from sewage treatment plant. *J Microbiol* **48**, 754-759.

Lee, J. S., Lee, K. C., Kim, K. K., Hwang, I. C., Jang, C., Kim, N. G., Yeo, W. H., Kim, B. S., Yu, Y. M. & Ahn, J. S. (2009). Acinetobacter antiviralis sp. nov., from Tobacco plant roots. *J Microbiol Biotechnol* **19**, 250-256.

Malhotra, J., Anand, S., Jindal, S., Rajagopal, R. & Lal, R. (2012). Acinetobacter indicus sp. nov., isolated from a hexachlorocyclohexane dump site. *Int J Syst Evol Microbiol* **62**, 2883-2890.

Vaz-Moreira, I., Novo, A., Hantsis-Zacharov, E., Lopes, A. R., Gomila, M., Nunes, O. C., Manaia, C. M. & Halpern, M. (2011). Acinetobacter rudis sp. nov., isolated from raw milk and raw wastewater. *Int J Syst Evol Microbiol* **61**, 2837-2843.

Acinetobacter equi sp. nov. isolated from horse faeces

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Suplementary Information S2

Materials & Methods Electron Microscopy

Negative staining electron microscopy was done by using native bacteria which were taken from a colony grown on nutrient agar overnight at 37°C and dissolved in distilled water. The suspension was adsorbed on a particular sample support (i.e. a copper grid coated with a plastic film) and negatively stained with 0.5% uranyl acetate (for details see Laue and Bannert 2010).

For ultrathin section electron microscopy, bacteria were taken from colonies which were grown on LB agar overnight at 37°C, resuspended in HEPES buffer (0.05 M, pH 7.2), centrifuged at low speed and finally resuspended in a fixative consisting of 4% paraformaldehyde and 2.5% glutaraldehyde in Hepes buffer (0.05 M, pH 7.2). Incubation was done for 15 min at 37° C using a particular microwave oven (REM, Milestone Inc., Sorisole, Italy) followed by 105 min at room temperature. Bacteria were embedded in low-melting point agarose, post-fixed in osmium tetroxide and uranyl acetate, dehydrated and embedded in LR White resin. Ultrathin sections (60-80 nm) were taken with an ultramicrotome (Leica, Ultracut S or UC7) which was equipped with a diamond knife. Sections were collected on plain sample supports, stained with uranyl acetate and lead citrate and stabilized with a thin layer of carbon.

Transmission electron microscopy (TEM) of negatively stained samples and ultrathin sections was performed with a Tecnai12 BioTwin (FEI Corp., The Netherlands) which was equipped with a 1x1.3k pixel CCD camera (MegaviewIII, OSIS, Muenster, Germany).

Sample preparation for scanning electron microscopy started with the extraction of small pieces of LB agar with colonies on top which then were transferred into 2.5% glutaraldehyde (in 0.05 M HEPES buffer) for chemical fixation. Follow-up preparation involved post-fixation in 1% osmium tetroxide (in distilled water), dehydration in ethanol and critical point drying (K850, Quorum Technologies, Laughton, United Kingdom). Dried samples were mounted on sample supports (stubs) and coated with 3 nm of gold/palladium. Scanning microscopy (SEM) was done with a field-emission microscope (1530 Gemini, ZEISS, Oberkochen, Germany) using-secondary electron imaging with an in-lense detector at low kV (3-5 kV).

Reference

Laue, M.; Bannert, N. (2010) Detection limit of negative-staining electron microscopy for the diagnosis of bioterrorism-related microorganisms. Journal of Applied Microbiology 109:1159-1168.