Acinetobacter nectaris sp. nov. and *Acinetobacter boissieri* sp. nov., isolated from floral nectar of wild Mediterranean insect-pollinated plants

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The taxonomic status of 14 strains of members of the genus *Acinetobacter* isolated from floral nectar of wild Mediterranean insect-pollinated plants, which did not belong to any previously described species within this genus, was investigated following a polyphasic approach. Confirmation that these strains formed two separate lineages within the genus *Acinetobacter* was obtained from comparative analysis of the partial sequences of the 16S rRNA gene and the gene encoding the β-subunit of RNA polymerase (*rpoB*), DNA–DNA reassociation data, determination of the DNA G+C content and physiological tests. The names *Acinetobacter nectaris* sp. nov. and *Acinetobacter boissieri* sp. nov. are proposed. The type strain of *A. nectaris* sp. nov. is SAP 763.2^T (=LMG 26958^T=CECT 8127^T) and that of *A. boissieri* sp. nov. is SAP 284.1^T (=LMG 26959^T=CECT 8128^T).

Members of the genus *Acinetobacter* are generally regarded as common, free-living saprophytes that show extensive metabolic versatility and potential to adapt to different human-associated and natural environments (Towner, 2006; Doughari *et al.*, 2011; Sand *et al.*, 2011). Apart from the well-known human and animal-pathogenic species of the genus *Acinetobacter*, several novel species within this genus have been described during recent years to accommodate isolates from agricultural soils (Kang *et al.*, 2011), activated sludge (Carr *et al.*, 2003), raw wastewater (Vaz-Moreira *et al.*, 2011), a hexachlorocyclohexane dumpsite (Malhotra *et al.*, 2012) and diverse natural environmental sources, such as forest soils (Kim *et al.*, 2008), seawater (Vaneechoutte *et al.*, 2009) and wetlands (Anandham *et al.*, 2010). Nevertheless, except for those species with clinical importance, the distribution and ecological role(s) of the 'acinetobacters' in most environments are largely unknown (Carr *et al.*, 2003; Towner, 2006). In particular, the possible associations of members of this bacterial group with plant hosts remain to be addressed.

Floral nectar is the key component in the mutualism between angiosperms and their animal pollinators, which take this sugary solution as a reward for their pollination services (Brandenburg *et al.*, 2009; Heil, 2011). While foraging on flowers, pollinators can contaminate floral nectar with different prokaryotic and eukaryotic micro-organisms, some of which are particularly well-adapted to thrive in this ephemeral habitat characterized by high osmotic pressure and the presence of plant secondary metabolites with defensive functions (Herrera *et al.*, 2010; Pozo *et al.*, 2012). Nectar micro-organisms can alter pollinators' foraging behaviour in different ways, for example by reducing the nutritional value of floral nectar

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Abbreviations: BI, Bayesian inference; ML, maximum-likelihood; NJ, neighbour-joining; OTU, operational taxonomical unit; PM, Phenotype MicroArray.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences determined in this study are JQ771129–JQ771142 and those for the partial *rpoB* gene sequences are JQ771143–JQ771156.

A supplementary figure and four supplementary tables are available with the online version of this paper.

and/or changing other physico-chemical conditions within the floral microenvironment, and thus potentially interfere with plant sexual reproduction, as has been recently suggested for nectar yeasts (Canto *et al.*, 2008; Herrera *et al.*, 2008; de Vega *et al.*, 2009; Herrera & Pozo, 2010; Peay *et al.*, 2012).

Recently, species of the genus *Acinetobacter* have been identified as representing the main bacterial genus inhabiting the floral nectar of some cultivated plant species from Northern Israel (Fridman *et al.*, 2012) and phylogenetically diverse wild Mediterranean plants from Southern Spain (Álvarez-Pérez & Herrera, 2013). In this latter study, nectar isolates of members of the genus *Acinetobacter* grouped into a single operational taxonomical unit (OTU) defined on the basis of a 3 % dissimilarity cut-off in the 16S rRNA gene sequence, but into two different OTUs when this threshold was lowered to 1 % (Álvarez-Pérez & Herrera, 2013). Such differentiation was also supported by some differences in colony morphology and growth rate in plate cultures, but no additional tests were conducted to further characterize those isolates. In this study we explore the taxonomic status of these two nectar groups of acinetobacters associated with wild Mediterranean plants.

The 14 strains investigated in this study are listed in Table S1, available in IJSEM Online. These strains were isolated on different dates from nectar samples of several plant species collected at different places within the surroundings of Doñana's Natural Park (Huelva province, southwest Spain), using the procedure described by Álvarez-Pérez *et al.* (2012). Additionally, for comparative taxonomic analysis, *Acinetobacter calcoaceticus* DSM 30006^T, *Acinetobacter baylyi* DSM 14961^T, *Acinetobacter gerneri* DSM 14967^T and *Acinetobacter radioresistens* DSM 6976^T were included in some phenotypic and genotypic assays. The inclusion of *Acinetobacter calcoaceticus* in those tests was justified by its status as the type species for the genus *Acinetobacter*, while the other three taxa were identified as the most closely related species to our nectar strains on the basis of 16S rRNA gene sequence data (see below).

Colonies of all the nectar strains grown on trypticase soy agar (TSA; Panreac) were circular, convex to umbilicate, smooth and slightly opaque with entire margins. After 5 days of incubation at 25 °C, colonies of these strains were variable in diameter, ranging from 0.5 to 2.5 mm, although smaller colonies were also observed for some strains. On microscope images, cells appeared as non-motile coccobacilli and commonly occurred in pairs, but also alone or in short chains. No spores were observed. The main phenotypic properties of the type strains of all studied taxa are summarized in Table 1, and those of all studied nectar isolates are shown in Table S2. All tests were carried out at 25 °C unless otherwise indicated. Catalase activity was determined by evaluating bubble production with a 3 % (v/v) hydrogen peroxide solution (Cappuccino & Sherman, 2002). Oxidase activity was tested using oxidase test strips (MB 0266 A; Oxoid,). All strains were aerobic, catalase-positive and oxidase-negative. Whereas no growth was observed on TSA in an anaerobic jar, all nectar strains were able to grow at decreased oxygen levels, as assessed by visual inspection of bacterial cultures grown on TSA in a candle jar for 5 days at 25 °C. Growth at 4, 25, 30, 37 and 41 °C, haemolysis of sheep blood, gelatin hydrolysis and production of acid from glucose and sucrose in Hugh–Leifson medium were examined as described previously (Hugh & Leifson, 1953; Skerman, 1959; Bouvet & Grimont, 1986; Carr *et al.*, 2003). All nectar strains grew at 25 and 30 °C, but not at 37 or 41 °C. Strains SAP219.2, SAP239.2, SAP240.2, SAP241.2, SAP242.2, SAP284.1^T and SAP320.1 were able to grow at 4 °C but strains SAP220.2, SAP249.1, SAP 305.1, SAP763.2^T, SAP 956.2, SAP970.1 and SAP971.1 could not. All strains were non-haemolytic on Columbia agar supplemented with sheep blood. Strains SAP 305.1 and SAP 970.1 were found to grow poorly on this medium. None of the tested isolates were able to hydrolyse gelatin. All nectar strains produced acid from sucrose and glucose.

Carbon source oxidation was determined by Phenotype MicroArray (PM) technology (Biolog) using PM plate 1. Using this technology, kinetic profiles are generated by continuously monitoring the metabolic activity during incubation (Bochner *et al.*, 2001). Plates were incubated in the OmniLog automated incubator-reader (Biolog) for 5 days at 25 °C and were read every 15 min. Interpretation of results was performed using OmniLog PM software according to the manufacturer's instructions. Clear differences were observed between the two groups of nectar strains and between these nectar strains and the type strains of *A. baylyi*, *A. calcoaceticus*, *A. gerneri* and *A. radioresistens*. In contrast to the type strains of the related species of the genus *Acinetobacter*, all nectar strains were able to oxidize sucrose and D-fructose as the only carbon source. D-mannose, on the other hand, was only oxidized by the group which included strain SAP763.2^T. In addition, D-glucose was only oxidized by some strains of that group. In addition to sucrose and D-fructose, L-malic acid was the only carbon source that could be oxidized by the majority of the strains of the group containing strain SAP284.1^T. For the group including strain SAP763.2^T, all strains were found to oxidize L-aspartic acid, bromosuccinic acid, fumaric acid, L-glutamic acid, L-malic acid, DL-malic acid, succinic acid, L-asparagine and L-proline; and some of the seven strains in this group were able to oxidize D-xylose, D-gluconic acid, α-ketoglutaric acid, mono-methylsuccinate or L-alanine (Table S2). In order to compare the results obtained by PM fingerprinting with more conventional assimilation tests commonly used for classification of members of the genus *Acinetobacter*, some key biochemical features were also assessed using the phenotypic system described by Bouvet & Grimont (1986) and adapted by Nemec *et al.* (2009). More specifically, tests were performed for sucrose, D-glucose, succinic acid and phenylacetate. Briefly, assimilation tests were performed using the basal mineral medium of Cruze *et al.* (1979)

Table 1. Differential phenotypic characteristics of the type strains of all studied species of the genus *Acinetobacter*

Strains: 1, *A. nectaris* sp. nov. SAP 763.2^T; 2, *A. boissieri* sp. nov. SAP 284.1^T; 3, *A. calcoaceticus* DSM 30006^T; 4, *A. baylyi* DSM 14961^T; 5, *A. gerneri* DSM 14967^T; 6, *A. radioresistens* DSM 6976^T. *A. nectaris* sp. nov. and *A. boissieri* sp. nov. can be separated from the type strains of *A. calcoaceticus*, *A. baylyi*, *A. gerneri* and *A. radioresistens* by some basic phenotypic characteristics, such as their ability to oxidize fructose and sucrose, their tolerance to sucrose concentrations above 30 % (w/v) and their inability to grow at ⩾37 °C and assimilate acetate. +, Positive reaction; −, negative reaction; W, weak growth; ND, no data available.

Characteristic	1	2	3	4	5	6
Growth on TSA at:						
4 °C	−	+	W	W	−	+
25 °C	+	+	+	+	+	+
30 °C	+	+	+	+	+	+
37 °C	−	−	+	+	+	+
41 °C	−	−	−	W	+	+
Anaerobic growth	−	−	−	−	−	−
Growth at decreased oxygen levels	+	+	ND	ND	ND	ND
Haemolysis on Columbia blood agar	−	−	−	−	−	−
Gelatin hydrolysis	−	−	−	−	−	−
Catalase activity	+	+	+	+	+	+
Oxidase activity	−	−	−	−	−	−
Growth on LB broth plus sucrose at:						
10 % (w/v)	+	+	+	+	+	+
20 % (w/v)	+	+	+	+	+	+
30 % (w/v)	+	+	+	+	+	−
40 % (w/v)	+	+	−	−	−	−
50 % (w/v)	+	W	−	−	−	−
Acid production from glucose	+	+	+	+	+	−
Acid production from sucrose	+	+	+	+	+	−
Oxidation of: *						
D-Glucose	−	−	−	+	−	−
D-Fructose	+	+	−	−	−	−
D-Mannose	+	−	−	−	−	−
Sucrose	+	+	−	−	−	−
D-Xylose	−	−	−	−	−	−
Acetic acid	−	−	+	+	+	+
D-Aspartic acid	−	−	−	+	−	−
L-Aspartic acid	+	−	−	+	−	−
Bromosuccinic acid	+	−	−	+	+	−
Citric acid	−	−	−	+	+	−
Fumaric acid	+	−	−	+	+	−
D-Galacturonic acid	−	−	+	−	−	−
D-Gluconic acid	+	−	−	+	−	−
L-Glutamic acid	+	−	−	+	+	−
α-Hydroxybutyric acid	−	−	−	+	+	−
m-Hydroxyphenyl acetic acid	−	−	−	−	+	−
p-Hydroxyphenyl acetic acid	−	−	−	−	+	−
α-Ketobutyric acid	−	−	−	+	+	−
α-Ketoglutaric acid	−	−	−	+	+	−
L-Lactic acid	−	−	−	+	+	−
D-Malic acid	−	−	−	−	−	−
L-Malic acid	+	+	−	+	+	−
DL-Malic acid	+	−	−	+	+	−
Mucic acid	−	−	−	+	−	−
Propionic acid	−	−	−	+	+	−
Pyruvic acid	−	−	−	+	+	−
D-Saccharic acid	−	−	−	+	−	−
Succinic acid	+	−	+	+	+	−
Tricarballylic acid	−	−	−	+	−	−
Methylpyruvate	−	−	+	+	+	−
Mono-methylsuccinate	−	−	−	+	+	−
α-Hydroxyglutaric acid-g-Lactone	−	−	−	−	+	−
2-Aminoethanol	−	−	−	−	−	−
Dulcitol	−	−	+	−	−	−
D-Alanine	−	−	+	+	+	−
L-Alanine	−	−	−	+	+	−
L-Asparagine	+	−	−	+	−	−
L-Glutamine	+	−	−	+	+	−
Gly–Pro	−	−	−	−	+	−
Phenylethylamine	−	−	−	−	+	−
L-Proline	+	−	−	+	+	−
L-Threonine	−	−	+	−	−	−
Tween 20	−	−	+	+	+	−
Tween 40	−	−	+	+	+	−
Tween 80	−	−	+	+	+	+

*Oxidation of carbon sources was determined by Phenotype MicroArray (PM) technology (Biolog) using PM plate 1. Further details on the procedure and the results obtained for all the novel nectar isolates characterized in this work are provided in Table S2.

supplemented with 0.1 % (w/v) of the tested carbon source. The basal medium consisted of the following (l^{-1}): 10.0 g KH_2PO_4, 5.0 g Na_2HPO_4, 2.0 g $(NH_4)_2SO_4$, 0.2 g $MgSO_4.7H_2O$, 0.001 g $CaCl_2.2H_2O$ and 0.001 g $FeSO_4.7H_2O$ (pH 7.0). 5 ml of this medium was dispensed into tubes, inoculated with washed bacterial cells and incubated at 25 °C under agitation. Growth on the different 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. In general, results obtained by these assays confirmed the results obtained by the PM technology assays (data not shown). From the two groups of nectar isolates, all isolates were again able to assimilate sucrose, whereas D-glucose and succinic acid were only assimilated by (some of) the isolates of the group which included SAP763.2^T. In contrast to other known species of the genus *Acinetobacter*, none of the nectar isolates were able to assimilate acetate.

Sucrose tolerance was determined by culturing the studied strains in transparent plastic vials containing Luria–Bertani (LB) broth (Difco) supplemented with 0 (positive control), 10, 20, 30, 40 or 50 % sucrose (w/v, Sigma–Aldrich). All these liquid media were filter-sterilized and kept at 4 °C until use. The range of sugar concentrations tested closely

matched the range of naturally occurring variation in floral nectars of the wild Mediterranean plant communities from which our nectar strains were recovered (S. Álvarez-Pérez & C. M. Herrera, unpublished results). Single colonies picked from 5-day cultures on TSA medium were used to inoculate the tubes and these were incubated at 25 °C for up to 10 days. The turbidity of the cultures with respect to negative controls (i.e. tubes containing no inoculated media) was recorded as a positive result. At the end of the experiment, an aliquot of each test tube was plated on TSA medium to check for possible contaminations. Furthermore, acid production by bacterial strains when growing at different sucrose concentrations was tested by adding 40 µl methyl red (Panreac) to each tube. All nectar strains were able to grow at sucrose concentrations ranging from 10 to 50 % (w/v), although the growth of strains of the group containing SAP284.1^T at 40 % and 50 % sucrose was very weak. Acidification of the culture media was observed in all tubes containing sucrose, but not in the positive control (no sucrose). In contrast, *A. baylyi* DSM 14961^T, *A. calcoaceticus* DSM 30006^T and *A. gerneri* DSM 14967^T only grew at sucrose concentrations up to 30 %, and *A. radioresistens* DSM 6976^T only tolerated 10 % and 20 % sucrose. Furthermore, these four reference strains did not acidify either sucrose-containing culture broths or the positive control.

Methods for genotypic characterization of the studied strains included comparative sequence analysis of the 16S rRNA and the β-subunit of RNA polymerase (*rpoB*)-encoding genes, assessment of overall genomic relatedness by DNA–DNA hybridizations and determination of the DNA G+C content.

An almost complete fragment of the 16S rRNA gene was amplified and subsequently sequenced as described by Álvarez-Pérez *et al.* (2012). Preliminary sequence comparisons with the 16S rRNA gene sequences stored in GenBank (http://www.ncbi.nlm.nih.gov/genbank/) and the Ribosomal Database Project (RDP, http://rdp.cme.msu.edu/) databases showed that the nectar strains belonged to the family *Moraxellaceae* in the *Gammaproteobacteria* subdivision, and the best hits for all sequences were the putative isolates of members of the genus *Acinetobacter* recovered by Fridman *et al.* (2012) from floral nectar of cultivated plants (99 % and 97–98 % overall similarity to strains SAP763.2^T and SAP284.1^T, respectively). The SimTable tool available at the EzTaxon server v. 2.1 (http://www.eztaxon.org/, last accessed 10 December 2011; Chun *et al.*, 2007) was used to search for neighbours among species of the genus *Acinetobacter* with validly published names on the basis of 16S rRNA gene sequences, identifying *A. baylyi* B2^T, *A. gerneri* 9A01^T and *A. radioresistens* DSM 6976^T as the species most closely related to the novel nectar strains, but with a sequence similarity value $\leqslant$96.3 % in all cases (Table S3). The sequence similarity value between strains SAP 763.2^T and SAP 284.1^T, as determined through the EzTaxon server, was 97.7 %.

The 16S rRNA gene sequences of the novel nectar strains and reference strains of members of the genus *Acinetobacter* and the family *Moraxellaceae* were included in a multiple alignment generated by CLUSTAL W (Chenna *et al.*, 2003). The resulting alignment was trimmed with BioEdit v. 7.0.9.0 (Hall, 1999) to ensure that all sequences had the same start and end point, and analysed with Gblocks (Castresana, 2000) to eliminate ambiguously aligned regions, using 'allow gap positions=with half', 'minimum length of a block=5' and default settings for all other options. Following these procedures, 1320 nt positions (98 % of the original alignment) remained for subsequent phylogenetic analysis using the neighbour-joining (NJ) method as implemented in the MEGA 5 software package (Tamura *et al.*, 2011). Pairwise evolutionary distances were computed by the Jukes–Cantor method, and reliability of nodes in the NJ phylogram was assessed by running 1000 bootstrap replicates. In the NJ phylogram based on 16S rRNA gene sequences (Fig. 1) the novel nectar strains clustered with other members of the genus *Acinetobacter*, but stood apart from the recognised species of this genus by forming a consistently differentiated group with 99 % bootstrap support. Furthermore, all strains of the group which included strain SAP 763.2^T clustered together with a 100 % bootstrap support, as did all those strains corresponding to the group which included strain SAP 284.1^T (Fig. 1).

Comparative sequence analysis of two variable regions – zone 1 (approximately 397 bp) and zone 2 (approximately 544 bp) – of the *rpoB* gene was used to confirm both the within-species relatedness of the two groups of novel strains, and their separation from each other and from previously described species of the genus *Acinetobacter*. Primer sequences and PCR conditions were as previously described (Khamis *et al.*, 2004; La Scola *et al.*, 2006), with some minor modifications: 250 µM of each dNTP (Sigma–Aldrich), 0.4 µM of each of the corresponding forward and reverse primers (Sigma–Aldrich) and 5×10^{-2} U *Taq* polymerase µl^{-1} (Bioline) were used in reaction mixtures and 52 °C was the temperature for primer annealing in PCR cycles. As with the 16S rRNA gene sequence, concatenated sequences of *rpoB* zones 1 and 2 of the nectar strains and type strains of other species of the genus *Acinetobacter* were included in a multiple alignment and analysed with Gblocks, which resulted in the selection of 843 nucleotide positions (99 % of the original alignment). A phylogenetic tree was inferred by the NJ method using the Jukes–Cantor method, and again, the two groups of nectar strains formed two different clusters with bootstrap values supporting their distinctness from each other and from the other acinetobacters (Fig. S1).

The genomic DNA–DNA relatedness between the strains SAP 763.2^T and SAP 284.1^T and between these and the type strains of *A. calcoaceticus*, *A. baylyi* and *A. gerneri* was evaluated by DNA–DNA hybridizations. High-molecular-mass total genomic DNA was extracted by the method of Wilson (1987). DNA–DNA hybridizations were carried out

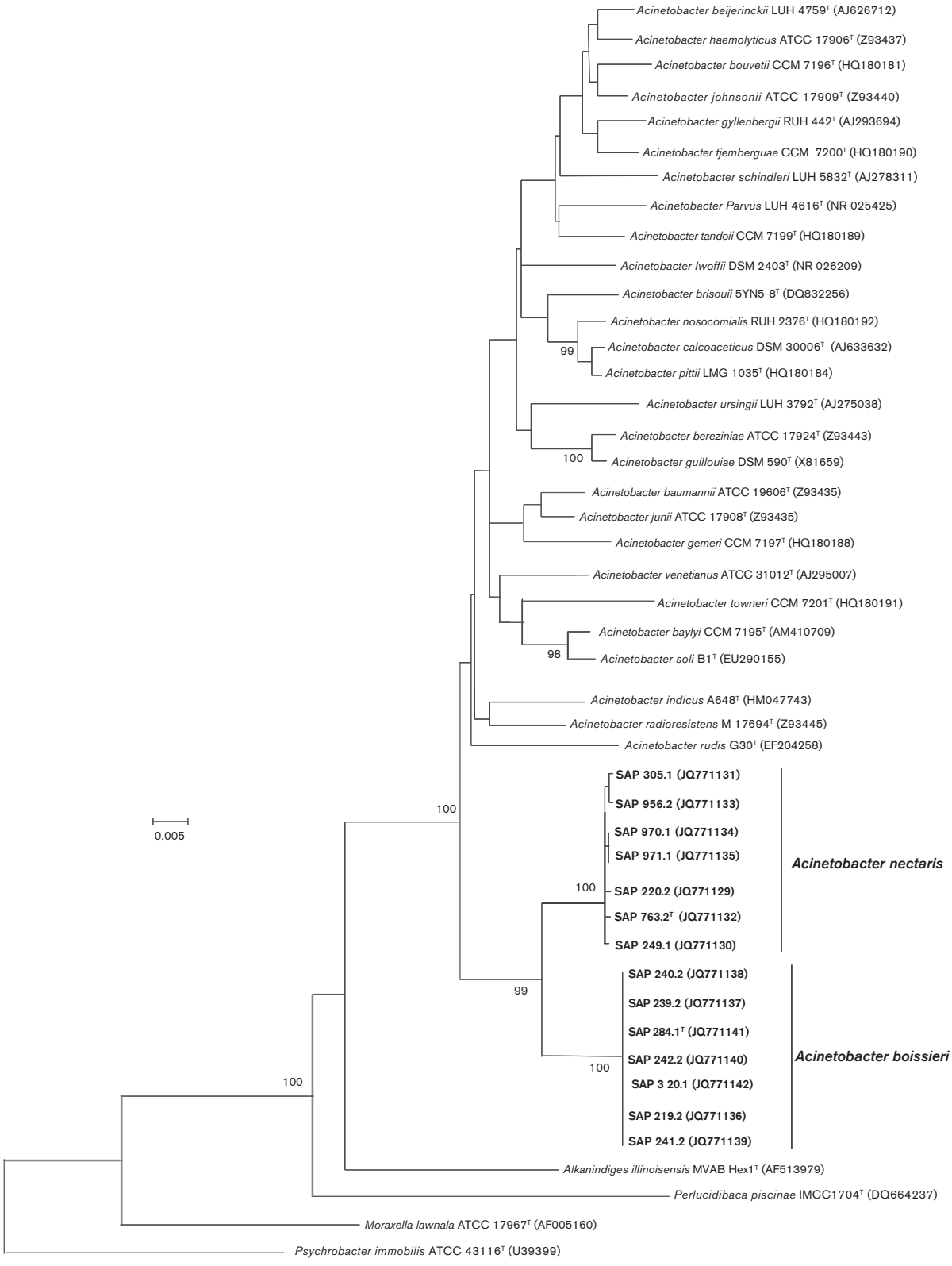

Fig. 1. Neighbour-joining tree, based on 16S rRNA gene sequences, showing the relationships of nectar strains of *A. nectaris* sp. nov. and *A. boissieri* sp. nov. with respect to other members of the genus *Acinetobacter* and representatives of closely related genera within the family *Moraxellaceae*. Evolutionary distances were computed using the Jukes–Cantor method and are in the units of the number of base substitutions per site. There were a total of 1320 positions in the final dataset. All positions

containing gaps and missing data were eliminated. Node support values (bootstrap percentages, based on 1000 simulations) ⩾90 % are shown next to the branches. GenBank accession numbers are given in parentheses. The tree was rooted with the 16S rRNA gene sequence of *Psychrobacter immobilis* ATCC 43116^T.

according to a modification of the microplate method described by Ezaki *et al.* (1989) (Goris *et al.*, 1998; Cleenwerck *et al.*, 2002). The hybridization temperature was 38 °C and the assay was repeated at least seven times for each pair of strains to calculate the mean percentages of DNA–DNA relatedness. Reciprocal reactions were performed and the variation between them was within acceptable limits (Goris *et al.*, 1998). Strain SAP 763.1^T and strain SAP 284.1^T showed very low DNA–DNA relatedness values to each other and with the type strains of the other species of the genus *Acinetobacter* tested (⩽30 % of relative binding in all cases; Table S4), thus supporting the conclusion that the novel nectar strains group into two novel genomic lineages within their genus.

Finally, the DNA G+C content was determined for strain SAP 763.2^T and strain SAP 284.1^T using the same DNA extract as in the DNA–DNA hybridizations. The DNA was enzymically degraded into nucleosides and the nucleotidic base composition was determined by HPLC, according to the method of Mesbah *et al.* (1989). Three independent analyses were conducted for each DNA sample, and mean DNA G+C content values were calculated. The DNA G+C content of strain SAP 763.2^T and strain SAP 284.1^T was 36.6 and 37.8 mol%, respectively. These values are slightly lower than those commonly found for strains of other species of the genus *Acinetobacter* (Bouvet & Grimont, 1986; Nishimura *et al.*, 1988; Nemec *et al.*, 2003; Vaneechoutte *et al.*, 2009; Kang *et al.*, 2011).

Taken together, the results of the present study support a clear genotypic and phenotypic differentiation of the two groups of acinetobacters isolated from floral nectar of wild Mediterranean plants, as well as their independence from other currently recognized species of the genus *Acinetobacter*. The studied strains should therefore be classified into two novel species, for which we propose the names *Acinetobacter nectaris* sp. nov. and *Acinetobacter boissieri* sp. nov. Furthermore, the habitat from which these species were recovered is also a novelty for the genus, as no other nectar-dwelling species of the genus *Acinetobacter* have been described so far. Future research should be aimed at clarifying if *A. nectaris* sp. nov. and *A. boissieri* sp. nov. have any relevant role within plant–pollinator systems, as suggested in the case of nectar yeasts. In this respect, the carbon source oxidation profiles observed in this work for *A. nectaris* sp. nov. and *A. boissieri* sp. nov. seem to be complementary to those of nectarivorous yeasts that quickly deplete glucose in nectar and enrich this floral reward in fructose (Canto *et al.*, 2008; Herrera *et al.*, 2008). If confirmed, such a scenario would suggest that interactions between fungal and bacterial kingdoms might be a relevant driving force in the ecology and evolution of nectar-inhabiting microbiota.

Description of *Acinetobacter nectaris* sp. nov.

Acinetobacter nectaris (nec.ta′ris. L. n. *nectar -aris* the drink of the gods, and also the sugary solution secreted by some flowers to attract pollinators; L. gen. n. *nectaris* of nectar, isolated from floral nectar of plants).

The description is based on the characteristics of seven strains which were isolated from the floral nectar of plants collected in the surroundings of Doñana's Natural Park (Huelva province, SW Spain). Cells are Gram-negative, aerobic, oxidase-negative, catalase-positive, non-motile coccobacilli, generally occurring in pairs. All strains tested can grow at decreased oxygen concentrations, but not under anaerobic conditions. Growth occurs at moderate temperatures (e.g. 25 and 30 °C), but not at 4, 37 and 41 °C. Colonies on TSA medium are round and smooth, convex to umbilicate, slightly opaque, with entire margins and (<)0.5–2.5 mm in diameter after 5 days of incubation at 25 °C. Some isolates grow poorly on Columbia agar supplemented with sheep blood, and all strains are non-haemolytic on this medium. Gelatin is not hydrolysed. All strains produce acid from sucrose and glucose and oxidize D-fructose, D-mannose and sucrose as the sole carbon source. Some isolates can grow on D-glucose, though at slow growth rates. In addition, all strains oxidize L-aspartic acid, bromosuccinic acid, fumaric acid, L-glutamic acid, L-malic acid, DL-malic acid, succinic acid, L-asparagine and L-proline. The type strain oxidizes D-gluconic acid but not D-xylose, α-ketoglutaric acid, mono-methylsuccinate or L-alanine. Other isolates showed different reactions for these latter substrates. None of the strains assimilated acetate. Sucrose is tolerated at concentrations ranging from 10 % to 50 % (w/v).

The type strain is SAP 763.2^T (=LMG 26958^T=CECT 8127^T), isolated from floral nectar of the plant species *Muscari comosum* (L.) Mill. (Hyacinthaceae) collected in the surroundings of Doñana's Natural Park (Huelva province, SW Spain). The DNA G+C content of the type strain is 36.6 mol%.

Description of *Acinetobacter boissieri* sp. nov.

Acinetobacter boissieri [bois.si′e.ri. N.L. masc. gen. n. *boissieri* of Boissier, named after the Swiss botanist Pierre-Edmond Boissier (1810–1885), in recognition of his great contribution to the description of the flora of Southern Spain].

The description is based on the characteristics of seven strains which were isolated from the floral nectar of plants

collected in the surroundings of Doñana's Natural Park (Huelva province, SW Spain). Cells are Gram-negative, aerobic, oxidase-negative, catalase-positive, non-motile coccobacilli, generally occurring in pairs. All strains can grow at decreased oxygen concentrations, but not under anaerobic conditions. Growth occurs between 4 °C and higher temperatures such as 30 °C, but not at 37 °C or 41 °C. Colonies on TSA medium are round, convex to umbilicate, smooth, slightly opaque, with entire margins and (<)0.5–2.5 mm in diameter after 5 days of incubation at 25 °C. Haemolysis on sheep blood agar is negative. Gelatin is not hydrolysed. All strains produce acid from sucrose and glucose and oxidize D-fructose and sucrose as the sole carbon source. In addition, the type strain and the majority of the other tested strains oxidize L-malic acid. None of the strains assimilated acetate. Sucrose is tolerated at concentrations ranging from 10 % to 50 % (w/v) but, for most strains, growth at 40 % or higher values is slow.

The type strain is SAP 284.1^T (=LMG 26959^T=CECT 8128^T), isolated from floral nectar of the plant species *Fritillaria lusitanica* Wikstr. (Liliaceae) collected in the surroundings of Doñana's Natural Park (Huelva province, SW Spain). The DNA G+C content of the type strain is 37.8 mol%.

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