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Acinetobacter apis sp. nov., Isolated from the Intestinal Tract of a Honey Bee, *Apis mellifera*[§]

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A novel Gram-negative, obligate aerobic, non-motile, and both coccobacillus- and bacillus-shaped bacterium, designated strain HYN18^T, was isolated from the intestinal tract of a honey bee (*Apis mellifera*). The isolate was oxidase-negative and catalase-positive. Strain HYN18^T showed optimum growth at 25°C, pH 6–7, and in the presence of 1% (w/v) NaCl in trypticase soy broth medium. The isolate was negative for hydrolyses of starch, casein, gelatin and urea, indole production from tryptone and hemolysis on sheep blood agar. A phylogenetic analysis based on the 16S rRNA gene and *rpoB* gene sequence showed that strain HYN18^T was most closely related to *Acinetobacter nectaris* SAP 763.2^T and *A. boissieri* SAP 284.1^T with 98.3% and 98.1% similarity (16S rRNA gene), respectively, and 84.4% similarity with *Acinetobacter nectaris* SAP 763.2^T (*rpoB* gene). The major cellular fatty acids were summed features 3 (comprising $C_{16:1}\omega7c/C_{16:1}\omega6c$), $C_{12:0}$ and $C_{16:0}$. The main isoprenoid quinone was ubiquinone-9 (Q-9). The polar lipids of strain HYN18^T were phosphatidylethanolamine, three unidentified lipids, an unidentified phospholipid and an unidentified glycolipid. The DNA G+C content was 40.6 mol%. DNA-DNA hybridization experiments indicated less than 33 ± 10% relatedness to the closest phylogenetic species, *Acinetobacter nectaris* SAP 763.2^T. Thus, the phenotypic, phylogenetic and genotypic analyses indicate that strain HYN18^T is a novel species within the genus *Acinetobacter*, for which the name *Acinetobacter apis* is proposed. The type strain is HYN18^T (=KACC 16906^T =JCM 18575^T).

Keywords: *Acinetobacter apis* sp. nov., honey bee intestinal tract, polyphasic taxonomy

Introduction

Recently, colony collapse disorder (CCD) has emerged as a serious problem in the honey bee (*Apis mellifera*), especially in the USA, which is characterized by the sudden disappearance of honey bees from the hive (Oldroyd, 2007). CCD has serious effects on pollination so the crops of various fruits, nuts, and vegetables may be affected by this catastrophe (Morse and Calderone, 2000). Although the aetiology of CCD is still unexplained, a metagenomic survey of gut microbiota in honey bee CCD has shown that increased gamma-proteobacterial taxa in the intestinal tract of CCD bees may contribute to development and exacerbation of the disease (Cox-Foster *et al.*, 2007). In this regard, several studies of bacteria from honey bee have been reported (Evans and Armstrong, 2006; Ahn *et al.*, 2012), but there is still a lack of knowledge about the transfer of pathogenic bacteria in bee colonies (Woolhouse *et al.*, 2005). Thus, investigating bacterial symbionts in honey bees might contribute to the treatment of CCD.

The genus *Acinetobacter*, which belongs to the family *Moraxellaceae*, was first described by Brisou and Prevot (1954). Members of the genus *Acinetobacter* are Gram-negative, aerobic, oxidase-negative, catalase-positive, and immotile bacteria. At present, the genus *Acinetobacter* comprises 30 validated species (Euzeby, 1997). Species in the genus *Acinetobacter* have been isolated from water, soil, sludge, and human specimens (Nishimura *et al.*, 1988; Carr *et al.*, 2003; Nemec *et al.*, 2003, 2009; Kim *et al.*, 2008; Vaz-Moreira *et al.*, 2011; Malhotra *et al.*, 2012). In current study, we described the taxonomic characterization of the bacterial strain, HYN18^T, and suggested that it represents a novel species of the genus *Acinetobacter*.

Materials and Methods

Bacterial strain and culture conditions

As part of an investigation into the microbial diversity of insects living in Korea, an *Acinetobacter*-like strain was isolated from the intestinal tract of a honey bee (*Apis mellifera* Linne) from Pocheon, Korea. A honey bee was washed with 100% ethanol twice, before dissection. After washing, intestinal tissue from the crop to the hindgut of honey bee was extracted using sterile forceps and homogenized by shaking in a tube containing glass beads (0.4-0.6 mm diameter) and 1 ml filtered phosphate-buffered saline (PBS) for 1 min using a vortex. Bacterial isolation from the homogenized honey bee gut sample was achieved by serial dilutions using PBS and spreading on tryptic soy agar (TSA; Bacto, USA)

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plates. The plates were incubated at 15°C for 1 week. Single colonies were transferred repeatedly to fresh medium to obtain pure cultures. After primary purification, the isolate was stored at -80°C in tryptic soy broth (TSB; Bacto) containing 40% (v/v) glycerol. To facilitate a more extensive characterization of strain HYN18^T, type strains of the closely related species, *A. nectaris* SAP 763.2^T (=LMG 26958^T), *A. boissieri* SAP 284.1^T (=LMG 26959^T), *A. indicus* A648^T (=DSM 25388^T), and *A. radioresistens* DSM 6976^T, were obtained from the Belgian Co-ordinated Collections of Microorganisms/Laboratorium voor Microbiologie, Universiteit Gent (BCCM/LMG) and Leibniz-Institut DSMZ-Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSMZ) and used as reference species.

Morphological, physiological, and biochemical characterization

Physiological and biochemical tests were examined with strain HYN18^T cultivated for 48 h at 25°C in TSB or TSA medium. A Gram staining kit (bioMérieux, France) was used according to the manufacturer's instructions. Gram staining and the cell morphology were observed using a light microscope (ECLIPSE 50*i*, Nikon, Japan) as described before (Roh *et al.*, 2013). A field-emission electron microscope (SUPRA VP55, Carl Zeiss, Germany) was also used to determine the detailed cellular morphology. A cellular motility test was performed using semi-solid agar (Tittsler and Sandholzer, 1936). Growth in anaerobic conditions was assessed after incubation for 7 days at 25°C on TSA plates in an anaerobic chamber (Sheldon Manufacturing Inc., USA), which contained a $N_2/CO_2/H_2$ (90:5:5) atmosphere. Hydrolysis of starch, casein and gelatin was identified by incubating strain HYN18^T and the reference species on TSA added with 0.5% soluble starch (Junsei, Japan), 5% skim milk (Difco, USA) and 2% gelatin (Sigma, USA), respectively (Benson, 1994; Smibert and Krieg, 1994). Urea hydrolysis was tested using TSB supplemented with 2% urea (Sigma) and phenol red (Sigma) (Mobley *et al.*, 1988). Indole production from tryptone was determined by cultivation of the isolate and the reference species on TSB with 1% tryptone (Bacto) and confirmed with James reagent (bioMérieux) (Benson, 1994). Haemolysis of sheep blood was determined by cultivations on TSA and brain heart infusion agar (BHIA; Bacto) plates supplemented with 5% sheep blood. The growth ranges and optimal growth conditions for strain HYN18^T were determined by cultivation at different temperatures (4, 10, 15, 20, 25, 30, 37, 45, 55, and 65°C) and with different pH values (pH 4.0–10.0 at intervals of 1.0 pH unit) in TSB medium. The pH values were adjusted using 10 mM MES (for pH 4, 5, and 6), 10 mM TAPS (for pH 7, 8, and 9) or 10 mM disodium hydrogen phosphate (Na_2HPO_4) (for pH 10). The NaCl requirements and tolerance were tested in TSB medium with different salt concentrations (0, 1, 2, 3, 4, 5, 8, 10, 12, and 15%, w/v). Salt-free TSB was prepared using the formula for Bacto medium but without NaCl. Tests for identification of ability to grow on a minimal (Cruze's) medium supplemented with the major carbon sources (sodium acetate, D-glucose, sucrose, and succinic acid) were performed according to the methods of Álvarez-Pérez *et al.* (2013) and Cruze *et al.* (1979). The turbidity of each culture was detected at 600 nm (OD_{600}) using a spectrophotometer (Synergy MX, BioTek, USA) after 24 h, 48 h, and 7 days of incubation. The biochemical assays were performed after cultivation in the optimum growth conditions for 48 h on TSA medium. Catalase activity was confirmed by bubble production in 3% (v/v) hydrogen peroxide. The oxidase activity was assessed using 1% (w/v) tetramethyl-*p*-phenylenediamine (bioMérieux). API ZYM test strips (bioMérieux) and API 20NE test strips (bioMérieux) with API suspension medium (bioMérieux) were used to assess the enzyme activities of the isolate and the related type strains, according to the manufacturer's instructions. Acid production from various carbohydrates and the metabolism of sole carbon source were tested using API 50 CH test strips (bioMérieux) with 50 CHB/E medium (bioMérieux) and GN2 MicroPlates (Biolog, USA) with GN/GP inoculating fluid (Biolog), respectively.

16S rRNA gene sequence determination and phylogenetic analysis

For the phylogenetic analysis, amplification of the 16S rRNA gene from strain HYN18^T was performed using colony PCR with a PCR pre-mix (iNtRon Biotechnology, Korea) and two universal bacterial-specific primers: forward primer 8F (5′-AGAGTTTGATCCTGGCTCAG-3′) and reverse primer 1492R (5′-GGYTACCTTGTTACGACTT-3′) (Lane, 1991). The PCR product was sequenced using a BigDye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems, USA), according to the manufacturer's instructions. The reaction mixture was analyzed using an automated DNA analyzer system (PRISM 3730XL DNA Analyzer, Applied Biosystems). The almost complete 16S rRNA gene sequence fragments of strain HYN18^T (1,497 bp) were assembled using SeqMan (DNASTAR). The assembled 16S rRNA gene sequence was compared with those of type strains in the EzTaxon-e server (http://eztaxon-e.ezbiocloud.net/; Kim *et al.* (2012)). Due to the limitations of the 16S rRNA gene (Dahllof *et al.*, 2000) and to consolidate the phylogenetic description, the protein-encoding gene RNA polymerase subunit β (*rpoB*) was amplified and sequenced, as described by La Scola (2006). The zone 1 and 2 of *rpoB* was amplified separately and assembled together (881 bp). The assembled *rpoB* gene sequence was compared with those in GenBank by using BLAST (http://www.ncbi.nlm.nih.gov/BLAST; Johnson *et al.* (2008)). The 16S rRNA gene and the protein-encoding gene of the isolate were aligned with those of the most closely related species using the multiple alignment program CLUSTALW (Thompson *et al.*, 1994). The Bioedit program was used to check the aligned sequences manually (Hall, 1999). The phylogenetic trees were constructed by MEGA5 (Tamura *et al.*, 2011) based on the 16S rRNA and *rpoB* gene sequences with 1,000 random bootstrap replicates using the neighbour-joining (Saitou and Nei, 1987), maximum-parsimony (Kluge and Farris, 1969), and maximum-likelihood (Felsenstein, 1981) methods.

Chemotaxonomic characterization

Strain HYN18^T and the closely-related species were grown on TSA for 48 h at optimum growth condition, before the chemotaxonomic analyses. The cells were standardized at the exponential phase. The cellular fatty acids were extracted from the isolate and the reference species, according to the protocol specified by the Sherlock Microbial Identification System (MIDI, 1999). The cellular fatty acids were analyzed by gas chromatography (Agilent 6890 gas chromatography, Agilent Technologies, USA) and identified using the Microbial Identification software package (Sherlock version 6.2B) (Sasser, 1990) in combination with the TSBA6 library. Isoprenoid quinones were extracted by steeping in chloroform-methanol (2:1, v/v), as described by Collins and Jones (1981). One-dimensional thin-layer chromatography (TLC) on a silica gel 60 F_{254} plate (Merck, Germany) was used for purification and the purified quinones were analyzed using reverse-phase high performance liquid chromatography (HPLC) with a Thermo ODS HYPERSIL (250 × 4.6 mm) column. To facilitate a more comprehensive analysis of isoprenoid quinones, liquid chromatography (LC) (Ultimate 3000, Dionex, Germany) was also performed using an ion trap-mass spectrometer equipped with an electrospray ionization probe (HCT, Bruker Daltonics, Germany), according to the protocol described by Taguchi *et al.* (2005). The polar lipids were extracted from strain HYN18^T and the reference species following the method described by Xin *et al.* (2000). Separation was achieved using two-dimensional TLC on a silica gel 60 F_{254} plate (Merck). The two solvents used for separation and the reagents sprayed to detect and identify the polar lipids were as follows: chloroform/methanol/water (65:25:4, v/v) for the first dimension (from left to right), chloroform/methanol/acetic acid/water (80:12:15:4, v/v) for the second dimension (from bottom to top), 10% ethanolic molybdatophosphoric acid reagent for total lipids, ninhydrin reagent for amino-containing lipids, α-naphthol reagent for glycolipids, and Zinzadze reagent for phospholipids (Tindall, 1990).

DNA G+C content determination and DNA-DNA hybridization

Genomic DNA was extracted from the isolate and the reference species for the genotypic analysis (Rochelle *et al.*, 1992). The G+C content was calculated using a fluorimetric method with SYBR Gold 1 (Invitrogen, USA) and a real-time PCR thermocycler (Bio-Rad Laboratories) (Gonzalez and Saiz-Jimenez, 2002). Genomic DNA from *Escherichia coli* K12, *Ruegeria pomeroyi* DSS-3^T, and *Ruminococcus obeum* ATCC 29174^T were used for calibration to overcome measurement limitations and to minimize errors. DNA-

Table 1. Differential characteristics of strain HYN18^T and closely-related species

Strains: 1, strain HYN18^T; 2, *A. nectaris* SAP 763.2^T; 3, *A. boissieri* SAP 284.1^T; 4, *A. indicus* A648^T; 5, *A. radioresistens* DSM 6976^T. All data were derived from the current study, except those indicated. Data for sole-carbon-source metabolism, acid production from carbohydrate, enzyme activity and reaction were obtained using GN2 MicroPlates (Biolog), API 50CH, API 20NE, and API ZYM, respectively. All strains were oxidase-negative and catalase-positive. All strains were negative for hemolysis on sheep blood agar, hydrolyses of starch, casein and gelatin, and indole production. All strains metabolized L-arabinose, D-fructose, D-galactose, gentiobiose, α-D-glucose, D-melibiose, sucrose, D-glucosaminic acid, and glucuronamide (GN2 MicroPlates). All strains were positive for alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, acid phosphatase and naphthol-AS-BI-phosphohydrolase (API ZYM). Symbols: +, positive; -, negative, ND, no data available.

Characteristic	1	2	3	4	5
Optimum temperature (°C)	25	25[a]	25[a]	28[b]	30[c]
Growth at 37°C	-	-	-	-	+[b]
Hydrolysis of:					
Urea	-	-	+	-	-
Metabolism of:					
Citric Acid, α-Ketobutyric Acid, D-Alanine, L-Alanine, D-Glucose-6-Phosphate	+	-	-	-	-
D-Mannitol, D-Psicose, Pyruvic Acid Methyl Ester, Succinic Acid Monomethyl Ester, D-Gluconic Acid, α-Ketoglutaric Acid, Succinic Acid, Bromosuccinic Acid, Succinamic Acid, L-Glutamic Acid, L-Proline, γ-Amino Butyric Acid	+	+	-	-	-
D-Raffinose	-	+	+	-	-
L-Asparagine	-	+	-	-	-
D-Cellobiose, α-D-Lactose, D-Mannose	-	-	-	+[b]	ND
Malonate, L-Leucine	-	-	-	ND	+[c]
Acid production from:					
D-Arabinose, L-Xylose, L-Rhamnose, *N*-Acetylglucosamine, Esculin, D-Maltose, L-Fucose	-	+	+	ND	ND
Potassium Gluconate	+	+	-	ND	ND
Enzyme activity and reaction					
α-Glucosidase	-	+	-	-	-
D-Glucose fermentation	-	+	+	-	-
Urease	-	-	+	-	-
Reduction of nitrates to nitrites, Valine arylamidase	+	-	-	+	+
Cystine arylamidase	-	-	-	+	+

[a] Data from Álvarez-Pérez *et al.* (2013)
[b] Data from Malhotra *et al.* (2012)
[c] Data from Nishimura *et al.* (1988)

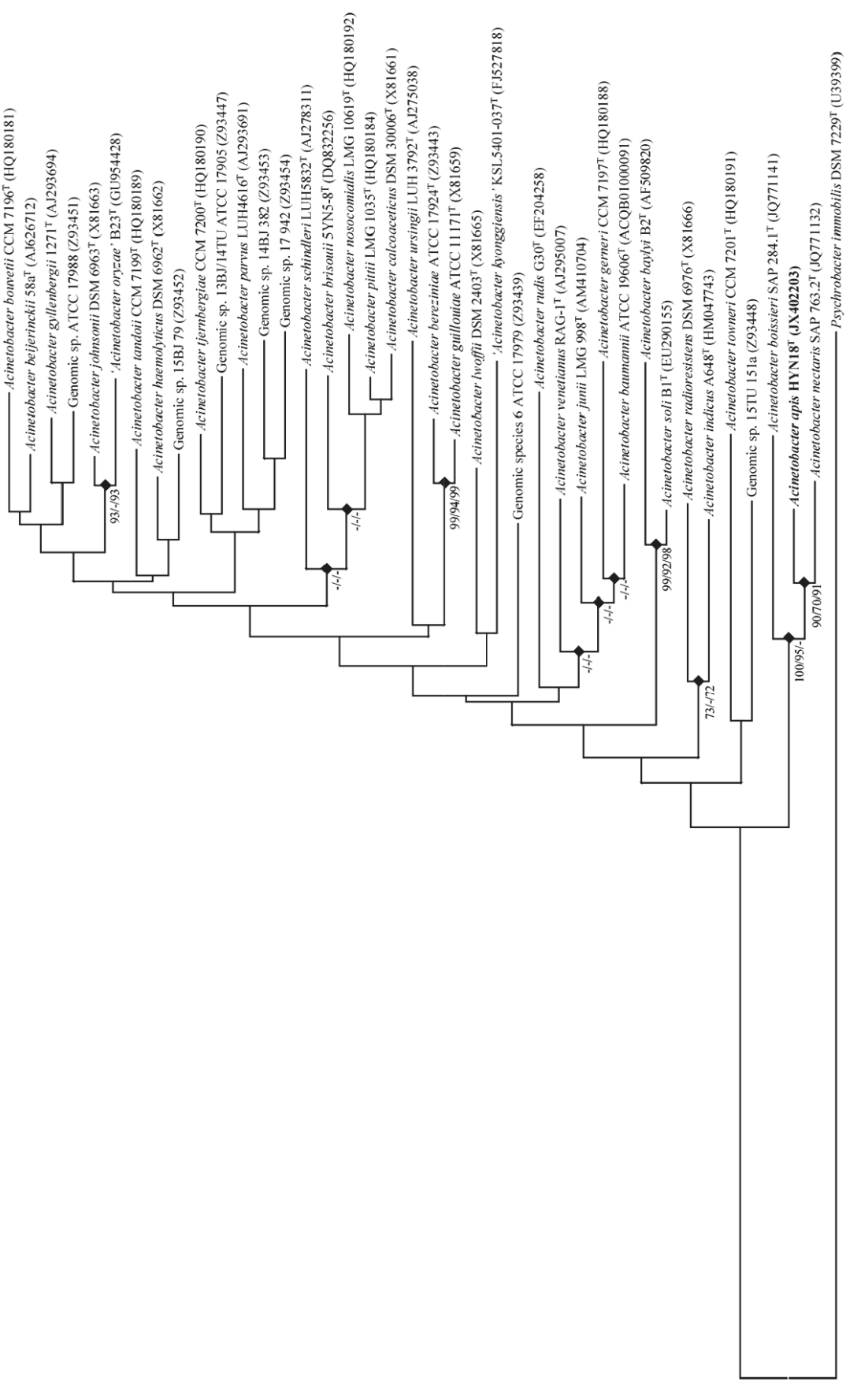

Fig. 1. Phylogenetic tree based on the 16S rRNA gene sequence, which was constructed using the neighbour-joining, maximum-parsimony and maximum-likelihood algorithms. Filled diamonds indicate branches present in the phylogenetic trees generated using the three different methods. The numbers on the nodes (>70%) represent the bootstrap values as percentages of 1000 replicates. *Psychrobacter immobilis* DSM 7229^{T} was used as an outgroup. Bar, 0.01 accumulated changes per nucleotide.

DNA hybridization (DDH) was performed using a genome-probing microarray to elucidate the genetic relatedness (Bae *et al.*, 2005; Chang *et al.*, 2008). The DDH values for the isolate and the reference species were calculated based on the signal-to-noise ratio of the probe (Loy *et al.*, 2005).

Results and Discussion

The tests showed that strain HYN18^T was Gram-negative, non-motile and an obligate aerobe. Cells are coccobacillus- or bacillus-shaped (1.8 μm long and 0.6 μm wide), and surface appendages (pili, fimbriae, or flagella) were observed (Supplementary data Fig. S1). Hydrolyses of starch, casein, gelatin and urea, and indole production from tryptone were negative. The isolate was non-haemolytic on BHIA and TSA supplemented with 5% sheep blood. Strain HYN18^T grew at 15–30°C, pH 6.0–8.0, and in the presence of 0–2% (w/v) NaCl, with optimal growth at 25°C, pH 6.0–7.0 and in the presence of 1% (w/v) NaCl. Strain HYN18^T showed growth on Cruze's media with supplemented succinic acid and D-glucose. Acid was produced by the isolate from L-arabinose, D-ribose, D-xylose, D-galactose, D-glucose, D-fructose, D-mannose, D-mannitol, D-cellobiose, D-lactose, D-melibiose, sucrose, D-raffinose, gentiobiose, D-lyxose, D-fucose, gluconate, and 5-ketogluconate. The isolate metabolited L-arabinose, D-fructose, L-fucose, D-galactose, gentiobiose, α-D-glucose, D-mannitol, D-melibiose, D-psicose, sucrose, pyruvic acid methyl ester, succinic acid monomethyl ester, citric acid, D-gluconic acid, D-glucosaminic acid, α-ketobutyric acid, α-ketoglutaric acid, succinic acid, bromosuccinic acid, succinamic acid, glucuronamide, D-alanine, L-alanine, L-glutamic acid, L-proline, γ-aminobutyric acid, and D-glucose-6-phosphate. The following enzyme activities and reaction were positive based on the API ZYM and API 20 NE test strip results: alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase, and reduction of nitrates to nitrites. The biochemical differences between the isolate and the closely-related species are shown in Table 1.

The 16S rRNA gene sequence comparison results showed that the isolate shared relatively high similarity with *Acinetobacter nectaris* SAP 763.2^T (98.3%) and *A. boissieri* SAP 284.1^T (98.1%). In BLAST comparison, the *rpoB* gene of the isolate showed the highest match with *A. nectaris* SAP 763.2^T between validated species (84.4% similarity). The phylogenetic consensus trees based on the 16S rRNA genes and the *rpoB* genes of strain HYN18^T and the type strains of the genus *Acinetobacter* are shown in Fig. 1 and Supplementary data Fig. S2, respectively. The isolate clustered phylogenetically with *A. nectaris* SAP 763.2^T and *A. boissieri* SAP 284.1^T.

The major cellular fatty acids (>10%) were summed features 3 (comprising $C_{16:1}\omega7c/C_{16:1}\omega6c$, 31.1%), $C_{12:0}$ (18.3%) and $C_{16:0}$ (18.0%). Although the majority of cellular fatty acids composition of strain HYN18^T was generally similar to those of the reference species, there are differences in the components presented in minor proportion. In particular, components included in unsaturated acids such as $C_{14:1}\omega5c$, $C_{16:1}\omega9c$, and $C_{20:4}\omega6,9,12,15c$ showed differences in their presence and absence. The complete cellular fatty acid compositions of strain HYN18^T and the reference species are shown in Table 2. The main isoprenoid quinone extracted from the isolate was ubiquinone Q-9, which is also the dominant isoprenoid quinone in *A. indicus* (Malhotra *et al.*, 2012) and *A. radioresistens* (Nishimura *et al.*, 1988), which have close phylogenic relatedness. The polar lipids from strain HYN18^T comprised phosphatidylethanolamine (PE), three unidentified lipids (L1-3), an unidentified phospholipid (PL1), and an unidentified glycolipid (GL1) (Supplementary data Fig. S3). The isolate shared all spots (PE, L1-3, PL1, and GL1) with the two reference species (*A. nectaris* and *A. boissieri*); however, two unidentified lipids other than L1-3 possessed in *A. nectaris* and *A. biossieri* were absent in strain HYN18^T.

Table 2. Cellular fatty acid composition (%) of strain HYN18^T and the reference species

Strains: 1, strain HYN18^T; 2, *A. nectaris* SAP 763.2^T; 3, *A. boissieri* SAP 284.1^T; 4, *A. indicus* A648^T; 5, *A. radioresistens* DSM 6976^T. All data were obtained from the current study. Values are percentages of total fatty acids. Fatty acids that comprised <0.1% were omitted for all species. Symbol: tr, trace (<0.5%); -, not detected.

Fatty acid	1	2	3	4	5
Saturated acids					
$C_{10:0}$	2.4	0.7	2.3	tr	0.9
$C_{11:0}$	-	tr	-	-	-
$C_{12:0}$	18.3	16.4	7.9	15.7	24.2
$C_{13:0}$	tr	0.5	-	-	-
$C_{14:0}$	3.3	3.3	3.4	1.8	tr
$C_{16:0}$	18.0	10.9	22.5	10.5	13.5
$C_{17:0}$	tr	tr	tr	tr	-
$C_{18:0}$	0.5	tr	1.0	1.0	1.3
Unsaturated acids					
$C_{14:1}\omega5c$	tr	tr	-	-	
$C_{16:1}\omega9c$	-	-	-	1.7	0.9
$C_{17:1}\omega8c$	0.6	0.6	tr	0.5	tr
$C_{18:3}\omega6c$ (6, 9, 12)	1.8	0.7	0.8	-	2.0
$C_{18:1}\omega9c$	6.1	2.5	1.0	19.1	19.1
$C_{20:4}\omega6,9,12,15c$	-	-	-	-	tr
Branched acids					
$C_{10:0}$ 3OH	-	tr	-	-	-
$C_{11:0}$ 3OH	-	tr	-	-	-
$C_{12:0}$ 2OH	tr	5.2	tr	1.3	2.9
$C_{12:0}$ 3OH	7.0	7.6	3.4	5.6	12.2
$C_{16:1}\omega7c$ alcohol	2.0	8.5	5.1	-	-
$C_{16:0}$ N alcohol	2.6	7.0	4.2	-	3.0
$C_{17:0}$ 10-methyl	1.5	1.4	0.8	-	-
$C_{16:0}$ 3OH	-	tr	tr	-	-
$C_{18:0}$ 10-methyl, TBSA	-	-	-	tr	-
Summed features[a]					
1	-	tr	-	-	-
2	2.3	1.9	2.8	2.7	1.4
3	31.1	29.9	42.5	37.8	15.6
8	1.4	0.7	1.0	1.4	1.5

[a] Summed features were used when two or three fatty acids could not be separated using the Microbial Identification System. Summed features 1 comprised iso-$C_{15:1}$ H/$C_{13:0}$ 3OH. Summed features 2 comprised iso-$C_{16:1}$ I/$C_{14:0}$ 3OH. Summed features 3 comprised $C_{16:1}\omega7c/C_{16:1}\omega6c$. Summed features 8 comprised $C_{18:1}\omega7c/C_{18:1}\omega6c$.

The genomic DNA G+C content of strain HYN18^T was 40.6 mol%, which was within the usual G+C content range for the genus *Acinetobacter* (38–47 mol%) (Navia *et al.*, 2002). The DDH values between the strain and the reference species were as follows: of 33 ± 10% (18 ± 4% for the reciprocal) relative to *A. nectaris* SAP 763.2^T and 17 ± 7% (20 ± 11% for the reciprocal) relative to *A. boissieri* SAP 284.1^T. Isolate was confirmed as a separate novel genotypic species since DDH values that were below a threshold of 70% (Wayne *et al.*, 1987).

The phenotypic, phylogenetic and genotypic analyses suggest that strain HYN18^T represents a novel species in the genus *Acinetobacter*, for which the name *Acinetobacter apis* sp. nov. is proposed.

Description of *Acinetobacter apis* sp. nov.

Acinetobacter apis (a'pis. L. n. *apis* -is, a bee, which is also the genus name of the honey bee, *Apis mellifera*; L. gen. n. *apis*, of a bee, of *Apis mellifera*, isolated from the intestinal tract of a honey bee).

Cells are obligate aerobes, oxidase-negative, catalase-positive, Gram-negative, non-motile and both coccobacillus- and bacillus-shaped (1.8 μm long and 0.6 μm wide) with fimbria- or pilus-like structures. Growth occurs at 15–30°C, 0–2% (w/v) NaCl and pH 6–8. Optimal growth occurs at pH 6.0–7.0, with 1% (w/v) NaCl and at 25°C. On TSA medium, colonies are round, opaque with a creamy white colour, smooth, and convex. Cells have ability to grow on minimal medium with succinic acid and glucose. Haemolysis on sheep blood agar is negative. Acid is produced from L-arabinose, D-ribose, D-xylose, D-galactose, D-glucose, D-fructose, D-mannose, D-mannitol, D-cellobiose, D-lactose, D-melibiose, sucrose, D-raffinose, gentiobiose, D-lyxose, D-fucose, gluconate, and 5-ketogluconate. The isolate metabolites L-arabinose, D-fructose, L-fucose, D-galactose, gentiobiose, α-D-glucose, D-mannitol, D-melibiose, D-psicose, sucrose, pyruvic acid methyl ester, succinic acid monomethyl ester, citric acid, D-gluconic acid, D-glucosaminic acid, α-ketobutyric acid, α-ketoglutaric acid, succinic acid, bromosuccinic acid, succinamic acid, glucuronamide, D-alanine, L-alanine, L-glutamic acid, L-proline, γ-aminobutyric acid, and D-glucose-6-phosphate. The following enzyme activities and reaction are positive based on the API ZYM and API 20 NE test strip results: alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase, and reduction of nitrates to nitrites. The major cellular fatty acids are summed features 3 (comprising $C_{16:1}\omega7c$/$C_{16:1}\omega6c$), $C_{12:0}$ and $C_{16:0}$. The main isoprenoid quinone is Q-9. The polar lipids consist of phosphatidylethanolamine, three unidentified lipids, an unidentified phospholipid and an unidentified glycolipid. The DNA G+C content is 40.6 mol%.

The type strain, HYN18^T (=KACC 16906^T =JCM 18575^T), was isolated from the intestinal tract of a honey bee, *Apis mellifera*.

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