Janibacter anophelis sp. nov., isolated from the midgut of *Anopheles arabiensis*

Peter Kämpfer,¹ Olle Terenius,² Jenny M. Lindh² and Ingrid Faye²

Correspondence

Peter Kämpfer
peter.kaempfer@agrar.
uni-giessen.de

¹Institut für Angewandte Mikrobiologie, Justus-Liebig-Universität Giessen, IFZ–Heinrich-Buff-Ring 26–32, D-35392 Giessen, Germany

²Department of Genetics, Microbiology and Toxicology, Stockholm University, S-106 91 Stockholm, Sweden

A Gram-positive, aerobic, non-motile strain, H2.16B^T, isolated from the midgut of the mosquito *Anopheles arabiensis* was investigated using a polyphasic approach. On the basis of 16S rRNA gene sequence similarity studies, strain H2.16B^T was shown to belong to the genus *Janibacter*, being most closely related to *Janibacter melonis* (98.3%), *Janibacter terrae* (98.5%) and *Janibacter limosus* (98.5%). Chemotaxonomic data (*meso*-diaminopimelic acid as the diagnostic diamino acid in the cell wall and major fatty acids of iso-C_{16:0}, C_{17:1}ω8c and C_{17:0}) supported the allocation of the strain to the genus *Janibacter*. The results of DNA–DNA hybridization and physiological and biochemical tests allowed the genotypic and phenotypic differentiation of strain H2.16B^T from closely related species. Thus, H2.16B^T represents a novel species of the genus *Janibacter*, for which the name *Janibacter anophelis* sp. nov. is proposed. The type strain is H2.16B^T (=CCUG 49715^T=CIP 108728^T).

The genus *Janibacter* was originally proposed by Martin *et al.* (1997) as a member of the family *Intrasporangiaceae*. At present, the genus comprises three species, *Janibacter limosus* (Martin *et al.*, 1997), *Janibacter terrae* (Yoon *et al.*, 2000) and *Janibacter melonis* (Yoon *et al.*, 2004). *Janibacter brevis*, originally described by Imamura *et al.* (2000), was shown to be a heterotypic synonym of *J. terrae* (Lang *et al.*, 2003).

Strain H2.16B^T was isolated during the characterization of organisms from the midgut of *Anopheles arabiensis* mosquitoes originating from Kenya (Lindh *et al.*, 2005). Subcultivation was done on nutrient agar (Oxoid) at 30 °C for 24 h. Gram-staining (Hucker method) was performed as described by Gerhardt *et al.* (1994). Cell morphology was observed under a Zeiss light microscope at ×1000 using cultures that had been grown for 10 h or for 3 days at 30 °C on nutrient agar. The growth ability test was performed in Luria–Bertani broth at 10–50 °C (at 5 °C intervals) with shaking at 160 r.p.m.

The 16S rRNA gene was analysed as described previously (Kämpfer *et al.*, 2003). Phylogenetic analysis was performed by using the ARB (Ludwig *et al.*, 2004) and MEGA version 2 (Kumar *et al.*, 2001) software packages, after multiple alignment of the data by CLUSTAL_X (Thompson *et al.*, 1997). Distances were obtained (using distance options according

to the Kimura two-parameter model; Kumar *et al.*, 2001) and clustering was performed using the neighbour-joining (Fig. 1) and maximum-parsimony methods by using bootstrap values based on 1000 replications. The 16S rRNA gene sequence of strain H2.16B^T was a continuous stretch of 1483 bp. Sequence similarity calculations after neighbour-joining analysis indicated that the closest relatives of strain H2.16B^T were *J. melonis* (98.3%), *J. terrae* (98.5%) and *J. limosus* (98.5%). Both the neighbour-joining tree (Fig. 1) and the maximum-parsimony tree (not shown) revealed that strain H2.16B^T clustered most closely with these species.

The results of chemotaxonomic analyses of diamino acid (as described by Kroppenstedt, 1985) and fatty acids (as described by Kämpfer & Kroppenstedt, 1996) are given in the species description. The diagnostic diamino acid in the cell-wall peptidoglycan was *meso*-diaminopimelic acid, essentially in accordance with the diamino acid type reported for other members of the genus (Martin *et al.*, 1997; Yoon *et al.*, 2000, 2004). The fatty acids (analysed from total cell hydrolysates grown on TSBA for 24 h at 28 °C) contained large amounts of iso-C_{16:0}, C_{17:1}ω8c and C_{17:0}, in addition to other fatty acids (Table 1). The results of the physiological characterization, performed using previously described methods (Kämpfer *et al.*, 1991), are given in Table 2 and in the species description. DNA–DNA hybridizations between strain H2.16B^T and the type strains of *J. limosus*, *J. terrae* and *J. melonis* were performed using the method described by Ziemke *et al.* (1998), except that for nick translation 2 µg DNA was labelled during a 3 h incubation at 15 °C. Strain H2.16B^T showed relatively low DNA–DNA

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The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of *Janibacter anophelis* sp. nov. H2.16B^T is AY837752.

Fig. 1. Phylogenetic analysis, based on 16S rRNA gene sequences available from the EMBL database (accession numbers are given in parentheses), constructed after multiple alignment of data by CLUSTAL_X (Thompson *et al.*, 1997). Distances were obtained with distance options according to the Kimura-2 model and clustering was performed with the neighbour-joining method using the software package MEGA, version 2.1 (Kumar *et al.*, 2001). Bootstrap values based on 1000 replications are given as percentages at branching points. Bar, 0.01 K_{nuc} unit.

Table 1. Fatty acid content of *Janibacter anophelis* H2.16B^T and other *Janibacter* species

Taxa: 1, *Janibacter anophelis* H2.16B^T; 2, *J. terrae* DPO strains (n=10); 3, *J. terrae* DPO 360 (=DSM 11214); 4, *J. brevis* DSM 13953^T; 5, *J. terrae* DSM 13876^T; 6, *J. limosus* DSM 11140^T; 7, *J. limosus* DSM 11141; 8, *J. melonis* CM2104^T. Data for taxa 2–7 are from Lang *et al.* (2003); data for *J. melonis* CM2104^T are from Yoon *et al.* (2004). Values are percentages of total fatty acid content. Fatty acids C_{17:1}ω6c, 10-methyl C_{16:0} and 10-methyl C_{19:0} were not present in any of the strains tested.

Fatty acid	1	2	3	4	5	6	7	8
C _{15:0}	3.3	—	—	0.66	0.20	0.92	0.68	12.1
C _{16:0}	2.5	0.2–0.9	0.2	1.55	0.55	2.12	0.92	2.4
C _{17:0}	16.4	0.8–1.6	0.8	2.18	0.52	17.79	4.51	13.4
C _{18:0}	2.7	—	—	0.78	0.35	3.94	0.91	1.5
C _{19:0}	1.0	—	—	—	—	1.90	—	0.5
C _{15:1} ω6c	—	0–0.7	—	0.36	0.28	—	—	1.4
iso-C _{16:1} H	—	2–3	3.07	0.54	2.08	—	1.06	—
iso-C _{17:1} ω9c	0.4	3–6	5.66	2.06	3.51	—	—	—
anteiso-C _{17:1} ω9c	—	0–1.4	1.36	—	0.68	—	—	—
C _{17:1} ω8c	13.8	12–15	11.98	20.90	11.92	26.15	24.98	20.0
C _{18:1} ω9c	3.9	8–14	8.53	15.08	13.34	17.48	11.63	7.6
C _{18:1} ω7c	—	—	—	1.25	1.28	—	—	—
iso-C _{19:1}	—	—	—	—	0.30	—	—	—
iso-C _{14:0}	4.7	0.7–1.5	1.02	0.50	1.06	—	0.59	2.8
iso-C _{15:0}	4.4	4–5	4.43	3.72	2.38	0.56	—	5.1
anteiso-C _{15:0}	0.6	0–0.6	0.55	—	0.44	—	—	1.2
iso-C _{16:0}	32.9	36–44	41.07	30.45	38.10	12.21	34.82	16.7
iso-C _{17:0}	3.3	4–5	4.17	6.56	3.86	1.47	0.73	4.1
anteiso-C _{17:0}	2.0	3–6	5.89	1.97	5.67	—	—	2.2
10-methyl C _{17:0}	2.1	4–5	5.16	2.68	5.97	2.84	5.97	0.4
iso-C _{18:0}	3.2	0–0.8	0.57	1.25	0.92	3.36	3.48	1.1
10-methyl C _{18:0}	—	—	—	—	0.20	0.78	—	0.3
Feature 4*	1.2	3–5	2.75	3.85	3.85	1.08	2.41	2.1
Feature 6*	—	—	—	3.66	2.32	—	—	—
Feature 7*	—	0–1.6	1.36	—	—	—	—	—
Feature 8*	0.9	1.5–2.3	1.60	—	—	7.40	4.96	3.7

*Unknown fatty acids; these compounds have no names listed in the peak library file of the MIDI system and therefore were not identified or included in the percentage calculation.

Table 2. Biochemical properties of *Janibacter anophelis* H2.16B^T and the type strains of other *Janibacter* species

Taxa: 1, *Janibacter anophelis* H2.16B^T; 2, *J. terrae* DSM 13876^T; 3, *J. limosus* DSM 11140^T; 4, *J. melonis* CM2104^T. All data are from this study. W, Weak reaction; D, delayed reaction.

Characteristic	1	2	3	4
Growth at 37 °C (on TSBA)	+	W	—	+
Utilization of:				
Acetate	+	+	—	+
N-Acetyl-D-glucosamine	—	D	D	W
cis-Aconitate	—	+	—	—
Adipate	—	+	—	W
4-Aminobutyrate	W	—	—	+
L-Azelate	—	W	—	—
D-Fructose	—	+	+	—
D-Gluconate	+	+	+	—
D-Glucose	+	+	+	W
i-Inositol	+	+	W	—
D-Maltose	+	D*	+	W
D-Mannose	+	+	W	W
2-Oxoglutarate	—	+	—	+
L-Proline	W	+	W	+
Putrescine	—	+	+	—
L-Suberate	—	+	W	W
Sucrose	+	+	+	W
D-Trehalose	+	D*	+	+

*Reaction also reported for *J. terrae* strain DPO 1361 (=DSM 11220) and *J. brevis* DSM 13953^T [a later synonym of *J. terrae* (Lang et al., 2003)].

relatedness to *J. melonis* DSM 16063^T (12.0%; reciprocal reaction, 9.8%), *J. terrae* DSM 13876^T (13.7%; reciprocal reaction, 32.4%) and *J. limosus* DSM 11140^T (34.0%; reciprocal reaction, 19.2%). For these reasons, it is clear that strain H2.16B^T represents a novel species of the genus *Janibacter*, for which we propose the name *Janibacter anophelis* sp. nov.

Description of *Janibacter anophelis* sp. nov.

Janibacter anophelis (a.no'phe.lis. N.L. gen. n. *anophelis* from a mosquito of the genus *Anopheles*, as the type strain was isolated from the midgut of *Anopheles arabiensis*).

Gram-positive and oxidase-positive; shows oxidative metabolism. Non-motile, non-endospore-forming cocci of 1.0–1.5 µm in diameter. Very young cultures show rod-like cells. Good growth (visible colonies with a diameter >1 mm) occurs after 3 days incubation on nutrient agar at 25–30 °C. Grows in Luria–Bertani broth at 20–40 °C, with an optimum at 35 °C. Generation time at 35 °C is 44 min. *meso*-Diaminopimelic acid is the diagnostic diamino acid in the cell-wall peptidoglycan. The fatty acid content is mainly iso-C_{16:0}, C_{17:1}ω8c and C_{17:0}. Different 10-methyl acids are present. Results of carbon source utilization tests (including

differentiating characteristics) are shown in Table 2. The type strain does not produce acids from the following sugars: glucose, lactose, sucrose, D-mannitol, dulcitol, salicin, adonitol, inositol, sorbitol, L-arabinose, raffinose, L-rhamnose, maltose, D-xylose, trehalose, cellobiose, methyl D-glucoside, erythritol, melibiose or arabinol. The following carbon sources are utilized as a sole source of carbon (after 48 h incubation): D-gluconate, D-glucose, D-maltose, D-mannose, sucrose, D-trehalose, i-inositol, acetate, propionate, 4-aminobutyrate, fumarate, glutarate, 3-hydroxybutyrate, DL-lactate, L-malate, pyruvate, L-alanine, L-aspartate, L-histidine, L-leucine, L-proline and L-serine. The following carbon sources are not utilized: N-acetyl-D-galactosamine, N-acetyl-D-glucosamine, L-arabinose, *p*-arbutin, D-cellobiose, D-fructose, D-galactose, melibiose, L-rhamnose, D-ribose, salicin, D-xylose, adonitol, maltitol, D-mannitol, sorbitol, putrescine, *cis*-aconitate, *trans*-aconitate, adipate, L-azelate, citrate, itaconate, mesaconate, 2-oxoglutarate, L-suberate, β-alanine, L-ornithine, L-phenylalanine, L-tryptophan, DL-3-hydroxybenzoate, DL-4-hydroxybenzoate or L-phenylacetate.

The type strain, H2.16B^T (=CCUG 49715^T = CIP 108728^T), was isolated from the midgut of *Anopheles arabiensis* from Kenya.

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