Arthrobacter equi sp. nov., isolated from veterinary clinical material

A. F. Yassin,1 C. Spröer,2 C. Siering,3 H. Hupfer3 and P. Schumann2

1Institut für Medizinische Mikrobiologie und Immunologie der Universität Bonn, Sigmund-Freud-Straße 25, 53127 Bonn, Germany
2DSMZ – German Collection of Microorganisms and Cell Cultures, Inhoffenstraße 7B, 8124 Braunschweig, Germany
3Kekulé-Institut für Organische Chemie und Biochemie der Universität Bonn, 53121 Bonn, Germany

A Gram-positive-staining, catalase-positive, non-spore-forming, rod-shaped bacterium, strain IMMIB L-1606T, isolated from genital swabs of a horse, was characterized using a polyphasic approach. Comparative 16S rRNA gene sequence analysis showed that the organism was related to members of the genus Arthrobacter, displaying sequence similarities of 93.5–99.1 % with the type strains of recognized species of the genus. Cell-wall analysis revealed peptidoglycan type A3x L-Lys–L-Ser–L-Thr–L-Ala. DNA–DNA hybridization data and biochemical characterization of strain IMMIB L-1606T enabled the isolate to be differentiated genotypically and phenotypically from phylogenetically closely related species of the genus Arthrobacter. Therefore, it is concluded that strain IMMIB L-1606T represents a novel species of the genus Arthrobacter, for which the name Arthrobacter equi sp. nov. is proposed. The type strain of Arthrobacter equi sp. nov. is IMMIB L-1606T (=DSM 23395T=CCUG 59597T).

The genus Arthrobacter consists of a group of Gram-positive, catalase-positive, aerobic, asporogenous bacteria. The main distinguishing morphological feature of species of the genus Arthrobacter is a growth cycle in which the irregular rods in young cultures are replaced by coccoid forms in older cultures (rod–coccus growth cycle). At the time of writing, the genus contains the A3x peptidoglycan variant and the A. nicotianae group possesses the A4x peptidoglycan variant. The majority of these species have been isolated from various environmental sources. Several species have been isolated from human clinical specimens, such as Arthrobacter albus (Wauters et al., 2000), Arthrobacter creatinolyticus (Hou et al., 1998), Arthrobacter cumminsii (Funke et al., 1996), Arthrobacter luteolus (Wauters et al., 2000), Arthrobacter sanguinis (Mages et al., 2008), Arthrobacter scleromae (Huang et al., 2005) and Arthrobacter woluwensis (Funke et al., 1996; Bernasconi et al., 2004). Two species, however, have been isolated from veterinary sources, namely Arthrobacter gandavensis (Storms et al., 2003) and Arthrobacter nasiphoacae (Collins et al., 2002). During the course of study of bacterial isolates encountered in animal clinical specimens, an unusual strain, designated IMMIB L-1606T, isolated from a horse, was characterized. In this paper, the results of a study of strain IMMIB L-1606T using a polyphasic taxonomic approach are reported and a novel species of the genus Arthrobacter is proposed.

Strain IMMIB L-1606T was isolated from genital swabs of a horse using Columbia agar (BD) supplemented with 5 % sheep blood. The strain was characterized taking into consideration the proposed minimal standards for the characterization of new members of the suborder Micrococccinae as suggested by Schumann et al. (2009). Motility was observed by the hanging drop technique for cells in exponential phase in brain-heart infusion (BHI) broth. Cell morphology was observed using a transmission electron microscope (Philips CM-10) with cultures grown on BHI broth medium at 35 °C for 17–190 h. Negative staining of cells was performed with 1 % uranyl acetate. Growth was tested on diagnostic sensitivity test agar (DST agar; Oxoid), BHI agar (BD), Columbia agar supplemented with 5 % sheep blood (BD) and tryptic soy agar (TSA;
Oxoid). Growth at various temperatures (5, 10, 22, 30, 37 and 42 °C) was determined on Columbia agar. Growth in NaCl was determined in nutrient broth (Difco). The pH range and optimum were determined by incubation in BHI broth at pH 4.0, 5.0, 6.0, 7.4, 8.0 and 9.0. Oxidase activity was determined using the BBL DrySlide oxidase test (BD). The presence of catalase was assessed using 3 % (v/v) H2O2.

The isolate and strains of phylogenetically related species (Arthrobacter defluvii DSM 18782T, Arthrobacter chlorophenolicus DSM 12829T, Arthrobacter polychromogenes DSM 20136T and Arthrobacter oxydans DSM 20119T) were characterized biochemically under the same laboratory conditions using the API Coryne and API ZYM systems according to the manufacturer’s instructions. The Voges–Proskauer reaction was carried out using the method of Barritt (1936). Hippurate hydrolysis was tested using the method of Luechtfeld & Wang (1982). Hydrolysis of adenine, casein, elastin, guanine, hypoxanthine, testosterone, tyrosine and xanthine was investigated as described previously (Gordon, 1966, 1967). Aesculin hydrolysis was tested as described by Gordon (1966) and gelatin hydrolysis was tested as detailed by Gordon & Mihm (1957). DNase test agar (Difco) was used for the DNase assay. Starch hydrolysis was assessed by using the method of Barrow & Feltham (1993). Tests to determine carbon source utilization were performed as described previously (Yassin et al., 1995).

Fatty acid methyl ester analysis was performed on the studied strains according to methods described previously (Minnikin et al., 1980; Yassin et al., 2007). The menaquinone composition of strain IMMIB L-1606T was determined as described by Yassin & Hupfer (2006). Phospholipids were extracted, purified and identified using two-dimensional TLC as described previously by Yassin et al. (1993). The DNA G+C content (mol%) was determined by HPLC according to Mesbah et al. (1989). The peptidoglycan was isolated after disruption of cells by shaking with glass beads and subsequent trypsin digestion according to Schleifer (1985). Amino acids and peptides in cell-wall hydrolysates were analysed by two-dimensional ascending TLC on cellulose plates by using previously described solvent systems (Schleifer, 1985). Amino acid identity was confirmed and their molar ratio was estimated by GC (GC 14A; Shimadzu) and GC-MS (320-MS Quadrupole; Varian) of N-heptafluorobutylrly amino acid isobutyl esters (MacKenzie, 1987; Groth et al., 1996).

The 16S rRNA gene of the isolate was amplified by PCR using previously described procedures (Rainey et al., 1996) and sequenced directly using a Taq DyeDeoxy terminator cycle sequencing kit (Applied Biosystems) and an automatic DNA sequencer (model 310; Applied Biosystems). The closest known relatives of the novel isolate were determined by performing database searches. The 16S rRNA gene sequence of strain IMMIB L-1606T and those of other known related bacteria, retrieved from GenBank, were added to the ARB database (Ludwig et al., 2004) and aligned using the respective tool from the ARB package. The resulting alignment was corrected manually and phylogenetic trees were constructed using maximum-parsimony (Fitch, 1971), neighbour-joining (Saitou & Nei, 1987) and maximum-likelihood (Felsenstein, 1981) methods. The evolutionary distance matrix for the neighbour-joining method was calculated using the correction of Jukes & Cantor (1969). Topologies of the neighbour-joining tree were evaluated using bootstrap analyses (Felsenstein, 1985) based on 1000 resamplings.

To investigate the phylogenetic relationships between strain IMMIB L-1606T and members of other taxa, comparative 16S rRNA gene sequencing was performed. The almost-complete 16S rRNA gene sequence of the strain (1487 nt) was determined in this study. Sequence database searches (BLAST) indicated that strain IMMIB L-1606T was most closely related to members of the genus Arthrobacter (sequence similarities of 93.5–99.1 %). A tree constructed using the neighbour-joining method depicting the phylogenetic position of strain IMMIB L-1606T among members of the genus Arthrobacter is shown in Fig. 1 and shows that the isolate represents a distinct subline within the genus. Strain IMMIB L-1606T clustered together with A. defluvii and Arthrobacter niigatensis, an association that was supported by a bootstrap resampling value of 74 % (Fig. 1). Highest 16S rRNA gene sequence similarities were shown with A. defluvii DSM 18782T (99.1 %), A. niigatensis LC4T (99.0 %), A. chlorophenolicus DSM 12829T (98.8 %) and Arthrobacter phenanthrenivorans DSM 18606T (98.3 %).

To investigate the genetic relationship between isolate IMMIB L-1606T, A. defluvii, A. niigatensis and A. chlorophenolicus, chromosomal DNA–DNA hybridizations were performed. Reassociation values for strain IMMIB L-1606T with respect to A. defluvii DSM 18782T and A. chlorophenolicus DSM 12829T were 32.3 % and 53.9 %, respectively. These results show that strain IMMIB L-1606T belongs to a separate genomic species (Wayne et al., 1987). DNA–DNA hybridization with A. niigatensis JCM 21826T (Ding et al., 2009) was not performed as this strain is not available since its authenticity has not been verified by the JCM or DSM culture collections.

Strain IMMIB L-1606T was aerobic, catalase-positive and oxidase-negative. Colonies were cream coloured on Columbia agar supplemented with 5 % sheep blood, BHI agar, DST agar and TSA. Strain IMMIB L-1606T exhibited a rod–coccus cycle. Cells were predominantly short rods or oval-shaped (0.56–0.72 × 2.0–2.3 μm) after 17–60 h growth. The rods fragmented into cocci (0.49–0.88 μm in diameter) after 190 h growth (Supplementary Fig. S2 in IJSEM Online). Cells were Gram-positive and grew between 10 and 35 °C. The Voges–Proskauer reaction was negative. The isolate hydrolysed adenine, casein, gelatin, hypoxanthine, tyrosine and urea, but did not reduce nitrate. Using the API Coryne system, the isolate did not produce acid from glucose or any other carbohydrates tested. Using the API Coryne and API ZYM systems, activity was detected for β-galactosidase, α-glucosidase, β-glucuronidase, leucine.
arylamidase, naphthol-AS-BI-phosphohydrolase and pyrazinamidase. All other enzyme tests in the API Coryne and API ZYM systems were negative. The isolate differed from *A. defluvii* and *A. chlorophenolicus* in the hydrolysis of hippurate and hypoxanthine (Table 1).

Cell wall analysis revealed that peptidoglycan hydrolysates (4 M HCl, 100 °C, 16 h) contained lysine, alanine, threonine, serine and glutamic acid in an approximate molar ratio of 0.9:2.6:0.9:0.7:1.0. The peptides L-Ala–D-Glu, L-Ala–D-Ala, L-Ala–L-Thr, L-Lys–L-Ser, L-Lys–D-Ala and 

![Fig. 1.](http://ijs.sgmjournals.org) Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing the position of strain IMMIB L-1606T within the radiation of species of the genus *Arthrobacter*. Numbers at the nodes indicate the level of bootstrap support >70% based on neighbour-joining analyses of 1000 resampled datasets; solid circles indicate that the corresponding nodes (groupings) were also recovered in maximum-likelihood and maximum-parsimony trees. Bar, 1.0% sequence divergence.
Table 1. Differential biochemical characteristics of strain IMMIB L-1606T and phylogenetically related species of the genus Arthrobacter

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydrolysis of:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aesculin</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>Hippurate</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Hypoxanthine</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Xanthine</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>DNA (2 days)</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Starch (3 days)</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Utilization as source of carbon and energy:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acetate</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>iso-Amylalcohol</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>L-Arabinose</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>2,3-Butanediol</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>D-Galactose</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>D-Glucose</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>p-Hydroxybenzoate</td>
<td>w</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>D-Mannitol</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Raffinose</td>
<td>+</td>
<td>w</td>
<td>+</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Sucrose</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>D-Sorbitol</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Utilization as source of carbon and nitrogen:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arginine</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>Production of:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acid phosphatase</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Alkaline phosphatase</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>β-Glucuronidase</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Gelatin liquefaction</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Pyrrolidinol arylamide</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Nitrate reduction</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

Strains: 1, IMMIB L-1606T; 2, A. deflavi DSM 18782T; 3, A. chlorophenolicus DSM 12829T; 4, A. polychromogenes DSM 20136T; 5, A. oxydans DSM 20119T. All strains were positive for: hydrolysis of adenine, casein, gelatin, tyrosine and urea; utilization of cellobiose, citrate, gluconate, myo-inositol, L-lactate, lactose, maltose, melizitose, rhamnose, trehalose and D-xylene as carbon sources; utilization of L-alanine, L-proline and L-serine as carbon and nitrogen sources; and β-galactosidase, α-glucosidase, leucine arylamidase, naphthol-AS-Bl-phosphohydrolase and pyrazinamidase activities. All strains were negative for: hydrolysis of elastin, guanine, keratin and testosterone; utilization of adipic acid, adonitol, mesoerythritol, m-hydroxybenzoate and 1,2-propanediol as carbon sources; utilization of acetamide and ornithine as sources of carbon and nitrogen; esterase C4, ester lipase C8, lipase C14, β-glucosidase, β-galactosidase, N-acetyl-β-glucosaminidase, valine arylamidase, cystine arylamidase, tryptophan, chymotrypsin, α-mannosidase, α-fucosidase and urease activities; and acetoin production. +, Positive; −, negative; w, weak utilization after 3 weeks. All data are from this study. Note that data on glucose utilization deviate from those originally published by Kim et al. (2008) due to the test methods.

Genomic and chemotaxonomic data confirm that strain IMMIB L-1606T belongs to the genus Arthrobacter. On the basis of low DNA–DNA relatedness with its closest phylogenetic neighbour and differences in biochemical and morphological traits with respect to all of the type strains described for the genus Arthrobacter, it can be concluded that strain IMMIB L-1606T represents a novel species within the genus, for which the name Arthrobacter equi sp. nov. is proposed.

Description of Arthrobacter equi sp. nov.

Arthrobacter equi (e’qui. L. gen. n. equi of the horse).

Cells are Gram-positive-staining, non-motile, non-sporulating and have a rod–coccus cycle. Cells are aerobic, catalase-positive and oxidase-negative. Colonies are cream-coloured, convex and smooth with entire margins, reaching 1–2 mm in diameter on Columbia blood agar supplemented with 5% sheep blood, BHI agar, DST agar and TSA after 2 days of incubation at 34 °C. No growth occurs at 37 °C. Tolerates 0–2% NaCl. Grows at pH 6.0–9.0, with optimum growth at pH 6.0; does not grow at pH 4.0–5.0. Nitrate reduction is negative. Acetoin is not produced. Hydrolyses adenine, casein, DNA (2 days of

l-Ser–l-Lys–d-Ala were detected in the partial hydrolysate of the peptidoglycan (4 M HCl, 100 °C, 0.75 h). Dinitrophenylation according to Schleifer (1985) revealed that alanine was at the N terminus of the interpeptide bridge. From the available data, it can be concluded that the peptidoglycan of strain IMMIB L-1606T is of type A3α l-Lys–l-Lys–l-Thr–l-Ala (Schleifer & Kandler, 1972; A11.23 according to http://www.dsmz.de/microorganisms/main.php?content_id=35). The fatty acid profile of strain IMMIB L-1606T was dominated by ai-C15:0 (47.5%), i-C15:0 (24.5%), i-C16:0 (8.5%) and ai-C17:0 (4.8%). These results are in accordance with the fatty acid compositions of other members of the genus Arthrobacter.
The major menaquinone is MK-9(H2). The polar lipids are glycolipids. The major fatty acids are ai-C15 : 0 and i-C15 : 0.

Table 2. Cellular fatty acid composition (%) of strain IMMIB L-1606T and the most closely related Arthrobacter species

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>i-C14 : 0</td>
<td>2.8</td>
<td>8.5</td>
<td>2.4</td>
<td>3.5</td>
<td>1.0</td>
</tr>
<tr>
<td>C14 : 0</td>
<td>0.9</td>
<td>1.9</td>
<td>1.1</td>
<td>1.0</td>
<td>0.4</td>
</tr>
<tr>
<td>i-C15 : 0</td>
<td>24.5</td>
<td>–</td>
<td>17.2</td>
<td>6.6</td>
<td>9.7</td>
</tr>
<tr>
<td>ai-C15 : 0</td>
<td>47.5</td>
<td>15.8</td>
<td>49.7</td>
<td>56.2</td>
<td>46.2</td>
</tr>
<tr>
<td>C15 : 0</td>
<td>0.3</td>
<td>0.6</td>
<td>0.7</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>i-C16 : 1o7c</td>
<td>1.2</td>
<td>5.9</td>
<td>0.4</td>
<td>1.8</td>
<td>1.3</td>
</tr>
<tr>
<td>i-C16 : 0</td>
<td>8.5</td>
<td>17.0</td>
<td>10.4</td>
<td>6.6</td>
<td>5.7</td>
</tr>
<tr>
<td>C16 : 1o7c</td>
<td>0.6</td>
<td>0.2</td>
<td>0.4</td>
<td>1.8</td>
<td>1.6</td>
</tr>
<tr>
<td>C16 : 0</td>
<td>2.3</td>
<td>9.5</td>
<td>4.4</td>
<td>3.1</td>
<td>4.5</td>
</tr>
<tr>
<td>i-C17 : 1</td>
<td>0.8</td>
<td>0.7</td>
<td>–</td>
<td>–</td>
<td>0.6</td>
</tr>
<tr>
<td>ai-C17 : 0</td>
<td>1.4</td>
<td>4.9</td>
<td>0.6</td>
<td>3.1</td>
<td>4.4</td>
</tr>
<tr>
<td>i-C17 : 0</td>
<td>2.3</td>
<td>1.9</td>
<td>1.6</td>
<td>0.7</td>
<td>1.2</td>
</tr>
<tr>
<td>ai-C17 : 0</td>
<td>4.8</td>
<td>23.5</td>
<td>8.5</td>
<td>7.8</td>
<td>17.5</td>
</tr>
<tr>
<td>i-C18 : 1</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>0.2</td>
</tr>
<tr>
<td>C18 : 1o9c</td>
<td>0.2</td>
<td>0.5</td>
<td>–</td>
<td>–</td>
<td>2.0</td>
</tr>
<tr>
<td>C18 : 0</td>
<td>0.1</td>
<td>0.9</td>
<td>0.2</td>
<td>–</td>
<td>0.5</td>
</tr>
</tbody>
</table>

Acknowledgements

We thank Professor Dr Hans-Georg Trüper for nomenclatural advice. We also thank Bettina Sträubler, Anika Wasner, Birgit Grün and Gabriele Pöpper from DSMZ for excellent technical assistance in analysis of the peptidoglycan, determination of the DNA G+C value and other experiments conducted on the strain.

References


