Camelimonas abortus sp. nov., isolated from placental tissue of cattle

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A Gram-negative, rod-shaped, non-spore-forming bacterium, isolated from placental tissue of a cow, was investigated for its taxonomic position. On the basis of 16S rRNA gene sequence similarities, strain UK34/07-5^T was shown to belong to the class *Alphaproteobacteria*, closely related to the type strain of *Camelimonas lactis* (96.0% sequence similarity). The polyamine pattern showed the major compound spermidine and moderate amounts of putrescine. The major quinone was ubiquinone Q-10. The polar lipid profile was composed of the major compounds phosphatidylethanolamine, phosphatidylcholine, phosphatidylglycerol and phosphatidylmonomethylethanolamine and moderate amounts of diphosphatidylglycerol, three unidentified aminolipids and an unidentified phospholipid. The profile of major fatty acids, consisting of C_{19:0} cyclo ω 8c and C_{18:1} ω 7c, with C_{18:0} 3-OH as the hydroxylated fatty acid, was very similar to that of *C. lactis* M 2040^T. The results of DNA–DNA hybridization and physiological and biochemical tests allowed both genotypic and phenotypic differentiation of the isolate from *C. lactis*. The relatively low 16S rRNA gene sequence similarity of 96.0% to *C. lactis* M 2040^T and marked differences in the polar lipid profiles as well as the results of physiological tests and the DNA–DNA hybridization data support the creation of a novel species, for which the name *Camelimonas abortus* sp. nov. is proposed, with the type strain UK34/07-5^T (=CIP 110303^T =CCUG 61094^T =DSM 24741^T =CCM 7941^T).

Strain UK34/07-5^T was initially cultured on Farrell's medium (Farrell, 1974) from placental tissue following a case of abortion in a Holstein–Friesian cow in Derbyshire, UK, in 2007. Farrell's medium was manufactured using a *Brucella* medium base (CM0169; Oxoid) and a *Brucella* selective supplement (SR0209E; Oxoid). However, initial identification by classical methods failed. Subsequent sequence analysis of the 16S rRNA gene revealed *Camelimonas lactis* M 2040^T as the closest phylogenetic neighbour. The genus *Camelimonas* was recently described by Kämpfer *et al.* (2010) for a group of Gram-negative, rod-shaped, non-spore-forming bacteria, isolated from milk of camels at a camel milk production farm in the United Arab Emirates. To investigate whether strain UK34/07-5^T is indeed

a member of the genus *Camelimonas*, the strain was studied by following a polyphasic approach.

Strain UK34/07-5^T showed a beige-coloured colony on nutrient agar (Oxoid) at 37 °C. Subcultivation for further analyses was done on tryptone soy agar (TSA; Oxoid) at 28 °C for 48 h. Gram-staining was performed as reported previously by Gerhardt *et al.* (1994) and the staining result, as well as the cell morphology, were observed with a Zeiss light microscope at $\times 1000$. Cells for these studies were grown for 3 days at 28 °C on TSA. The 16S rRNA gene was sequenced as reported by Kämpfer *et al.* (2003). A 'phylogenetic' analysis was performed using the ARB software package (Ludwig *et al.*, 2004) and the corresponding SILVA SSURef 100 database (release August 2009; Pruesse *et al.*, 2007) as well as with the software package MEGA version 4 (Tamura *et al.*, 2007). Multiple alignment of the data was performed by using CLUSTAL_X (Thompson *et al.*, 1997). Pairwise distances were calculated and clustering with the neighbour-joining method was performed by using bootstrap values

Abbreviations: oNP, o-nitrophenyl; pNA, p-nitroanilide; pNP, p-nitrophenyl.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain UK34/07-5^T is FR851926.

Fig. 1. Phylogenetic analysis based on 16S rRNA gene sequences of strain UK34/07-5^T and selected type strains of the class *Alpha-proteobacteria*. Accession numbers are shown in parentheses. The phylogenetic tree was calculated with the maximum-likelihood method without filters. Bar, 0.05 substitutions per nucleotide position.

based on 1000 replications. A maximum-likelihood tree was reconstructed with fastDNAmL (Olsen *et al.*, 1994) without filters.

These calculations showed that the 16S rRNA gene sequence of isolate UK34/07-5^T showed the highest similarity to that of *C. lactis* M 2040^T (96.0%). Similarities to the type strains of other related genera, like *Chelatococcus*, *Bosea* and *Microvirga*, were below 96%. A maximum-likelihood tree is shown in Fig. 1. The branching pattern was confirmed by maximum-parsimony and neighbour-joining analyses (not shown).

For quinone, polar lipid and polyamine cell-wall diamino acid analyses, cells were grown on 3.3 × PYE medium (1.0% peptone from casein, 1.0% yeast extract, pH 7.2) for 72 h at 28 °C. Polar lipids and quinones were extracted and analysed as described previously (Tindall, 1990a, b; Altenburger *et al.*, 1996). Polyamines were extracted and analysed as described by Busse & Auling (1988). HPLC analyses were carried out using the equipment described by Stolz *et al.* (2007). The polar lipid profile of UK34/07-5^T (Fig. 2) consisted of the major compounds phosphatidylethanolamine, phosphatidylmonomethylethanolamine, phosphatidylglycerol and phosphatidylcholine and moderate amounts of diphosphatidylglycerol, three unidentified aminolipids and an unidentified phospholipid. The quinone system was ubiquinone Q-10 (99%) with traces of Q-9 and Q-11 (both less than 1%). The polyamine pattern consisted of spermidine [49.4 μmol (g dry weight)⁻¹], putrescine [11.5 μmol (g dry weight)⁻¹], spermine [1.0 μmol (g dry weight)⁻¹] and traces of cadaverine [≤0.1 μmol (g dry weight)⁻¹]. The quinone system and polyamine pattern are in excellent agreement with those of *C. lactis* (Kämpfer *et al.*, 2010). Also, the polar lipid profile of isolate UK34/07-5^T showed most of the lipids reported for *C. lactis*, but the presence of an unidentified phospholipid and absence of an unidentified glycolipid distinguish isolate UK34/07-5^T from *C. lactis*.

The fatty acid profile of the strain is shown in Table 1, together with that of *C. lactis* M 2040^T. No pronounced

differences in fatty acid profiles were found between the strains; however, slight differences could be observed. The major fatty acids were C_{19:0} cyclo ω8c, C_{18:1} ω7c, C_{16:0} and C_{18:0} 3-OH as the hydroxylated fatty acid. The profile was very similar to that reported for *C. lactis* M 2040^T (Kämpfer *et al.*, 2010).

The results of the biochemical and physiological characterization are given in the species description and in Table 2 (differential characters), obtained using methods described previously (Kämpfer *et al.*, 1991). The presence of urease was determined as recommended by the manufacturer (Merck; cat. no. 1.08492).

Fig. 2. Polar lipid profile of UK34/07-5^T after two-dimensional TLC and detection with molybdatophosphoric acid. DPG, Diphosphatidylglycerol; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PME, phosphatidylmonomethylethanolamine; AL1–3, unidentified aminolipids; PL, unidentified phospholipid.

Table 1. Cellular fatty acid contents of strain UK34/07-5^T and *C. lactis* M 2040^T

Values are percentages of total fatty acids. The fatty acid pattern for strain M 2040^T was taken from Kämpfer *et al.* (2010), but was generated using the same method, as described by Kämpfer & Kroppenstedt (1996) using an HP gas chromatograph HP 6890 with Sherlock MIDI software version 2.11 and the TSBA peak-naming table version 4.1. —, Not detected.

Fatty acid	Strain UK34/07-5 ^T	<i>C. lactis</i> M 2040 ^T
Unknown 14.959	—	0.8
Summed feature 2*	4.8	4.6
Summed feature 3*	—	0.6
C _{16:0}	1.8	7.9
C _{17:1} ω8c	—	0.8
C _{18:1} ω7c	27.2	11.5
C _{18:0}	5.9	1.3
C _{19:0} cyclo ω8c	52.5	67.3
11-Methyl C _{18:1} ω7c	2.6	1.4
10-Methyl C _{19:0}	—	1.0
C _{18:0} 3-OH	3.3	1.7
C _{20:2} ω6,9c	1.9	1.0

*Summed features are groups of two or three fatty acids that cannot be separated using the MIDI System. Summed feature 2 contained iso-C_{16:1} and/or C_{14:0} 3-OH; summed feature 3 contained C_{16:1}ω7c and/or iso-C_{15:0} 2-OH.

DNA–DNA hybridization experiments with *C. lactis* M 2040^T were performed with the method reported by Ziemke *et al.* (1998). This investigation resulted in DNA–DNA relatedness values of 18.0 and 13.6 % (reciprocal analysis).

Phylogenetic analysis and 16S rRNA gene sequence similarity suggested that isolate UK34/07-5^T is a representative of a novel species of the genus *Camelimonas*, and results from DNA–DNA hybridizations confirmed its status as a member of a novel species. Isolate UK34/07-5^T exhibited several physiological traits and a polar lipid profile that were different from those of *C. lactis*. Hence, we propose a novel species for isolate UK34/07-5^T, *Camelimonas abortus* sp. nov.

Description of *Camelimonas abortus* sp. nov.

Camelimonas abortus (a.bor'tus. L. gen. n. *abortus* of a miscarriage, an abortion, referring to the isolation of the type strain from the placental tissue of a cow following a case of abortion).

Cells are non-motile, non-spore-forming rods (approx. 2 µm long and 1 µm wide). Gram-stain-negative, oxidase- and catalase-positive, showing an oxidative metabolism. Good growth occurs on R2A agar, TSA, 3.3 × PYE agar and nutrient agar but not on MacConkey agar at 25–30 °C. Beige-coloured, translucent and shiny colonies with entire edges are formed within 24 h, with a diameter of approx. 2 mm. Predominant compound in the polyamine pattern is

Table 2. Distinguishing characteristics of strain UK34/07-5^T and *C. lactis* M 2040^T

All data were obtained in this study. Both strains were positive for the utilization of D-glucose, acetate, propionate, adipate, suberate, azelate, glutarate, DL-3-hydroxybutyrate, oxoglutarate and L-aspartate. Both strains were negative for the utilization of N-acetyl-D-galactosamine, N-acetyl-D-glucosamine, L-arabinose, p-arbutin, cellobiose, D-fructose, D-galactose, D-gluconate, D-mannose, maltose, melibiose, L-rhamnose, D-ribose, salicin, sucrose, D-xylose, D-adonitol, myo-inositol, maltitol, D-mannitol, D-sorbitol, putrescine, *cis*- and *trans*-aconitate, L-malate, pyruvate, β-alanine, D-histidine, L-leucine, L-ornithine, L-phenylalanine, L-proline, L-serine, L-tryptophan, 3-hydroxybenzoate, 4-hydroxybenzoate and phenylacetate. Both strains hydrolysed aesculin, L-alanine pNA, bis-pNP phosphate, pNP phenylphosphonate and L-proline pNA. Neither strain hydrolysed oNP β-D-galactopyranoside, pNP β-D-glucuronide, pNP α-D-glucopyranoside, pNP β-D-glucopyranoside, pNP β-D-xyloside, p-phosphorylcholine or L-glutamate-γ-3-carboxy pNA.

Characteristic	Strain UK34/07-5 ^T	<i>C. lactis</i> M 2040 ^T
Growth on MacConkey agar	—	+
Utilization of:		
Trehalose	+	—
4-Aminobutyrate	—	+
Citrate	—	+
Fumarate	—	+
Itaconate	—	+
DL-Lactate	—	+
Mesaconate	—	+
L-Alanine	—	+
Presence of:		
Unidentified glycolipid	—	+
Unidentified phospholipid	+	—

spermidine and putrescine is found in moderate amounts. The quinone system is ubiquinone Q-10. The polar lipid profile is composed of the major compounds phosphatidylethanolamine, phosphatidylmonomethylethanolamine, phosphatidylglycerol and phosphatidylcholine and moderate amounts of diphosphatidylglycerol, three unidentified aminolipids and an unidentified phospholipid. The major fatty acids are C_{19:0} cyclo ω8c, C_{18:1}ω7c and C_{16:0} and C_{18:0} 3-OH as hydroxylated fatty acid. Utilizes the following carbon sources: D-glucose, trehalose, acetate, propionate, adipate, suberate, azelate, glutarate, DL-3-hydroxybutyrate, oxoglutarate and L-aspartate. Does not utilize N-acetyl-D-galactosamine, N-acetyl-D-glucosamine, L-arabinose, p-arbutin, cellobiose, D-fructose, D-galactose, D-gluconate, D-mannose, maltose, melibiose, L-rhamnose, D-ribose, salicin, sucrose, D-xylose, D-adonitol, myo-inositol, maltitol, D-mannitol, D-sorbitol, putrescine, *cis*- or *trans*-aconitate, citrate, fumarate, DL-lactate, L-malate, itaconate, mesaconate, pyruvate, L- or β-alanine, D-histidine, L-leucine, L-ornithine, L-phenylalanine, L-proline, L-serine, L-tryptophan, 3-hydroxybenzoate, 4-hydroxybenzoate or phenylacetate.

Hydrolyses aesculin, L-alanine *p*-nitroanilide (pNA), bis-*p*-nitrophenyl (bis-pNP) phosphate, pNP phenylphosphonate and L-proline pNA and does not hydrolyse *o*-nitrophenyl (oNP) β -D-galactopyranoside, pNP β -D-glucuronide, pNP α -D-glucopyranoside, pNP β -D-glucopyranoside, pNP β -D-xyloside, *p*-phosphorylcholine or L-glutamate- γ -3-carboxy pNA. Results of carbon source utilization tests that differentiate the *Camelimonas* species are indicated in Table 2.

The type strain, UK34/07-5^T (=CIP 110303^T =CCUG 61094^T =DSM 24741^T =CCM 7941^T), was isolated from placental tissue following a case of abortion in a Holstein-Friesian cow in Derbyshire, UK, in 2007.

Acknowledgements

We thank Dr J. Euzéby for his nomenclatural advice.

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