Camelimonas lactis gen. nov., sp. nov., isolated from the milk of camels

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Three strains of Gram-negative, rod-shaped, non-spore-forming bacteria (M 2040^T, M 1973 and M 1878-SK2), isolated from milk of camels at a camel-milk production farm in the United Arab Emirates, were investigated for their taxonomic allocation. On the basis of 16S rRNA gene sequence similarities, all three strains were shown to belong to the *Alphaproteobacteria* and were most closely related to *Chelatococcus asaccharovorans* and *Chelatococcus daeguensis* (95.1 and 95.2 % sequence similarity to the respective type strains). *meso*-Diaminopimelic acid was detected as the characteristic peptidoglycan diamino acid. The predominant compound in the polyamine pattern was spermidine, and *sym*-homospermidine was not detectable. The quinone system was ubiquinone Q-10. The polar lipid profile included the major compounds phosphatidylcholine and diphosphatidylglycerol and moderate amounts of phosphatidylethanolamine, phosphatidylglycerol, an unidentified glycolipid and two unidentified aminolipids. Minor lipids were also detected. The major fatty acid profile, consisting of C_{19:0} cyclo ω 8c and C_{18:1} ω 7c, with C_{18:0} 3-OH as the major hydroxylated fatty acid, was similar to those of the genus *Chelatococcus*. The results of DNA–DNA hybridization experiments and physiological and biochemical tests allowed both genotypic and phenotypic differentiation of the isolates from described *Chelatococcus* species. Isolates M 2040^T, M 1973 and M 1878-SK2 were closely related on the basis of DNA–DNA reassociation and therefore represent a single novel species. In summary, low 16S rRNA gene sequence similarities of 95 % with *Chelatococcus asaccharovorans* and marked differences in polar lipid profiles as well as in polyamine patterns support the description of a novel genus and species to accommodate these strains, for which the name *Camelimonas lactis* gen. nov., sp. nov. is proposed. The type strain of *Camelimonas lactis* is M 2040^T (=CCUG 58638^T =CCM 7696^T).

During routine bacteriological testing for *Brucella* species, three isolates (M 2040^T, M 1973 and M 1878-SK2) were cultivated from milk of different camels of a camel-milk production farm in the United Arab Emirates. The strains grew well on *Brucella* agar (Difco) but were biochemically markedly distinct from *Brucella* species. Further analysis by

16S rRNA gene sequencing revealed *Chelatococcus* species as the closest relatives, but with only 95 % sequence similarity. The alphaproteobacterial genus *Chelatococcus* was described by Auling *et al.* (1993) and, at present, the genus comprises the two species *Chelatococcus asaccharovorans* (Auling *et al.*, 1993) and *Chelatococcus daeguensis* (Yoon *et al.*, 2008). Based on these results, strains M 2040^T, M 1973 and M 1878-SK2 were subjected to detailed analyses in order to determine their exact taxonomic position.

Strains M 2040^T, M 1973 and M 1878-SK2 were initially cultivated on *Brucella* agar and showed beige-coloured colonies on nutrient agar (Oxoid) at 37 °C. Subcultivation for further analyses was done on tryptone soy agar (TSA;

Abbreviations: pNA, *p*-nitroanilide; pNP, *p*-nitrophenyl.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains M 2040^T, M 1878-SK2 and M 1973 are respectively FN430422, FN430423 and FN430424.

Results of genomic fingerprinting of the camel isolates after ERIC-PCR are available as supplementary material with the online version of this paper.

Fig. 1. Phylogenetic analysis based on 16S rRNA gene sequences available from the EMBL database (accession numbers in parentheses) constructed after multiple alignment of the data by CLUSTAL_X (Thompson *et al.*, 1997). Distances were calculated (distance options according to the Kimura-2 model) and clustering with the neighbour-joining method was performed by using the software package MEGA version 2.1 (Kumar *et al.*, 2001). Bootstrap values based on 1000 replications are listed as percentages at branching points. Bar, 0.02 substitutions per nucleotide position.

Oxoid) at 28 °C for 48 h. Gram staining was performed as described by Gerhardt *et al.* (1994). Cell morphology was observed under a Zeiss light microscope at $\times 1000$, with cells grown for 3 days at 28 °C on TSA. The 16S rRNA gene was analysed as described by Kämpfer *et al.* (2003a). Phylogenetic analysis was performed using the ARB software package (Ludwig *et al.*, 2004) and also the software package MEGA version 2.1 (Kumar *et al.*, 2001) after multiple alignment of the data by CLUSTAL_X (Thompson *et al.*, 1997). Distances were calculated (distance options according to the Kimura-2 model) and clustering with the neighbour-joining and maximum-likelihood methods was performed by using bootstrap values based on 1000 replications. The almost-complete 16S rRNA gene sequences of all strains were compared by sequence similarity calculations after a neighbour-joining analysis. The results of these calculations showed that the sequences of isolates M 1973 and M 2040^T were identical and were 99.8 % similar to that of isolate M 1878-SK2. The closest relatives of strains M 2040^T, M 1973 and M 1878-SK2 were the type strains of *Chelatococcus asaccharovorans* (94.9–95.0 %) and *Chelatococcus daeguensis* (95.0–95.2 %).

A neighbour-joining tree is shown in Fig. 1. The branching pattern was confirmed by maximum-parsimony and maximum-likelihood analyses (not shown). In these trees, strains M 2040^T, M 1973 and M 1878-SK2 clustered together in proximity to the two *Chelatococcus* species but clearly formed a separate branch representing a potential novel genus.

After genomic fingerprinting employing ERIC-PCR, which was carried out as described by Wieser & Busse (2000), identical banding patterns were observed for isolates M 1973 and M 2040^T in the size range 150–1700 bp. Isolate M 1878-SK2 shared the most prominent bands of approximately 780 and 370 bp but exhibited some additional characteristic bands, clearly differentiating it from the other two isolates. These results indicate that isolates M 1973 and M 2040^T might be clonally related, whereas isolate M 1878-SK2 is genetically more distantly related (see Supplementary Fig. S1, available in IJSEM Online).

For quinone, polar lipid, polyamine and cell wall diamino acid analyses, cells were grown on PYE medium (0.3 %

peptone from casein, 0.3 % yeast extract, pH 7.2) 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 profiles of isolates M 2040^T (Fig. 2), M 1973 and M 1878-SK2 (results not shown) consisted of the major components phosphatidylcholine and diphosphatidylglycerol and moderate amounts of phosphatidylethanolamine, phosphatidylglycerol and two aminolipids (AL1, AL2). These two aminolipids showed chromatographic behaviour similar to that of two aminolipids (AL1, AL2) detected in representatives of the genus *Ochrobactrum* (Kämpfer *et al.*, 2003b, 2007,

Fig. 2. Polar lipid profile of strain M 2040^T after two-dimensional TLC and detection with molybdatophosphoric acid. DPG, Diphosphatidylglycerol; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; AL1–3, unidentified aminolipids; GL, unknown glycolipid; L1–3, unidentified polar lipids.

2008; Huber *et al.*, 2010). Furthermore, moderate amounts of a glycolipid and three unidentified polar lipids (L1–L3) and trace amounts of a third aminolipid were detected. The polar lipid profile of *Chelatococcus asaccharovorans* DSM 6462^T, analysed in parallel in this study, exhibited the major components phosphatidylcholine, phosphatidylmonomethylethanolamine, diphosphatidylglycerol and unidentified aminolipid AL2, moderate amounts of phosphatidylglycerol and minor amounts of unidentified aminolipid AL1 and four unidentified polar lipids (L1–L4), of which two showed highly hydrophobic chromatographic behaviour. In addition to some quantitative differences, the amounts of certain lipids and absence/presence of some minor lipids, the presence of

moderate amounts of phosphatidylethanolamine and the absence of phosphatidylmonomethylethanolamine clearly distinguish the three isolates from *Chelatococcus asaccharovorans* DSM 6462^T. The quinone system of the three isolates was composed predominantly of ubiquinone Q-10 (Table 1). In the polyamine pattern of the three isolates, spermidine was the major compound (Table 1). This polyamine pattern is clearly distinct from that of *Chelatococcus asaccharovorans* DSM 6462^T, which was reported to contain the major compound *sym*-homospermidine and moderate amounts of spermidine and putrescine (Auling *et al.*, 1993). The diamino acid was analysed as described by Schleifer (1985) and *meso*-diaminopimelic acid was detected.

Table 1. Profiles of cellular fatty acids, quinones, polyamines and characteristic polar lipids of the novel strains and the type strains of *Chelatococcus* species

Strains: 1, M 2040^T; 2, M 1973; 3, M 1878-SK2; 4, *Chelatococcus asaccharovorans* DSM 6462^T; 5, *Chelatococcus daeguensis* CCUG 54519^T. Data in parentheses were taken from Yoon *et al.* (2008); other data are from this study unless indicated. +, Present; –, not detected; NR, not reported.

Compound	1	2	3	4	5
Fatty acids (%)					
Unknown 14.959	0.8	0.8	–	0.9 (0.8)	– (0.8)
Summed feature 2*	4.6	0.3	3.5	3.1 (4.0)	2.2 (3.4)
Summed feature 3*	0.6	–	–	– (1.1)	0.8 (0.7)
C _{15:0}	–	–	–	– (–)	1.9 (–)
C _{16:0}	7.9	8.2	8.8	9.7 (8.5)	1.2 (2.2)
C _{17:0}	–	–	–	– (0.4)	0.6 (0.9)
C _{17:0} cyclo	–	–	0.7	2.6 (3.2)	– (0.3)
C _{17:1} ω8c	0.8	0.9	0.7	– (–)	(–)
C _{18:1} ω7c	11.5	12.6	8.2	21.0 (47.4)	51.3 (64.4)
C _{18:0}	1.3	1.3	1.5	2.1 (1.7)	– (1.7)
iso-C _{18:1} H	–	–	–	1.2 (–)	– (–)
C _{19:0} cyclo ω8c	67.3	65.8	72.0	53.2 (29.7)	20.4 (16.7)
11-Methyl C _{18:1} ω7c	1.4	1.4	–	1.5 (–)	– (–)
10-Methyl C _{19:0}	1.0	0.9	–	–	– (–)
C _{18:1} 2-OH	–	–	–	– (0.9)	3.0 (3.7)
C _{18:0} 3-OH	1.7	1.6	1.4	– (0.8)	2.5 (3.4)
C _{20:2} ω6,9c	1.0	1.4	1.2	1.8 (–)	5.9 (–)
C _{20:1} ω7c	–	–	–	2.7 (1.4)	0.6 (0.4)
Quinones (%)					
Q-8	2	2	8	NR	(15)
Q-9	4	3	3	NR	NR
Q-10	94	95	89	+†	(83)
Q-11	<0.5	<0.5	<0.5	NR	NR
Polyamines [μmol (g dry weight)^{–1}]					
Putrescine	0.1	4.9	10.2	7.3†	NR
Spermidine	66.8	61.1	53.0	13.3†	NR
<i>sym</i> -Homospermidine	–	–	–	29.9†	NR
Spermine	1.5	0.4	0.4	3.5†	NR
Polar lipids					
Phosphatidylethanolamine	+	+	+	–	NR
Phosphatidylmonomethylethanolamine	–	–	–	+	NR

*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.

†Data taken from Auling *et al.* (1993).

The fatty acid profiles of the novel strains and the type strains of *Chelatococcus* species are shown in Table 1. No pronounced differences in the fatty acid profiles were found; however, slight differences could be observed. The major fatty acids were C_{19:0} cyclo ω 8c, C_{18:1} ω 7c and C_{16:0}, with C_{18:0} 3-OH as the major hydroxylated fatty acid. The profiles were similar to those reported for *Chelatococcus* species.

Results of physiological characterization carried out using methods described previously (Kämpfer *et al.*, 1991) are given in the species description and in Table 2 (differential characters). The presence of urease was determined using urea agar base (Merck; catalogue no. 1.08492) as recommended by the manufacturer according to Christensen (1946).

DNA–DNA hybridization experiments were performed with all three novel strains and reference strains *Chelatococcus asaccharovorans* DSM 6462^T and *Chelatococcus daeguensis* CCUG 54519^T using the method described by Ziemke *et al.* (1998).

These studies resulted in DNA–DNA relatedness values ranging from 89 to 100 % (reciprocal analyses) among the three strains. The hybridization results for strains M-2040^T, M 1973 and M 1878-SK2 with *Chelatococcus asaccharovorans* DSM 6462^T were 49.2 % (37.1 %), 24.2 % (22.3 %) and 29.6 % (25.7 %), respectively (results of reciprocal analysis in parentheses), and hybridization results for M-2040^T, M 1973 and M 1878-SK2 with *Chelatococcus daeguensis* CCUG 54519^T were 49.6 % (42.2 %), 29.2 % (18.5 %) and 28.5 % (21.7 %), respectively.

On the basis of these results, we propose a novel genus and species to accommodate the three strains from camel's milk, with the name *Camelimonas lactis* gen. nov., sp. nov.

Description of *Camelimonas* gen. nov.

Camelimonas (Ca.me.li.mo'nas. L. n. *camelus* a camel with either one or two humps; L. fem. n. *monas* a unit, in bacteriology, a monad; N.L. fem. n. *Camelimonas* a monad isolated from camels).

Cells are Gram-negative, non-motile, non-spore-forming rods, approx. 2 μ m long and 1 μ m wide. Oxidase-positive, showing an oxidative metabolism. Good growth occurs on R2A agar, TSA, PYE agar, nutrient agar and MacConkey agar at 25–30 °C. Beige, translucent and shiny colonies with entire edges are formed within 24 h, with a diameter of approx. 2 mm. The characteristic peptidoglycan diamino acid is *meso*-diaminopimelic acid. The predominant compound in the polyamine pattern is spermidine, and *sym*-homospermidine is absent. The quinone system is ubiquinone Q-10. The polar lipid profile includes the major compounds phosphatidylcholine and diphosphatidylglycerol and moderate amounts of phosphatidylethanolamine, phosphatidylglycerol, an unidentified glycolipid and two unidentified aminolipids. The major fatty acids are C_{19:0} cyclo ω 8c, C_{18:1} ω 7c and C_{16:0}, with C_{18:0} 3-OH as

Table 2. Physiological characteristics of the novel strains and the type strains of *Chelatococcus* species

Strains: 1, M 2040^T (strains M 1973 and M 1878-SK2 gave identical results); 2, *Chelatococcus asaccharovorans* DSM 6462^T; 3, *Chelatococcus daeguensis* CCUG 54519^T. Data were obtained in this study. All strains were positive for utilization of D-glucose*, acetate, propionate, adipate*, 4-aminobutyrate, glutarate, DL-3-hydroxybutyrate, DL-lactate, oxoglutarate, L-alanine and L-aspartate. All strains were negative for utilization of N-acetyl-D-galactosamine, N-acetyl-D-glucosamine, D-mannose†, maltose, melibiose, L-rhamnose†, salicin, maltitol, D-mannitol, D-sorbitol, putrescine, β -alanine, D-histidine, L-leucine, L-ornithine, L-phenylalanine, L-tryptophan, 3-hydroxybenzoate and 4-hydroxybenzoate. All strains hydrolysed aesculin and L-alanine pNA. None of the strains hydrolysed o-nitrophenyl β -D-galactopyranoside, pNP β -D-glucuronide, pNP α -D-glucopyranoside*, pNP β -D-glucopyranoside*, pNP β -D-xyloside*, 2-deoxythymidine-5'-pNP phosphate, pNP phosphorylcholine or L-glutamate- γ -3-carboxy pNA. +, Positive; (+), weakly positive; –, negative; v, strains may be positive or negative.

Test	1	2	3
Hydrolysis of:			
bis-pNP phosphate	+	–	+
pNP phenylphosphonate	+	–	+
L-Proline pNA	v	–	–
Utilization of:			
L-Arabinose	–	(+)*	+
p-Arbutin	–	–*	(+)*
Cellobiose	–	–*	+
D-Fructose	–	–*	+
D-Galactose	–	–*	+
Gluconate	–	(+)	–
D-Ribose	–	+	+
Sucrose	–	–	+
Trehalose	–	–	+
D-Xylose	–	+	–
Adonitol	–	–*	+
myo-Inositol	–	–*	(+)*
cis-Aconitate	–	–	+
trans-Aconitate	–	–	+
Azelate	+	–	–
Citrate	+	–*	+
Fumarate	+	–	+
Itaconate	+	–	–
L-Malate	–	(+)*	(+)*
Mesaconate	+	–	+
Oxoglutarate	+	+	–
Pyruvate	–	+	+
Suberate	+	+	–
L-Proline	–	(+)	+
L-Serine	–	–	(+)
Phenylacetate	–	(+)*	–*

*Data congruent with those reported by Yoon *et al.* (2008).

†Data not congruent with those reported by Yoon *et al.* (2008).

the major hydroxylated fatty acid. The G + C content of the DNA of the type strain of the type species is 65 mol%. The type species is *Camelimonas lactis*.

Description of *Camelimonas lactis* sp. nov.

Camelimonas lactis (lac'tis. L. gen. n. *lactis* from milk, referring to milk as the isolation source of the first reported strains).

Cell and colony morphology and growth characteristics are as given in the genus description. Growth is detected on TSA at 15–37 °C. No growth is observed at 10 or 45 °C. Growth is observed in TS broth at pH 5.5–10.5. Positive for urease. In addition to Q-10, minor amounts of Q-8 and Q-9 and traces of Q-11 are detected. In addition to the polar lipids listed in the genus description, minor amounts of three unidentified polar lipids and a third aminolipid are detectable. Putrescine is detectable in minor to moderate amounts. Utilizes the following carbon sources: D-glucose, acetate, propionate, adipate, 4-aminobutyrate, azelate, fumarate, glutarate, DL-3-hydroxybutyrate, itaconate, DL-lactate, mesaconate, oxoglutarate, L-alanine and L-aspartate. Does not utilize N-acetyl-D-galactosamine, N-acetyl-D-glucosamine, L-arabinose, D-fructose, D-galactose, gluconate, D-mannose, maltose, melibiose, L-rhamnose, D-ribose, salicin, sucrose, trehalose, D-xylose, adonitol, inositol, maltitol, D-mannitol, D-sorbitol, *cis*- or *trans*-aconitate, putrescine, β -alanine, D-histidine, L-leucine, L-ornithine, L-phenylalanine, L-tryptophan, 3-hydroxybenzoate or 4-hydroxybenzoate. Hydrolyses aesculin and L-alanine *p*-nitroanilide (pNA) and does not hydrolyse *o*-nitrophenyl β -D-galactopyranoside, *p*-nitrophenyl (pNP) β -D-glucuronide, pNP α -D-glucopyranoside, pNP β -D-glucopyranoside, pNP β -D-xyloside, 2-deoxythymidine-5'-pNP phosphate or L-glutamate- γ -3-carboxy pNA. Further details of carbon source utilization and hydrolysis of chromogenic substrates (including differentiating characters from *Chelatococcus* species) are indicated in Table 2.

The type strain, M 2040^T (=CCUG 58638^T =CCM 7696^T), was isolated from milk of a camel. Other strains of this species are M 1973 and M 1878-SK2.

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