Staphylococcus microti sp. nov., isolated from the common vole (*Microtus arvalis*)

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Two strains of Gram-positive cocci were isolated from viscera of common voles (*Microtus arvalis* Pallas) with generalized *Brucella microti* infection in the Czech Republic. Biochemical features and phylogenetic analysis based on 16S rRNA gene sequences showed that the strains are representatives of the genus *Staphylococcus* and assigned *Staphylococcus muscae* as the nearest relative. A detailed characterization done by ribotyping, *rpoB* and *hsp60* gene sequencing, whole-cell protein analysis and rep-PCR using the (GTG)₅ primer differentiated the two strains from all described staphylococci. DNA–DNA hybridization with the type strain of *S. muscae* demonstrated that the two strains should be considered as members of a novel species (26.8% reassociation). The two analysed strains were found to be coagulase-negative, novobiocin-susceptible, oxidase-negative cultures, phenotypically close to one another, but showing differences in ribotype profiles. The major fatty acids were iso-C_{15:0}, iso-C_{17:0}, anteiso-C_{15:0}, C_{18:2ω6,9c}/anteiso-C_{18:0}, C_{18:0} and C_{18:1ω9c}. MK-7 was the predominant isoprenoid quinone, with minor amounts of MK-6 and MK-8. The polar lipid profile was composed of the major lipids diphosphatidylglycerol and phosphatidylglycerol and several unknown lipids. These results proved that the two isolates represent a novel staphylococcal species. The name proposed for this novel taxon is *Staphylococcus microti* sp. nov.; the type strain is 4005-LJ(m)^T (=CCM 4903^T =CCUG 55861^T =DSM 22147^T).

At the time of writing, the genus *Staphylococcus* (family *Staphylococcaceae*) includes 50 species and subspecies that are common inhabitants of different mammals, humans, birds, cold-blooded animals and the environment.

Abbreviation: DDH, DNA–DNA hybridization.

The GenBank/EMBL/DDBJ accession numbers for 16S rRNA gene, *rpoB* and *hsp60* gene sequences of strain CCM 4903^T are EU888120–EU888122. The GenBank/EMBL/DDBJ accession numbers for other sequences determined in the study are EU888123–EU888134.

Cluster analysis based on (GTG)₅ PCR and whole-cell protein SDS-PAGE profiles and results of 2D TLC of polar lipids of strain CCM 4904 are available as supplementary material with the online version of this paper.

Staphylococci are isolated mainly from skin, glands, mucous membranes and, occasionally, from animal or human blood or viscera (Kloos *et al.*, 1992). A study of staphylococcal distribution in different mammals (other than humans) and birds showed the predominant species to be the novobiocin-resistant, coagulase-negative species *Staphylococcus xylosus* and *Staphylococcus sciuri* (Nagase *et al.*, 2002). These species were also predominant in tested laboratory mice. *Staphylococcus intermedius* is found frequently in a wide range of animal species, e.g. dogs, cats, mink and horses, and *S. intermedius* and *Staphylococcus schleiferi* subsp. *coagulans* are isolated as common pathogens in sick dogs. Natural populations of staphylococci in laboratory animals have been studied in

the past (Kloos *et al.*, 1992), but we still know little about their natural distribution in free-living animals, which could represent a reservoir of potentially pathogenic bacteria (Hauschild, 2001). A significant number of strains of *Staphylococcus saprophyticus* and *Staphylococcus equorum* were observed by Hauschild (2001) in wild insectivores and rodents, in addition to *S. xylosus* and *S. sciuri*. On the other hand, only a few *Staphylococcus simulans* and *Staphylococcus hominis* strains were isolated as the only novobiocin-susceptible, coagulase-negative staphylococci found in these animals. A preliminary study by Kloos *et al.* (1976) described a very small number of *Staphylococcus aureus* and *S. intermedius* strains isolated from grey squirrels. Different potentially pathogenic bacteria besides staphylococci have also been shown to inhabit rodents. Recently, the novel species *Brucella microti* has been isolated (Hubálek *et al.*, 2007) and described (Scholz *et al.*, 2008) from clinical samples of diseased common voles (*Microtus arvalis*). This paper deals with a description and characterization of a novel staphylococcal species isolated from two of these *M. arvalis* individuals.

The strains originated from the liver and kidney of diseased, free-living common voles (one adult male, one adult female) during an epizootic caused by *B. microti* (Hubálek *et al.*, 2007) in the Břeclav district of South Moravia, Czech Republic, in 1999–2002. The animals, live-trapped and euthanized on 21 September 2000, were apparently ill, with oedematous legs, visceral lesions including granulomas, abscesses, orchitis (in the male) and enlarged lymphatic inguinal nodes. Samples were cultivated on meat-peptone agar at 37 °C for 72 h under aerobic conditions. In addition to strains of *B. microti*, strains 4005-LJ(m)^T (=CCM 4903^T) and 4334-LJ(m) (=CCM 4904) were isolated from the male and the female, respectively. The following type cultures of phylogenetically related staphylococcal taxa were obtained from the Czech Collection of Microorganisms (CCM; <http://www.sci.muni.cz/ccm/>) and used for comparative analyses: *Staphylococcus muscae* CCM 4175^T (=MB4^T), *Staphylococcus chromogenes* CCM 3387^T, *Staphylococcus hyicus* CCM 2368^T, *Staphylococcus lutrae* CCM 4681^T, *Staphylococcus delphini* CCM 4115^T, *S. intermedius* CCM 5739^T, *Staphylococcus pseudintermedius* CCM 7315^T, *Staphylococcus felis* CCM 4196^T, *Staphylococcus schleiferi* subsp. *schleiferi* CCM 4070^T and *S. schleiferi* subsp. *coagulans* CCM 4313^T.

Colony size, morphology and pigment production were determined on P agar after 5 days (72 h at 37 °C and 48 h at room temperature) as recommended by Freney *et al.* (1999). Haemolysis was examined on Columbia blood agar (Oxoid) supplemented with 7% sheep blood at 37 °C (pH 7.2) after 24 h in a synergy test with a producer of β -haemolysin (*S. intermedius* CCM 4710). Phenotyping was done using API Staph, ID32 Staph and API ZYM kits (bioMérieux) according to the manufacturer's instructions and using additional conventional tube and plate tests as described previously (Freney *et al.*, 1999; Mannerová *et al.*,

2003). Susceptibility to antibiotics as well as to lysostaphin and lysozyme was tested by the disc diffusion method on Mueller–Hinton agar (Oxoid) (Freney *et al.*, 1999; Woods & Washington, 1995). The results of phenotyping were evaluated using *apiweb* software (<https://apiweb.biomerieux.com>) and phenotype characteristics published in individual species descriptions.

Both cultures were Gram-stain-positive, catalase-positive and oxidase- and coagulase-negative. Circular, slightly concave, smooth, pearly, whitish small colonies, 0.8–1.8 mm in diameter, were observed on P agar. During initial isolation, the colonies were numerous, very small and only visible after 3 days on meat-peptone agar plates. Biotyping data in comparison with 10 related species and subspecies are summarized in Table 1. The results of all other examined phenotypic features are listed in the species description.

Both strains were clearly members of the genus *Staphylococcus* on the basis of their biochemical traits. However, they showed a unique phenotypic profile that differed from those of all closely related staphylococcal species, particularly in aesculin hydrolysis. In addition, they could be clearly differentiated from related coagulase-positive staphylococci (*S. delphini*, *S. intermedius*, *S. pseudintermedius*, *S. schleiferi* subsp. *coagulans*, *S. hyicus* and *S. lutrae*) on the basis of the absence of coagulase production and from related coagulase-negative taxa on the basis of tests summarized in Table 1. *S. muscae*, the species that was most closely related phylogenetically as well as phenotypically, can be differentiated from the novel strains by negative reactions for acid production from lactose and mannose, pyrrolidonyl arylamidase formation and aesculin hydrolysis. Further, in contrast to strains CCM 4903^T and CCM 4904, *S. muscae* strains usually do not grow at 45 °C.

Isolation of genomic DNA and ribotyping with either *EcoRI* or *HindIII* restriction endonucleases (Takara) as well as hybridization with a digoxigenin-labelled probe complementary to the 16S and 23S rRNA were done as described by Švec *et al.* (2001). The obtained ribotype profiles were digitized and analysed using BioNumerics software version 4.601 (Applied Maths). Dendrograms were constructed using the Dice similarity coefficient and the UPGMA clustering method. Position tolerance of 1% was allowed for the bands.

Strains CCM 4903^T and CCM 4904 generated unique *EcoRI* fingerprints, containing 17 and 19 fragments, respectively, and were well separated from all staphylococcal taxa with validly published names (data not shown; in-house ribotype database). The similarity between isolates CCM 4903^T and CCM 4904 was 88.9%. All phylogenetically related staphylococci showed similarity levels lower than the cut-off of 62% that was used for species differentiation among the genus *Staphylococcus* by Chesneau *et al.* (2000). *S. lutrae* CCM 4681^T and *S. muscae* CCM 4175^T showed the highest similarity to the novel

Table 1. Biochemical features of strains CCM 4903^T and CCM 4904 (*Staphylococcus microti* sp. nov.) and phylogenetically related species

Strains: 1, *S. microti* sp. nov. CCM 4903^T and CCM 4904; 2, *S. muscae* CCM 4175^T; 3, *S. intermedius* CCM 5739^T; 4, *S. pseudintermedius* CCM 7315^T; 5, *S. hyicus* CCM 2368^T; 6, *S. schleiferi* subsp. *coagulans* CCM 4313^T; 7, *S. schleiferi* subsp. *schleiferi* CCM 4070^T; 8, *S. delphini* CCM 4115^T; 9, *S. lutrae* CCM 4681^T; 10, *S. felis* CCM 4196^T; 11, *S. chromogenes* CCM 3387^T. Results in columns 3–11 may relate to multiple strains and were obtained from Devriese *et al.* (1978, 2005), Foster *et al.* (1997), Freney *et al.* (1988), Hájek (1976), Hájek *et al.* (1986, 1992), Igimi *et al.* (1989, 1990) and Varaldo *et al.* (1988) unless indicated. +, Positive; –, negative; w, weakly positive; d, positive reaction in 11–89% of strains.

Characteristic	1	2	3	4	5	6	7	8	9	10	11
Production of:											
Plasma coagulase	–	–	+	+	d	+	–	+	+	–	–
Clumping factor	–	–	d	–	–	–	+	–	–	–	–
Oxidase	–	–	w	–	–	–	–	–	–	–	–
Arginine dihydrolase	–	–	d	+	+	+	+	+	–	+	+
Acetoin	w	–	–	+	–	+	+	–	–	–	–
Pyrrolidonyl arylamidase	+	–	+	+	–	d	+	+	–*	+	–*
Urease	–	–	+	+	+	+	–	+	+	+	+
Hydrolysis of:											
Aesculin	+	–	–	–*	–	–	–	–	–*	–*	–
Tween 80	+	+	+	+	+	–*	+	–*	–*	–*	–
Casein	–	–	d	–*	+	w*	–*	+	+	+	+
Gelatin	–	–	+	+	+	+	+	–	–*	–*	+
Acid production from:											
Lactose	+	–	+	+	+	d	–	+	+	+	+
D-Mannose	w	–	+	+	+	+	+	+	+	+	+
D-Mannitol	–	–	+	w	–	d	–	w	d	d	w
Maltose	–	–	–	+	–	–	–	+	+	–	w
Trehalose	+	+	+	+	+	–	d	–	+	+	+
Growth at 45 °C	+	–	+	+	w	+	+	+	+	w	w

*Not given in the original species description; results obtained in this study using the strains listed.

isolates (54.6 and 48.2% respectively; Fig. 1). *Hind*III ribotype similarity of the novel isolates was 79% (Fig. 2); thus, differentiation of the two tested strains was more evident than with *Eco*RI ribotype analysis. The ribotyping results showed isolates CCM 4903^T and CCM 4904 to be different strains but members of the same staphylococcal species.

Almost-complete 16S rRNA gene sequences (positions 55–1495 of *Bacillus subtilis*) of isolates CCM 4903^T and CCM 4904 were obtained by PCR and sequencing as described previously (Pantůček *et al.*, 2005). Partial *rpoB* gene sequences (positions 1444–1928 of the *rpoB* gene sequence of *S. aureus* RN4220; GenBank accession no. X64172) were determined for both strains and also *S. pseudintermedius* CCM 7315^T and *Staphylococcus simiae* CCM 7213^T as described previously (Mellmann *et al.*, 2006). Anticipated 554 bp fragments for the *hsp60* genes of strains CCM 4903^T and CCM 4904 and *S. muscae* CCM 4175^T and the as-yet unsequenced strains *Staphylococcus carnosus* subsp. *utilis* CCM 4752, *S. condimentii* CCM 4753^T, *S. fleurettii* CCM 4922^T, *S. nepalensis* CCM 7045^T, *S. pettenkoferi* CCM 7495^T and *S. saprophyticus* subsp. *bovis* CCM 4410^T were amplified with degenerate primers designed by Goh *et al.*

(1996). The obtained amplicons were purified with a QIAquick PCR purification kit (Qiagen), digested with *Eco*RI and *Bam*HI (Roche Diagnostics) and cloned in pBluescript II SK(+) vector (Stratagene). Both strands of the DNA were sequenced with sequencing primers M13 uni(–21) and M13 rev(–29) at MWG Biotech. The sequences were analysed using BioNumerics software version 5.10 (Applied Maths). Pairwise alignment similarities were calculated and phylogenetic trees were constructed using the neighbour-joining method. No correction factor was used for the distance matrices. Bootstrap analysis based on 1000 randomly generated trees was performed to investigate the stability of the trees obtained (Fig. 3). To infer phylogenetic analysis, the maximum-likelihood method was also applied using the BioNumerics software.

Phylogenetic analysis based on 16S rRNA, *rpoB* and *hsp60* gene sequences included the type strains of all *Staphylococcus* species with validly published names and showed that strains CCM 4903^T and CCM 4904 exhibited identical sequences for the three genes and are representatives of the genus *Staphylococcus*. For the 16S rRNA, *rpoB* and *hsp60* gene sequences, the novel strains shared the

Fig. 1. *EcoRI* ribotype cluster analysis of strains CCM 4903^T and CCM 4904 and the type strains of the most closely related staphylococcal species.

highest similarity values with the corresponding sequences of the *S. muscae* type strain (99.3, 92.5 and 88.1%, respectively). The next most closely related taxa based on sequence analyses were *S. intermedius*, *S. pseudintermedius*, *S. delphini*, *S. lutrae*, *S. schleiferi* subsp. *schleiferi*, *S. chromogenes*, *S. felis*, *S. hyicus* and *S. schleiferi* subsp. *coagulans*, showing similarity values to the respective type strains of 98.4–98.1% for the 16S rRNA gene, 91.5–85.2% for *rpoB* and 86.8–81.0% for *hsp60*. Based on the 16S rRNA gene sequence, the tested strains were placed in the *S. hyicus*–*S. intermedius* cluster group proposed by Takahashi *et al.* (1999) and constituted, together with *S. muscae*, a distinct '*S. muscae*' species group within this monophyletic clade (94% bootstrap probability). The neighbour-joining tree calculated from 16S rRNA gene sequences had a topology identical to that obtained using the maximum-likelihood analysis (not shown). A comparison of the neighbour-joining trees (Fig. 3) and maximum-likelihood trees generated from the *hsp60* and *rpoB* gene sequences showed agreement in the *S. hyicus*–*S. intermedius*–*S. muscae* complex except for *S. chromogenes*, which was clustered with *S. intermedius* on the basis of the *hsp60* gene sequence and maximum-likelihood method (not shown).

rep-PCR with the (GTG)₅ primer was done according to Gevers *et al.* (2001) with a minor modification described previously by Švec *et al.* (2008). rep-PCR fingerprints were analysed using Pearson's correlation coefficient and UPGMA clustering method (BioNumerics; Applied Maths). Optimization of 1% was allowed for the densitometric curves. The tested strains showed very similar (GTG)₅ fingerprints (98.2% similarity) and were clearly separated from all related species used for cluster analysis (Supplementary Fig. S1, available in IJSEM Online) as well as from other staphylococcal taxa (in-house rep-PCR database). The type strains of *S. muscae* and *S. lutrae* showed the highest similarity to strains CCM 4903^T and CCM 4904 (34.5 and 30.7%, respectively), as for ribotyping.

Preparation of whole-cell protein extracts, SDS-PAGE, densitometric analysis of protein profiles and their numerical analysis were performed as reported by Pot *et al.* (1994). The bacteria were grown under standard conditions (37 °C for 24 h) on CM3 nutrient agar (Oxoid). A whole-cell protein extract of *Psychrobacter immobilis* CCM 4923 was used as a reference profile and Sigma Wide Range markers (6.5–205 kDa) were used as molecular mass

Fig. 2. Comparison of *HindIII* ribotypes of strains CCM 4903^T and CCM 4904.

Fig. 3. Neighbour-joining trees based on 16S rRNA (a), *rpoB* (b) and *hsp60* (c) gene sequence comparisons, showing the phylogenetic relationships of strains CCM 4903^T and CCM 4904 (*Staphylococcus microti* sp. nov.) and selected staphylococcal reference strains. Bootstrap probability values (percentages of 1000 tree replications) are indicated at branch-points. Bars, 0.5 (a) and 2.0 (b, c) substitutions per 100 nucleotide positions.

markers. Densitometric analysis of digitized protein fingerprints was done with BioNumerics software version 4.601 using Pearson's correlation coefficient, with 1% position tolerance being allowed. Construction of the dendrogram was performed with the UPGMA clustering method. The two strains generated unique protein patterns, and the similarity between the isolates was

95.6%. Strains of related *Staphylococcus* species and other species and subspecies with validly published names showed unambiguously separated protein profiles. *S. felis* CCM 4196^T and *S. hyicus* CCM 2368^T showed protein patterns similar to those of CCM 4903^T and CCM 4904 (55.6% similarity); other reference type cultures exhibited protein pattern similarity below 52% (Supplementary Fig. S2).

For analysis of fatty acids, bacteria were cultivated on Columbia II agar base (BBL) with 5% horse blood at 37 °C for 24 h. Cell saponification, conversion of fatty acids to methyl esters and their extraction and GC analysis (Hewlett Packard HP 5890) as well as numerical analysis were done as described previously by Eerola & Lehtonen (1988) and Sasser (2001). The fatty acids observed in cells of strains CCM 4903^T and CCM 4904 differed from those of described staphylococcal taxa both qualitatively and quantitatively. As major fatty acids, iso-C_{15:0}, iso-C_{17:0} and anteiso-C_{15:0} and unsaturated C_{18:2}ω_{6,9c}/anteiso-C_{18:0}, C_{18:0} and C_{18:1}ω_{9c} were found in both strains. Detailed results are summarized in Table 2.

Peptidoglycan type was examined using purified cell walls obtained after mechanical disruption of cells with glass beads and subsequent trypsin digestion. Amino acids and peptides of cell-wall hydrolysates were analysed by two-dimensional ascending TLC on cellulose plates using previously described solvent systems (Schleifer, 1985). Amino-terminal amino acids of the interpeptide bridge were detected by dinitrophenylation (Schleifer, 1985). Molar amino acid ratios were determined using GC and GC-MS of *N*-heptafluorobutyl amino acid isobutyl esters (MacKenzie, 1987). The analyses revealed the occurrence of the peptidoglycan type A3α L-Lys-Gly₂₋₃-L-Ser-Gly

Table 2. Fatty acid contents of strains CCM 4903^T and CCM 4904

Values are percentages of total fatty acids. —, Not detected.

Fatty acid	CCM 4903 ^T	CCM 4904
iso-C _{13:0}	0.5	0.5
C _{14:0}	0.6	—
iso-C _{15:0}	51.7	50.2
anteiso-C _{15:0}	12.6	12.5
iso-C _{16:0}	0.5	—
C _{16:0}	3.7	3.3
iso-C _{17:0}	13.7	14.0
anteiso-C _{17:0}	3.9	4.3
C _{18:2} ω _{6,9c} /anteiso-C _{18:0}	4.0	4.9
C _{18:1} ω _{9c}	1.5	2.5
C _{18:0}	4.4	4.2
iso-C _{19:0}	1.1	1.0
C _{20:0}	1.2	0.8
Unidentified peak ECL 14.924	0.6	1.1
Unidentified peak ECL 19.418	—	0.9

according to Schleifer & Kandler (1972) (type A11.3 according to http://www.dsmz.de/microorganisms/main.php?content_id=35) in the tested strains, which is typical of the genus *Staphylococcus*. Total hydrolysates (100 °C for 16 h, 4 M HCl) of the peptidoglycan contained amino acids Ala/Gly/Ser/Glu/Lys in the following approximate molar ratios: 1.5:3.0:0.8:1.0:0.9 for CCM 4903^T and 1.8:3.7:0.9:1.0:1.0 for CCM 4904.

Quinones and polar lipids were extracted as described previously (Altenburger *et al.*, 1996; Stolz *et al.*, 2007; Tindall, 1990a, b). Biomass used for extraction was grown at 28 °C on PYE medium (0.3 % peptone, 0.3 % yeast extract, pH 7.2). Strains CCM 4903^T and CCM 4904 showed a quinone system with menaquinone MK-7 (95 and 97 %, respectively) predominating and minor amounts of MK-6 (5 and 3 %) and MK-8 (both <1 %). *S. chromogenes* CCM 3387^T and *S. muscae* CCM 4175^T showed rather similar quinone systems (96 and 95 % MK-7, 1 and 5 % MK-6 and 3 % and <1 % MK-8). Similar quinone systems have been reported for several other staphylococci, including *Staphylococcus capitis*, *S. cohnii*, *S. epidermidis*, *S. haemolyticus*, *S. hominis*, *S. saprophyticus*, *S. simulans*, *S. warneri* and *S. xylosus* (Nahaie *et al.*, 1984). The polar lipid profiles of strains CCM 4903^T and CCM 4904 were identical, consisting of the major lipids diphosphatidylglycerol and phosphatidylglycerol. In addition, moderate amounts of an unknown aminophospholipid (APL1) and an unknown glycolipid (GL1) and minor to trace amounts of a second glycolipid (GL2), a second aminophospholipid (APL2), a phospholipid and an unknown polar lipid were found (Supplementary Fig. S3). A third α -naphthol-positive spot showing highly hydrophobic chromatographic behaviour was also detected, but is not visible in Supplementary Fig. S3. The chromatographic behaviour of GL1 corresponded well to that of glycolipid G₁, which was assumed to be β -gentiobiosyl diacylglycerol (Nahaie *et al.*, 1984). Polar lipid profiles of *S. chromogenes* CCM 3387^T and *S. muscae* CCM 4175^T showed the same major characteristics. However, in the profile of *S. muscae* CCM 4175^T, the content of the unknown phospholipid PL1 was significantly higher than in CCM 4903^T and CCM 4904 and, in *S. chromogenes* CCM 3387^T, PL1 and glycolipid GL2 could not be detected (not shown).

DNA–DNA hybridization (DDH) was carried out between strain CCM 4903^T and *S. muscae* CCM 4175^T, the closest relative on the basis of the results of sequence analyses of three genes. The strains were cultivated in BHI (Oxoid) for DNA isolation before DDH. Wet biomass (3 g) was resuspended in isopropanol/water (1:1, v/v). Isolation of DNA as well as the DDH procedure was performed at the DSMZ. DNA–DNA relatedness between strain CCM 4903^T and *S. muscae* CCM 4175^T was 26.8 %. This value indicates that CCM 4903^T does not belong to *S. muscae*.

Identical 16S rRNA gene, *rpoB* and *hsp60* sequences and identical or almost-identical banding patterns in (GTG)₅

PCR, ribotyping and SDS-PAGE of whole-cell proteins as well as their biochemical and physiological traits, polar lipid profiles and fatty acid profiles demonstrated that the two strains are members of the same species. Clearly different banding patterns obtained in ribotyping for the two strains suggest that this method would be useful for their discrimination. On the basis of the results obtained in this study, we conclude that the analysed isolates are not members of any *Staphylococcus* species with a validly published name and, in the case of the most closely related species, *S. muscae*, this was confirmed by the results from DDH. Hence, we propose the name *Staphylococcus microti* sp. nov. to accommodate the two staphylococcal isolates.

Description of *Staphylococcus microti* sp. nov.

Staphylococcus microti (mi.cro'ti. N.L. gen. n. *microti* of *Microtus*, the zoological name for a genus of vole, referring to the isolation of the first strains from individuals of *Microtus arvalis*).

Cells are Gram-stain-positive cocci, occurring singly or in irregular clusters. Colonies are smooth, circular, slightly concave, opalescent whitish with continuous margins, 0.8–1.8 mm in diameter. Catalase-positive and oxidase- and coagulase-negative. Clumping factor is not detected by the latex test. Growth occurs at 15, 37 and 45 °C and in the presence of 10 % NaCl, but not 15 % NaCl. Produces δ -haemolysin on blood agar with sheep blood in synergy with a producer of β -haemolysin. Produces alkaline and acid phosphatases, pyrrolidonyl arylamidase, β -galactosidase, arginine arylamidase and lecithinase. Produces acid from D-glucose, D-fructose, lactose, trehalose, D-xylose, sucrose and N-acetylglucosamine, reduces nitrates and hydrolyses aesculin, Tween 80 and DNA. Weak reactions are observed for esterase lipase (C 8) and leucine arylamidase in the API ZYM kit. Other weak reactions are found for acetoin production and acid production from D-mannose. Does not produce arginine dihydrolase, β -glucuronidase, β -glucosidase or ornithine decarboxylase or acid from maltose, D-mannitol, xylitol, melibiose, raffinose, methyl α -D-glucoside, D-ribose, cellobiose, turanose or L-arabinose. Urea, starch, casein and gelatin are not hydrolysed. Cultures show negative responses for esterase (C4), lipase (C14), valine arylamidase, cystine arylamidase, trypsin, chymotrypsin, naphthol-AS-BI-phosphohydrolase, α -galactosidase, α -glucosidase, N-acetyl- β -glucosaminidase, α -mannosidase and α -fucosidase. Resistant to bacitracin (0.04 U) and lysozyme (400 mg l⁻¹) but susceptible to novobiocin (5 μ g), furazolidone (100 μ g), amoxicillin/clavulanic acid (20/10 μ g), oxacillin (1 μ g), tetracycline (30 μ g), trimethoprim/sulfamethoxazole (23.75/1.25 μ g), erythromycin (15 μ g), clindamycin (2 μ g), vancomycin (30 μ g), ciprofloxacin (5 μ g), gentamicin (10 μ g), chloramphenicol (30 μ g), rifampicin (5 μ g), teicoplanin (30 μ g) and lysostaphin (50 mg l⁻¹). Dominant fatty acids are iso-C_{15:0}, iso-C_{17:0}, anteiso-C_{15:0}, C_{18:2} ω 6,9c/anteiso-C_{18:0}, C_{18:0} and C_{18:1} ω 9c. Peptidoglycan type is A3 α L-Lys-Gly₂₋₃-L-Ser-Gly and the quinone system includes the

major component menaquinone MK-7 and minor quantities of MK-6 and MK-8. The polar lipid profile consists of the major compounds diphosphatidylglycerol and phosphatidylglycerol. Additionally, moderate amounts of an unknown glycolipid and an unknown aminophospholipid and minor amounts of another glycolipid, an aminophospholipid, a phospholipid and a polar lipid are detectable.

The type strain is 4005-LJ(m)^T (=CCM 4903^T =CCUG 55861^T =DSM 22147^T), isolated from the liver of a free-living common vole in the Czech Republic. Its characteristics are in full agreement with those given in the species description.

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