Moraxella pluranimalium sp. nov., isolated from animal specimens


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Four unusual Gram-negative, catalase-positive, oxidase-positive, coccus-shaped bacteria isolated from one sheep and three pigs were characterized using phenotypic and molecular genetic methods. On the basis of cellular morphology and biochemical criteria, the isolates were tentatively assigned to the genus Moraxella, although the organisms did not appear to correspond to any recognized species. Comparative 16S rRNA gene sequencing studies demonstrated that the isolates represent a novel subline within the genus Moraxella. The most closely related species in phylogenetic terms was Moraxella cuniculi, with 16S rRNA gene sequence similarity of 97.9 % to the type strain CCUG 2154T, although the DNA–DNA relatedness value was only 29 %. The novel isolates were readily distinguished from all recognized Moraxella species by means of physiological and biochemical tests. On the basis of molecular genetic and phenotypic evidence, therefore, the four isolates represent a novel species of the genus Moraxella, for which the name Moraxella pluranimalium sp. nov. is proposed. The type strain is 248-01T (=CECT 7295T =CCUG 54913T).

The genus Moraxella, originally described by Lwoff (1939), contains both coccoid and rod-shaped bacteria that are genetically related. At the time of writing, the genus comprises 17 species (Euzéby, 1997; Juni & Bøvre, 2005). Over the past two decades, clinical microbiologists have become increasingly aware of the importance of members of this genus as emerging pathogens. In humans, Moraxella catharralis is of clinical relevance, being responsible for respiratory infections, endocarditis and meningitis, especially in immunocompromised patients (Verduin et al., 2002), although other Moraxella species have also been implicated (Berrocal et al., 2001). Although various Moraxella species have been isolated from animals, only Moraxella bovis has been traditionally considered of clinical relevance in veterinary medicine, being responsible for ocular and respiratory infections (Brown et al., 1998; Quinn et al., 1999; Lavin et al., 2000). In this study, we report the phenotypic and phylogenetic features of a novel bacterial species of the genus Moraxella.

The bacterial strains studied, designated 248-01T, M1364, 1387-02, CD12CA4, were isolated from the brain of a sheep with meningitis (M1364), the nasal turbinates of a healthy pig (248-01T) and the pleura (1387-02) and peritoneal cavity fluid (CD12CA4; Olvera et al., 2007) of two different pigs with lesions of pleuritis and polyserositis, respectively. Samples were collected and frozen at −40 °C until processing in the laboratory. Strains were isolated on Columbia blood agar plates (bioMérieux) after incubation at 37 °C for 24–48 h under aerobic conditions.

Gram stain, oxidase and catalase tests were performed as described by Barrow & Feltham (1993). DNA hydrolysis was tested with DNase test agar. Growth in brain heart infusion broth was assessed at 4 and 15 °C for up to 14 days, at 22 °C for up to 7 days and at 30, 37 and 42 °C for 48 h. Growth in the presence of 0.5, 1.5, 3.0, 5.0 and 6.5 % NaCl and under anaerobic (with 4–10 % CO2) and microaerobic (with 5–15 % O2 and 5–12 % CO2)
conditions, using the GasPak Plus and CampyPak Plus systems (BBL), respectively, was assessed at 37 °C for 48 h. Growth on MacConkey agar (bioMérieux) and chocolate agar (bioMérieux) was tested at 37 °C for 24 and 48 h. Additional biochemical tests were performed by using the API 20NE, API 20E and API ZYM systems (bioMérieux) according to the manufacturer’s instructions (except that the API 20NE incubation was performed at 37 °C for 72 h). The four isolates exhibited similar phenotypic characteristics, except with regard to the assimilation of glucose (isolate CD12CA4 was negative), L-arabinose and D-mannose (only M1364 was positive in both tests), the production of alkaline phosphatase (M1364 and 1387-02 were positive) and growth in presence of 3 % NaCl (248-01T and CD12CA4 did not grow). Details of the morphological, physiological and biochemical characteristics of the isolates are given in the species description and in Table 1.

To establish the phylogenetic affinities of the unknown isolates, their 16S rRNA gene sequences were determined as described previously (Vela et al., 2005) and were subjected to a comparative analysis. Pairwise analysis of the almost-complete sequences (1395 nt) determined revealed 99.3–99.9 % sequence similarity among the four strains. Sequence searches of GenBank performed using the program FASTA (Pearson, 1994) showed that the isolates were most closely related to members of the genus Moraxella. The isolates exhibited the highest levels of 16S rRNA gene sequence similarity with Moraxella cuniculi CCUG 2154T (97.9 % sequence similarity). These sequences and those of strains of representative species within the genus Moraxella with validly published names were retrieved from GenBank and aligned with the newly determined sequences by using the program DNATools (Rasmussen, 1995). Phylogenetic trees were constructed according to three different algorithms: neighbour joining (Saitou & Nei, 1987), with the programs DNATools and TreeView (Page, 1996), maximum likelihood, with PHYML software (Guindon & Gascuel, 2003), and maximum parsimony, with the software package MEGA version 3.1 (Kumar et al., 2004). Genetic distances for the neighbour-joining and maximum-likelihood algorithms were calculated using Kimura’s two-parameter model (Kimura, 1980); close-neighbour interchange (search level=2, random additions=100) was applied in the maximum-parsimony analysis. The stability of the groupings was estimated by using bootstrap analysis (based on 1000 replications). The phylogenetic trees obtained using neighbour joining (Fig. 1) and the other two methods (data not shown) revealed a clear affiliation between the isolates (exemplified by strain 248-01T) and the genus Moraxella: the novel strains were positioned as a separate branch within the genus. It is evident from Fig. 1 that strain 248-01T is phylogenetically related to M. cuniculi. Bootstrap resampling revealed a strong association (100 %) between the novel isolates and the aforementioned species.

### Table 1. Characteristics useful in differentiating strain 248-01T from recognized Moraxella species isolated from animals

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<td>Naphthol-AS-BI phosphohydrolase</td>
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*C, Coccus; R, rod; SR, short rod.
†Data are from this study and from Bøvre (1984), Rossau et al. (1991) and Kodjo et al. (1995, 1997).
The novel isolates had a sequence similarity lower than 98 % with respect to members of the genus Moraxella, a value that falls below the cut-off point (98.7 %) specified by Stackebrandt & Ebers (2006) for the delineation of genomic species. Nevertheless, DNA–DNA reassocation experiments were performed between the relevant strain (248-01T) and its closest phylogenetic neighbour (M. cuniculi CCUG 2154T). DNA was extracted and purified (248-01T was 46.4 mol%, which is within the range reported for members of the genus Moraxella). DNA–DNA reassociation experiments were performed between the relevant strain (248-01T) and its closest phylogenetic neighbour (M. cuniculi CCUG 2154T). DNA was extracted and purified (248-01T was 46.4 mol%, which is within the range reported for members of the genus Moraxella). Nevertheless, DNA–DNA reassocation experiments were performed between the relevant strain (248-01T) and its closest phylogenetic neighbour (M. cuniculi CCUG 2154T). DNA was extracted and purified (248-01T was 46.4 mol%, which is within the range reported for members of the genus Moraxella). The G+C content of strain 248-01T and M. cuniculi CCUG 2154T was 29 %, clearly confirming that the novel isolate represents a separate species (Wayne et al., 1987).

The G+C content of the DNA of strain 248-01T was determined from the mid-point value (Tm) of the thermal denaturation profile (Marmur & Doty, 1962) obtained with a Perkin-Elmer UV-Vis Lambda 20 spectrophotometer at 260 nm. The DNA G+C content of strain 248-01T was 46.4 mol%, which is within the range reported for members of the genus Moraxella (Juni & Bøvre, 2005).

Therefore, on the basis of phenotypic, phylogenetic and genotypic data, it is clear that the four strains isolated from pigs and a sheep merit classification within a novel species of the genus Moraxella, for which the name Moraxella pluranimalium sp. nov. is proposed. Tests that are useful in differentiating M. pluranimalium sp. nov. from its closest phylogenetic relative and from other Moraxella species isolated from animals are shown in Table 1.

**Description of Moraxella pluranimalium sp. nov.**


Cells are Gram-negative cocci (1.5–2.0 μm in diameter). Non-spore-forming. Catalase-positive, oxidase-positive and aerobic. Colonies are circular, non-pigmented, smooth and entire and do not produce a diagnostic odour on Columbia blood agar after 48 h incubation at 37 °C. Growth occurs at 22–37 °C but not at 42, 15 or 4 °C. Growth is enhanced in 5–10 % CO2 (demonstrating capnophily) on 5 % sheep blood agar and chocolate agar. Growth does not occur on MacConkey agar. Growth occurs in brain–heart infusion broth containing 0.5 or 1.5 % NaCl, but not with 5.0 or 6.5 % NaCl. Growth with 3 % NaCl is variable (strain 248-01T does not grow). Colonies are non-haemolytic. With the API 20E and API 20NE kits, aesculin, gelatin and urea are not hydrolysed. Indole and acetoin are not produced (API 20E). Nitrate is not reduced (API 20NE). Acid is not produced from D-glucose, D-mannitol, inositol, D-sorbitol, L-rhamnose, sucrose, melibiose, amygdalin or L-arabinose (API 20E). Assimilates glucose and malate, but not citrate, phenyl acetate, L-arabinose, D-mannose, D-mannitol, N-acetyl-D-glucosamine, maltose, gluconate, caprate or adipate (API 20NE). Ester lipase (C8), esterase (C4), leucine arylamidase and naphthol-AS-BI-phosphohydrolase are detected (API ZYM). Production of alkaline phosphatase is variable (strain 248-01T is negative). Valine arylamidase, lipase (C14), cystine arylamidase, acid phosphatase, arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, tryptophan deaminase, α-glucosidase, N-acetyl-β-glucosa-
minidase, β-glucosidase, β-glucuronidase, α-galactosidase, β-galactosidase, α-mannosidase, α-fucosidase, α-chymotrypsin and trypsin are not detected (API ZYM). The clinical significance is unclear. Strains have been isolated from pigs and a sheep. The DNA G+C content of the type strain is 46.4 mol% (Tm).

The type strain, 248-01T (=CECT 7295 = CCUG 54913T), was isolated from the nasal turbinate of an apparently healthy pig. Additional strains of the species are CD12CA4 (=CECT 7296 = CCUG 54914), M1364 and 1387-02.

Acknowledgements

The authors thank A. Casamayor for technical assistance and Juncal Fernández-Garayzábal for her assistance with English reviewing of the manuscript.

References


