Polyphasic Taxonomy Leading to the Proposal of *Moraxella canis* sp. nov. for *Moraxella catarrhalis*-Like Strains

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The taxonomic position of a group of 16 *Moraxella catarrhalis*-like strains, isolated mainly from dogs, was examined by using morphological tests, biochemical tests, serology, ribotyping with oligonucleotide probes, polymerase chain reaction typing of the 16S rRNA gene and the 16S-23S rRNA gene spacer region, polyacrylamide gel electrophoresis of total proteins, fatty acid profiles, moles percent G+C, dot spot and in-solution DNA-DNA hybridizations, and DNA-rRNA hybridizations. It was found that these organisms constitute a distinct cluster within the genus *Moraxella*. Since they differ genotypically as well as phenotypically from previously described *Moraxella* species, a new species, *Moraxella canis*, is proposed to accommodate these isolates. The type strain is LMG 11194 (= N7 = CCUG 8415A).

During the testing of the specificity and reliability of DNA probes for the detection of *Moraxella catarrhalis*, some strains presumptively identified as *M. catarrhalis* on the basis of biochemical and morphological data did not hybridize with some of the probes tested. Additional preliminary genotypic and phenotypic studies indicated that two of these strains were asaccharolytic neisseriae. The other strains, however, resembled *M. catarrhalis* strains but were not identical. This observation prompted us to collect more of these *M. catarrhalis*-like strains and to study this group in more detail.

In this paper we describe the results of a polyphasic taxonomic study in which these strains were compared with other organisms belonging to the recently proposed bacterial family *Moraxellaceae* (28). This family includes the genera *Moraxella*, *Acinetobacter*, and *Psychrobacter*. It has also been shown that the subgenus *Branhamella* does not correspond to a genotypic entity and that its four species constitute separate groups in the genus *Moraxella*. A proposal for the reestablishment of the name *M. catarrhalis* was formulated by Rossau et al. (28).

The results of this study show that the *M. catarrhalis*-like strains, which were isolated mainly from dogs, belong to a new species in the genus *Moraxella*. The name *Moraxella canis* is proposed and is used below. Since no genotypic relationship between the genus *Moraxella* and taxa belonging to the emended family *Neisseriaceae* has been demonstrated (27), the possibility of a relationship between *M. canis* and genuine *Neisseria* species could be excluded.

M. catarrhalis NCTC 4103 was also included in this study. Since this strain seems to be neither an unequivocal *M. canis* strain nor an unequivocal *M. catarrhalis* strain, no species name is used below when we refer to this strain.

MATERIALS AND METHODS

Organisms and media used. Data on the origins of the strains included in this study are listed in Table 1. The culture collection strains of *M. catarrhalis* tested were

selected on the basis of different criteria. Strain ATCC 25238 is the type strain. Strains D6, J9, and W3 are lipopolysaccharide (LPS) serotype strains (i.e., strains against which sera were raised and which are serotype strains for serogroups A, B, and C, respectively) (37). Strain U35, which produces β -lactamase, was kindly provided by C. Kamme (17). The following *M. canis* strains, described by the donors as *M. catarrhalis*, were used: CCUG 8415A^T (= N7^T) (T = type strain), supplied by E. Falsen (Culture Collection of the University of Göteborg, Göteborg, Sweden); N4518 (= P37), supplied by J. Y. Riou (Institut Pasteur, Paris, France); and BLU 8387 subclone U33 with large colonies and BLU 8387 subclone U34 with small colonies, supplied by J. Wüst (Zürich University, Zürich, Switzerland). The origin of strain O18 could not be traced. Other *M. canis* strains were isolated by L. A. Devriese (Laboratory of Veterinary Bacteriology, University of Ghent, Ghent, Belgium) after screening of the muzzles of a total of 124 dogs and 36 cats on a selective medium for *M. catarrhalis* (36). Four strains from two dogs (N47 and N48 from one dog and N49 and N50 from the other dog) were withheld for further study since they had a different colony morphology. Type strains and/or representative strains of other *Moraxella* species were also included. Strains were routinely cultured on Mueller-Hinton agar containing 5% sheep blood (BBL Microbiology Systems, Cockeysville, Md.) unless indicated otherwise.

Serology. An inhibition enzyme-linked immunosorbent assay (ELISA) with LPS as the specific antigen was performed as described previously (37). Briefly, microtiter plates were coated with LPS type A, B, or C. The ELISA was carried out with homologous antisera previously absorbed with whole-cell suspensions of the strains to be tested. The optical density values measured after inhibition of the LPS-antiserum reactions by the homologous strains were defined as 100% homology. Inhibition by *Moraxella ovis* ATCC 19575 was defined as 0% homology. Percentages of LPS homology for the other strains were calculated from the optical density values measured after inhibition of the LPS-antiserum reactions by these strains by using the following equation: percentage of LPS homology for test strain = $[1 - (\text{optical density of test strain} - \text{optical density of}$

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TABLE 1. Strains used

Species	Culture collection no. ^a			Country of origin (year)	Source
	UZG	LMG	Others		
<i>M. canis</i>	N7 ^T	11194 ^T	CCUG 8415A ^T	Sweden (1979)	Dog bite, female
<i>M. canis</i>	U33	11183	Wüst BLU 8387	Switzerland	Blood, male
<i>M. canis</i>	U34	11184	Wüst BLU 8387	Switzerland	Blood, male
<i>M. canis</i>	O18	11185			
<i>M. canis</i>	P37	11186	Riou N4518	France	Dog sputum
<i>M. canis</i>	V44	11187		Belgium (1991)	Dog muzzle
<i>M. canis</i>	V45	11188		Belgium (1991)	Dog muzzle
<i>M. canis</i>	W40	11189		Belgium (1991)	Dog muzzle
<i>M. canis</i>	W4	11190		Belgium (1991)	Dog muzzle
<i>M. canis</i>	W10	11191		Belgium (1991)	Dog muzzle
<i>M. canis</i>	N47			Belgium (1991)	Dog muzzle
<i>M. canis</i>	N48			Belgium (1991)	Dog muzzle
<i>M. canis</i>	N49			Belgium (1991)	Dog muzzle
<i>M. canis</i>	N50			Belgium (1991)	Dog muzzle
<i>M. canis</i>	N51			Belgium (1991)	Dog muzzle
<i>M. canis</i>	N53			Belgium (1991)	Cat muzzle
<i>M. catarrhalis</i>		5128 ^T	CCUG 353 ^T , ATCC 25238 ^T		
<i>M. catarrhalis</i>		11192 ^T	ATCC 25238 ^T		
<i>M. catarrhalis</i>	B13		Nagatake NNBR O43	Japan	Pneumonia
<i>M. catarrhalis</i>	J9		Calder A1079	Scotland (1986)	
<i>M. catarrhalis</i>	W3			Belgium (1984)	Sputum
<i>M. catarrhalis</i>	D6		Calder 55504	Scotland (1986)	
<i>M. catarrhalis</i>	G25	11177		Belgium (1987)	Sputum
<i>M. catarrhalis</i>	J27	11178		Belgium (1987)	Sputum
<i>M. catarrhalis</i>	O29	11182		Belgium (1988)	Sputum
<i>M. catarrhalis</i>	U35	11179	Kamme BC002 Pc ^R	Sweden	Nasopharynx, laryngitis
<i>M. catarrhalis</i>	V30	11180			
<i>M. catarrhalis</i>	V31	11181			
<i>M. catarrhalis</i>			ITM 4197	Belgium	Blood, male
<i>Moraxella</i> sp.	X31	11193	NCTC 4103		
<i>Moraxella</i> sp.		1133	NCTC 4103		
<i>M. lacunata</i>		5301 ^T	ATCC 17967 ^T , CCUG 4441 ^T		Conjunctivitis
<i>M. lacunata</i>		1008	ATCC 17951		
<i>M. lacunata</i>		1009	ATCC 17952		
<i>M. lacunata</i>		1020	ATCC 17972		Throat, child
<i>M. nonliquefaciens</i>			ATCC 19975 ^T		
<i>M. nonliquefaciens</i>		1010	ATCC 17953		
<i>M. nonliquefaciens</i>		1011	ATCC 17954		Throat
<i>M. bovis</i>		5299 ^T	ATCC 10900 ^T		Cattle, pinkeye
<i>M. bovis</i>		986 ^T	CCUG 2133 ^T , ATCC 10900 ^T		Cattle, pinkeye
<i>M. bovis</i>		1006	ATCC 17947		Cattle, keratitis
<i>M. caviae</i>		5129			
<i>M. ovis</i>		8381 ^T	ATCC 33078 ^T		Ovine, keratoconjunctivitis
<i>M. cuniculi</i>		8382 ^T	ATCC 14688 ^T		Rabbit, rhinopharynx
<i>M. osloensis</i>		6916	CCUG 63	Sweden	Urine
<i>M. osloensis</i>		5131 t ₁ ^T	CCUG 350 ^T		Cerebrospinal fluid
<i>M. osloensis</i>		1043	ATCC 19961		Urine
<i>P. immobilis</i>		6784 t ₁	CCUG 9702		Lemon sole, gills
<i>A. calcoaceticus</i>		1046 ^T	ATCC 23055t ₁ ^T		Soil
<i>N. gonorrhoeae</i>			NCTC 8375 ^T		
<i>N. canis</i>			ATCC 14687 ^T		

^a Abbreviations: ATCC, American Type Culture Collection, Rockville, Md.; CCUG, Culture Collection, Department of Clinical Bacteriology, University of Göteborg, Göteborg, Sweden; ITM, Institute for Tropical Medicine, Antwerp, Belgium; LMG, Culture Collection, Laboratorium voor Microbiologie, Universiteit Gent, Ghent, Belgium; NCTC, National Collection of Type Cultures, Central Public Health Laboratory, London, England; UZG, Universitair Ziekenhuis, Ghent, Belgium.

FIG. 1. Sequences and locations of the oligonucleotides used as probes and/or primers. The sequences of these primers and probes are quasiconserved in eubacteria. The cross-hatched area represents the spacer region between the 16S and 23S rRNA genes.

homologous strain)/(optical density of *M. ovnis* strain – optical density of homologous strain) $\times$ 100.

Levels of total antigenic homology were determined as follows. ELISA microtiter plates were coated with tryptic soy broth culture supernatants (called whole-cell antigen preparations) of each strain to be tested. The ELISA was carried out as described by Voller et al. (39) with anti-J9 serum. The optical density value measured for the coated whole-cell preparation of *M. catarrhalis* J9 was defined as 100% homology. The levels of total antigenic homology for the other strains were calculated as follows: percentage of total antigenic homology for test strain = (optical density of test strain/optical density of strain ATCC 25238^T) $\times$ 100.

Biochemical characteristics. Butyrate esterase activity was tested by using 4-methylumbelliferyl butyrate as the substrate (35). γ -Glutamyl-aminopeptidase disks (Rosco Diagnostics, Taastrup, Denmark) were used to test for the presence of this enzyme. The presence of extracellular DNase was determined on DNase agar (Oxoid, Ltd., London, United Kingdom). Growth on a medium containing acetazolamide was used to evaluate the absence of carbonic anhydrase (36). All other tests were performed as described by McFaddin (23).

Oligonucleotide synthesis, purification, and labelling. The oligonucleotides used as primers or probes were synthesized by the phosphite-triester method with a Cyclone 8400 DNA synthesizer (New Brunswick Scientific, Edison, N.J.). The oligonucleotides were deprotected and precipitated by using a double volume of ethanol. The oligonucleotides used as hybridization probes were 5' labelled by using [γ -³²P]ATP and T4 polynucleotide kinase (Pharmacia, Uppsala, Sweden) (20). The sequences and locations of the oligonucleotides used as probes or primers are shown in Fig. 1.

PCR analysis. Purified whole-cell DNA (approximately 100 ng per reaction) was amplified by using the polymerase chain reaction (PCR) technique with conserved primers 1 and 2 and conserved primers 3 and 4, resulting in the amplification of the 16S rRNA gene and the 16S-23S rRNA gene spacer region, respectively (Fig. 1). The PCR reactions were performed in 50 μ l of a solution containing 200 μ M dATP, 200 μ M dCTP, 200 μ M dGTP, 200 μ M dTTP (all four deoxynucleoside triphosphates were obtained from Boehringer Mannheim, Mannheim, Germany), 50 pmol of each of the primers, 1 $\times$ PCR buffer (Stratagene Cloning Systems, La Jolla, Calif.), and 2 U of *Taq* polymerase (Stratagene). Initial denaturation at 95°C for 5 min was followed by 30 cycles consisting of annealing at 50°C for 1 min, extension at 72°C

for 1 min, and denaturation at 95°C for 1 min. Finally, additional extension occurred at 72°C for 7 min, after which the samples were cooled to room temperature and extracted with CHCl₃. The amplified products from the reactions with primers 1 and 2 and the reactions with primers 3 and 4 were electrophoresed in 1.6 and 1.8% (wt/vol) agarose gels, respectively, at 8 V/cm for 90 min. Restriction endonucleases were purchased from Boehringer Mannheim or New England Biolabs, Beverly, Mass., and were used according to the manufacturers' recommendations.

Standard molecular genetic techniques. High-molecular-weight whole-cell DNA was purified essentially as described by Marmur (22). Nick translation was performed as described by Maniatis et al. (20). A 1- μ g portion of purified whole-cell DNA was digested to completion with *Hind*III restriction endonuclease (Boehringer Mannheim), loaded onto a 1% (wt/vol) agarose gel, and electrophoresed at 8 V/cm for 90 min. The DNA fragments were transferred to Zeta-Probe nylon membranes (Bio-Rad Laboratories, Richmond, Calif.) under alkaline conditions (24). Genomic DNA (1 μ g per spot) was dot spotted as described previously (29). The membranes were hybridized as described below. Prehybridizations were performed in a solution containing 3 $\times$ SSC (1 $\times$ SSC is 0.15 M NaCl plus 0.015 M sodium citrate [pH 7.0]), 20% (vol/vol) deionized formamide, 25 mM sodium phosphate buffer (pH 7.1), 1% (wt/vol) sodium dodecyl sulfate (SDS), 0.02% (wt/vol) Ficoll, 0.02% (wt/vol) bovine serum albumin, 0.02% (wt/vol) polyvinylpyrrolidone, and 0.1 mg of sheared, denatured salmon sperm DNA per ml for 0.5 to 1 h at 45°C. Hybridizations were performed at the same temperature for 4 h to overnight in a mixture that was the same as the prehybridization mixture except that approximately 10⁶ cpm of ³²P-labelled oligonucleotide probe or nick-translated genomic DNA per ml was added. After a brief rinse at room temperature in wash solution (3 $\times$ SSC, 20% formamide, 25 mM sodium phosphate buffer [pH 7.1]) containing 0.1% SDS, the membranes were finally washed for 30 min at the hybridization temperature in wash solution containing 1% SDS. The membranes were then rinsed at room temperature in 1.5 $\times$ SSC–0.1% SDS for about 10 min and autoradiographed by using Fuji RX film (Fuji Photo Film, Tokyo, Japan) at –70°C and an intensifying screen.

PAGE of whole-cell proteins. All strains were grown for 48 h on heart infusion medium (Difco Laboratories, Detroit, Mich.) at 28°C in Roux flasks. Whole-cell protein extracts were prepared, and SDS-polyacrylamide gel electrophoresis

(PAGE) was performed by using the procedure of Laemmli (18), slightly modified as described previously (34).

Numerical analysis of the protein gel electropherograms. Densitometric analysis, normalization, and interpolation of the protein profiles and numerical analysis were performed by using the GelCompar 1.3 software package (Helix C.V., Biotechnology Business Development, Ghent, Belgium). The profiles were recorded and stored on a PC-AT computer. The similarity between pairs of traces was expressed by the Pearson product moment correlation coefficient (31), converted for convenience to a percentage.

DNA base composition. Melting curves were determined by the thermal denaturation method (11), and the average G+C contents were calculated by using the equation of Marmur and Doty (21), as modified by De Ley (8).

DNA-DNA hybridizations. The degree of binding, expressed as a percentage, was determined spectrophotometrically by using the initial renaturation method (9). Renaturations were performed in $1 \times$ SSC at the optimal renaturation temperature (64.9°C) with a total DNA base pair concentration of 0.92 mM. Degrees of binding of 25% and less were not significant.

DNA-rRNA hybridizations. DNA was further purified by CsCl gradient centrifugation, thermally denatured, and fixed on cellulose nitrate filters (type SM11358; Sartorius, Göttingen, Germany), as described previously (10). ^3H -labeled 23S rRNA from *Moraxella lacunata* subsp. *liquefaciens* ATCC 17952 was prepared as described by Rossau et al. (28). Hybridizations and determinations of $T_{m(e)}$ (the temperature at which one-half of a DNA-rRNA duplex was denatured) were performed as described elsewhere (28).

Gas chromatographic analysis of cellular fatty acid methyl esters. Bacterial strains were precultured on heart infusion agar slants, streaked onto plates containing 3.0% (wt/vol) Trypticase soy broth (BBL) supplemented with 1.5% (wt/vol) Bacto Agar (Difco), and incubated at 28°C for 24 h. Approximately 60 mg (wet weight) of cells was harvested from the most dilute quadrant showing confluent growth. The extraction of fatty acids, preparation of methyl esters and separation of methyl esters by gas-liquid chromatography were performed as described previously (38). Fatty acid methyl ester fingerprints were identified by using the Microbial Identification System software package (version 3.2; Microbial ID, Inc., Newark, Del.).

RESULTS AND DISCUSSION

A random selection of 60 strains of *M. catarrhalis* out of a collection of about 1,000 strains (Universitair Ziekenhuis, Ghent, Belgium) from different clinical and geographical origins included 2 strains which were oxidase- and catalase-positive, gram-negative diplococci and exhibited butyrate esterase and extracellular DNase activities. However, colony morphology on Mueller-Hinton agar containing 5% sheep blood more closely resembled that of members of the *Enterobacteriaceae* (large, smooth colonies) than that of typical *M. catarrhalis* strains (gliding, butyrous colored colonies). Consequently, these strains (N7^T, isolated from a dog bite, and O18, of unknown origin) were considered atypical *M. catarrhalis* strains. Subsequent genetic investigations showed that these strains did not hybridize to some of the rRNA-derived DNA probes designed for specific detection of *M. catarrhalis* strains, indicating that these strains may belong to a different, possibly undescribed, taxon.

In the meantime, three other *M. catarrhalis* isolates (U33,

U34, and P37) described in the literature were supplied by different investigators. Strain P37 was isolated from dog sputum and was described as "atypical" (26). U33 and U34 were subclones from the same strain isolated from the blood of a 61-year-old male human in Switzerland (40). The aberrant characteristics of strains U33 and U34 were noted by the authors, but Wüst et al. presumptively assigned these strains to *M. catarrhalis* on the basis of chemotaxonomic data. Since strains U33 and U34 had been found to be γ -glutamyl-aminopeptidase positive (40), the other atypical strains were tested for this characteristic and found to be equally positive, in contrast to typical *M. catarrhalis* strains. Also, another aberrant *M. catarrhalis* strain (NCTC 4103) described in the literature (3, 7) was found to produce γ -glutamyl-aminopeptidase. This extra biochemical characteristic enabled us to search for more similar isolates.

Since it was striking that one strain (P37) came from dog sputum and that another strain (N7^T) was associated with a dog bite wound, we used a medium selective for *M. catarrhalis* (36) to culture swabs taken from the mouths of dogs and cats attending a university hospital day care clinic for pets. Different colony types were picked, Gram stained and tested for oxidase, butyrate esterase, extracellular DNase, and γ -glutamyl aminopeptidase activities. From the cultured salivas of 124 dogs and 36 cats, a total of 11 strains were found to be gram-negative cocci which were positive for the four biochemical tests. All strains which were isolated from animals were recovered in low numbers (<50 CFU per selective plate). Their presence would probably have been overlooked if nonselective media had been used. No pathogenic role could be ascertained. All 11 isolates from dogs and cats, together with strains N7^T, O18, P37, U33, and U34, were examined in more detail and compared with typical *M. catarrhalis* strains, strain NCTC 4103, and strains belonging to other *Moraxella* species by using a variety of techniques, as described below.

Colony morphology. *M. catarrhalis* strains produce typical colonies when they are grown on solid media; the butyrous colored colonies can be shifted around (hockey puck test) and can be picked from the agar without losing their semi-convex shape. Strain NCTC 4103 produces small, greyish colonies which more closely resemble those of nonhemolytic *Neisseria* spp. The colonies of strain W40 resemble colonies of *M. catarrhalis*, while other *M. canis* strains produce large, smooth colonies that look more like colonies of members of the *Enterobacteriaceae*. Some strains (N49, P37, U33, and U34) produce very slimy colonies resembling colonies of *Klebsiella pneumoniae*.

The production of a brown pigment when the organisms are grown on starch-containing Mueller-Hinton agar is also typical of most *M. canis* strains (except U34). Strain NCTC 4103 produces a faint brown pigment, while the *M. catarrhalis*, "false *Neisseria*" spp., and the *Moraxella* strains tested never showed pigmentation. Furthermore, most *M. catarrhalis* strains and strain NCTC 4103 show strong autoagglutination when colonies are suspended in a physiological solution. This autoagglutination is weakened but persists after boiling for 5 min. Autoagglutination, which disappeared after boiling, was observed for only three *M. canis* strains (N48, N53, and W4).

Biochemical characteristics. Biochemical characteristics were determined for all of the *M. canis* strains examined. The following characteristics are common to *M. catarrhalis*, *M. canis*, strain NCTC 4103, and most *Moraxella* species: strains are positive for oxidase and catalase activities; carbonic anhydrase is not present; no acid is produced from

TABLE 2. Differentiating phenotypic characteristics of *Moraxella* species^a

Taxon	Cell shape		γ -Glutamyl-aminopeptidase activity	DNase activity	Growth on medium containing mineral salts and ammonium acetate	Growth stimulated by bile salts	Growth inhibited by bile salts	Growth on Mueller-Hinton agar at room temp	Nitrate reduction	Nitrite reduction	Brown pigment on Mueller-Hinton agar
	Rods	Cocci									
<i>M. lacunata</i>	+ ^b	-	-	-	-	-	+	-	+	-	-
<i>M. bovis</i>	+	-	-	-	-	-	+	-	(-)	-	-
<i>M. nonliquefaciens</i>	+	-	-	-	-	-	+	-	+	-	-
<i>M. atlantae</i>	+	-	NT	-	-	+	NT	NT	-	-	-
<i>M. phenylpyruvica</i>	+	-	(-)	-	(-)	+	NT	NT	(+)	-	-
<i>M. osloensis</i>	+	-	(-)	-	(+)	-	d	+	d	-	-
<i>M. canis</i>	-	+	+	+	(+)	-	(-)	+	(+)	d	(+)
<i>Moraxella</i> sp. strain NCTC 4103	-	+	+	+	-	-	+	+	-	+	w
<i>M. catarrhalis</i>	-	+	-	+	-	-	+	-	(+)	(+)	-
<i>M. caviae</i>	-	+	-	+	-	-	+	-	+	(+)	-
<i>M. ovis</i>	-	+	-	+	-	-	+	-	(+)	-	-
<i>M. cuniculi</i>	-	+	-	+	-	-	+	-	-	-	-

^a Data from this study and reference 5.^b +, all strains positive; (+), most strains positive; d, results differ among strains; -, all strains negative; (-), most strains negative; w, weak reaction; NT, not tested.

glucose, maltose, saccharose, or lactose; strains do not hemolyze human blood (except *Moraxella bovis*, *Moraxella caviae*, and *M. ovis*) or sheep blood (except *M. bovis* and *M. ovis*); gelatin is not liquefied; serum is not liquefied (except *M. bovis* and *M. lacunata*); no growth occurs at 5°C or in the presence of 6% NaCl; growth is not stimulated by bile salts; no phenylalanine deaminase or urease activity is present; no indole is produced; strains are susceptible to 1.0 U of penicillin per ml (except β -lactamase-producing strains, which are found in most species [*M. canis* W4 is β -lactamase positive]); no polysaccharides are synthesized from saccharose; butyrate esterase activity is present; no lipase or lecithinase activity can be detected; and strains are unable to hydrolyze starch.

The most prominent phenotypic features differentiating *M. canis* strains from strains of other *Moraxella* species are summarized in Table 2. Since *M. canis* is the only *Moraxella* species besides *M. catarrhalis* and the "false neisseriae" which produces extracellular DNase, *M. canis* may be presumptively misidentified as one of these taxa by using conventional criteria. However, *M. canis* strains can be clearly distinguished from *M. catarrhalis*, *M. ovis*, *M. cuniculi*, and *M. caviae*. In general, *M. canis* strains are less fastidious than strains of other authentic *Moraxella* species, and in this respect they resemble *Moraxella osloensis* strains more closely. Like *M. osloensis* strains and in contrast to other authentic moraxellae, all *M. canis* strains grow on Mueller-Hinton agar at room temperature, and most strains are able to grow on medium containing mineral salts and ammonium acetate (except U34 and N47), lactose bromothymol blue agar (except U34), eosine-methylene blue agar (except U34), and MacConkey agar (except U34). Furthermore, the growth of *M. canis* strains except U33 and U34 is not inhibited by bile salts. Unlike *M. catarrhalis* strains, *M. canis* strains exhibit a rather slow butyrate esterase reaction (15 min, compared with 5 min), when they are tested with 4-methylumbelliferyl butyrate as the substrate.

However, typical for *M. canis* strains is the production of γ -glutamyl-aminopeptidase, which is considered to be absent in most *Moraxella* species (6), although Riou et al. (25) found γ -glutamyl-aminopeptidase activity in two *Moraxella*

phenylpyruvica strains examined and the presence of this enzyme could also be demonstrated in one of six *M. osloensis* strains examined in our laboratories (strain ATCC 19961). These criteria, together with colony morphology, can be used for differentiation of *M. canis* from *M. catarrhalis* and other authentic *Moraxella* species. Cell shape and DNase production allow differentiation of *M. canis* from *M. osloensis* strains. Phenotypic features that differentiate *M. canis* from *Neisseria* species, including *Neisseria canis* and asaccharolytic true neisseriae, are the presence of butyrate esterase and DNase activities and the absence of carbonic anhydrase activity.

Habitat. The habitats of *M. canis* and *M. catarrhalis* seem to be well separated. Normally, *M. catarrhalis* occurs in the upper respiratory tracts of humans, while most *M. canis* strains were found in the upper respiratory tracts of dogs and cats. Strains N7^T, U33, and U34, which were isolated from humans, were obtained from unusual sources; association with the muzzle of a dog is clear in the case of strain N7^T, which was isolated from a dog bite wound. During this and previous studies we could not isolate genuine *M. catarrhalis* strains from the upper respiratory tracts of dogs or cats, nor could we isolate *M. canis* from human upper respiratory tracts. Yet, the isolation of *M. catarrhalis* from dogs was previously reported by Saphir and Carter (30) and Bailie et al. (2). However, it should be noted that these authors probably used conventional identification criteria by which *M. catarrhalis* strains and *M. canis* strains cannot easily be differentiated. Bailie et al. (2) also described the isolation of *M. osloensis* from dogs, although the normal habitat of *M. osloensis* is probably the human upper respiratory tract.

Serology. (i) **LPS.** On the basis of their LPS antigenicities, *M. catarrhalis* strains can be subdivided into three types (types A, B, and C); about 7% of the strains are nontypeable (37). When this typing system was used, it was found that the LPS type of *M. canis* strains is closely related to the most common *M. catarrhalis* LPS type (type A). Strains N7^T, O18, P37, V44, V45, W40, and NCTC 4103 gave between 60 and 80% inhibition in an inhibition ELISA specific for LPS type A. Although the levels of inhibition with strains U33 and U34 were less than 30% with respect to *M. catarrhalis*

LPS type A, B, or C and these strains seem to possess the least closely related LPS among the *M. canis* strains tested. Wüst et al. (40) showed that the LPS of these strains still exhibited sufficient antigenic similarity to immunoprecipitate anti-*M. catarrhalis* serum. *M. catarrhalis* type B and C strains gave less than 50% inhibition, and hence, their LPS are serologically more distant from the LPS of type A strains than the LPS of *M. canis* strains are. The levels of inhibition observed with the other members of the *Moraxellaceae* tested (11 strains belonging to the following six species: *M. lacunata*, *M. osloensis*, *M. nonliquefaciens*, *M. ovis*, *M. caviae*, and *M. cuniculi*) and *N. canis* did not exceed 20% for all three *M. catarrhalis* LPS types.

(ii) **Total antigenic homology.** The optical density measured after an ELISA carried out with a whole-cell preparation of *M. catarrhalis* type strain ATCC 25238 as the coating antigen was defined as 100%. The optical densities for the 11 other *M. catarrhalis* strains and for strain NCTC 4103 were more than 85%. The optical densities for *M. canis* strains ranged between 20 and 70%, and the optical densities were 60% for *M. ovis*, 35% for *M. cuniculi*, and 35 and 25% for *M. caviae*. The optical densities for other *Moraxella* species and for *M. canis* U34 were less than 20% of the optical density obtained when the *M. catarrhalis* ATCC 25238^T broth culture supernatant was the coating antigen.

(iii) **Serological cross-reactivity with *Neisseria meningitidis* serum.** Wüst et al. (40) also reported that strains U33 and U34 cross-agglutinate with *N. meningitidis* ACYW135 antiserum. We found that this characteristic was present in 12 of the 16 *M. canis* strains tested. Because of autoagglutination of *M. catarrhalis* strains and strain NCTC 4103, agglutination with *N. meningitidis* antiserum was not interpretable.

From these data it can be concluded that *M. canis* strains are serologically more closely related to *M. catarrhalis* than to most other *Moraxella* species, including the "false neisseriae." *Moraxella* spp. possess the rough type of LPS, and thus, when the low number of possible epitopes on these small molecules is taken into account, serological cross-reactions could be expected to occur between closely related species. However, total antigenic homology, as determined by an ELISA, enables differentiation between *M. catarrhalis* and *M. canis*. Strain NCTC 4103 is serologically indistinguishable from *M. catarrhalis* strains.

Comparative protein gel electrophoresis (SDS-PAGE). Figure 2 shows a dendrogram based on an average-linkage cluster analysis of SDS-PAGE protein patterns from *M. canis* strains and representative strains of *M. catarrhalis* and other *Moraxella* species. Figure 3 shows the corresponding electropherograms of the *M. canis* and *M. catarrhalis* strains and the two subcultures (LMG 1133 and LMG 11193) of *Moraxella* sp. strain NCTC 4103. All of the *M. catarrhalis* strains studied belong to a separate cluster, and *M. ovis* type strain LMG 8381 is the closest neighbor of this cluster (Fig. 2). Eight strains of *M. canis* constitute another electrophoretic cluster. U33 and U34 cluster separately. However, a visual comparison of the protein profiles of the latter two strains reveals several similarities with the protein profiles of *M. canis* strains belonging to cluster I (Fig. 3). The heavy protein band in the 34,000-molecular-weight region is at the origin of a separate position on the dendrogram. Deletion of this dense protein band from the numerical analysis resulted in the grouping of all *M. canis* strains in a single cluster (data not shown). All other *Moraxella* species occupy gel electrophoretic positions outside clusters I, II, and IV. Subcultures LMG 1133 and LMG 11193 of *Moraxella* sp. strain NCTC 4103 also constitute a separate gel electrophoretic cluster

(cluster III), confirming the intermediate position of these taxa. The two subcultures produced very similar electropherograms (Fig. 3).

Fatty acid analysis. The fatty acid profiles of strains of seven *Moraxella* species (*M. bovis*, *M. canis*, *M. catarrhalis*, *M. lacunata*, *M. nonliquefaciens*, *M. osloensis*, and *M. ovis*) and of strain LMG 1133 are shown in Table 3. The major fatty acids of the strains studied are C_{10:0}, C_{12:0} 3-OH, C_{16:1} *cis*-9, C_{16:0}, C_{18:3} *cis*-6,12,14, and C_{18:1} *cis*-9. Our results largely corroborate previously reported results (13, 15, 16, 19, 40). Mostly quantitative differences in fatty acid composition were observed; these differences may have been due to the cultivation conditions used.

In our study, oleic acid (C_{18:1} *cis*-9) was detected as one of the major fatty acid components of all species studied. Sugimoto et al. (33) divided *Moraxella* species into two groups, the oleic acid group and the *cis*-vaccenic acid (C_{18:1} *cis*-11) group. The latter group is characterized by the absence of oleic acid and includes the type strains of *M. catarrhalis* and *M. ovis*. When the Microbial Identification System was used, only *M. canis* and *M. catarrhalis* showed trace amounts of summed feature 7 (which includes *cis*-vaccenic acid [Table 3]). Like all of the strains studied, *M. catarrhalis* and *M. ovis* were characterized by high amounts of oleic acid. It is not clear whether different cultivation conditions are responsible for these discrepancies. As shown in Table 3, *M. canis* strains contain the highest mean percentage of C_{18:1} *cis*-9 (67.8%) and the lowest amounts of C_{16:0} (1.2%) and C_{16:1} *cis*-9 (8.8%). Strains of this species can be further differentiated from *M. catarrhalis* strains by the presence of small amounts of C_{17:0} iso. The fatty acid pattern of strain LMG 1133 is similar to the profile of *M. catarrhalis* strains. Minor quantitative differences and the presence of small amounts of C_{16:0} iso and C_{17:0} iso and the absence of C_{17:1} B differentiate strain LMG 1133 from *M. catarrhalis* strains. Also, the profiles of *M. osloensis* strains are quantitatively very similar to the profiles of *M. catarrhalis* strains, but can be distinguished by the absence of C_{12:0} and C_{14:0}. *M. ovis* has the highest percentage of summed feature 3 (6.6%). The fatty acid profiles of the type strain of *M. bovis* and two strains of *M. lacunata* are very similar; compared with other species, considerable quantitative differences in the major fatty acids are observed. These two species can be differentiated by the presence or absence of some minor fatty acids. *M. nonliquefaciens* had the lowest amount of C_{18:1} *cis*-9 (20.7%) and the highest amounts of C_{12:0} (8.3%), C_{12:0} 3-OH (14.4%), ECL 15549 (5.5%), C_{16:0} (12.4%), and C_{18:3} *cis*-6,12,14 (7.4%). The fatty acid profile of the *M. canis* strains differs significantly from that of the Centers for Disease Control group NO-I strains, which are fastidious, nonoxidative, gram-negative organisms associated with dog and cat bites (14).

rRNA gene restriction patterns. Ribotyping (12) has proven to be an interesting tool for taxonomic purposes. In this study, we used a slightly modified technique, as outlined below. Genomic DNAs of different bacterial strains were cleaved to completion with *Hind*III, electrophoresed in agarose gels, and transferred to membranes. The fragments carrying rRNA gene sequences were localized by hybridization with oligonucleotide probes hybridizing to conserved regions on the 16S or 23S rRNA gene instead of with an *Escherichia coli* 16S+23S rRNA probe, which is what was used in most of the previous studies dealing with ribotyping. In this study a mixture of three oligonucleotide probes (probes 1, 5, and 6) was used. The locations of these probes on the 16S and 23S rRNA gene are shown in Fig. 1. Figure

FIG. 2. Dendrogram based on average-linkage cluster analysis of SDS-PAGE protein patterns from representative strains of *M. catarrhalis*, *M. canis*, and other *Moraxella* species. The scale represents the Pearson product moment correlation coefficient converted to percentages.

4 shows the hybridization patterns of a number of representative strains. Among the eight genuine *M. catarrhalis* strains tested, only the rRNA gene restriction pattern of strain V31 differed slightly from the others and exhibited some resemblance to the strain NCTC 4103 (LMG 1133) pattern. Among the 15 *M. canis* isolates, seven different restriction fragment types could be recognized. However, the profiles had some common features which enabled us to distinguish the *M. canis* strains from *M. catarrhalis* strains and from other the bacterial species tested (data not shown). The doublet at the left of the *M. canis* restriction patterns is characteristic for *M. canis* strains. The only exception is strain N49. In strain N49 the upper band of the doublet is absent and two higher-molecular-weight bands are present (Fig. 4). None of the *M. canis* strains exhibited the low-molecular-weight band at about 350 bp (single band at the left) typically found in *M. catarrhalis* strains and strain NCTC 4103. The following eight strains belong to the restriction fragment length polymorphism type 1 group: V44, V45, N50, W10, N7^T, U33, U34, and O18. Strains N51 and N53 belong to type 7, and strains N47 and N48 belong to type 5. Strains W40, W4, P37, and N49 stand apart and

belong to the restriction fragment length polymorphism type 2, 3, 4, and 6 groups, respectively (Fig. 4). From these results it is obvious that considerably more heterogeneity is found in *M. canis* than in *M. catarrhalis*. However, it is not uncommon that different banding patterns are observed in one species while some other species are extremely homogeneous (1, 12, 32).

PCR analysis of the 16S rRNA gene and the 16S-23S rRNA spacer region. The use of PCR typing for taxonomic purposes is rather new and is still under development. However, this procedure might be preferred to classical restriction fragment length analyses, such as ribotyping, since there are fewer technical constraints and it is easier to perform while the informational value seems to be comparable. The results obtained after we separately amplified the 16S-23S rRNA spacer region and the 16S rRNA gene are shown in Fig. 5 and 6, respectively.

Enzymatic amplification of the 16S-23S rRNA gene spacer region with primers 3 and 4 revealed a difference in fragment length between *M. catarrhalis* strains and *M. canis* strains. All eight *M. catarrhalis* strains examined yielded a fragment about 620 bp long, while the fragments of the 15 *M. canis*

FIG. 3. Electropherograms of *M. catarrhalis* and *M. canis* strains and *Moraxella* sp. strain NCTC 4103 subclones LMG 1133 and X31. The low-molecular-weight markers (lane LMWM) were (from left to right) lysozyme (molecular weight, 14,500), trypsin inhibitor (20,100), trypsinogen (24,000), carbonic anhydrase (29,000), glyceraldehyde-3-phosphate dehydrogenase (36,000), egg albumin (45,000), and bovine albumin (66,000).

strains and of strain NCTC 4103 were about 580 bp long. When DNA of *M. nonliquefaciens* ATCC 19975^T was used as the target, a 670-bp fragment was observed. *M. osloensis* ATCC 19961 yielded a fragment about 750 bp long. In addition, *M. canis* strains differed from *M. catarrhalis*, *M. nonliquefaciens*, and *M. osloensis* strains with respect to the presence of restriction sites for *Eco*RI and *Nco*I. A restriction site for *Eco*RI was observed in *M. catarrhalis* V31, but the spacer region fragments of other *M. catarrhalis* strains contained a restriction site for neither *Eco*RI nor *Nco*I. *M. nonliquefaciens* ATCC 19975^T also did not possess recognition sites for *Eco*RI or *Nco*I, while in *M. osloensis* ATCC 19961, a restriction site for *Eco*RI was observed. In contrast, the spacer region fragments of strain NCTC 4103 and *M. canis* W40, N51, and N53 possessed restriction sites for both enzymes and produced identical restriction patterns. The other *M. canis* strains lacked the *Nco*I recognition site.

Evidently no length polymorphism could be observed on an agarose gel when the 16S rRNA genes were enzymatically amplified. When primers 1 and 2 were used, all of the strains examined produced a DNA fragment about 1,500 bp long (data not shown). However, as illustrated in Fig. 6, after restriction endonuclease digestion with *Hind*III or *Eco*RI, DNA fragments originating from *M. canis* strains could readily be differentiated from DNA fragments originating from *M. catarrhalis* strains. When either *Hind*III or *Eco*RI was used, no differences in restriction patterns were observed among the *M. catarrhalis* strains studied. Strain NCTC 4103 could not be distinguished from genuine *M. catarrhalis* strains. Within *M. canis*, two types, both different from *M. catarrhalis* or *M. nonliquefaciens*, were observed when *Eco*RI was used. Strains N7^T, O18, V45, N47, N48, N49, and N50 belong to one group, while strains U33, U34, P37, W40, V44, W4, W10, N51, and N53 belong to a second group (Fig. 6). *M. osloensis* could not be differenti-

ated from the first group of *M. canis* strains when *Eco*RI was used. When *Hind*III was used, all *M. canis* strains exhibited the same pattern and possessed one recognition site approximately 30 bp from the beginning of the amplicon, in contrast to *M. osloensis*, which did not possess any sites for *Hind*III. Because of the small fragment cleaved off, the difference between the two species was more apparent when *Eco*RI-*Hind*III double digestion was used (data not shown).

By combining the results obtained with the *Hind*III and *Eco*RI digests of the 16S rRNA gene fragment and the *Eco*RI and *Nco*I digests of the spacer region fragment, a total of seven restriction sites could be examined. Among the *M. canis* strains, we ended up with three different types. Type 1 included strains N7^T, O18, V45, N47, N48, N49, and N50, each of which had two *Eco*RI sites and one *Hind*III site. Type 2 (three *Eco*RI sites and one *Hind*III site) included strains U33, U34, P37, V44, W4, and W10. The strains exhibiting a type 3 pattern (one *Hind*III site, three *Eco*RI sites, and one *Nco*I site) were W40, N51, and N53. Type 2 and 3 strains and type 1 and 2 strains differ in only one restriction site, while type 1 strains differ from type 3 strains in two sites.

All of the *M. catarrhalis* strains except V31 are the same type. At least three to five of the seven sites examined differ between typical *M. catarrhalis* strains and strains of *M. canis*. For strain NCTC 4103, a total of two differences were observed compared with typical *M. catarrhalis* strains; with *M. canis* strains we found three to five differences. Two to four differences occurred between *M. canis* strains and *M. osloensis* ATCC 19961. It is also noteworthy that only one difference between *M. catarrhalis* strains and the type strain of *M. nonliquefaciens* was detected. However, the exact taxonomic consequences of these observations cannot be fully evaluated at this time since it is not known at which taxonomic level the observed differences are relevant. Nev-

TABLE 3. Fatty acid compositions of *Moraxella* species

Taxon	No. of strains ^a	% of:															
		C _{10:0}	C _{12:0}	C _{12:0} 3-OH	C _{14:0}	Summed feature 3 ^b	ECL 1554 ^c	C _{16:0}	C _{16:0} iso	C _{16:0} cis-9	C _{16:1} B ^d	C _{17:0} iso	C _{17:1} B ^d	C _{18:0}	C _{18:1} cis-9	Summed feature 7 ^e	C _{18:3} cis-6,12,14
<i>M. canis</i>	10	3.2 (0.6) ^f	4.0 (0.9)	5.1 (1.3)		3.2 (0.9)		1.2 (0.5)	8.8 (2.4)	1.0 (0.6)	1.8 (0.7)	tr ^g	tr	67.8 (3.1)	tr	1.7 (0.5)	
<i>M. ovis</i>	1	4.0	2.5	11.1		6.6	1.8	9.1	9.6	1.2		1.3	2.1	45.3		4.4	
<i>M. catarrhalis</i>	7	4.2 (0.9)	2.8 (0.6)	4.2 (1.0)	1.1 (0.3)	tr	tr	6.4 (1.6)	20.7 (3.4)	1.0 (0.3)		4.1 (1.5)	1.3 (0.6)	51.4 (6.4)	tr	1.6 (0.4)	
<i>Moraxella</i> sp.	1	3.9	4.3	6.7	2.0	tr	tr	4.1	1.4	16.7	1.7		tr	53.7		1.7	
<i>M. nonliquefaciens</i>	2	5.9 (0.6)	8.3 (1.3)	14.4 (1.4)	tr		5.5 (2.9)	12.4 (0.1)	23.5 (0.1)				tr	20.7 (2.8)		7.4 (0.1)	
<i>M. lacunata</i>	2	3.5 (1.5)	4.5 (0.8)	4.6 (1.5)	tr	3.0 (0.4)	tr	5.4 (4.2)	35.0 (4.0)			1.2 (0.2)	tr	38.3 (0.3)		2.2 (0.4)	
<i>M. bovis</i>	1	2.6	5.4	7.9			1.3	2.4	36.7			5.6		36.0		2.2	
<i>M. osloensis</i>	3	6.4 (0.9)		3.6 (0.6)		1.6 (0.3)		3.1 (1.4)	19.6 (3.0)			1.6 (0.7)	3.2 (2.6)	61.0 (1.3)	tr		

^a Number of strains analyzed. The following strains were included: *M. catarrhalis* O29, G25, V31, V30, J27, LMG 11192, and LMG 5128^T; *M. canis* O18, W40, U34, P37, U33, W10, V45, V44, W4, and N7^T; *Moraxella* sp. strain LMG 1133; *M. osloensis* LMG 1043, LMG 5131^T, and LMG 6916; *M. ovis* LMG 8381^T; *M. lacunata* LMG 1009 and LMG 1020; and *M. nonliquefaciens* LMG 1010 and LMG 1011.

^b Fatty acids C_{16:1} iso-1 and C_{14:0} 3-OH could not be separated from each other by gas chromatography by using the Microbial Identification System and together were considered summed feature 3.

^c ECL, equivalent chain length.

^d The double bond position (indicated by the capital letter) is unknown.

^e Fatty acids C_{18:1} cis-11, C_{18:1} trans-9, and C_{18:1} trans-6 could not be separated from each other by gas chromatography by using the Microbial Identification System and together were considered summed feature 7.

^f The numbers in parentheses are standard deviations.

^g tr, trace (the relative percentage of the fatty acid was less than 1%).

ertheless, the results again illustrate the existence of some genotypic heterogeneity within *M. canis* strains at the rRNA gene level, but it is obvious that the three different types are more similar to each other than to the typical *M. catarrhalis* strains, strain NCTC 4103, or strains of other *Moraxella* species.

Mol% G+C. The G+C contents of *M. canis* P37, U33, and N7^T were 45.9, 47.4, and 47.5 mol%, respectively. This is within the range reported for the genus *Moraxella* (40 to 47.5 mol% [4]). The G+C contents of three *M. catarrhalis* strains (LMG 5128^T, G25, and V31) were between 43.3 and 43.7 mol%.

DNA-rRNA hybridizations. DNA-rRNA hybridization experiments in which DNAs from *M. canis* O18 and U33 and ³H-labeled 23S rRNA from *M. lacunata* ATCC 17952 were used yielded a *T_m(e)* of 75.4°C for both *M. canis* strains. Since similar *T_m(e)* values (75.6 and 75.7°C) were found by using DNAs from *M. catarrhalis* ATCC 25238^T and NCTC 4103, respectively, it could be concluded that strains O18 and U33 are members of the genus *Moraxella* and consequently members of the family *Moraxellaceae*. The fact that the *T_m(e)* values obtained with strains ATCC 25238^T and NCTC 4103 are somewhat lower than those reported previously (28) may be attributed to the gradual decomposition of the ³H-labeled rRNA during storage. Previously published DNA-rRNA hybridization studies (27, 28) demonstrated that *Moraxella* species, including *M. catarrhalis*, are not related to unequivocal members of the emended family *Neisseriaceae*. For *Moraxella* species *T_m(e)* values as low as 60 to 65°C were found with *Neisseria flavescens* rRNA (27). The available data also proved that the type strain of *N. canis* undoubtedly belongs to the *Neisseriaceae* (*T_m(e)* versus *N. flavescens* rRNA, 76.3°C). In combination with our results, it can be concluded that *M. canis* and *N. canis* are clearly separate bacterial species.

DNA-DNA hybridizations. The degrees of binding obtained by using the initial renaturation rate DNA-DNA hybridization method with DNAs from *M. canis* P37, U33, and N7^T ranged from 82 to 91%. Between these strains and strain NCTC 4103 the degrees of binding were between 52 and 61%. Low to moderate DNA homology values (degrees of binding between 9 and 38%) were found between the *M. canis* strains and *M. catarrhalis* ATCC 25238^T, G25, and V31, as well as *M. osloensis* ATCC 19961. Data obtained under stringent conditions for hybridization between dot-spotted DNAs of a variety of strains and ³²P-labeled DNA of *M. canis* O18 confirmed that strains O18, N7, U33, and U34 are more closely related to each other than to *M. catarrhalis* strains, strain NCTC 4103, and one strain each of *M. ovis*, *M. caviae*, *M. cuniculi*, *M. lacunata*, *M. nonliquefaciens*, *M. osloensis*, *Psychrobacter immobilis*, *Acinetobacter calcoaceticus*, and *Neisseria gonorrhoeae* (data not shown). These results indicate that *M. canis* strains are not particularly closely related to *M. catarrhalis* or other *Moraxella* species and thus belong to a separate species.

Taxonomic implications. Biochemical as well as genotypic heterogeneity among *M. canis* strains was observed by using several techniques. The phenotypic heterogeneity was particularly obvious when the SDS-PAGE profiles of the cellular proteins were compared. However, this technique attempts to detect differences at a rather fine taxonomic level. Ribotyping and PCR analysis of the 16S rRNA gene and the 16S-23S rRNA spacer region also revealed heterogeneity. In fact, DNA-DNA hybridization results showed that strains belonging to different ribotype groups or strains having different PCR types may still be related above the species

FIG. 4. Southern hybridization of *Hind*III-digested genomic DNAs from representative strains of *M. catarrhalis* and *M. canis* and from *Moraxella* sp. strain NCTC 4103 with a mixture of oligonucleotide probes 1, 5, and 6.

level (e.g., strains N7^T and P37, which exhibit a degree of binding of 90%). Alternatively, these techniques also revealed some common properties among the *M. canis* strains examined which were not found in the other taxa studied. The fact that *M. canis* strains constitute a single cluster is further corroborated by the fatty acid analysis results. Strains U33 and U34 are undoubtedly phenotypically and serologically the most aberrant strains. However, these strains are indistinguishable from certain other *M. canis* strains by PCR analysis and ribotyping. Moreover, strain U33 exhibits degrees of binding between 82 and 91% with strains N7^T and P37. Thus, U33 and U34 are genuine *M. canis* strains.

The combined data indicate that *M. canis* strains are not related to *M. catarrhalis* above the species level, despite the fact that *M. canis* strains phenotypically resemble *M. catarrhalis* strains (DNase-producing, gram-negative, asaccharolytic cocci) and show some serological cross-reactivity. However, serology should not be considered a highly reliable taxonomic technique. The available data also exclude the possibility of a relationship with other *Moraxella* species. From these results it can be concluded that the *M. canis* strains are a separate phenotypic and genotypic entity, different from any species described previously. Consequently, the creation of a new species for this group of organisms can be justified. As these strains can be assigned to the genus *Moraxella* on the basis of DNA-rRNA hybridization results (which also exclude the possibility of a relationship with *N. canis* or other *Neisseria* species), and since most of the isolates originate from dogs, the name *M. canis* is proposed. Also, the phenotypic and chemotaxonomic characteristics of these strains are consistent with the descriptions of the genus *Moraxella* (5) and the family *Moraxellaceae* (28).

Position of strain NCTC 4103. Catlin and Cunningham (7), Bøvre (4), and Baumann et al. (3) provided genotypic and phenotypic evidence that strain NCTC 4103 is related to *M. catarrhalis* but cannot be considered a genuine *M. catarrhalis* strain. Our data cannot unequivocally elucidate the

exact taxonomic position of this strain. The fatty acid profiles and DNA restriction patterns of strain NCTC 4103 resemble those of *M. catarrhalis* strains but are not entirely identical. No DNA-DNA homology was found between *M. catarrhalis* strains and strain NCTC 4103. However, these results are in conflict with previously reported DNA-DNA hybridization results showing a significant level of DNA

FIG. 5. Ethidium bromide-stained agarose (1.8%) gel of PCR products of the 16S-23S rRNA gene spacer region. Lanes b through h contained undigested material; lanes j through p contained material digested with *Eco*RI and *Nco*I. The following strains were used: *M. osloensis* ATCC 19961 (lanes b and j), *M. nonliquefaciens* ATCC 19975^T (lanes c and k), *M. catarrhalis* LMG 5128^T (lanes d and l), *M. catarrhalis* V31 (lanes e and m), *M. canis* N7^T (lanes f and n), *M. canis* W40 (lanes g and o), and *Moraxella* sp. strain NCTC 4103 (lanes h and p). Lanes a, i, and q contained the molecular weight marker (ϕ X 174 DNA digested with *Hae*III).

FIG. 6. Ethidium bromide-stained agarose (1.6%) gel of PCR products of the 16S rRNA gene. Lane a contained undigested material from *M. osloensis* ATCC 19961; lanes c through i contained amplicons digested with *EcoRI*; and lanes k through q contained amplicons digested with *HindIII*. The following strains were used: *M. osloensis* ATCC 19961 (lanes c and k), *M. nonliquefaciens* ATCC 19975^T (lanes d and l), *M. catarrhalis* LMG 5128^T (lanes e and m), *M. catarrhalis* V31 (lanes f and n), *M. canis* N7^T (lanes g and o), *M. canis* W40 (lanes h and p), and *Moraxella* sp. strain NCTC 4103 (lanes i and q). Lanes b, j, and r contained the molecular weight marker (λ DNA digested with *HindIII* mixed with ϕ X 174 DNA digested with *HaeIII*). Agarose gel electrophoresis of all of the undigested amplicons (data not shown) proved that the smallest fragment (ca. 280 bp) in lane e is not a result of digestion of the 16S rRNA gene of *M. catarrhalis* LMG 5128^T but is an aspecific PCR product that itself is cut by *HindIII* (lane m).

homology (59%) (28). Moreover, in this study we found significant levels of DNA homology (52 to 61%) between strain NCTC 4103 and *M. canis* strains, which suggests that strain NCTC 4103 might be a member of this species. However, in many other aspects (fatty acid profile and restriction and protein patterns) strain NCTC 4103 differs from *M. canis* strains. In conclusion, strain NCTC 4103 seems to be situated somewhere between *M. catarrhalis* and *M. canis*. In our opinion the assignment of *Moraxella* sp. strain NCTC 4103 to a species should be postponed until more unambiguous data are available.

Description of *Moraxella canis* sp. nov. *Moraxella canis* (ca'nis. L. gen. n. *canis*, of a dog). Cells are cocci (diameter, about 0.8 to 1.3 μ m) and usually occur in pairs or short chains. Gram-negative, but there may be a tendency to resist decolorization. A brown pigment may be observed on Mueller-Hinton agar. Cells are nonmotile in liquid medium. Chemoorganotrophic and aerobic. Mesophilic. Cell growth is usually observed on medium containing mineral salts and ammonium acetate, lactose bromothymol blue agar, eosine-methylene blue agar, Mueller-Hinton agar, and MacConkey agar at 37°C. Growth occurs on Mueller-Hinton agar at room temperature. No growth occurs in the presence of 6% NaCl or at 5°C. Usually indifferent to bile salts. Oxidase, catalase, extracellular DNase, butyrate esterase, and γ -glutamyl-aminopeptidase are produced. Phenylalanine deaminase, indole, urease, and carbonic anhydrase are not produced. No acid is produced from glucose. Tween 80 and starch are not hydrolyzed. Autoagglutination in physiological solution

is usually not observed. Gelatin and serum are not liquefied. No hemolysis of human and sheep blood occurs. The major fatty acids are oleic acid (C_{18:1} *cis*-9) and palmitoleic acid (C_{16:1} *cis*-9). Isoheptadecanoic acid (C_{17:0} iso) is present in moderate amounts, and myristic acid (C_{14:0}) is absent. Isolated from the saliva of dogs or cats and occasionally from humans (dog bite wounds or blood). Pathogenicity is not known. The G+C content ranges from 45.9 to 47.5 mol%. The type strain is LMG 11194 (= N7 = CCUG 8415A).

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