Moraxella caprae sp. nov., a New Member of the Classical Moraxellae with Very Close Affinity to *Moraxella bovis*

ANGELI KODJO,^{1*} TONE TØNJUM,² YVES RICHARD,¹ AND KJELL BØVRE²

Ecole Nationale Vétérinaire de Lyon, F-69280 Marcy l'Etoile, France,¹ and Kaptein W. Wilhelmsen og Frues Bakteriologiske Institutt, University of Oslo, Rikshospitalet, N-0027 Oslo 1, Norway²

Eight phenotypically homogenous *Moraxella*-like strains were isolated from the nasal flora of healthy goats. Total genomic DNA-DNA hybridization, DNA base composition determination, and genetic transformation studies were performed to determine the relationships of these bacteria to the classical moraxellae. The eight new isolates exhibited very high levels of genetic affinity to *Moraxella bovis*, as shown by quantitative and qualitative genetic transformation data, and exhibited high DNA-DNA relative binding ratios to each other (63% or more) but lower levels of DNA homology with all of the other species investigated, including the closely related classical moraxellae. Our results, combined with the general morphologic and phenotypic profiles of these organisms, indicate that they should be classified with the classical moraxellae, and we propose the name *Moraxella caprae* for them. Strain 8897 (= CCUG 33297 = NCTC 12877) is the type strain of *M. caprae*.

The group of bacteria called the classical moraxellae, which is also called the "*Moraxella lacunata* group" (5), comprises three species, *Moraxella lacunata*, *Moraxella bovis*, and *Moraxella nonliquefaciens*, which exhibit strong phenotypic and genetic affinities to each other. These organisms are rod shaped, gram negative, oxidase positive, and nonmotile. Phenotypic differentiation of these bacteria is commonly based on the ability to produce hemolysis on blood agar, the ability to liquefy gelatin and coagulated serum, and the ability to reduce nitrates (5). *M. bovis* is the cause of infectious bovine keratoconjunctivitis (5, 7, 22). *M. bovis* has also been isolated from healthy cattle and other animals, including horses (9, 10). *M. bovis* isolates obtained from cattle are characterized by hemolysis and liquefaction of coagulated serum and gelatin (4). This species is usually nitrate reduction negative. However, nitrate-positive strains have also been reported (4). *M. lacunata* exhibits all of the properties of *M. bovis* except hemolysis and nitrate reduction. This species is a causative agent of human conjunctivitis and keratitis, as well as chronic sinusitis and endocarditis (22), but it has also been isolated from healthy eyes, noses, and throats (4, 5, 18). It is most often encountered in humans, but has also been found in guinea pigs (5). *M. nonliquefaciens*, a nonhemolytic species, is regularly found to be nitrate reduction positive and gives negative reactions in tests for liquefaction of coagulated serum or gelatin. *M. nonliquefaciens* is part of the normal flora in the human upper respiratory tract and is frequently isolated from nasal cavities (4, 5, 20, 22). It has also been isolated from blood (sometimes associated with septicemia), from eyes, from cerebrospinal fluids, from lower respiratory tracts, and from other local sites (18, 20). *M. lacunata*, *M. bovis*, and *M. nonliquefaciens* are considered low-pathogenicity species that depend on reduced host resistance for invasion and production of clinical manifestations.

The three species described above may also be differentiated by other methods, such as total genomic DNA-DNA hybridization (20, 22) or quantitative genetic transformation when the high-level streptomycin or spectinomycin resistance marker is used. In DNA-DNA hybridization experiments, members of

one species usually exhibit relative binding ratios (RBRs) of about 70% or more (25). Ratios of interstrain transformation to intrastrain transformation of 0.3 to 1.0 are commonly obtained within each species (2, 3, 7, 21, 23); this ratio usually ranges from about 10^{-1} to 10^{-3} for different species of the classical moraxellae (2, 3). Qualitative transformation with mutant auxotrophic recipient strains has also been used for rapid identification of *Moraxella* species (11–13).

In recent years, we have isolated from healthy goats eight *Moraxella*-like strains that have phenotypic characteristics intermediate between those of *M. bovis* and those of *M. nonliquefaciens*. We have found previously that these goat strains (called group 2) are all nitrate reduction positive and hemolytic, but are not able to liquefy gelatin or coagulated serum (14). The results of sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) of whole proteins have also indicated that they are related to the classical moraxellae, particularly *M. nonliquefaciens* (14). The results of preliminary qualitative genetic transformation studies have confirmed these findings and that these organisms belong to the genus *Moraxella* and are very closely related to the "*M. lacunata* group."

The aim of this study was to analyze the new *Moraxella*-like strains by performing genetic transformation and total genomic DNA-DNA hybridization experiments, as well as a DNA base composition analysis, to determine their taxonomic position within the "*M. lacunata* group."

MATERIALS AND METHODS

Bacterial strains and growth conditions. The strains which we investigated are listed in Table 1. We included various *Moraxella* species and other fastidious gram-negative bacteria for comparison. *M. bovis* MB 4 and MB 21 were used as the recipient strains in a qualitative genetic transformation assay. Strains were grown on 5% human blood agar plates (Difco) and chocolate agar plates at 35°C in the presence of 5% CO₂. Defined MB medium, which was shown to support growth of *M. bovis*, was used in qualitative genetic transformation experiments (see below).

DNA-DNA hybridization. (i) DNA dot blot experiments. DNA was extracted and blotted for use in dot blot hybridization experiments essentially as described previously (22). Briefly, DNA extracted by a modification of the method of Marmur (15) was heat denatured at 95°C for 10 min, cooled on ice, and mixed with an equal volume of cold 2 M ammonium acetate. The DNA was applied to nitrocellulose filters by using a Hybri-Dot System apparatus (Bethesda Research Laboratories). Eight parallel dots (1 µg each) and a Tris-EDTA buffer control were used for each strain. The filters were washed in 6× SSC buffer (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) and then soaked in 2× Denhardt's

* Corresponding author. Mailing address: Ecole Nationale Vétérinaire de Lyon, BP 83, 1 Avenue Bourgelat, F-69280 Marcy l'Etoile, France. Phone: 33 78 87 25 91. Fax: 33 78 87 25 94.

TABLE 1. List of bacterial strains used in this study

Taxon	Strain(s) ^a	Other designation(s) ^a	Source (year isolated)	Reference(s)	Received from ^a
<i>Moraxella caprae</i> sp. nov.	8897 ^T , 8898, 88100, 88101, 88102, 88103, 88104, 88105		Goats (1988)	14	
<i>Moraxella bovis</i>	ATCC 10900 ^T CIP 649 9 NCTC 11012 ^{Tb} MB 4 ^c MB 21 ^d	ATCC 17947 ATCC 25576 ^T CIP 103741, ATCC 43759 CIP 103743, ATCC 43760	Cattle pink eye Cattle pink eye Cattle Eye of horse Mutant of strain ATCC 10900 ^T Mutant of strain ATCC 10900 ^T	2, 20 20 20 12 12	CIP
<i>Moraxella lacunata</i>	ATCC 17967 ^T A947 50136	NCTC 11011 ^T		2, 20 20	ATCC
<i>Moraxella nonliquefaciens</i>	ATCC 19975 ^T NCTC 7784	ATCC 17953	Human nasal swab	1, 2, 20 3, 5, 20	
<i>Moraxella osloensis</i>	CIP 6835 ^T 5873	ATCC 20776 ^T	Human spinal fluid	20	CIP
<i>Moraxella atlantae</i>	CIP 8225 ^T	ATCC 29525 ^T	Human blood		CIP
<i>Moraxella phenylpyruvica</i>	CIP 8227 ^T	ATCC 23333 ^T	Human blood		CIP
<i>Moraxella lincolnii</i>	CCUG 9405 ^T		Human nasopharynx	24	
<i>Moraxella (Branhamella) catarrhalis</i>	Ne 11 ^T	ATCC 23238 ^T		22	
<i>Moraxella (Branhamella) ovis</i>	199/55 ^T	ATCC 33078 ^T		22	
<i>Kingella kingae</i>	4177/66 ^T	ATCC 23330 ^T	Human nose	22	
<i>Kingella denitrificans</i>	NCTC 10995 ^T			22	
<i>Neisseria elongata</i>	6171/75			22	
<i>Neisseria weaveri</i> (formerly CDC group M5)	B5522			1	
<i>Eikenella corrodens</i>	ATCC 23834 ^T	NCTC 10596 ^T	Sputum	22	
<i>Psychrobacter immobilis</i>	NCMB 98	CCUG 9702	Gills of lemon sole		
<i>Oligella urethralis</i>	ATCC 17960 ^T		Human ear	20	
<i>Haemophilus aphrophilus</i>	9574/86		Sinusitis	21	
<i>Haemophilus paraphrophilus</i>	NCTC 10557 ^T	ATCC 29241 ^T	Paronychia	21	
<i>Pasteurella multocida</i>	646		Pig	21	
<i>Pasteurella haemolytica</i>	NVI (serotype 1)			21	
<i>Escherichia coli</i> K-12				22	

^a ATCC, American Type Culture Collection, Rockville, Md.; CIP, Collection Institut Pasteur, Paris, France; CCUG, Culture Collection, University of Göteborg, Göteborg, Sweden; NCTC, National Collection of Type Culture, London, United Kingdom; NCMB, National Collection of Marine Bacteria, Aberdeen, Scotland.

^b This organism is also called *Moraxella equi*.

^c Proline auxotroph.

^d Tryptophan auxotroph.

solution for 1 h before they were baked in a vacuum oven at 80°C for 2 h. The filters were stored dry until they were used.

(ii) **Labelling of probes.** Mechanically fragmented genomic DNA probes were labelled with [³²P]dCTP to a specific activity of 10⁷ cpm/μg of DNA by using a random priming labelling kit (Amersham International, Inc., Buckinghamshire, England) according to the manufacturer's recommendations.

(iii) **Hybridization.** Hybridization was performed by using a previously described protocol (20). The sodium salt concentration in the prehybridization and hybridization fluids was 0.1 M. The hybridization results of the dot blot experiments were obtained by performing overnight autoradiography (Hyperfilm MP; Amersham) and scintillation counting (Packard Instrument Co., Meriden, Conn.) of standard pieces of the nitrocellulose filters which were cut with a vacuum cutting device designed by us.

(iv) **Quantitation of dot blot hybridization reaction mixtures.** We determined the mean counts per minute for the eight parallel experiments minus the control counts per minute obtained with salmon sperm DNA for each of the strains. In this study the mean value obtained for the autologous strain represented a DNA homology value or RBR of 100%. The mean RBR for each strain was calculated by dividing the mean value for the strain by the mean value for the autologous reaction and multiplying the result by 100. The 95% confidence interval for the sample mean of the RBRs was estimated for each experiment (data not shown).

Determination of G+C contents of DNAs. The overall base compositions of the DNAs of the new *Moraxella* strains were determined by high-performance liquid chromatography as described by Peyret et al. (17). *M. bovis* ATCC 10900^T (T = type strain) DNA was included in the study as a control. The DNA base composition of a strain was expressed as its DNA guanine-plus-cytosine (G+C) content, which was calculated as follows: [(guanine content + cytosine content)

× 100]/(guanine content + cytosine content + adenine content + thymine content).

Genetic transformation. (i) Preparation of high-level streptomycin resistance DNA and quantitative transformation assays. The media and reagents used in transformation assay mixtures have been described previously (22). Mutants were selected for high-levels of streptomycin resistance. Briefly, a 12-h culture on a blood agar plate was harvested with broth. Then 10 to 20 blood agar plates were each inoculated with 3 drops of the suspension, the inoculum was spread with a glass triangle, and the plates were incubated at 35°C for about 5 to 6 h until microcolonies were visible. The blood agar plates were then transferred with the growth surface up onto streptomycin agar plates containing a final streptomycin concentration of 1,000 μg/ml after diffusion. These plates were incubated at 35°C for at least 4 days until colonies resistant to high levels of streptomycin appeared. Each presumed resistant colony was picked and streaked onto another blood agar plate containing 1,000 μg of streptomycin per ml to confirm the high level of streptomycin resistance. DNA was then extracted, and the DNA concentration was adjusted to 200 μg/ml. A quantitative transformation assay was performed by exposing 0.5 ml of the recipient suspension to 0.05 ml of each DNA for 20 min. After DNase treatment, 0.1-ml aliquots of each final suspension and appropriate dilutions were inoculated onto blood agar plates. These plates were preincubated for 7 h before they were placed on top of an agar layer containing a final streptomycin concentration of 50 μg/ml after diffusion. The plates were then incubated for 3 to 5 days before colonies were counted and ratios of interstrain transformation to intrastrain (autologous) transformation were calculated. None of the eight new *Moraxella* strains was found to be competent.

(ii) **Qualitative genetic transformation.** Cells of *M. bovis* MB 4 (= CIP 103741 = ATCC 43759), a proline auxotroph, and *M. bovis* MB 21 (= CIP 103743 =

ATCC 43760), a tryptophan auxotroph, were used as competent recipient cells. These two mutants were derived from *M. bovis* ATCC 10900^T following chemical mutagenesis (13) and were found to be extremely stable; their frequencies of reversion to prototrophy were less than 10^{-9} . Crude DNAs from donor strains were used to induce reversion of the two *M. bovis* auxotrophic mutant assay strains to prototrophy, which made them able to grow on an *M. bovis* defined medium without proline or tryptophan (medium MB). The composition of the medium and the method which we used were adapted from the composition of the medium and method used by Juni et al. (13). The auxotrophic mutant strains were grown overnight on a brain heart infusion agar plates at 35°C for use as recipients. Loopfuls of cell pastes of these recipient strains were placed in the six squares of another brain heart infusion agar plate. Then 50 µl of each crude transforming DNA, which was obtained by lysis at 70°C in a lysing solution (0.025% sodium dodecyl sulfate, 0.15 M sodium chloride, 0.015 M trisodium citrate; sterilized at 121°C for 15 min), was spread over the recipient cell paste in five squares. The lysing solution present in the drops diffused into the agar, whereas the high-molecular-weight DNA remained with the auxotrophic recipient cells on the agar surface and could transform some of the cells during growth when the preparations were incubated at 35°C overnight. One drop of each DNA tested was placed on a bacterium-free area that was just to the right of the square in which recipient cells were mixed with that DNA so that subsequent incubation could verify that the DNA preparation was sterile. The remaining square on the left was treated with sterile lysis solution and was used as a non-DNA-containing control. After overnight incubation at 35°C, treated and nontreated cells from each square were streaked uniformly onto suitably labeled sections of an MB medium plate, which was then incubated at 35°C for at least 48 h. Transformation results were defined as positive when more than 100 colonies were observed on an MB medium plate.

RESULTS

DNA-DNA hybridization. The results of the DNA-DNA hybridization experiments are shown in Table 2. Our DNA-DNA dot blot hybridization analysis showed that the DNAs of all eight new *Moraxella* strains exhibited mean RBR with *Moraxella* sp. strain 8897^T probe DNA ranging from 63 to 100%. Because of their high levels of homology, only one of the DNAs of these strains was used as a genomic probe to test the affinity of this group to the other *Moraxella* species and other fastidious gram-negative bacteria included in the study (Table 2).

The new *Moraxella* strains could be clearly distinguished from the other *Moraxella* species and other gram-negative bacterial species included in the study on the basis of the interspecific RBR obtained in hybridization experiments (Table 2). However, comparatively high values were obtained within the classical moraxellae group, particularly with *M. bovis* species; for example, an RBR of 42% was obtained between *Moraxella* sp. strain 8897^T probe DNA and *M. bovis* 9 filter-bound DNA. When *M. bovis* ATCC 10900^T was used as the reciprocal probe with three strains of the new *Moraxella* species, RBR ranging from 36 to 42% were obtained.

DNA base composition. The results of the DNA G+C content determination experiments are shown in Table 3. The G+C contents of the DNAs of the new *Moraxella* strains were similar, ranging from 40 to 41.5 mol%. The G+C content of the *M. bovis* ATCC 10900^T DNA included in this study was 40 mol%.

Genetic transformation. The results of the genetic transformation experiments are shown in Table 3. As in the DNA-DNA hybridization experiments, strain 8897^T was used as a representative of the new *Moraxella* species. None of the eight strains of the new *Moraxella* species was naturally competent; therefore, these organisms were used only as donors of DNA in genetic transformation experiments. All of the strains were tested in qualitative genetic transformation experiments in which *M. bovis* auxotrophic mutants MB 4 and MB 21 were the recipients. In the quantitative genetic transformation experiments, DNA from a streptomycin-resistant mutant of representative strain 8897^T was used as the donor DNA for the new *Moraxella* species. Because this new entity exhibited pheno-

TABLE 2. RBR from DNA-DNA hybridization experiments

Filter DNA source	Mean RBR with ³² P-labeled DNA from:	
	<i>M. caprae</i> 8897 ^T	<i>M. bovis</i> ATCC 10900 ^T
<i>Moraxella caprae</i> sp. nov. strains		
8897 ^T	100	42
8898	66	38
88100	63	36
88101	89	ND ^a
88102	77	ND
88103	>100	ND
88104	93	ND
88105	87	ND
<i>Moraxella bovis</i> strains		
ATCC 10900 ^T	15–25 ^b	100
CIP 64.9	20–29 ^b	>100
9	42	>100
NTCT 11012 ^T (<i>M. equi</i>)	20–21 ^b	47
<i>Moraxella lacunata</i> strains		
ATCC 17967 ^T	13	
A947	3	
50136	5	
<i>Moraxella nonliquefaciens</i> strains		
ATCC 19975 ^T	6–8 ^b	
NCTC 7784	4	
<i>Moraxella osloensis</i> strains		
CIP 6835 ^T	2	
5873	3	
<i>Moraxella atlantae</i> CIP 8225 ^T	4	
<i>Moraxella phenylpyruvica</i> CIP 8227 ^T	<1	
<i>Moraxella lincolnii</i> CCUG 9505 ^T	1	
<i>Moraxella</i> (<i>Branhamella</i>) <i>ovis</i> 199/55 ^T	4–13 ^b	
<i>Moraxella</i> (<i>Branhamella</i>) <i>catarrhalis</i> Ne11 ^T	1	ND
Other gram-negative strains		
<i>Kingella kingae</i> 4177/66 ^T	<1	
<i>Kingella denitrificans</i> NCTC 10995 ^T	<1	
<i>Neisseria elongata</i> 6171/75	<1	
<i>Neisseria weaveri</i> B5522	<1	
<i>Oligella urethralis</i> ATCC 17960 ^T	<1	
<i>Eikenella corrodens</i> ATCC 23834 ^T	<1	
<i>Psychrobacter immobilis</i> NCMB 98	<1	
<i>Haemophilus aphrophilus</i> 9574/86	<1	
<i>Haemophilus paraphrophilus</i> NCTC 10557 ^T	<1	
<i>Pasteurella multocida</i> 646	<1	
<i>Pasteurella haemolytica</i> NVI	<1	
<i>Escherichia coli</i> K-12	<1	

^a ND, not determined.

^b Range of values from three sets of experiments.

typic properties intermediate between those of *M. bovis* (hemolysis positive) and those of *M. nonliquefaciens* (serum and gelatin liquefaction negative), only *M. bovis* and *M. nonliquefaciens* were used as recipient strains. All of the strains of the new *Moraxella* species assayed were found to be positive in qualitative genetic transformation experiments performed with both *M. bovis* MB 4 and MB 21 (Table 3). They could also be grown on MB medium.

Quantitative genetic transformation assays performed with *M. bovis* ATCC 10900^T as the recipient and strain 8897^T DNA as the donor DNA gave high transformation values, which ranged from 5×10^{-2} to 4.2×10^{-1} , whereas the ratio of interspecies transformation to intrastrain transformation obtained with reference to *M. nonliquefaciens* NCTC 7784 was as low as 1×10^{-3} (Table 3).

TABLE 3. DNA base compositions and affinities of *M. caprae* strains to other *Moraxella* species as determined by qualitative and quantitative genetic transformation

DNA donor	G+C content (mol%) ^a	Qualitative transformation with the following recipient strains:		Quantitative transformation ratios with the following recipient strains:		
		<i>M. bovis</i> MB 4	<i>M. bovis</i> MB 21	<i>M. bovis</i> ATCC 10900 ^T	<i>M. bovis</i> 9	<i>M. nonliquefaciens</i> NCTC 7784
<i>M. bovis</i>	41–44.5					
<i>M. bovis</i> MB 4		– ^b	+			
<i>M. bovis</i> MB 21		+	–			
<i>M. bovis</i> ATCC 10900 ^T	40	+	+	1	7 × 10 ^{–1}	1.8 × 10 ^{–3c}
<i>M. bovis</i> 9		+	+		1	
<i>M. bovis</i> NCTC 11012 ^T (<i>M. equi</i>)		w ^d	w ^d	8 × 10 ^{–2}		
<i>M. nonliquefaciens</i>	40–44					
<i>M. nonliquefaciens</i> NCTC 7784		–	w	2.6 × 10 ^{–3}		1
<i>M. caprae</i>	40–41.5					
<i>M. caprae</i> 8897 ^{T e}	41.5	+ ^f	+ ^f	5 × 10 ^{–2} –4.2 × 10 ^{–1}	1.3 × 10 ^{–1}	1 × 10 ^{–3}

^a The data for *M. bovis* ATCC 10900^T and *M. caprae* 8897^T are from this study. All other G+C content data are from references 3, 4, and 17.

^b –, negative; +, positive; w, weak.

^c Data from reference 1.

^d Data from reference 12.

^e Only one streptomycin-resistant mutant was used as a representative of *M. caprae*.

^f DNAs from all *M. caprae* strains were positive.

DISCUSSION

The eight new bacterial strains which we investigated were recovered from the nasal cavities of healthy goats during a survey conducted in the Rhône-Alpes region of France (14). These organisms were provisionally assigned to the genus *Moraxella* on the basis of their Gram reaction, cellular morphology, absence of motility, and reactions in a panel of conventional biochemical and hydrolytic enzymatic tests (14). Because they exhibited most of the properties of *M. nonliquefaciens*, the occurrence of hemolysis on blood agar led us originally to consider these goat isolates hemolytic variants of *M. nonliquefaciens*, as suggested by their SDS-PAGE protein patterns. The results of SDS-PAGE were analyzed by an unweighted average-linkage method based on the DICE coefficient and showed that the goat isolate group was quite homogeneous and did not cluster with strains of *M. bovis*, *M. lacunata*, and *M. nonliquefaciens* (the closest neighbor) (14). However, the goat isolates could also be considered variants of *M. bovis* which had lost the ability to liquefy serum or gelatin. Therefore, it was important to define by methods other than conventional phenotypic characterization methods the genetic affinities of these isolates (formerly group 2) with respect to members of the classical moraxellae group, particularly their relationships to *M. bovis*, a nonhemolytic variant of *M. bovis* (*Moraxella equi*), and *M. nonliquefaciens*. Genetic transformation in which the high-level streptomycin or spectinomycin resistance marker is used has been shown by Henriksen and Bøvre (7), Bøvre (2, 3), Tønnum et al. (20, 21, 23), and Høke and Vedros (8) to be a powerful and specific tool for species differentiation in the genus *Moraxella*. The usefulness of genetic transformation for *Moraxella* species differentiation was further confirmed by Tønnum et al. (20, 22) in experiments in which they used DNA-DNA dot-blot hybridization, multilocus enzyme electrophoresis, and pilin-specific PCR probes. Previous findings (i.e., SDS-PAGE results), supplemented with the results of this study, clearly indicate that the new isolates constitute a cluster of strains different from *M. bovis* in cattle and *M. nonliquefaciens*. These organisms exhibited high RBR with each other (63% or more), but distinctly lower RBR with all of the other species investigated, including the closely related classical moraxellae, indicating that they belong to a new ge-

netic entity in the genus *Moraxella*. The base compositions of their DNAs were very similar to the base compositions determined for the genus *Moraxella* (4, 5, 19). The ratios of inter-strain transformation to intrastrain transformation were also consistent with the description of a separate, quite homogeneous new cluster with very close affinities to *M. bovis*. On the basis of this information, we propose that these strains belong to a new *Moraxella* species, *Moraxella caprae*. This name reflects the origin of the organisms (goats). Strains of a *Moraxella* species named "*M. caprae*" have been described previously by Pande and Sekariah (16). However, the description of caprine isolates published by these authors is compatible with typical *M. bovis* isolates. The species described by Pande and Sekariah cannot be studied further because representative strains are not extant.

The close affinity of *M. caprae* sp. nov. to *M. bovis* raises a question concerning the influence of the host (and habitats in general) on bacteria and the adaptation of bacteria to these habitats. In many cases, adaptation of bacteria to their habitats induces significant phenotypic, serologic, and/or pathogenetic modifications that result from minor modifications in genomes which usually are not detectable by total DNA-DNA hybridization and can be detected only at the DNA sequence level. *Neisseria gonorrhoeae*, which is found mainly in human genitourinary tracts, and *Neisseria meningitidis*, which is most often found in human oral cavities, are typical examples of such adaptation. These species exhibit levels of DNA-DNA homology that are greater than 93% (8), which is consistent with the genomic definition of a bacterial species (25). However, on the basis of their specific habitats and patterns of pathogenicity, they are still considered two separate species. Another example is *M. bovis* and related organisms; typical *M. bovis* is found in cattle, *M. equi* is found in horses (9, 10), and *M. caprae* sp. nov. is found in goats. In this case, although the three taxa are genetically closely related, as shown by genetic transformation data and, to some extent, by DNA-DNA hybridization data (22; this study), *M. equi* is still nonhemolytic and *M. caprae* sp. nov. is nonproteolytic, in contrast to *M. bovis* strains isolated from cattle. In previous experiments (22), an RBR as high as 92% was found between *M. equi* NCTC 11012 and *M. bovis* ATCC 10900^T when ATCC 10900^T was the probe, but an RBR

of only 47% was obtained in this study. In general, Tønnum et al. obtained higher RBR values than the values found in this study. The less stringent hybridization conditions which were used in the experiments of Tønnum et al. could explain the discrepancies. However, *M. equi* appeared to be more closely related to *M. bovis* than *M. caprae* is in both sets of experiments.

There is still some controversy concerning whether the three previously described classical *Moraxella* species (*M. lacunata*, *M. bovis*, and *M. nonliquefaciens*) should be placed in a single species, as they are particularly closely related (6, 20, 22). On the other hand, recent enzyme genotype, hybridization (RBR), and transformation ratio data have demonstrated that strains of these species still appear to be separate entities. The data even indicate that the previous designations *M. lacunata* and *Moraxella liquefaciens* should be retained to distinguish the two entities within the current taxon *M. lacunata* (22). Phylogenetic studies in which 16S ribosomal DNA sequence analysis is used would help us understand more about the evolution of these groups of bacteria and would help us make a final decision concerning the taxonomy and species of the genus *Moraxella*, including *M. caprae*.

Description of *Moraxella caprae* sp. nov. *Moraxella caprae* (ca'prae. L. gen. n. *caprae*, of a goat). Phenotypic characteristics are described in reference 14. Cells are gram-negative, medium to large straight rods with a tendency to grow as diplobacilli or in short chains. They are nonmotile, aerobic, and grow well at temperatures between 30 and 37°C. Colonies are greyish white, smooth, and variable in size. Colonies that are 1 to 1.5 mm in diameter after 18 h are smooth and convex; colonies become large and flat and are surrounded by a wide area of hemolysis after 36 to 48 h of incubation in an aerobic atmosphere with or without CO₂ on 5% sheep or human blood agar plates. Weak growth under the same incubation conditions occurs on nutrient agar. The oxidase and catalase reactions are strongly positive. All strains hydrolyze Tween 80 and tributyrin and reduce nitrate. No acid is produced from carbohydrates. All strains are nonproteolytic (Loeffler slant and gelatin negative). Indole and urease reactions are also negative. The main traits that distinguish *M. caprae* from *M. bovis* are reduction of nitrate and absence of proteolysis. Hemolysis on human or sheep blood agar is a clear-cut feature which differentiates *M. caprae* from all other *Moraxella* species. The DNA G+C content is 40 to 41.5 mol%. The clinical significance of this organism is unknown. Strain 8897 (= CCUG 33297 = NCTC 12877) is the type strain; it was isolated in 1988 from the nasal cavity of a healthy goat.

ACKNOWLEDGMENTS

We are indebted to Eva Løvstad and Hélène Meugnier for excellent technical assistance.

This work was supported by a grant from the Anders Jahres Foundation to T.T. and by an FEMS fellowship to A.K.

REFERENCES

- Andersen, B. J., A. G. Steigerwalt, S. P. O'Connor, D. G. Hollis, R. S. Weyant, R. E. Weaver, and D. J. Brenner. 1993. *Neisseria weaveri* sp. nov., formerly CDC group M-5, a gram-negative bacterium associated with dog bite wounds. *J. Clin. Microbiol.* 31:2456-2466.
- Bøvre, K. 1965. Studies on transformation in *Moraxella* and organisms assumed to be related to *Moraxella*. III. Quantitative streptomycin resistance transformation between *Moraxella bovis* and *Moraxella nonliquefaciens* strains. *Acta Pathol. Microbiol. Scand.* 63:42-50.
- Bøvre, K. 1965. Studies on transformation in *Moraxella* and organisms assumed to be related to *Moraxella*. V. Streptomycin resistance transformation between serum-liquefying, nonhaemolytic moraxellae, *Moraxella bovis* and *Moraxella nonliquefaciens*. *Acta Pathol. Microbiol. Scand.* 65:435-449.
- Bøvre, K. 1984. Genus II. *Moraxella* Lwoff 1939, 173 emend. Henriksen and Bøvre 1968, 391^{AL}, p. 296-303. In N. R. Krieg and J. B. Holt (ed.), *Bergey's manual of systematic bacteriology*, vol. 1. The Williams & Wilkins Co., Baltimore.
- Bøvre, K., and N. Hagen. 1980. The family *Neisseriaceae*: rod-shaped species of the genera *Moraxella*, *Acinetobacter*, *Kingella*, and *Neisseria*, and the *Branhamella* group of cocci, p. 1506-1529. In M. P. Starr, H. G. Stolp, H. G. Trüper, A. Balows, and H. G. Schlegel (ed.), *The prokaryotes. A handbook on habitats, isolation, and identification of bacteria*, vol. 2. Springer-Verlag, Berlin.
- Catlin, B. W. 1991. *Branhamaceae* fam. nov., a new proposed family to accommodate the genera *Branhamella* and *Moraxella*. *Int. J. Syst. Bacteriol.* 41:320-323.
- Henriksen, S. D., and K. Bøvre. 1968. The taxonomy of the genera *Moraxella* and *Neisseria*. *J. Gen. Microbiol.* 51:387-392.
- Hoke, C., and N. A. Vedros. 1982. Taxonomy of the *Neisseriaceae*: deoxyribonucleic acid base composition, interspecific transformation, and deoxyribonucleic acid hybridization. *Int. J. Syst. Bacteriol.* 32:57-66.
- Hughes, D. E., and G. W. Pugh. 1970. Isolation and description of a *Moraxella* from horses with conjunctivitis. *Am. J. Vet. Res.* 31:457-459.
- Huntington, P. J., P. J. Coloe, J. D. Bryden, and F. MacDonald. 1987. Isolation of a *Moraxella* sp. from horse with conjunctivitis. *Aust. Vet. J.* 64:118-119.
- Juni, E. 1974. Simple genetic transformation assay for rapid diagnosis of *Moraxella osloensis*. *Appl. Microbiol.* 27:16-24.
- Juni, E., G. A. Heym, M. J. Maurer, and M. L. Miller. 1987. Combined genetic transformation and nutritional assay for identification of *Moraxella nonliquefaciens*. *J. Clin. Microbiol.* 25:1691-1694.
- Juni, E., G. A. Heym, and R. D. Newcomb. 1988. Identification of *Moraxella bovis* by qualitative genetic transformation and nutritional assays. *Appl. Environ. Microbiol.* 54:1304-1306.
- Kodjo, A., A. Moussa, E. Borges, and Y. Richard. 1993. Identification of *Moraxella*-like bacteria isolated from caprine and ovine nasal flora. *J. Vet. Med. Ser. B* 40:97-104.
- Marmur, J. 1961. A procedure for the isolation of deoxyribonucleic acid from micro-organisms. *J. Mol. Biol.* 3:208-218.
- Pande, P., and P. C. Sekariah. 1960. A preliminary note on the isolation of *Moraxella caprae* nov. sp. from an outbreak of infectious keratoconjunctivitis in goats. *Curr. Sci.* 29:276-277.
- Peyret, M., J. Freney, H. Meugnier, and J. Fleurette. 1989. Determination of G+C content using high-performance liquid chromatography for the identification of staphylococci and micrococci. *Res. Microbiol.* 140:467-475.
- Ringvold, A., E. Vik, and L. S. Bevanger. 1985. *Moraxella lacunata* isolated from epidemic conjunctivitis among teenaged females. *Acta Ophthalmol.* 63:427-431.
- Rossau, R., A. Vanlandschoot, M. Gillis, and J. De Ley. 1991. Taxonomy of *Moraxellaceae* fam. nov., a new bacterial family to accommodate the genera *Moraxella*, *Acinetobacter*, and *Psychrobacter* and related organisms. *Int. J. Syst. Bacteriol.* 41:310-319.
- Tønnum, T., G. Bukholm, and K. Bøvre. 1989. Differentiation of some species of *Neisseriaceae* and other bacterial groups by DNA-DNA hybridization. *Acta Pathol. Microbiol. Immunol. Scand.* 97:395-405.
- Tønnum, T., G. Bukholm, and K. Bøvre. 1990. Identification of *Haemophilus aphrophilus* and *Actinobacillus actinomycetemcomitans* by DNA-DNA hybridization and genetic transformation. *J. Clin. Microbiol.* 28:1994-1998.
- Tønnum, T., D. A. Caugant, and K. Bøvre. 1992. Differentiation of *Moraxella nonliquefaciens*, *M. lacunata*, and *M. bovis* by using multilocus enzyme electrophoresis and hybridization with pilin-specific DNA probes. *J. Clin. Microbiol.* 30:3099-3107.
- Tønnum, T., N. Hagen, and K. Bøvre. 1985. Identification of *Eikenella corrodens* and *Cardiobacterium hominis* by genetic transformation. *Acta Pathol. Microbiol. Immunol. Scand. Sect. B* 93:386-394.
- Vandamme, P., M. Gillis, M. Vancanneyt, B. Hoste, K. Kersters, and E. Falsen. 1993. *Moraxella lincolnii* sp. nov. isolated from the human respiratory tract and reevaluation of the taxonomic position of *Moraxella osloensis*. *Int. J. Syst. Bacteriol.* 43:474-481.
- Wayne, L. G., D. J. Brenner, R. R. Colwell, P. A. D. Grimont, O. Kandler, M. I. Krichevsky, L. H. Moore, W. E. C. Moore, R. G. E. Murray, E. Stackebrandt, M. P. Starr, and H. G. Trüper. 1987. Report of the Ad Hoc Committee on Reconciliation of Approaches to Bacterial Systematics. *Int. J. Syst. Bacteriol.* 37:463-464.